Production of Bioelectricity from Microalgae Microbial Fuel Cell (MMFC) Using *Chlorella pyrenoidosa* and Batik Wastewater

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Abstract. Nowadays, the usage of batik clothes increased would make batik wastewater more desecrate the rivers in Indonesia. Batik wastewater contained much of chemical substances, so that very dangerous. One of environmentally alternatives energy that could solve this problem was Microbial Fuel Cell (MFC) which utilizes organic matter as an energy source of microbes in carrying out its metabolic activities to produce electricity. MFC system with microalgae named Microalgae Microbial Fuel Cell (MMFC). In this study, it would investigate the electrical energy produced by MMFC using *Chlorella pyrenoidosa* as catholyte and batik wastewater as anolyte. This study aims to evaluate performance of MMFC based on the influence of yeast variations (2 gr/L and 8 gr/L), concentration of wastewater (100%v and 50%v), and number of graphite electrode (1:1 and 2:2). The methods started from culturing microalgae. Then, MMFC simulation operated for 7 days. Every 24 hours, voltage and current were measured to be processed into polarization curve. The value of absorbance and COD level of wastewater too. The results of this study showed that 100%v batik wastewater, 2:2 bars graphite electrode, and 8 gr/L yeast addition was the best results in MMFC simulation.

1. Introduction

Nowadays, Indonesia was faced with a crucial problem regarding water and soil pollution to the environment [1]. One type of industry which very potential to cause environmental pollution was textile industry, especially batik industry. The process of making batik in industry consisted of painting, waxing, coloring, wax cleaning, washing, and coloring with synthetic dyes. In staining, this compound was only used around 5% while the remaining 95% would be disposed as wastewater. This means that only 5% of the dye effectively bind the clothes while the rest was being waste. Therefore, wastewater treatment of batik industry generally consumed 0,6 kWh of energy per m3 of domestic wastewater, or consumed approximately 1 kWh of energy per kg of COD [2].

One of the alternative energies which was environmentally friendly is Microbial Fuel Cell (MFC) by utilizing organic material (substrate) as energy source of bacteria in carrying out its metabolic activities to generate electricity [3]. This system utilized wastewater as a substrate so that it could be used as an ideal tool for processing the wastewater through a system which generated electricity also. By utilizing batik wastewater, it could convert biochemical compounds directly into electrical energy.
and yeast was used as a biocatalyst in improving the performance of MFC’s which were capable of converting energy. Chemical substances in the substrate became electrical energy in MFC process [4].

MFC was a device that worked to convert chemical energy into electrical energy utilizing the catalytic action of microorganisms. It consisted of an anode and cathode chamber, connected by an external electric circuit separated internally by a cation-specific membrane or a salt bridge [5]. MFC was the technology that utilized microbes to produce energy taken in the form of electricity. Microbes can convert various kinds of organic compounds into CO₂, water, and energy. One of MFC technologies which being developed at this time was Microalga Microbial Fuel Cell (MMFC) technology [6]. In this study, MMFC was constructed in an attempt to place microalgae at the MMFC anolyte as donor electrons for power generation. Anode chamber was containing the microalgae cultures that catalyzed into electrons and protons. Then, batik wastewater would save in cathode chamber. Power would be produced by reduction of the wastewater at the cathode chamber.

![Figure 1. MMFC operating system [7].](image)

*Chlorella pyrenoidosa* as one of microalgae was introduced in the anode of MFC to act as an electron donor. By controlling the oxygen content, light intensity, and algae cell density, microalgae would generate electricity without requiring externally added substrates. *Chlorella pyrenoidosa* was a photosynthetic organism that had a great CO₂ fixation ability [8]. One of studies about *Chlorella pyrenoidosa* was the conducted research in application of *Chlorella* sp. grew well in wastewater, this algae has the ability to reduce levels of pollutants very well, such as BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) with a range between 89 – 96% in the logarithmic phase and 80 – 95% in the stationary phase [9].

