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The Removal of Cu (II) and Pb (II) on Wastewater using Microbial Fuel Cells (MFCs) with Graphite/Chitosan Electrode

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Abstract

Heavy metal contamination in water has become a major problem in the environment as a result of very rapid industrial development. The presence of heavy metal contamination is becoming toxic, carcinogenic, and able to harm living organisms in ecosystem. Various methods have been applied for reducing heavy metals in water with high efficiency results, but most of them are still require complicated equipment and procedure. Microbial Fuel Cell (MFC) is a new technology for reducing heavy metals using biosorption activity of microorganisms. This study aimed at analyze the concentrations reduction of Cu (II) and Pb (II) using MFC on river sediments and sludge substrate. At the initial stages, graphite/chitosan electrodes were prepared and characterized. Subsequently, the exoelectrogenic microorganisms in the substrate were identified by the Gram staining method and biochemical tests. The processes were carried out in MFC dual chamber reactor with open circuit system for 120 hours. Heavy metal concentrations were analyzed using Atomic Absorption Spectrometry (AAS) periodically at wavelengths 250 nm (Cu) and 283 nm (Pb). COD (Chemical Oxygen Demand) removal, power density, DO (Dissolved Oxygen), and pH were also analyzed. The concentration of Cu (II) metal experienced the biggest decrease in wetland mud substrate that was 75%, followed by Pb (II) which was 45%. Whereas on the river sediment substrate, the concentration of Pb (II) experienced the largest decrease of 81%, followed by Cu (II) of 67%. In addition, measurements of power density values on both substrates were tend to be fluctuated.

Keywords: *heavy metal, MFC, biosorption, biosorption, graphite/chitosan electrodes*

Introduction

Heavy metal pollution has spread to all parts of the world due to the rapid development of the industry (Singh, 2001). In general, several kinds of heavy metal contamination produced by industry wastewater are Pb, Cr, Cu, Ni, Zn, Cd, and Hg (Gottsching and Pakarinen, 2000). These metals can be a threat to human health and the environment because of their non-biodegradable, toxic and carcinogenic properties and their accumulation in the food chain. Various methods of reducing heavy metals have been carried out, such as chemical precipitation, membrane filtration, ion exchange, coagulation, and inverse osmosis have been widely developed (Fu and Wang, 2011). Although the advantages of this method are high efficiency, expensive and sophisticated equipment is also needed, and requires considerable energy and chemicals (Atkinson et al, 1998). A biotechnological approach using bacteria is an alternative that can be done for the future because technically and economically it is very profitable (Malik, 2004).

Microbial Fuel Cell (MFC) is one of the prospective alternative technologies to be developed in neutralizing the amount of heavy metals, while producing electricity. MFC is a device that uses active microorganisms (bacteria) as biocatalysts in anodes with anaerobic processes to produce bioelectricity (Logan et al, 2006). MFCs can be run using microorganisms to produce electricity from organic materials. MFC has several advantages, such as resistance to poisoning and can oxidize organic matter with high COD (Catal et al, 2008).

Several important studies have been carried out regarding the reduction by MFC in various types of heavy metals, such as Cu, Se, V, Au, As, Zn, Cd, Mo, Sr, Ba, and Cr with various types of organic substrates. In a study conducted by Jiang et al (2013) it was successful in reducing eight types of heavy metals: 97.8% selenium, 96.8% barium, 94.7% strontium, 81.3% zinc, 77.1% molybdenum, 66.9% copper, 44.9% chromium, and 32.5% lead in the MFC device used a tailings oil sand substrate to produce a current density of 392 ± 15 mW/m^2. In addition, the research conducted by Abourached (2014) in reducing Cd and Zn metals using a single-chamber MFC and has a reduction efficiency of 96% Cd and 97% Zn with a current density of 3.6 W/m^2.

The choice of electrode type is one of the important design stages in the preparation of MFC devices. Graphite is a material commonly used in electrodes because it has high electrical conductivity, large surface areas, good

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electrocatalytic activity, and low production costs. In addition, the use of graphite in MFCs can maintain current densities six times higher than copper (Geim. A. K. 2009). The need for an effort to modify graphite in order to improve the performance of the electric power generation. Chitosan is a polysaccharide with a second abundance in nature after cellulose derived from shrimp shells or crabs. Chitosan has several beneficial properties such as, good adhesion, high permeability in electrolyte and non-electrolyte water, good mechanical strength, film-forming ability, and is a good matrix for enzymes and immobilization of biomachromolecules.

