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Development of Microbial Fuel Cell in Tofu Liquid Waste in Producing Clean Energy

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Abstract

The high need of Indonesian people for electrical energy has led to an increase in energy demand. This has triggered research efforts based on renewable technologies that are efficient and environmentally friendly. One of the renewable energy sources that is widely developed is Microbial Fuel Cell (MFC). MFC works by utilizing organic matter used by microbes as an energy source in carrying out metabolic activities. This study aims to determine the effect of *Bacillus subtilis* and *Escherichia coli* bacteria with different concentrations of 10% (v/v) and 15% (v/v) on the electrical energy generated from the tofu liquid waste substrate and observe changes in pH and Biological Oxygen Demand (BOD) in the MFC system. This research method consists of several steps, namely the preparation of electrolysis equipment, electrodes, substrates, bacterial cultures, electrolytes and MFC processes. The maximum value of voltage and current in *Bacillus subtilis* bacteria occurred at the 10 hours with 15% (v/v) bacterial concentration which amounted to 394 mV and 10.6 mA with a bacterial population of 16.15 × 10^9 CFU/mL and a power density value of 126.67 × 10^{-4} watt/m^2. Furthermore, the maximum value for *Escherichia coli* bacteria occurred at the 10 hours with a voltage and current of 266 mV and 5.3 mA with a bacterial population of 15.60 × 10^9 CFU/mL and a power density value of 55.67 × 10^{-4} watts/m^2. The pH value of the substrate variations with the addition of *Bacillus subtilis* bacteria ranged from 5-6, while with the addition of *Escherichia coli* bacteria variations ranged from 4-5. This system also reduces the value of biological oxygen demand.

INTRODUCTION

Energy use in Indonesia has experienced very rapid growth in line with economic and population growth. Rapid technological developments trigger an increase in energy needs, as if electricity is a primary need for society (Asrib et al., 2016). The people's need for electrical energy which continues to increase causes an energy crisis which will later become a problem on an ongoing basis. Energy sources such as fossil energy which are generally the main fuel for power plants are non-renewable energy and have a negative impact on the environment. One way to overcome and anticipate the use of fossil energy is to use alternative energy. Alternative energy is energy that can be renewed and is also environmentally friendly, one of the alternative energies that can be used is Microbial Fuel Cells.

Microbial Fuel Cell (MFC) is an alternative energy that utilizes organic materials used by microbes as an energy source in carrying out metabolic activities (Syafaati, 2015). Microorganisms can convert chemical energy stored in organic components into electrical energy during incubation in Microbial Fuel Cells, these microorganisms deliver electrons to the anode. Electrons originating from the anode flow to the cathode through a wire which generates an electric current (Rabaey et al., 2003). Besides being

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environmentally friendly and having the potential to generate electrical energy, MFC is also able to reduce the impact of environmental pollution resulting from organic waste. One of the organic wastes that can be utilized is tofu liquid waste.

The tofu manufacturing industry is widely spread across Indonesia, but there are still many tofu industries that do not have waste disposal channels that meet quality standards. Tofu liquid waste contains quite high organic matter so that if it is directly disposed of into rivers or water bodies it can cause an unpleasant odor, become a pollutant for the environment and reduce the quality of clean water (Putri & Fatimah, 2021). Environmental pollution by tofu liquid waste can be avoided by utilizing the liquid waste itself. To reduce the impact of environmental pollution, efforts are made to utilize tofu liquid waste as a substrate in the Microbial Fuel Cell (MFC) system.

MATERIALS AND METHODS

Materials and Instruments

The materials used: tofu liquid waste taken from the tofu processing home industry in Guntung Manggis, Kelurahan Landasan Ulin, bunsen burner, copper rods, carbo stems, distilled water, 1 M NaCl, 3% H_2O_2, 0.5 M H_2SO_4, 0.1 N $KMnO_4$, NBA, plastic wrap and aluminum foil.

The equipment used was MFC reactor consisting of dual chambers and connected to an alligator clip test lead, digital multimeter, analytical balance, hotplate, magnetic stirrer, beaker glass, erlenmeyer, stove wick, glass stirrer.

Proton exchange membrane preparation

Preparation of the proton exchange membrane, in this study, used a stove wick that had been boiled for one hour with 3% H_2O_2 and then rinsed with distilled water. Then boiled for one hour with 0.5 M H_2SO_4 and then rinsed with distilled water three times. The membrane was stored in distilled water and then dried before being applied to the MFC reactor.

