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The Effects of Biofilm and Selective Mixed Culture in the Electricity Outputs and Wastewater Quality of Tempe Liquid Waste Based Microbial Fuel Cell

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Abstract

Microbial Fuel Cell (MFC) technology is highly prospective to be developed because it could be utilized as the alternative electricity sources and simultaneously as the wastewater treatment unit using microorganism as catalyst. Industrial Tempe wastewater has the potential to be used as MFC substrate since it still contains high nutrition for microbe and could pollute the environment if it disposed before being processed first. This study focused on investigating the effect of selective mixed culture addition and biofilm formation on the electricity production and the wastewater treatment aspects with tubular single chamber membraneless reactor and industrial Tempe wastewater substrate. The result showed that, with the addition of selective mixed culture, the optimum electricity production obtained with addition of 1 ml gram-negative bacteria with increase in electricity production up to 92.14% and average voltage of 17.91 mV, while the optimum decreased levels of COD and BOD obtained with addition of 5 ml gram-negative bacteria which are 29.32% and 51.32%. On the biofilm formation experiment, optimum electricity production obtained from biofilm formation time for 14 days with increase in electricity production up to 10-folds and average voltage of 30.52 mV, while the optimum decreased levels of COD and BOD obtained from biofilm formation time for 7 days which are 18.2% and 35.9%.

Keywords: *biofilm; Microbial Fuel Cell; selective mixed culture; Tempe wastewater; tubular reactor*

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INTRODUCTION

MFC is a bio-electrochemical system which capable to convert energy stored in organic material into electrical energy by employing bacteria as biocatalyst and has been known as an alternative energy source technology (Ryckelynck *et al.*, 2005; Picioreanu *et al.*, 2010; Deng *et al.*, 2010). MFC can utilize various substrates derived from renewable sources, convert them into non-hazardous by-products, and simultaneously generate electricity (Jiang *et al.*, 2010). MFC technology can utilize existing microorganisms in liquid waste as a catalyst to generate electricity, and simultaneously also functions as a liquid waste processing unit itself (Liu *et al.*, 2004). MFC system becomes a new alternative in organic waste treatment. This system can produce 50-90% less sediment to be disposed, compared to other waste treatment methods (Holzman, 2005).

One of the waste that is abundant and can be processed with MFC system is waste of tempe industry. Tempe industry generally is a household industry that directly drains its waste water into sewers or rivers without being processed first. Tempe industrial wastewater is one of the food waste water causing many problems to the surrounding environment, because of high BOD and COD levels (Wiryani, 2010). Tempe industrial wastewater can be utilized as a substrate in MFC systems for electricity production. MFC that is used in this study used mixed culture bacteria derived from tempe industrial waste, which in previous studies has been shown that it could degrade the waste with a decrease in COD level up to 27.34%. Microorganisms (bacteria) have a large contribution in generating electricity in the MFC system, so it is necessary to further investigate the relationship of bacteria to MFC performance. The effect of bacteria on the production of alternative electrical energy from MFC can be reviewed through the addition of selective mixed culture, by adding the gram population of bacteria which capable of transferring electrons into a liquid waste substrate.

The MFC related research also underscores the ability of biofilm to transfer electrons directly with electrodes. Biofilm plays an important role in generating high electrical density, because it allows all the bacteria either directly or indirectly contact with the anode to be actively involved in transferring electrons (Lovley, 2011; Malvankar *et al.*, 2012; Reguera *et al.*, 2006; Torres *et al.*, 2010). The biofilms that are grown on the electrode surface of a bioelectrochemical system can increase the electric current up to 1.5-9.5 times (Bergel *et al.*, 2007). Based on the aforementioned explanation, this study aims to examine the effect of selective mixed culture addition and time span of biofilm formation on electricity production and wastewater quality in single-chamber MFC tubular reactor with tempe industry liquid waste as substrate.

MATERIALS AND METHOD

Electrodes Preparation

The graphite electrode is immersed in a 0.1 M HCl solution for 1 day, then rinsed with aquadest. The electrodes were then immersed in 0.1 M NaOH solution for 1 day and rinsed again with aquadest.