Through this, it would solve the problem about pollution from batik wastewater and decreased the chemical oxygen demand in the wastewater made it more friendly to the environment. In this study, the research was conducted for reducing the levels of pollutants in batik wastewater and proving the electrical energy produced by MMFC using *Chlorella pyrenoidosa*. The results of this study are also expected to be one step forward to obtain renewable energy sources, so that the use of MMFC systems with wastewater substrate can reduce fossil energy consumption [10].

Much of the research involving the use of MMFC for combined electricity production and wastewater treatment was focused on producing the most power through improved designs. However, estimates of the amount of power that could be produced in MMFC were a function of technique used to obtain polarization data. An alternate approached was to vary the circuit resistances at fixed time intervals, ranging from 10 s to 24 h. Polarization was performed once the voltage stabilized in the multimeter [11]. Power curves produced from polarization data could indicate the power producing capabilities from MMFC operation. The shape of the curves gives an insight into where the losses of resistance were occurred in the case of MMFC operation, could be broken down into three general areas; activation, ohmic, and mass transfer losses to Figure 2 [12].

A common problem often encountered when evaluating polarization curves was “power overshoot”. Power overshoot referred to the response of the system at high current densities (past the maximum power) in a power density curve where the cell voltage and current dropped very quickly resulting in a doubling back of the power density curve, producing lower than previously measured for
the lower current densities [11]. Two types of power overshoot were commonly observed in polarization measurements of MMFC. The first type produces maximum power densities that were much higher than those that could be sustained at a fixed resistance over long times, named Type M. As a result, Type M overshoot was easily controlled by lowering the scan rate or by increasing the time at a fixed resistance. The second type of occurred when the power density curves double back unexpectedly toward lower current densities, named Type D [17].

![Figure 2.](image)

**Figure 2.** Polarization data is used to produce (a) power density curve; indicating the power producing capabilities where * is the point of maximum power density and (b) polarization curve; relating to the losses that occur in a fuel cell; 1. Activation losses, 2. Ohmic losses, 3. Mass transfer losses [12].

Power overshoot was identified in numerous MMFC studies. Power overshoot could limit maximum power generated by MMFC, as demonstrated by this study. Few studies had attempted to explain why power overshoot occurred [17]. This present study, therefore, examined power overshoot too in MMFC operation, besides bioelectricity production and COD analyzation, identified the resistances corresponding to this overshoot and demonstrated that such an overshoot could restrict maximum power generated by MMFC.

2. Materials and Methods

2.1. Materials

The materials used in this study were the microalgae *Chlorella pyrenoidosa* as catholyte which obtained from C-Biore (Center of Biomass and Renewable Energy) Diponegoro University Integrated Laboratory. Then, batik wastewater with concentration by 100%v and 50%v as anolyte from Batik 16 industry Semarang, Meteseh, Semarang. Graphite electrode with length of 10 cm and diameter of 10 mm, used 1:1 and 2:2 bars, from an online store in Jakarta. The addition of yeast by 2 gr/L and 8 gr/L to the wastewater given. Then NaHCO₃ 0,32 gr/L, Urea 0,05 gr/L, and TSP 0,05 gr/L as nutrition of microalgae’s living. Besides, the utilization used in this study were glass box, digital multimeter, connecting wire, CO₂ wire, aeration wire, aerator, lamp, ballast, and luxmeter.

2.2. Methods

2.2.1. Cultivation of *Chlorella pyrenoidosa*. A litre of *Chlorella pyrenoidosa* was diluted to two litres, then the nutrients were added by 0,64 gr NaHCO₃, 0,1 gr Urea, 0,1 gr TSP. During cultivation, lighting and oxygen were given through aerator and then covered them with clear plastic. The absorbance (OD – Optical Density) had been checked everyday with spectrophotometer. If the absorbance had approached the value of 0.6, then *Chlorella pyrenoidosa* was ready to use.
2.2.2. MMFC Making Process. Batik wastewater was diluted into concentration according to the variable, 100%v and 50%v. Anode chamber (anolyte) was filled with a litre of wastewater. Meanwhile, the cathode chamber (catholyte) was filled with a litre of *Chlorella pyrenoidosa*. 1:1 and 2:2 bars of graphite electrode were put into the both chambers according to the variable. Yeast addition were given about 2 gr/L and 8 gr/L of the volume in the chamber according to the variable. Then, the both chambers were closed tightly, and the CO$_2$ wire were connecting the anolyte and catholyte to make CO$_2$ from wastewater flew to the microalgae for its photosynthesis. The catholyte was given a lighting, where 20 cm far from the reactor.

