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Effect of biofilm and selective mixed culture on microbial fuel cell for the treatment of tempeh industrial wastewater

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Abstract. Microbial Fuel Cell (MFC) provides a new alternative in the treatment of organic waste. MFC produces 50-90\% less sludge to be disposed than other methods. MFC technology can utilize existing microorganisms in the waste as a catalyst to generate electricity and simultaneously also serves as a wastewater treatment unit itself. Tempeh wastewater is one of the abundant industrial wastewater which can be processed using MFC. Research using the selective mixed culture is very likely to do due to the good result on COD removals by adding mixed culture. Microorganisms in tempeh wastewater consist of bacteria gram positive and gram negative. This study focused on the aspects of waste treatment which is determined by decreased levels of COD and BOD. Variations in this study are the formation time of biofilm and the addition of selective gram. MFC operated for 50 hours. For a variation of biofilm formation, experiments were performed after incubation by replacing incubation substrates used in the formation of biofilms. Biofilm formation time in this study was 3 days, 5 days, 7 days and 14 days. Gram positive and gram negative bacteria were used in selective mixed culture experiments. Selective mixed culture added to the reactor by 1 mL and 5 mL. Selection of gram-positive or gram-negative bacteria carried by growing mixed culture on selective media. COD and BOD levels were measured in the wastewater before and after the experiment conducted in each variation. Biofilm formation optimum time is 7 days which decrease COD and BOD levels by 18.2\% and 35.9\%. The addition of gram negative bacteria decreases COD and BOD levels by 29.32\% and 51.32\%. Further research is needed in order to get a better result on decreasing levels of COD and BOD.

Keywords: Biofilm; BOD; COD; Microbial fuel cell; Tempeh wastewater

1. Introduction
Industrial wastewater normally would be processed and send to the centralized waste treatment facility. The centralized facility mostly use aerobic waste treatment like activated sludge, trickling filter, or an oxidation ditch and pond aeration which consumes energy for the aeration process about 70\% of the total energy required for waste treatment. The conventional method still produces considerable excess sludge (Du, 2007).

MFC (Microbial Fuel Cell) is a device that uses bacteria to generate electricity from organic and non-organic compounds. MFC provides a new alternative in the treatment of organic waste. MFC produces 50-90\% less sludge to be disposed of than other methods (Holzman, 2005). MFC technology
can utilize existing microorganisms in the waste as a catalyst to generate electricity and simultaneously also serves as a wastewater treatment unit itself (Liu et al., 2004).

Tempeh wastewater is one of the abundant industrial wastewater which can be processed using MFC. Tempeh industry generally is a household industry which disposes its wastewater directly into sewers or rivers without being processed first. Tempeh industrial wastewater is one of wastewater that causes a lot of problems to the environment. Whereas it still contain high BOD and COD levels (Wiryani, 2007). In utilization effort, tempeh industrial wastewater can be used as a substrate in the MFC system for the production of electricity.

The bacteria used in the MFC system can be either a pure culture of bacteria added to a medium or mixed culture of bacteria that is naturally contained in the material that has high organic matter content. Tempeh wastewater contains nutrients such as nitrogen, phosphorus, and organic materials such as carbohydrates, vitamins, and protein that act as a nutrient for bacteria. MFC operated using a mixed culture on the addition of 5 mL consortia to the reactor could decrease COD levels by of 27.34% (Mulyana, 2015). Previous research on MFC more emphasis to focus on optimizing the power and electricity generated. The focus of research in decreased levels of COD and BOD is necessary due to the electricity output of the MFC is still not as high as expected. This study examines the potential MFC as an efficient waste processing.

Research using the selective mixed culture is very likely to do due to the good result on COD removals by adding mixed culture. Microorganisms in tempeh wastewater consist of bacteria gram positive and gram negative (Nout et al., 1990). One of a group of bacteria will have a more dominant role in the treatment of tempeh wastewater compared to the other.

One of important factor in biological wastewater treatment is the ability to separate the biomass from the effluent in the process of clarification. At the sewage treatment using activated sludge, existing biomass in the form of a single cell (bacteria), micro colonies, and sets that form the floc (Lear and Lewis, 2012). Excess sludge in large amounts that need to be removed and separated. Disposal of sludge is quite expensive and spend considerable funds on waste management. Biofilm formation potentially reduces the amount of sediment cells.

Based on the above considerations, the present study will focus on waste treatment based on MFC using biofilm and addition of selective mixed culture. In this article, we study the effect of biofilm time formation and selective mixed culture on waste removal.

2. Material and methods

Tubular reactor which made of acrylic was used in this experiment. Volume of reactor is 650 mL. The anode and cathode that used was made from carbon in a certain size. Four cylindrical anodes were used. Each anode has diameter and length of 8x10^{-3} m 8x10^{-2} m. A plat cathodes are used which has length and width of 4x10^{-2} metre and 5x10^{-3} metre. Cathode contact directly with the air called air-cathode. The anode and cathode are connected with external circuit using copper wire and 820-ohm resistor.