Bioreactor Configuration

The reactor in this study is made of acrylic material, a tubular single chamber MFC without membrane capacity 650 mL with anode and cathode graphite. Four cylindrical anodes with a length of $11x10^{-2}$ meters and a diameter of $8x10^{-3}$ meters. A direct contact cathode with external air (air cathode), plate-shaped with length $35x10^{-3}$, $48x10^{-3}$ wide and $5x10^{-3}$ meters thick. Anode and cathode are connected with copper wire and 820 Ω resistor. The tubular reactor scheme is shown in Figure 1.

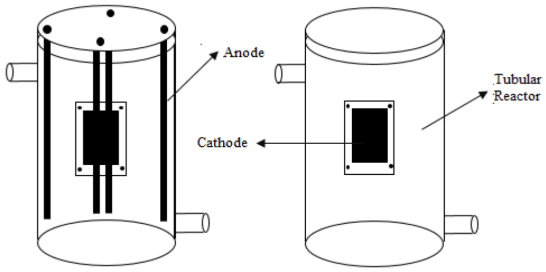


Figure 1. Tubular MFC reactor scheme

Biofilm Formation

Reactor which has been equipped with anode and cathode is filled with tempe liquid waste sample, electrolyte solution (0.03 M potassium persulfate), and phosphate buffer pH 7, with a 4.5:4.5:1 of volume ratio. Mixed culture Bacteria from tempe waste was added as much as 5.8 x 10^9 CFU/ml to the reactor. The reactor is then assembled in a closed-circuit condition with a resistor of 820 ohms and allowed to stand for 3, 5, 7, and 14 days to give time span for biofilm formation.

Characterization of Extracellular Polymeric Substances (EPS)

The analysis of formed biofilm was performed through EPS characterization which included the EPS extraction from biofilm step and analysis of carbohydrate and protein content in EPS. The four biofilm-generated anodes are released from the reactor and immersed into an EDTA buffer pH 7.5 which contains 10 mM Trizma base, 10 mM EDTA, and 2.5% NaCl, thus the biofilm detached from the anode. The biofilm mixture and EDTA were separated using a centrifuge at a speed of 3500 rpm for 20 minutes. The supernatant was then suspended in 10 ml 0.85% NaCl solution containing 0.22% formaldehyde solution at 80 for 30 minutes to extract the EPS. The soluble EPS in formaldehyde solution is separated using a centrifuge at a speed of 3500 rpm for 30 minutes. Carbohydrate content of extracted EPS was measured by phenol sulfuric acid method and the protein content was measured by Lowry method (Gong *et al.*, 2009). The sum of the carbohydrate and EPS proteins represents the total EPS in the sample. Xanthan gum and bovine serum albumin were used to create calibration curves in the determination of carbohydrate and protein content in EPS

MFC Experimental

The reactor is filled with artificial tempe liquid waste. The artificial tempe liquid wastes are made from boiled-soya water mixed with distilled water, with a ratio of 3:5 (w/v) (Nout and Rombouts, 1990). Boiled-soya water was then incubated for 3 days. Each experiment was performed, using 292.5 mL of tempe liquid waste, 292.5 mL of electrolyte solution (0.03 M potassium persulfate), 65 mL phosphate buffer pH 7. Selective mixed culture of tempe liquid waste was added as much as 1, 2, 3, 4 and 5 mL of gram bacteria that have been selected using Agitol Salt Agar (gram-positive) medium and Mac Conkey Agar (gram-negative) medium. Electrodes are coupled with 820 Ω resistors and connected to a digital multimeter device. The voltage data collection was conducted using APPA 109N multimeter for 50 hours connected directly to CPU/PC. All experiments were conducted at room temperature (27°C). The electrical circuit scheme of the MFC system can be seen in Figure 2.