Electrode Preparation

The electrodes were immersed in 1 M NaCl solution for 24 hours and then immersed back into distilled water when they were to be used.

Substrate Preparation

The substrate was obtained from the tofu processing household industry in the form of liquid waste as much as 5 liters, then placed in a container. Then incubated for 48 hours.

Electrolyte Preparation

The electrolyte solution used was 79.02 grams of $KMnO_4$ 1 M (Merck, Jerman) and dissolved in 500 mL of distilled water.

Bacterial Culture Preparation

Pure culture of *Bacillus subtilis* and *Escherichia coli* was grown on 24 grams of NBA media and 1% glucose in 1 liter of distilled water in erlenmeyer flasks. The broth is then filtered and sterilized in an autoclave for 15 minutes at 121°C and cooled to 28°C. After cooling, the pure culture of bacteria is transferred to the new medium using an aseptically ose needle. Then the new media (starter) was incubated at 30°C for 1 day.

Microbial Fuel Cell (MFC) Process

Prior to observing the substrate and inoculum in the reactor, they were incubated for 12 hours. Experiments were carried out by observing the voltage (mV), the current (mA) and counting the number of *Bacillus subtilis* bacterial cells every two hours over a twelve-hour period of treatment. The multimeter was connected to the MFC system with alligator clamps for each treatment variation.

RESULTS AND DISCUSSION

Bacterial Population

Bacteria are the main factor in the Microbial Fuell Cell (MFC) system, the growth of bacterial cells shows the optimization of the ability of bacteria to degrade liquid waste substrates to be converted into electrical potential. In this research, the bacteria utilized are *Bacillus subtilis* and *Escherichia coli*, these two bacteria are varied with different concentrations of 10% (v/v) and 15% (v/v). The value of the bacterial population in each variation is shown in Figures 1 – 2.

Figure 1, shows that the growth of *Bacillus subtilis* bacterial substrate variation with 15% (v/v) concentration has the highest bacterial population value of 16.15×10^9 CFU/mL at 10 hours and the

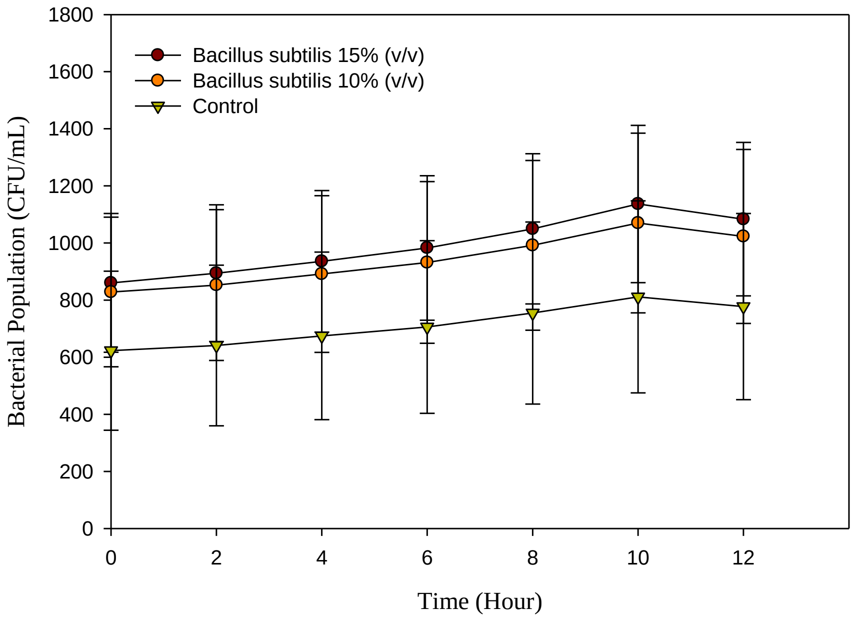

Figure 1. Comparison of number of growth of bacterial cells against time in variations substrate *bacillus subtilis.*