2.2.3. Simulation of MMFC. The MMFC system was operated for seven days, then every 24 hours, the voltage and current were measured with a multimeter. The absorbance (OD – optical density) of the microalgae also measured with spectrophotometer every day. Meanwhile, COD level of the wastewater were measured in early and at the end of MMFC simulation, to see the value of COD difference in wastewater before and after simulation.

2.2.4. Measurement of COD Level. COD (Chemical Oxygen Demand) was defined as the amount of oxygen equivalent consumed in oxidizing agents. The higher the COD value, the higher the amount of pollution contained in a wastewater. Batik wastewater would be measured its COD level with COD photometer, after being processed in COD reactor with operating condition of 150°C for two hours.

2.2.5. Polarization Curve Analysis. To estimate the amount of power that could be produced in a MMFC system were a function of the technique used to obtain polarization data. Polarization and power density curves, calculated based on the project area of the anode, were obtained by varying external resistances (10, 47, 100, 220, 470, 1000, 4700, 8200, 10.000, 47.000, 82.000, 100.000, 150.000, 200.000, 270.000, 330.000, 390.000, 510.000, 680.000, 820.000, and 1.000.000 $\Omega$) with a time interval of 24 hours. Data in form of current (i) and voltage (V) would calculate into current density (I) and power density (P), the power per unit area of electrode, by using equation:

$$I = \frac{V}{R \times A}$$  \hspace{1cm} (1)

$$P = V \times I$$  \hspace{1cm} (2)

Where I is current density (mA/m$^2$), V is voltage (V), R is resistant ($\Omega$), A is surface area of the electrode (m$^2$), and P is power density (mW/m$^2$).

3. Results and Discussion

3.1. The Effect of The Number of Electrode on MMFC Performance

3.1.1. Bioelectricity Production

Based on Figure 3, the trend of absorbance obtained was increased. Besides, from Figure 4, the voltage value tended to increase every day, but there was a voltage drop on the second day for both variables with 1:1 bar and 2:2 bars electrode, became 0.025 V and 0.036 V. it was happened because the effect of growth of algae which also decreased due to the algae were still adapt to their environment, so not to significantly produced the electricity. The optimum result was obtained on variable 2:2 bars electrodes on the sixth day with a voltage of 0.115 V. Both variables showed the fluctuating electricity production trend. The increasing of measured electrical value was thought to occur when microorganisms were breaking down simple substrates in the wastewater. The decreasing of electrical value could be thought to be caused when microorganisms were adapting to break down
more complex substrates into simpler ones [23]. A microorganism could become a substrate for other microorganism which caused free electrons and H⁺ ions not to be generated optimally so that the electrons flowing into the cathode were reduced and electricity produced was fluctuated [24].

![Figure 3](image1.png)  ![Figure 4](image2.png)

**Figure 3.** Growth of Chlorella pyrenoidosa every day on the influence of the number of electrodes  
**Figure 4.** Bioelectricity production every day on the influence of the number of electrodes

3.1.2. Chemical Oxygen Demand Analyzation.

The results of COD analyzation from batik wastewater showed that the COD value decreased from 824 mg/l to 752 mg/l (percentage reduce = 8,73%) after MMFC operation was carried out. It indicated that the dissolved oxygen amount in the wastewater decreased approach the maximum threshold which was 150 mg/l. Based on the research, it had been proven in MMFC process, there was a synergetic relationship between degradation of components in wastewater with algae’s growth to produce environmentally friendly bioelectricity [7]. The value of the voltage and electric current produced has a relationship to the level of waste product, include the number of electrodes used in MMFC process. The higher COD percentage reduce, the higher number of electrodes used in MMFC [3]. There was a linear correlation between the value of organic material in the waste and the electrical energy produced due to bacteria attached to the electrode surface, which degrades organic material under anaerobic conditions. This process causes the formation of degradation reaction of carbon dioxide, protons, and electrons in the waste [19].