In this study, the design of Microbial Fuel Cell (MFC) was built in a dual chamber using graphite/chitosan electrodes. Organic substrate consisting of lowland rice and river sediments to determine the optimum reduction efficiency in heavy metals Cu (II) and Pb (II). In addition, operating temperature, Dissolved Oxygen (DO) levels, power density, COD, and the presence of types of bacteria will also be identified.

Materials and Methods

Chemicals and Apparatus

The materials used in this study are wetland mud samples, Tanah Mas Semarang River sediment samples, distilled water, graphite, phosphoric acid 85%, HCl 0.1 M, NaOH 0.05 M, eucalyptus glue (FOX), chitosan (technical grade, obtained from CV. ChiMultiGuna, DDA 97%), acetic acid, Agar (Swallow), NaCl (Merck), $PbNO_3$ (Merck), $CuCl_2$ (Merck), $KMnO_4$ (Merck), Glucose (Merck), KNO_3 (Merck), concentrated H_2SO_4 (Merck), KH_2PO_4 (Merck), high digestion solutions. While the equipment used in this study are, aluminum plate electrodes (2.5 cm × 1.3 cm × 0.5 mm), salt bridges made of silicon with a length of 7 cm and a diameter of 1.5 cm, 250 mL glass compartments, Multimeter (Heles UX 369 TR), cable, alligator clamp, brush, glue gun, solder, Regulated Power Supply (MONTANA), Eikmann Grab, digital scales (APTK461), blenders, Atomic Absorption Spectrophotometry (Buck Scientific-210VGP), UV Spectrophotometer-Vis, and Dissolved Oxygen (DO) Meters (WalkLab), and pH meters.

MFC Preparation

In this study a microbial fuel cell with two compartments (dual-chamber) was used. Anode and cathode compartments are made using a cylindrical container with a volume of 250 mL. Anode is kept anaerobic (closed-air) while the cathode is conditioned in contact with air (air-cathode). The cathode compartment contains 0.5 M $KMnO_4$ solution. While the anode compartment contains a mixture of organic substrates with heavy concentrations of certain Pb (II) and Cu (II) metals. The salt bridge used originates from NaCl and KCl 1 M. Operation is maintained at room temperature. Both compartments are isolated with aluminum foil to reduce the intensity of light exposure. The reactor is operated at a constant resistance, which is 600 Ω.

MFC Performance Characterization

Performance parameters analyzed in the experiment are COD (Chemical Oxygen Demand). The metabolic activity of bacteria on organic substrates can be analyzed through the percentage reduction in COD. The COD test was carried out according to the SNI 6989.2: 2009 method, which was preceded by the addition of 98% sulfuric acid solution (w/w) to the substrate solution then analyzed by UV-Vis spectrophotometer at a wavelength of 660 nm. Biosorption activity of microorganisms were analyzed using Atomic Absorption Spectrometry (AAS).

Result and Discussion

Microorganism Analysis

Identification of bacterial species was carried out to determine the concentration of microorganism species contained in organic sludge substrate. The microorganisms that are expected in the MFC process are species of ecsoelectrogenic bacteria, namely E. coli, Clostridium sp., Micrococcus sp., And Bacillus sp. with a minimum concentration of 104 CFU / mL each. The test was carried out by the Gram staining method and biochemical tests according to SNI 2897: 2008.

The location of rice field sampling is on Jalan R. Martodinata, Semarang (6.9591° South 110.41014° East). While the location of river sediment retrieval is in the downstream area, Tanah Mas River, Semarang (6.981462° South 110.381534° East). Both locations are located not far from the industrial area in Semarang City which is considered to have a high level of pollution. The results of bacterial testing can be seen in Table 1.

Table 1. Microorganism Identification Results.