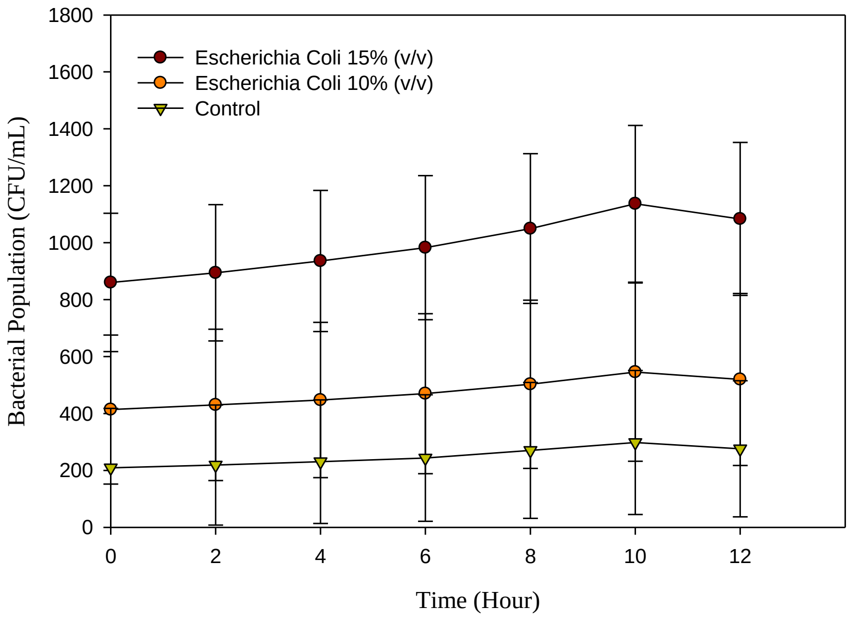

Figure 2. Comparison of number of growth of bacterial cells against time in variations substrate *Escherichia coli.*

lowest value in the 10% (v/v) variation with a value of 12.34 × 10^9 CFU/mL at 0 hours. In Figure 2, the value of the bacterial population in the substrate variation of *Escherichia coli* bacteria was highest in the 15% (v/v) concentration with a value of 15.60 × 10^9 CFU/mL at 10 hours and the lowest value was in the 10% (v/v) concentration with a value of 12.30 × 10^9 CFU/mL at 0 hours. For the value of the bacterial population in the substrate variation without the addition of bacteria, it has a relatively small population value, with the highest value being at the 10 hours of 4.28 × 10^9 CFU/mL and the lowest value being at the 0 hour with a value of 1.91 × 10^9 CFU/mL. Based on Figure 1 and Figure 2, it shows that the bacteria *Bacillus subtilis* and *Escherichia coli* experienced an increase in growth, this increase indicates that the bacterial cells are experiencing a stationary phase, where both bacteria can properly degrade substrates so that they can produce electrical energy. The maximum increase in the number of bacterial cells causes a rapid substrate degradation process so that nutrient availability will decrease. This will cause a decrease in the number of bacteria where this phase is called

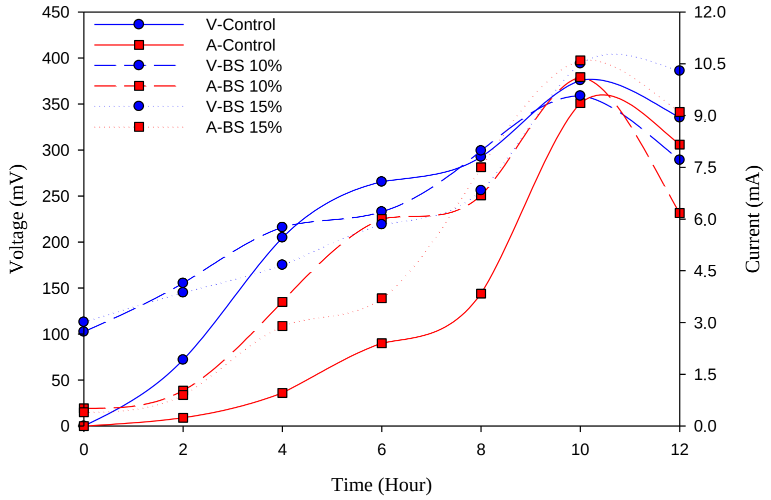

Figure 3. Comparison of voltage (mV) values against time and current (mA) in Variations substrate *Bacillus subtilis*.