3.2. The Effect of The Concentration of Batik Wastewater on MMFC Performance

3.2.1. Bioelectricity Production

Figure 5 showed that on the influence of concentration of batik wastewater on MMFC process, the absorbance of microalgae was increasing. Besides, Figure 6 showed that the highest voltage was obtained on the 6th day, 0.11 V for 50%v wastewater concentration and 0.095 V for 100%v concentration. From the data obtained, electricity production had been decreased on the last day of MMFC process because microalgae could produce biofilms which had the effect of increasing the resistance in anode and caused a decreasing in the electric voltage and current generated [13]. In the last day of 100%v wastewater variable showed the decreasing of voltage. The electricity production decreases according to the increase in concentration of wastewater due to factors, such as biofilm thickness and pH that affected the electron transfer and the activity of microalgae cell in producing electrons [20]. The electron gained at the anode must be the same as the number of electrons acceptors at the cathode. Anode temperature, pH discharge, and conductivity were also factor that would affect anode performance. Even so, in this study was estimated that biofilm thickness was the major factor in
decreasing electricity production. Internal barriers that occur due to the thick of biofilm could be minimized by increasing catalyst activation, maintaining pH and stability of temperature [21].

Figure 5. Growth of *Chlorella pyrenoidosa* every day on the influence of wastewater concentration

Figure 6. Bioelectricity production every day on the influence of wastewater concentration

### 3.2.2. Chemical Oxygen Demand Analyzation
After COD analyzation, the highest COD level decreased was obtained in 100%v concentration of wastewater. The value before MMFC operated was 460 mg/l and decreased until 266 mg/l. The percentage of COD value reducing about 42%. It was higher than [14] with substrate from pulp and paper wastewater. Meanwhile, 50%v concentration of batik wastewater, the COD level decreased not to significantly. It meant that MMFC operation more applicable in 100%v concentration of wastewater. Production of electricity in the MMFC process was influence by several factor, such as substrate oxidation, electron transfer, internal resistances, and reactions at the cathode. In the acclimatization process, cell metabolism was still a limiting factor that affected the output of electricity where microbial metabolism was proportional to the electrical energy produced. So, the COD value would decrease more in the higher concentration of wastewater [22]. But, in this study was estimated that biofilm thickness was the major factor in decreasing electricity production and caused a decreasing in COD which was not proportional to the increase in electricity production, in order to the voltage value decreased showed in the last day of MMFC process [21].

### 3.3. The Effect of The Addition of Yeast on MMFC Performance

#### 3.3.1. Bioelectricity Production
Figure 7 showed that the absorbance of microalgae also increased until 1.15. From Figure 8, the voltage increased every day except for 2 gr/l addition yeast, the voltage value decreased on the last day. The optimum value from the addition of 2 gr/l yeast was 0.103 V, while the addition of 8 gr/l yeast was 0.322 V. The decreasing voltage was caused by the metabolism of bacteria producing hydrogen, where hydrogen gas would accumulate over time and cover the electrodes, thus reducing electron transfer and decreased electricity production [4]. Meanwhile, the optimum bioelectricity value was obtained by adding yeast 8 gr/l where the voltage increased every day. Large amounts of CO₂ were produced by yeast fermentation and anaerobic microbes through fermentation where NADH was oxidized, the presence of a mediator would greatly increase the transfer of electrons to electrons for microalgae growth and got an increasing absorbance of microalgae. Then with the addition of 8 g/l yeast with a higher concentration in the experiment, had made the greater the anode-to-cathode electron transfer as an electron acceptor. So that, with the higher addition yeast, the greater the chance of oxidizing waste and electron exchange, the more often the result of voltage value increased significantly [25].
3.3.2. Chemical Oxygen Demand Analyzation