Organic Substrate	DO (mg/L)	Microorganism Identification Results			
		Clostridium sp.	*Bacillus sp.*	*E. coli*	*Micrococcus sp.*
Wetland Mud	4,7	-	+	+	-
Sediment	7,9	+	+	+	-

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Based on Table 1. the presence of bacteria that can be tested in river sediment sources is *Escherichia coli*, while in soil sources *Clostridium sp*. Based on the test results, it is known that the bacteria *Escherichia coli* and *Clostridium sp* belong to the phylum Proteobacteria and phylum Firmicutes. Protobacteria phylum is a gram-negative type bacterium and is facultative anaerobic, which that bacteria can live in oxygen conditions that are available or not, this is in line with the running process carried out on the MFC reactor, the anaerobic process. *Clostridium sp* belongs to the phylum Firmicutes, a Gram-positive type of bacteria. This type is considered beneficial in the formation of electron-active (Wrighton et al., 2011) and is obligate anaerobic, meaning that the bacterium Clostridium sp does not need oxygen for its life and if there is oxygen the bacteria will die.

Morphology Analysis of Electrode Material

Microstructure observations were also observed using a Scanning Electron Microscope with magnifications 1000, 3000, 5000, and 10000 times. Observations were made at the Universitas Diponegoro Integrated Laboratory. The results of observations using SEM can be seen in Figure 2.

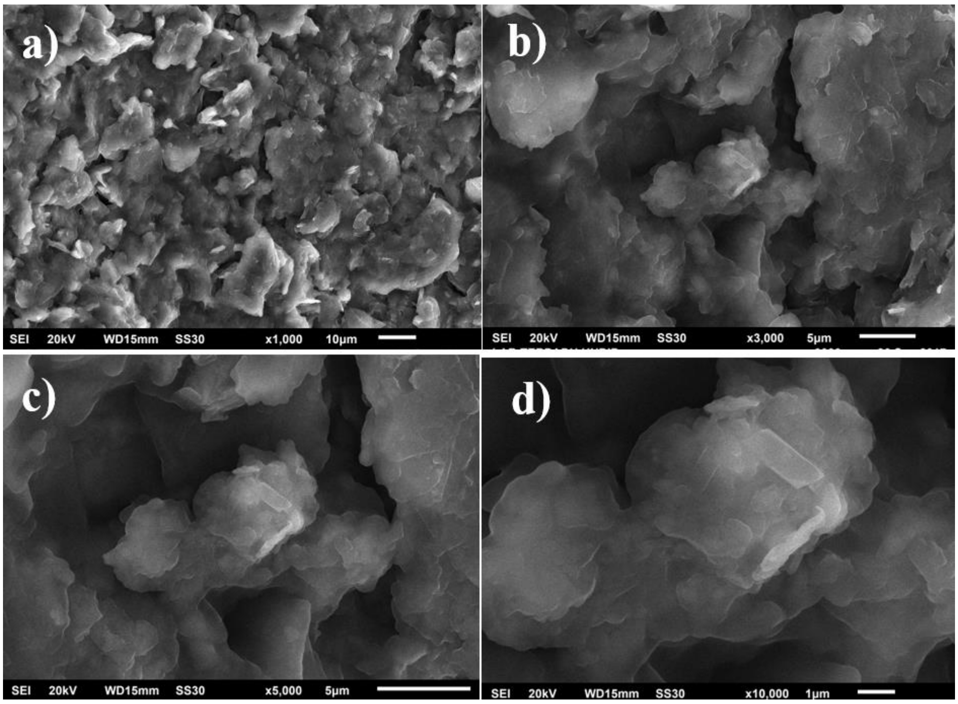

Figure 1. Morphological analysis of graphite/chitosan electrode with various magnification a) 1000 b) 3000 c) 5000 d) 10000 times.

Based on the Fig.1, the layered structure of graphite was clearly seen in Fig. 1a. Chitosan covered these layers which was the expected results. The chitosan modification made the graphite layer become softer. This results also confirmed by Congur et al. (2019), where The appearance of chitosan modified graphite morphology for electrode applications showed similar results.

Power Density and Biosorption Activity

The method for testing changes in Cu metal is based on SNI 6989.6: 2009. Changes in Cu metal concentrations in organic substrate paddy fields and sediments are shown in the Fig. 3. Based on the graph, it can be seen that the concentration of Cu metal tends to decrease in organic substrate of wetland mud after 24 hours but has the biggest increase that occurs at 96 hours which is equal to 49 ppm. Whereas the river sediment substrate tends to always increase until the 96th hour. This can be caused by the condition of the experimental media which is concentrated due to evaporation of diluents or distilled water. Cu levels will likely decrease due to dilution, whereas if there is water evaporation Cu levels will increase due to concentration (Komari, 2013).