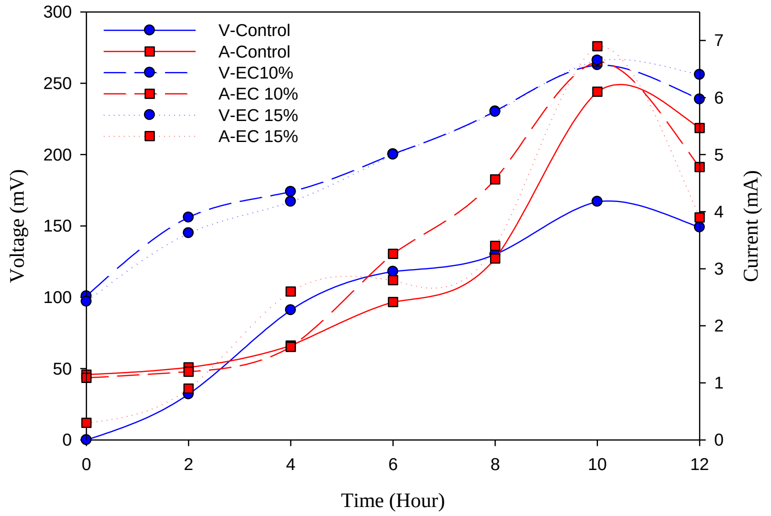

Figure 4. Comparison of voltage (mV) values against time and current (mA) in variations substrate *Escherichia coli*.

the death phase which is characterized by a decrease in the number of bacterial cells caused by a decrease in the amount of nutrients (Prayogo et al., 2017).

MFCs Power

The production of clean energy using Mirobial Fuell Cell (MFC) with the use of *Bacillus subtilis* and *Escherichia coli* bacteria with 10% (v/v) and 15% (v/v) concentrations respectively on the tofu liquid waste substrate can produce electrical energy. The MFC system used in this research is an MFC system with dual chamber, where there are two anode and cathode compartments, each of which is installed with an electrode. The anode compartment contains tofu liquid waste while the cathode compartment uses a conductive electrolyte solution, namely $KMnO_3$. The production of electrical energy generated during MFC operation is measured using a multimeter connected to both electrodes on the MFC reactor. The anode is connected to the negative cap on the multimeter and the cathode is connected to the positive cap. Changes in voltage and current values obtained over 12 hours are shown in Figures 3 – 4.

The voltage and current values obtained with different concentration variations are shown in Figure 3, at 10% (v/v) bacterial concentration, the highest voltage and current values occurred at hour 10 which amounted to 319 (mV) and 3.9 (mA) with a power density value of 0.005709 (Watt/m^2) and the lowest value occurred at hour 0 which amounted to 97 (mV) and 0.3 (mA) with a power density value of 0.000088 (Watt/m^2). As for the

15% (v/v) bacterial concentration, the highest voltage and current values were obtained at hour 10, which amounted to 394 (mV) and 10.6 (mA) with a power density value of 0.012667 (Watt/m^2) and the lowest value occurred at hour 0, which amounted to 113 (mV) and 0.4 (mA) with a power density value of 0.000137 (Watt/m^2). As for the voltage and current values without bacterial concentration, the highest value was obtained at hour 10 which amounted to 167 (mV) and 3.9 (mA) with a power density value of 0.001975 (Watt/m^2) and the lowest value occurred at hour 0 which amounted to 0 (mV) and 0 (mA) with a power density value of 0 (Watt/m^2).

In the variation with the addition of *Escherichia coli* bacteria, the power density value obtained from the concentration variation is shown in Figure 4, where the voltage and current values with 10% (v/v) bacterial concentration variation at hour 10 have the highest voltage and current values of 219 (mV) and 5.2 (mA) with a power density value of 0.003454 (Watt/m^2) and the lowest value occurs at hour 0 which is with a voltage and current value of 84 (mV) and 0.1 (mA) with a power density value of 0.000025 (Watt/m^2). As for the variation of 15% (v/v) bacterial concentration, the highest voltage and current were obtained at hour 10, namely 266 (mV) and 6.9 (mA) with a power density value of 0.005567 (Watt/m^2) and the lowest value occurred at 0 hour, namely with a voltage and current value of 91 (mV) and 0.3 (mA) with a power density value of 0.000083 (Watt/m^2). As for the voltage and current values without variation in bacterial concentration, the highest value occurred at hour 10 which was 167 (mV) and 3.9 (mA) with a power density value of 0.001975 (Watt/m^2) and the lowest value occurred at 0 hour which was equal to the voltage value and current strength of 0 with a power density value of 0 (Watt/m^2) The maximum increase in voltage and current values occurred at 10 hours and decreased in the next hour. The increase in voltage and current values indicates that the microbes are in exponential phase. In this phase the microbial cells divide rapidly and constantly. Thus, the microbes will become abundant, with a greater number of microbes, the oxidation process will run faster. So that it will increase voltage and electricity production (Utami et al., 2018). While the decrease in voltage is due to the formation of biofilm on the salt bridge which causes microbial activity at the anode to be inhibited, the biofilm can also block the process of transferring protons from the anode to the cathode. The retained protons will cause a change in pH at the anode so that it can interfere with bacterial growth. The biofilm layer also results in a lack of protons in the cathode chamber so that the resulting voltage is small (Li et al., 2010).