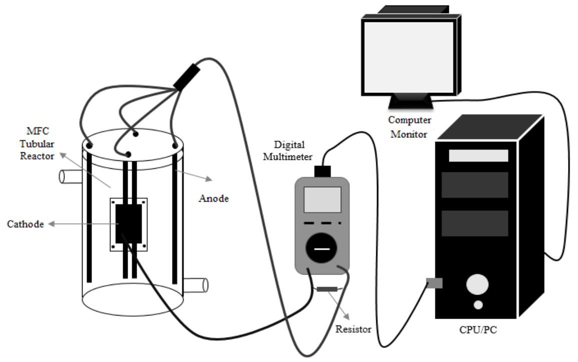


Figure 2. Scheme of Electrical circuit of MFC system

Measurement of COD and BOD Levels

The measurement of COD concentration was carried out by spectrophotometry method. The reagents used are CACH HACH Cat. 2125925 20-1500 mg/L. Standard solutions which are made to determine the accuracy and to generate the calibration curves then for determining the amount of COD level in tempe liquid waste. Measurement of BOD concentration was performed by BOD_5 method. Oxygen solubility was measured with a Dissolved Oxygen (DO) meter device of Hanna HI 7041s.

Data Processing

The data obtained from multimeter as voltage value (mV or V) were processed become the value of electrical current with resistance of 820 Ω. The calculations were performed using equation (1).

$$I = \frac{V}{R} \quad (1)$$

Where I (ampere) electrical current, V (volt) voltage, and R (Ω) electrical resistance. The electrical currents data were used to compute the power density to determine the power produced for every square meter of anode surface area by equation (2).

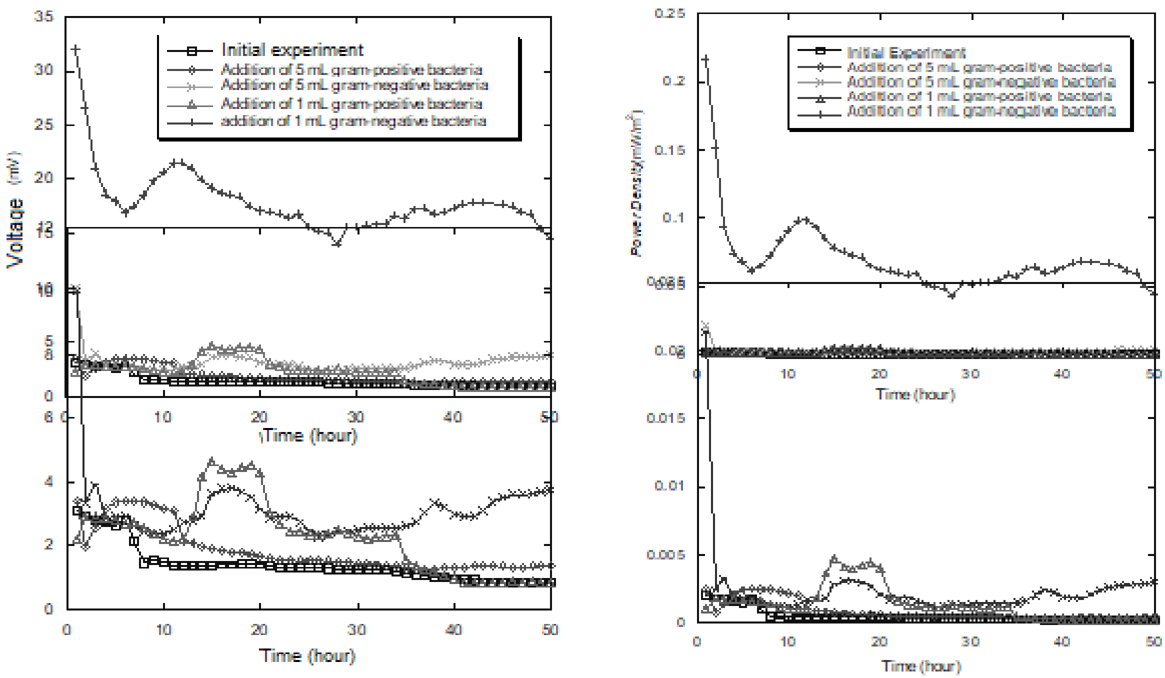
$$P = \frac{V \, x \, I}{A} \quad (2)$$

Where is P power density (mW/m^2) and A anode surface area (m^2).