The results of COD measurement showed that 2 gr/l yeast addition to batik wastewater gave 40.64% decreasing of COD level. While, in 8 gr/l yeast addition gave very high COD level reduced, it was 48.5%. Yeast consumed the biochemical component wastewater such as carbon, nitrogen, and phosphate to produce protons and electrons. During the process, the biochemicals inside the wastewater which represented by COD value could be degraded effectively by yeast *S. cerevisiae* compared to sugar [26]. It meant that the addition of yeast to the wastewater increased MMFC’s ability to degrade components of the wastewater. The higher addition of yeast gave to the MMFC process, the higher COD level reduce in wastewater. Yeast became a catalyst in oxidizing waste which made electron transfer more frequent.

3.4. Polarization Curve Analyzation

The test of polarization analysis was given to all variables, so that the polarization curves had made as figures below. Polarization data obtained from external resistance given between 10 – 1.000.000 Ω.

![Figure 7. Growth of *Chlorella pyrenoidosa* every day on the influence of addition of yeast](image1)

![Figure 8. Bioelectricity production every day on the influence of addition of yeast](image2)
Figure 9. Polarization curves on MMFC operation each variable with the effect of varying number of graphite electrode, concentration of batik wastewater, and yeast addition (a) 1:1 bar, 100%v, 2 gr/l; (b) 1:1 bar, 100%v, 8 gr/l; (c) 1:1 bar, 50%v, 2 gr/l; (d) 1:1 bar, 50%v, 8 gr/l; (e) 2:2 bars, 100%v, 2 gr/l; (f) 2:2 bars, 100%v, 8 gr/l; (g) 2:2 bars, 50%v, 2 gr/l; (h) 2:2 bars, 50%v, 8 gr/l.

From Figure 9, all polarization curves showed the power overshoot phenomenon. In Figure 2.b, 2.d, 2.e, 2.f, 2.g, and 2.h showed that the voltage value was steady at higher current density. Besides, from the last day of MMFC operation gave high power density and not so close to the power curves from Figure 2, when at last power density measured to be decreased significantly and reach the value of zero. The reason for power overshoot had been explored in several studies [15]. The overshoot phenomenon and the overestimation of power density could be attributed to the microalgaes’s inability to adjust to each new and more demanding resistance value. Therefore, the voltage was still decaying when the next resistance value was prematurely introduced and thus the value recorded at that particular resistance was inflated. The overshoot shape was produced when deterioration of the voltage began to slow down (as might as occur when the size of resistance change was reduced, e.g. the decrements of 100 Ω become decrements of 10 Ω) [16].
It had been showed that the resistances of MMFC was increased at higher current densities when power overshoot occurs [15]. The overshoot resulted from a rapid increase in the anode potential as resistance to current flow was decreased, indicating electron transfer limitation at the anode. The limitation was most likely related to a slow response from the microbes to adjust to the new resistance [11]. Hence, power overshoots were generated by limiting electron generation, rather than by limiting electron flow, so electron depletion underlies power overshoot in all MMFC operation [18]. Presence of the overshoots could therefore indicate that the length of sample time was insufficient and that the maximum power produced in that curve might not be accurate [16].

4. Conclusion
The influence of the number of electrodes, concentration of wastewater, and yeast addition in MMFC operation using Chlorella pyrenoidosa and batik wastewater gave the best result on 2:2 bars graphite electrode, 100%v concentration of batik wastewater, and 8 gr/l yeast addition. The addition of yeast to the wastewater increased MMFC’s ability to degrade components of the waste. Yeast became a catalyst in oxidizing waste which made electron transfer more frequent. It had been proven by 48.5% COD level reduced after MMFC operation, with the highest voltage by 0.322 V.

In polarization curve analysis, it showed that all the variables had overshoot phenomenon. Its presence because microalgae’s inability to adjust to each new resistance value. Therefore, voltage still decaying and began to slow down when size of resistance change was reduced. It indicated that the length of sample time was insufficient and that the maximum power produced in that curve might not be accurate.

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