The results obtained showed that the substrate with the addition of 15% (v/v) Bacillus subtilis bacteria produced high voltage, current and power density compared to the addition of other bacteria. This happened because Bacillus subtilis bacteria have better characteristics than Escherichia coli. The characteristics and properties of the bacteria determine their ability to degrade the tofu wastewater substrate. Bacillus bacteria are cellulotic bacteria that can produce cellulase and hydrolyze cellulose into simpler products, namely glucose (Prihantoro & Zulaika, 2015). It makes the tofu waste substrate a suitable place for degradation. The genus Bacillus is a bacterium characterized by aerobic to facultative anaerobic and gram-positive bacteria that can help the process of decomposing organic matter. Bacillus subtilis is rod-shaped, one-celled, approximately 0.5-2.5 μm long and 1.2-10 μm wide. While Eschericia coli is a short rod-shaped gram-negative bacterium that is approximately 2 μm long, and 2.0-6.0 μm wide. (Yoganathan & Ganesh, 2015).

The amount of electrical energy in the MFC system is influenced by the rate of metabolism carried out by bacteria, other than that the size of bacterial cells is also related to the amount of electrical energy produced. This is in accordance with research (Lee et al., 2010), that bacteria with smaller sizes can produce greater electrical energy than large bacteria. The phenomenon is caused by the difference in density of bacteria around the electrode, where small bacteria can make more contact with the electrode. From both bacteria used in this research, Bacillus subtilis has a smaller bacterial size than Escherichia coli. Therefore, the energy gain from the tofu liquid waste substrate with the addition of 15% (v/v) Bacillus subtilis bacteria is greater than the addition of other bacteria.

The pH changes of the tofu effluent in the anode compartment during the MFC process are shown in Figures 5 – 6.

In Figure 5, it is shown that the pH value in the variation of tofu liquid waste substrate with the addition of *Bacillus subtilis* bacteria concentrations of 10% (v/v) and 15% (v/v) ranged

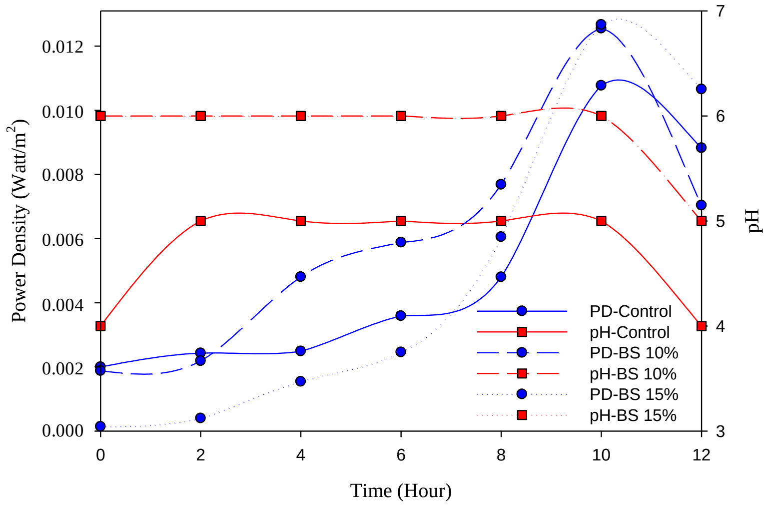

Figure 5. Comparison of pH with power density value against time for *Bacillus subtilis* substrate variations.