RESULTS AND DISCUSSION

The Effect of Gram-Bacteria Addition to the Production of Electricity from MFC

The experiment was conducted by adding 5 mL and 1 mL of gram-bacteria. Figure 3 shows that the addition of 5 mL and 1 mL of both gram-positive and gram-negative bacteria have a good effect on increasing the production of electricity in the tubular reactor MFC system. In both experiments, gram-negative bacteria were superior to the gram-positive bacteria, with best results given by the addition of 1 mL of gram-negative bacteria. Friman *et al.* (2013) concluded that MFCs that is using gram-negative bacteria cells produce higher electrical current than those using gram-positive bacteria.



(a) (b)

Figure 3. Comparison of voltage profile (a) and power density (b) in experimental by addition of 5 mL and 1 mL of gram bacteria

Gram-negative bacteria are superior and can have an effect on improved electricity production due to having thin peptidoglycan walls, thus facilitating the transfer of electrons to the anode. Gram negative bacterial cell membranes consist of peptidoglycan (about 10%), outer membrane (containing lipopolysaccharide, lipophosphate, and lipoprotein), outer membrane proteins, and periplasm, so generally gram-negative bacteria have a lower Zeta potential surface. Gram-positive bacterial cell membranes contain a simpler chemical composition, which is close to 90% peptidoglycan and 10% of the teichoic acid. A covalent bond between peptidoglycans and teichoic acid causes a high surface Zeta potential (negative surface charge). This potential Zeta surface affects the cell surface charges, which leads to differences in the electrogen ability of gram negative and positive bacteria. The high potential Zeta potential in gram-positive bacteria will complicate the transfer of electrons (Juang *et al.*, 2011).

The addition of 1 mL of gram-negative bacteria provides better electricity production than the addition of 5 mL. At the addition of 5 mL, the amount of existing nutrients are less so as to cause a decrease in the rate of bacterial cell metabolism. Hassan *et al.*, (2011) stated that the power density will decrease as the cellulose concentration decreases within the anode compartment.

The Effect of Gram-Bacteria Addition to the Quality of Wastewater

Decreased levels of COD and BOD were analyzed after the reactor was operated for 50 hours. Based on Figure 4a, the largest decrease in COD level was obtained at the reactor with the addition of 5 mL of gram-negative bacteria, which was 29.39%. In addition of 5 mL of gram bacteria, the yield of COD level decrease by addition of gram-negative bacteria is much greater than the gram-positive bacteria. The percentage decrease in BOD as shown in Figure 4b, has the same pattern as the decrease in COD levels. Addition of 5 mL of gram-negative bacteria resulted in the greatest decrease in BOD content of 51.32%.

Based on the bacteria coloring test that has been performed, the most dominant bacteria present in tempe liquid waste is gram-negative bacteria. The greater number of gram-negative bacteria indicates that gram-negative bacteria are more susceptible to adapt to tempeh liquid waste media. In the wastewater of tempe, there are species of bacteria of *Citrobacter diversus*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Klozaenae* which are classified as gram-negative bacteria and *Lactobacillus casei*, *Enterococcus faecium*, *Streptococcus Dysgalactiae* and *Staphylococcus Epidermidis* which are classified as gram-positive bacteria (Nout and Rombouts, 1990).

The better adaptability provides better gram-negative bacteria growth as well, so that more amounts are present in the reactor. The more bacteria the more degraded the waste will be. Some species of gram-negative bacterial groups in tempe liquid waste such as *Enterobacter cloacae*, are known to have resistance to chromate (Fujie *et al.*, 1996). Gram-negative bacteria *Pantoea agglomerans* one of the bacteria mixed culture, has been used also in the processing of paper mill waste (Dewit, 2006).

