Performance of microbial fuel cell double chamber using mozzarella cheese whey substrate

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Abstract. Nowadays the availability of electric energy is decreasing, hence there is a need for innovation of electric energy producer alternative; one of them is microbial fuel cell (MFC). MFC is a bioelectrochemical system generated by bacterial metabolism that utilizes organic substrate. One of the substrates that can be used is whey, a waste generated from cheese production. Therefore, this study aimed to determine the power of potential current and voltage generated from the use of whey cheese as a substrate for bacterial metabolism. In this research, double chamber system was used in microbial fuel cell reactor by using cheese whey as substrate at anode and potassium permanganate as cathode and utilizing membrane nafion 212 as membrane of proton exchange. The variable of experiment was bacteria type. The types of bacteria used in this study were Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus casei. While the operating time used was 100 hours. The highest current produced was 74.6 μA and the highest voltage was 529.3 mV produced by Lactobacillus bulgaricus bacteria. In this study, it was also found that the death phase of the three bacteria was at 70-80 hours.

1. Introduction
Electrical energy is a basic requirement in the community today, where almost all human activities require electrical energy. The vital importance of electricity for humankind, in case of Indonesia increase electricity demand is estimated to continue growing by 4.6% annually and will be tripled in 2030. If this is not accompanied by efforts to increase energy production, it is alarming that Indonesia will experience an energy crisis. Therefore, an effort should be made to generate sustainable alternative energy sources. Some innovations in renewable energy are available today; one of them is microbial fuel cell or MFC. Microbial Fuel Cell (MFC) is one of the prospective technological alternatives to be developed. MFC is a system that uses bacteria to produce electricity from organic or non-organic compounds. Various types of microbes have been used in the MFC, among others Shewanella oneidensis [1], Saccharomyces cerevisiae [2], Lactococcus lactis [4], Geobacter sulfurreducens [6], and Escherichia coli [7]. As well as some existing studies utilizing a variety of substrates such as tofu and other substrates that can be substrate for bacterial metabolism. One of the substrates that have the potential to be developed with MFC technology is whey, a waste generated from cheese production. Currently, cheese whey has not been further utilized. Cheese industries are beginning to grow along with the increasing interest of the community in processing food by adding cheese. The existing cheese industry only discharges whey through waste channels. Based on the
potential of MFC as an electricity supplier and the potential of whey for bacterial substrate, the present study aimed to utilize whey as a substrate in MFC reactors to generate electrical energy. In this study, two variables were used to determine the effectiveness of cheese whey as a substrate in generating electricity through the MFC reactor. The first variable observed was bacterial type, because bacteria are the main factor in electricity production using MFC reactor. It is important to know the most optimal bacteria for electricity production. Another variable tested was MFC reactor operation time to determine the optimum time for electricity production in MFC reactor.

2. Materials and Method
Whey in this research was obtained from CV. Brawijaya Dairy Industry, Batu. The cultures of *L. bulgaricus*, *S. thermophilus* and *L. casei* were obtained from Microbia Laboratory, Jakarta. MFC reactors were constructed in Mechatronics and Agroindustrial Machine Laboratory University of Brawijaya. The voltage and current were measured using digital multimeter (Sanwa Electric Instrument Co., Ltd., Japan).

2.1. MFC configuration
The set up comprised of a dual- chambered MFC consisted of an anode and a cathode with a reactor volume of 700 mL in each chamber and the reactors were connected in series. The two compartments were connected with a proton exchange membrane (PEM; Nafion 212, Lyntech, USA) and joined tightly. The reactor that used in this research is shown in Figure 1.

![MFC Double Chamber Reactor](image_url)

2.2 MFC experiments
The anode chamber was filled with three different inoculums that were *L. bulgaricus, S. thermophilus*, and *L. casei*. As well as added glucose, aquadest, and phosphate buffer (pH 7). The number of initial bacteria was $5.7 \times 10^4$ CFU/ml. The cathode chamber was filled with potassium permanganate and a phosphate buffer (pH 7). The MFC was operated for 3 hours, 30 hours and 100 hours.