Before used, the electrode was prepared. Graphite electrode immersed in a solution of 0.1 M HCl for 1 day and then rinsed with distilled water. It was soaked again into a solution of 0.1 M NaOH for 1 day and then rinsed again using distilled water. The reactor is filled with artificial tempeh wastewater. Artificial tempeh wastewater was made from the boiled soybeans using water at a ratio of 3:5 (Nout et al., 1990). Tempeh boiled water was incubated for 3 days. Each experiment was conducted using 292.5 mL tempeh wastewater, 292.5 mL of electrolyte solution (potassium persulfate 0.03 M), and 62.5 mL phosphate buffer pH 7.
MFC operated for 50 hours. For a variation of biofilm formation, experiments were performed after incubation by replacing incubation substrates used in the formation of biofilms. Biofilm formation time in this study was 3 days, 5 days, 7 days, and 14 days. Gram positive and gram negative bacteria were used in selective mixed culture experiments. Selective mixed culture added to the reactor by 1 mL and 5 mL. Selection of gram-positive or gram-negative bacteria carried by growing mixed culture on selective media. To cultivate gram-negative bacteria, MacConkey agar was used as growth media. To cultivate gram-positive bacteria, Mannitol Salt agar was used as growth media.

COD and BOD levels were measured in the wastewater before and after the experiment conducted in each variation. COD content measurement was conducted by spectrophotometric method. Reagents used were HACH COD reagents Cat. 2125925 20-1500 mg/L. Before measured COD, calibration curve was made to determine the amount of COD in tempeh wastewater. Standard solution was made to determine the calibration curve. BOD measurement was conducted using BOD5(20) methods. The solubility of oxygen was measured using Dissolved Oxygen (DO) meter Hanna HI 7041s instrument.

3. Results
In Figure 2, 7 days’ biofilm formation resulting the highest COD removal by 18.2%. COD removals on 14 days’ biofilm formation was lower than 7 days’ biofilm formation. BOD removals of waste have the same pattern with COD removals. 7 days’ biofilm formation resulted highest BOD removals by 35.9% and followed by 14 days’ biofilm formation by 34.26%.
Figure 2. Effect of biofilm formation time to (a) COD and (b) BOD removals

After the reactor operated for 50 hours, the largest reduction in COD levels based on Figure 3 was the addition of gram negative bacteria as much as 5 mL of 29.39%. The result was higher than previous research conducted by Mulyana (2014) and Citrasari (2015) with the addition of 5 mL mixed culture of bacteria which decreased COD levels by 26.19% and 27.35%.

Figure 3. Effect of addition of selective mixed culture on (a) COD and (b) BOD removals

4. Discussion

4.1. Effect of biofilm formation time to waste removals
At 14 days’ biofilm formation, substrate transfer into biofilm more restricted than 7 days’ biofilm. Substrate consumption rate increases as biofilm thickness and density increases. Number of bacteria which is catalyst in biochemical reaction increases as biofilm thickened (Seker et al., 1995). The
thicker the biofilm the bacteria in biofilms have a better survival (Watnick and Kolter, 2000). So that the bacteria in the 14 days’ biofilm has a better shelf life. However, at a certain thickness and density, the diffusion factor is more influential than the rate of reaction in substrate degradation of biofilm. The diffusion rate of the substrate to the bacteria in biofilm will decrease due to the decreasing diffusion coefficient (Seker et al., 1995). At 14 days the effect of biofilm diffusion rate is greater than the influence of the reaction rate of bacterial metabolism.

4.2. Effect of selective mixed culture addition to waste removals

On the addition of 1 mL selective mixed culture, waste removals of gram-negative just a bit larger than waste removals of gram-positive. However, the addition of 5 mL gram-negative resulted much greater waste removals than gram-positive bacteria. BOD removals of waste have the same pattern. Addition of gram-negative bacteria as much as 5 mL decreased BOD levels by 51.32%.

Based on the bacterial staining test which has been done, the most dominant bacteria in the tempeh wastewater are gram-negative bacteria. The high number of gram-negative showed that gram-negative bacteria more easily adapt to media tempeh wastewater. Tempeh wastewater contained diverse bacterial species such as Citrobacter, Enterobacter agglomerans, Enterobacter cloacae, Klebsiella pneumoniae and Klozaena which is a gram-negative bacteria and Lactobacillus casei, Enterococcus faecium, Staphylococcus epidermidis, and Streptococcus Dysgalactiae which is gram-positive bacteria (Nout, 1990). Even though the addition of selective bacteria in the same amount, gram-negative bacteria resulted better COD removal. With the better adaptation ability, the growth of gram-negative bacteria was also better so its number will have increased in the reactor. The more bacteria, the more waste is degraded (Citasari, 2014). Some species of a group of gram-negative bacteria in tempeh wastewater such as Enterobacter cloacae is resistant to Chromate (Fujie et al., 1996). Gram negative bacteria Pantoea agglomerans also commercially used in the paper mill waste treatment (Dewit et al., 2006).

5. Conclusion

Addition of gram-negative bacteria resulted greater waste removals than the addition of gram-positive bacteria. Addition of gram-negative bacteria as much as 5 mL decreased COD and BOD levels by 29.32% and 51.32%. The optimal Biofilm formation time for waste removal was 7 days’ biofilm. It decreased COD and BOD levels by 18.2% and 35.9%.

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