The Effect of Gram-Negative Bacteria Addition on Production of MFC Electricity

Figure 5 shows the highest voltage and power density values obtained on the addition of 1 mL of gram-negative bacteria. The addition of 1 mL of gram-negative bacteria could increase electricity production by 16.99 mV or 94.86% against negative control and 16.50 mV and 92.14% against the initial experiment. The highest voltage value reaches 31.90 mV and the power density is 0.22 mW/m^2, with an average voltage of 17.91 mV. In addition of 5 mL of gram-negative bacteria, it is obtained the most stable voltage and power density after the 20 h with the value is 2.5 mV. Figures 6a and b show that the voltage increases as the number of bacteria increases until a certain point because the electrons that flow into the electric current come from the bacterium degrades the substrate (Zhang *et al.*, 2013). The whole results of this study show that the addition of 1 mL of gram-negative bacteria is the maximum volume that can affect the increase of electricity production. In addition of 2 mL, 3 mL, 4 mL,

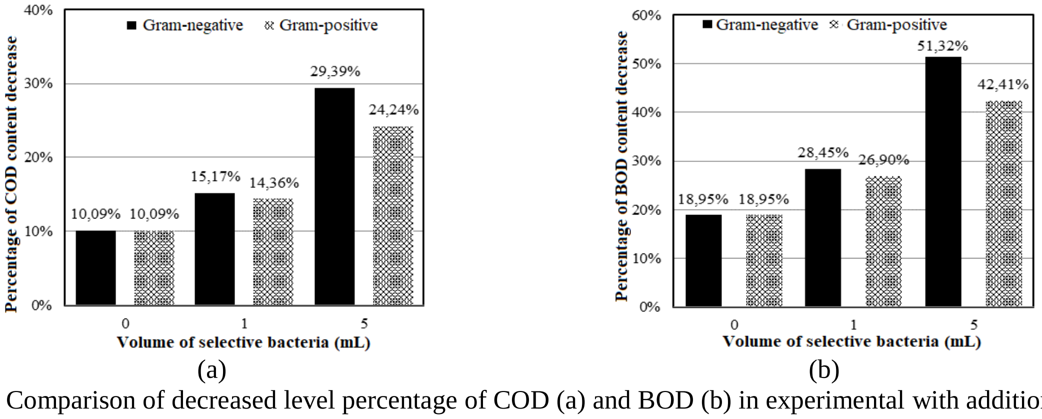
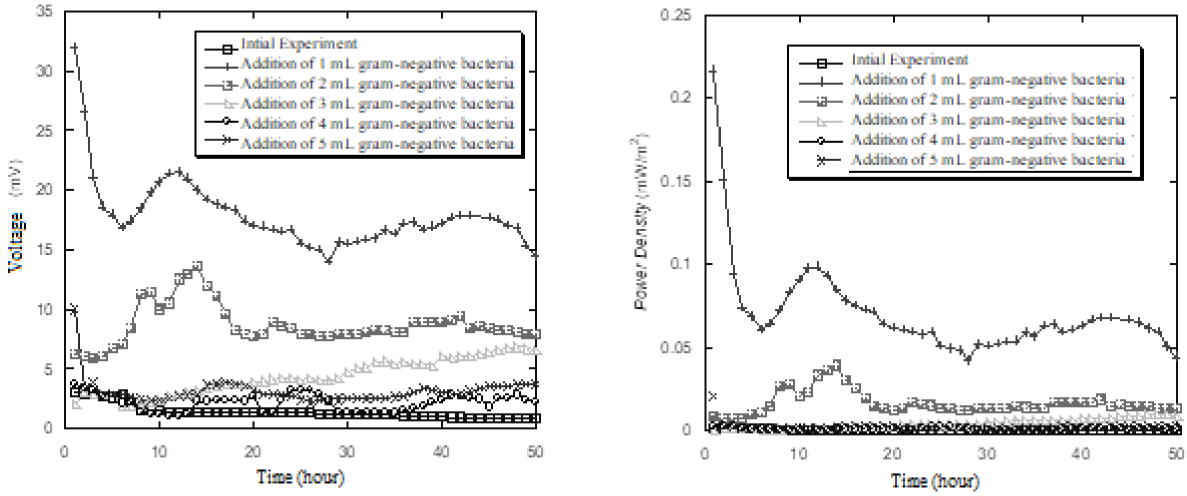
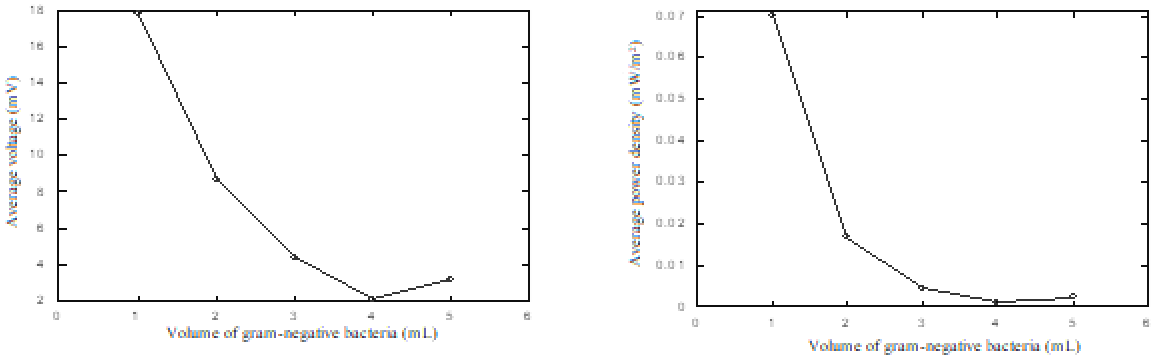