From the graph above, it can be seen that there is instability in current density in all three types of treatment. In all three types of treatment, power density tends to increase until the 96th hour and the largest value occurs in the Cu metal treatment that is equal to 142.69 mW/m^2. This is consistent with the study of Guerrero-Larrosa (2010) which explains that at the beginning of the measurement, the substrate concentration is still sufficient to increase the speed of the electrochemical process in MFC. The increased speed at the start of measurement is related to the activity of breaking down complex organic matter by bacteria. The longer, metabolic products will cause a reduction in electricity

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production. This happens until the metabolic rate and oxidation rate at the anode are in equilibrium. This situation makes the electrical energy continues to be stable until the substrate concentration decreases.

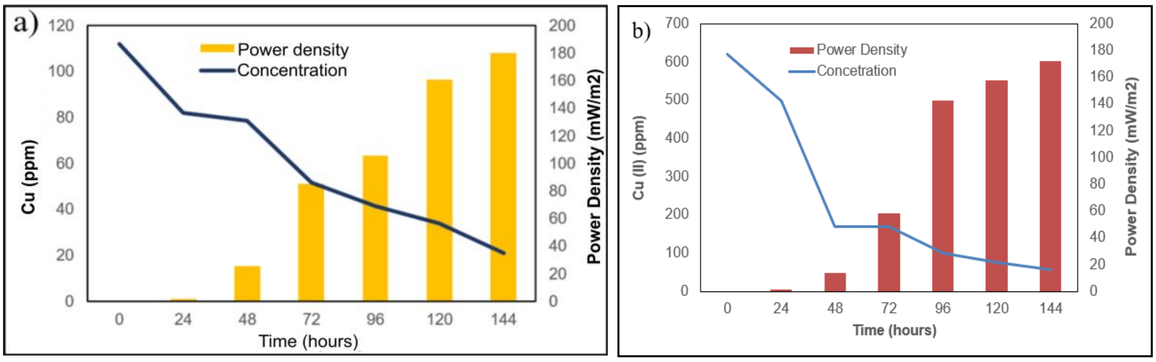

Figure 2. Cu (II) biosorption activities in, a) Wetland mud (MFCW-Cu) and b) River Sediments (MFCS-Cu).

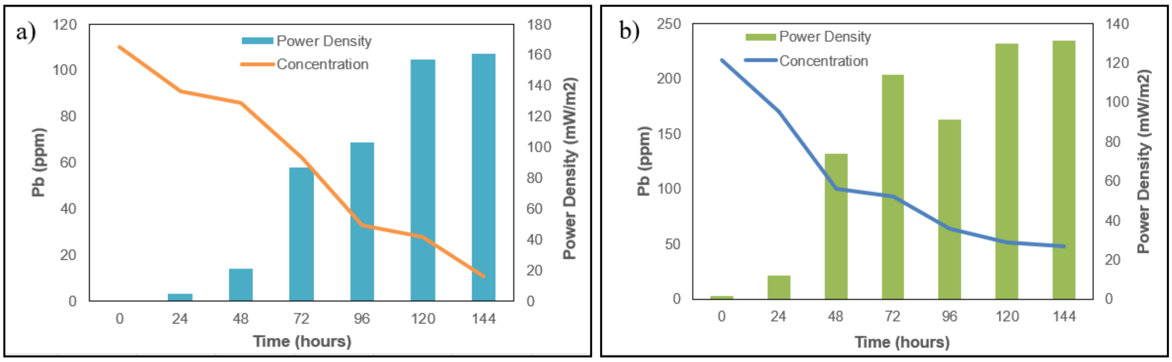

Figure 3. Pb (II) biosorption activities in, a) Wetland mud (MFCW-Pb) and b) River sediments (MFCS-Pb).

Based on these graphs, it can be seen that the concentration of Pb metal tends to decrease in the organic substrate of wetland mud until the 96th hour. Whereas there was a decrease in Pb metal concentration at the 48th hour, an increase at the 72nd hour, and decreased again at the 96th hour. The instability of Pb metal concentrations may be due to the condition of the experimental media which is concentrated due to evaporation of diluents or distilled water. Pb levels are likely to decrease due to dilution, whereas if there is water evaporation Pb levels will increase due to concentration (Komari, 2013).