3. Results and Discussion
The performance of microbial fuel cell double chamber reactor was evaluated by current strength and voltage. The main goal of this research was to find out the electrical output generated by microbial fuel cell double chamber reactor using mozzarella cheese whey
substrate. Figure 2 shows current produced and Figure 3 shows voltage output from 100 hours of reaction time.

Figure 2. Current strength of 100 hours reaction time

Figure 3. Voltage of 100 hours reaction time

Figure 2 shows the results of the current produced by each type of bacteria with reaction time of 100 hours. Based on the measurements, the three types of bacteria showed that current strength was increasing until 30 hours but afterwards undergoing stationary phase until 70 hours, after 70 hours the current strength was decreasing. The highest current strength value was generated by *Lactobacillus bulgaricus* bacteria with an average maximum current of 74.6 μA. The second highest current strength was produced by *Lactobacillus casei* with average maximum current strength at this treatment was 69 μA. While the lowest current strength produced by *Streptococcus thermophilus* with average
maximum current strength at this treatment was 62.3 μA. It is shown at Figure 2- that the three bacteria had maximum current strength at 40 hours and then undergo stationary phase but current value fluctuates about 1-3 μA. Then the three bacteria entering the death phase when reaction time was reaching around 70-80 hours where the value of the voltage decreased significantly even when it reached 100 hours there was still a voltage generated.

Figure 3 shows the voltage generated by each type of bacteria used in this study with a variation of reaction time 100 hours. The measurement indicated that the three types of bacteria were able to generate an increased current and voltage until 30 hours, next the bacteria undergoing stationary phase until 70-80 hours then it was dropped afterwards. The highest voltage value was generated by Lactobacillus bulgaricus with a maximum voltage of 529.3 mV. The second highest voltage was generated by Lactobacillus casei with a maximum voltage at this treatment was 486.6 mV. While the lowest voltage producer was Streptococcus thermophilus with a maximum voltage generated of 383.6 mV. The three bacteria were experiencing maximum current strength at 35-40 hours, then undergoing stationary phase until 70-80 hours and the bacteria entering death phase where the value decreased significantly. Interestingly, at 100 hours there was still a low voltage generated. These phenomena were in accordance with the bacterial growth phase consisted of four phases of growth namely, lag phase, log phase or exponential, stationary phase and phase of death. Lag phase is a phase of bacterial adaptation to the environment. Log or exponential phase is the phase of exponential increase in the number of bacteria. Increasing the number of these bacteria occur due to binary division in bacteria up to two cells. Then bacterial division occurs again in each cell and so on [3]. Increasing electric voltage and electric current strength at 0-40 hours was the result of bacterial log phase. Then at 40-70 hours the bacteria entering stationary phase i.e., a phase in which the growth rate equals to the death rate consequently the cell number was constant. The last phase undergone by bacteria was the death phase or declining phase where the amount of substrate was no longer meets the needs of bacteria resulting in bacterial death. In this case declining phase began to occur from 70 hours until the end of the observation at 100 hours but still a low voltage was generated.

Based on the results of the present study, the growth phase is the phase when Lactobacillus bulgaricus, Streptococcus thermophilus and Lactobacillus casei convert most lactose into electrical energy in microbial fuel cell. In addition, the results of this study were in accordance with other studies on growth of lactic acid bacteria using whey from milk fermentation using Lactobacillus bulgaricus [8]. The declining in the currents strength may be due to the decline in the number of bacteria as a consequence of the reduced availability of substrate. Another factor that occurred the decline phase was biofouling on proton exchange membrane. According to other studies biofilm enrichment during long-operating times may pollute the membrane fouling which decreases some factors such as concentration, ion transfer capacity and diffusion coefficient [5].

4. Conclusion
Whey, a waste that generated from cheese factory can be used as a substrate of lactose for bacteria in the microbial fuel cell double chamber reactor. The maximum voltage and current strength obtained from the use of Lactobacillus bulgaricus was 529.3 mV and 74.6 μA respectively. Lactobacillus bulgaricus maximum electricity at 40 hours and entering stationary phase with fluctuating values until 70 hours. While Streptococcus thermophilus peak point was reached at 50 hours and entering stationary phase until 80 hours, Lactobacillus casei reached the peak electricity at 45 hours.

5. References
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