Figure 4. Comparison of decreased level percentage of COD (a) and BOD (b) in experimental with addition of 5 mL and 1 mL of gram bacteria



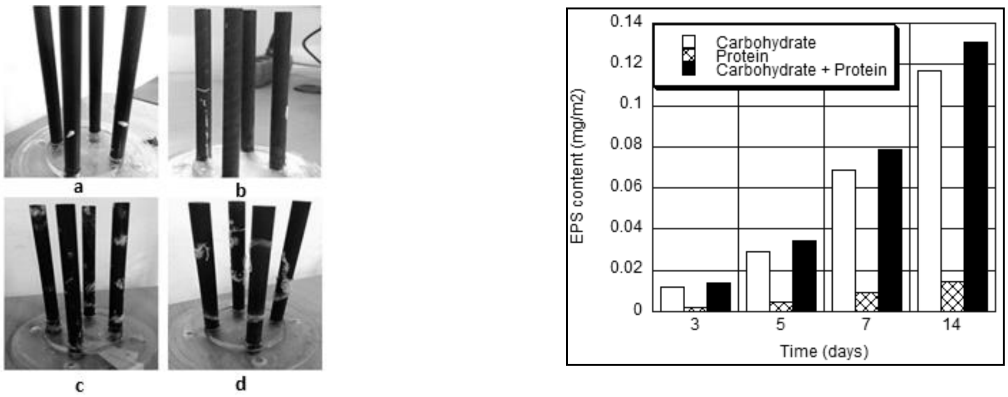
(a) (b)

Figure 5. Comparison of voltage profile (a) and power density (b) in the experiment with the addition of gram-negative bacteria



(a) (b)

Figure 6. average voltage profile (a) and power density (b) with the addition of gram-negative bacteria



(i) (ii)

Figure 7. Effect of incubation time for (a) 3 days, (b) 5 days, (c) 7 days, and (d) 14 days on biofilm formation in anode (i) and EPS content in biofilm (ii**)**

and 5 mL of bacteria, the resulting electricity decreased in terms of average voltage and average power density. The decline also occurs due to the toxic generation by bacteria metabolism by-products, thus inhibiting the growth (Madigan *et al.*, 2010) or due to the formation of intermediate compounds that block the transfer of electrons to electrodes (Rakesh *et al.*, 2014).

Effect of Incubation Time Span on Biofilm Formation in Anodes

Figure 7 (i) shows the formed biofilms on the anode with an incubation time of biofilm formation for 3, 5, 7, and 14 days. The longer the incubation time, the greater surface area of the anode was coated with biofilm. From Figure 7 it can be seen that the formation of biofilms on the anode surface is not homogeneous.

This is due to the difference in pore size on the anode surface. Surfaces with larger pore sizes are preferred by bacterial cells (Feng *et al.*, 2015). The anode that is used in this study is cylindrical graphite, so that the heterogeneous pillar structures are formed on biofilms with more observed living cells located near the anode surface (Nevin *et al.*, 2008).