From the graph above, it can be seen that there tends to be an increase in current density over 5 days. The decrease in power density occurs due to bacterial activity in the anode which can form a long time to form biofilms on the surface of the electrode (Kim et al, 2007; Nevin et al, 2008). The formation of biofilms can lead to increased obstacles in the anode (Zahara, 2011) and can cause a decrease in power density (Kim et al, 2007). The efficiency of electron transfer from bacteria to the electrode is proportional to the number of bacteria that come into contact with the electrode (Lee et al, 2010). If the surface of the electrode is already filled with biofilms, the amounts of electrons that can be transferred to the electrode will be small, resulting in a decrease in power density.

COD Removal Efficiency

Besides generating electricity, MFC can also be used for wastewater treatment. Chemical Oxygen Demand (COD) which is one the wastewater indicators, can be determined by comparing the values before and after operation. A higher COD removal efficiency indicates higher wastewater potential to be used for MFC operation. Table 1. Shows the COD removal efficiencies of all various conditions.

Table 1. COD Removal Efficiency during MFC Operation for Different Substrate and Heavy Metals.

	MFCW-Cu	**MFCS-Cu**	**MFCW-Pb**	**MFCS-Pb**
COD before operation (mg/L)	762.34	212.34	172.34	355.67
COD after operation (mg/L)	55.67	69.00	25.67	62.34
ΔCOD removal (mg/L)	706.67	143.34	146.67	293.33
COD removal efficiency (%)	92.70	67.50	85.11	82.47

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Based on the Table 1, it can be seen that the efficiency of COD reduction in wetland mud has a tendency to be higher than that of wetland muds. The highest efficiency is found in wetland mud with Cu metal, which is 92.7%. This is caused by the higher number of organisms that live in dynamic aquatic waters than static aquatic waters (Latief, 2003). The high decrease in COD efficiency can also be influenced by bacterial metabolic activity on organic substrates (Logan, 2006). The higher the bacterial growth at the anode, the higher the probability of electrons and protons that are being generated by the bacteria.

Conclusion

The biggest decrease in Cu (II) metal concentration in the organic substrate of wetland mud is 35% while in organic sediment the river sediment tends to always increase until the 96th hour. The decrease in the concentration of metal Pb (II) is greatest in organic substrate of wetland muds which tends to decrease until the 96th hour but in organic substrate the river sediment tends to fluctuate. Electricity production which is based on the value of power density tends to increase in the type of rice soil and sediment substrate. The highest power density value of river sediment substrate occurs in Cu (II) metal. Whereas the highest value of power density substrate of wetland mud in Pb (II) metal. The greatest value of COD reduction efficiency is found in the variable degradation of Cu metal with a river sediment substrate of 92.7%. For further research, it is recommended to conduct absorbance testing with a certain number of repetitions to meet statistical requirements. In addition, it is also advisable to test salinity, Optical Density (OD), and nutrient content (C, N, P) in each sample.

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Lembar Tanya Jawab

Moderator : Wibiana Wulan Nandari (UPN "Veteran" Yogyakarta)
Notulen : Yuli Ristianingsih (UPN "Veteran" Yogyakarta)

1. Penanya : Wibiana Wulan Nandari (UPN "Veteran" Yogyakarta)
   Pertanyaan : Apakah peranan bakteri dalam fuel cell?
   Jawaban : Bakteri mempunyai kemampuan biosurpsi untuk logam-logam tertentu, sehingga kandungan logam-logam tersebut dalam air yang semula toksik menjadi kurang toksik.

2. Penanya : Wibiana Wulan Nandari (UPN "Veteran" Yogyakarta)
   Pertanyaan : Bagaimana mekanisme penurutnan logam Cu dan Pb dengan bakteri?
   Jawaban : Belum sampai mempelajari lebih lanjut tentang mekanisme penurunan konsentrasi logam Cu dan Pb dengan bakteri.

3. Penanya : Wibiana Wulan Nandari (UPN "Veteran" Yogyakarta)
   Pertanyaan : Setelah dianalisis apakah hasil penelitian sudah sesuai baku mutu atau belum?
   Jawaban : Konsentrasi akhir Cu pada air limbah sudah berada dibawah baku mutu sekitar ±20 ppm, sehingga aman dilingkungan.