The biofilm EPS content is determined by measuring the carbohydrate and protein content that are the main components of EPS. The EPS content increase as the incubation time increased. This phenomenon happens because during the growth of biofilm, microbes consume large amounts of organic substrate for EPS synthesis which is useful for biofilm formation, and some of the resulting electrons are released out of cells to generate electricity (Li *et al.*, 2010). EPS can contribute up to 50-90% of the total biofilm organic carbon and has many functions, including the initial cell attachment to the surface, maturing the biofilm structure, and increasing the biofilm resistance (Zhang *et al.*, 2011).

Effect of Biofilm Formation Time on MFC Electricity Production

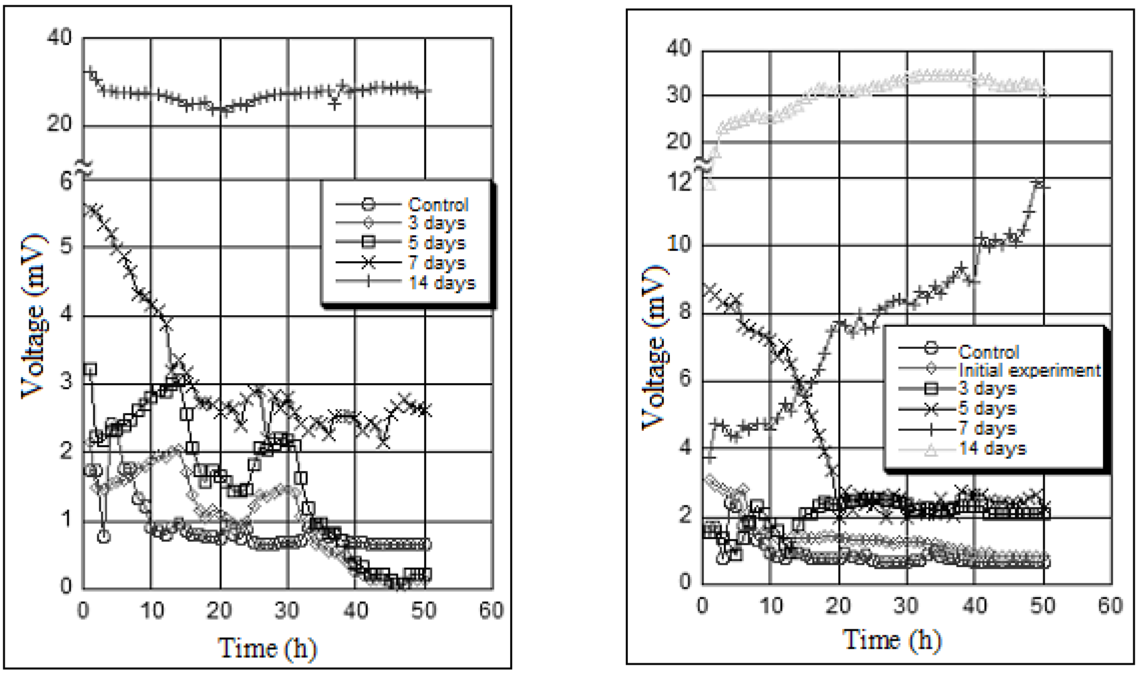
The Experiments of the biofilm formation time effects on MFC electricity production was performed by using 2 types of substrate ie glucose and tempe liquid waste. The use of glucose substrates aims to investigate the performance of cells that exist only in biofilms to generate electricity. The results show that the formation of biofilms on the anode surface can increase electricity production up to 10 times. The longer time of biofilm formation, the generated volatage are greater (Figure 8a).

The highest voltage was obtained at the incubation time of 14 days for biofilm formation with a maximum value of 32.45 mV and a power density of 0.22 mW/m^2 (Figure 8b). The production of MFC electricity is directly proportional to the increase of biofilms and biomass thickness on the anode, which indicates that cells which are not directly contacted with the anode also play a role in the electron transfer process (Malvankar *et al.*, 2012). The mechanism of electron transfer from biofilm to anode can occur through cytochrome c or conductive pili which are present in the biofilm matrix. Pili and cytochrome c can act as a medium of electrons transfer from one cell to another so that the electrons reach the anode.

The effect of biofilm formation on electricity production using the tempe liquid waste substrate was conducted to determine how far the effect of cells on the biofilm in increasing the production of electricity compared to cells in the bulk phase. The results obtained have the same trend as the results using glucose as a substrate. The highest voltage is generated at 14 days incubation time with a maximum value of 34.81 mV and a power density of 0.26 mW/m^2. Larger electricity production is produced by the MFC with the tempe liquid waste substrate, with the maximum produced voltage is slightly different. This proves that cells in the liquid phase also play a role in generating electrical current, although the greater influence is given by the cells in the biofilm.

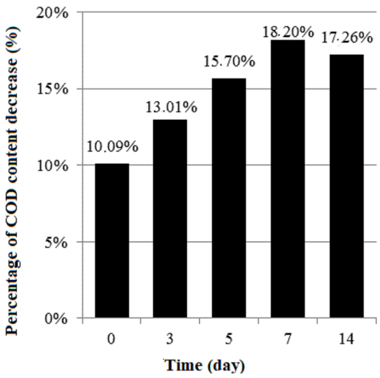
The Effect of Biofilm Formation Time on Wastewater Quality

The highest decrease of COD content was 18.2% which was obtained at biofilm incubation time for 7 days (Figure 9). The percentage of BOD content

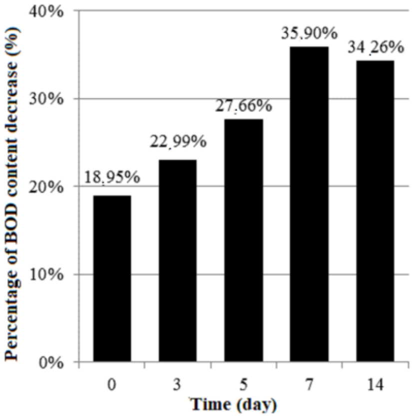


(a) (b)

Figure 8. Electrical voltage profile on variation of incubation time using biofilm glucose substrate (a) and tempe liquid waste (b)



(a)



(b)

Figure 9. The decreased percentage of COD (a) and BOD (b) level on variation of biofilm incubation time

decrease in wastewater has the same pattern with COD content. The formation of biofilm for 7 days resulted a greater decrease in BOD content of 35.9% and and 34.26% for 14 days incubation time. At 14 days biofilm incubation time the amount of substrate transfer into biofilm is more limited than 7 days.

The rate of substrate consumption increases with the increasing of density and biofilm thickness since the number of bacteria also increases the reaction rate (Seker *et al.*, 1994). The thicker biofilm, the bacteria on the biofilm have better survival ability to grow. As the result, the bacteria on 14 days biofilm have better survival ability. However, in a certain thickness and density the diffusion factor is more influential than the reaction rate. The rate of substrate diffusion to bacteria will decrease due to the decrease of diffusion coefficient (Watnick *et al.*, 2000). In the 14 days biofilm the effect of the diffusion rate is greater than the effect of bacterial metabolism rate.

CONCLUSION

The addition of selective mixed culture significantly increases the production of generated electricity in MFC tubular system up to 94.86% against negative control. The Increased electricity production given by gram-negative bacteria againts gram-positive bacteria is 85.33%. The addition of 1 mL of gram-negative bacteria produces a maximum voltage of 31.90 mV with a power density of 0.22 mW/m^2. The addition of gram-negative bacteria decreased the pollutants content greater than that gram-positive bacteria did. The addition of gram-negative bacteria as much as 5 mL decrease COD and BOD level by 29.32% and 51.32%, respectively. The formation of biofilms on MFC anodes can increase the electricity production up to 10 times. The highest electricity production was obtained at the time of biofilm formation for 14 days with EPS concentration of 0.131 mg/m^2 and the maximum voltage and density values were 34.81 mV and 0.26 mW/m^2, respectively. The optimum biofilm formation time for waste treatment was 7 days, with the percentage of COD and BOD levels decrease was 18.2% and 35.9%, respectively.

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