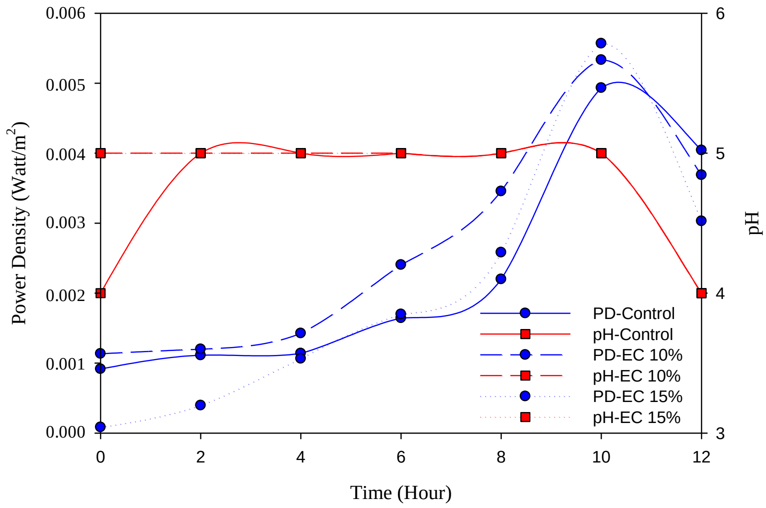

Figure 6. Comparison of pH with power density value against time for *Escherichia coli* substrate variations.

from 5-6, the initial pH of the waste before treatment was 6 until 10 hours constant, then decreased to 5 at 12 hours. While in Figure 4 the pH value in the variation of tofu liquid waste substrate with the addition of *Escherichia coli* bacteria concentrations of 10% (v/v) and 15% (v/v). While in Figure 4 the pH value in the variation of tofu liquid waste substrate with the addition of *Escherichia coli* bacteria concentrations of 10% (v/v) and 15% (v/v) ranged from 4-5, the initial pH of the waste before treatment was 6 until 10 hours constant, then decreased to 5 at 12 hours. While in Figure 4 the pH value in the variation of tofu liquid waste substrate with the addition of *Escherichia coli* bacteria concentrations of 10% (v/v) and 15% (v/v) ranged from 4-5, the initial pH of the waste before treatment was 5 until 10 hours constant, then decreased to 4 at 12 hours. The pH value obtained in each substrate variation tends to decrease. The decrease in pH conditions indicates the formation of acid by acidogenic bacteria in the reactor. (Bouallagui et al., 2005) stated that acidogenic (acid-forming) bacteria grow rapidly and decompose glucose into acetic, propionic and butyric acids. The decrease in pH is also caused by sulfate oxidation reactions, nitrification, oxidation of organic carbon. Changes in pH value in the anode compartment during the MFC process on each substrate are directly proportional to the power density produced, if the pH of the substrate is too low or high it can affect bacterial metabolism which affects proton transfer and cathode reactions so that it has an impact on MFC performance in producing electrical energy. MFC performance and pH conditions have a close relationship in the MFC system, where when the pH value decreases significantly, the output voltage and current produced accordingly becomes low.

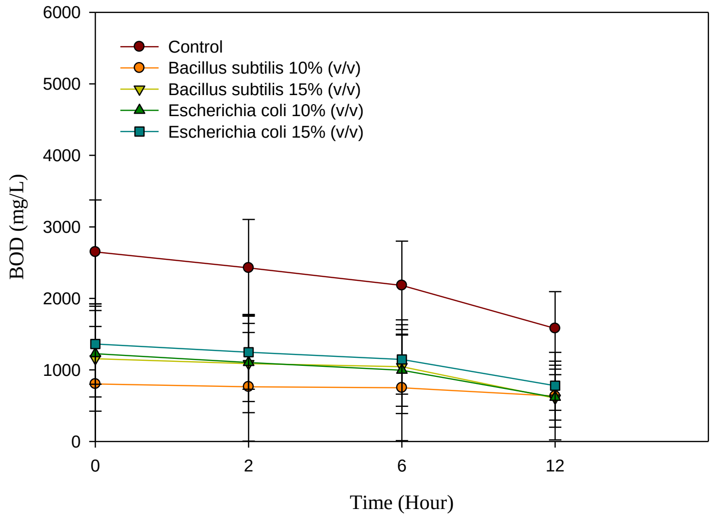

Figure 7. Comparison of BOD values (mg/L) against time at substrate variations

Biological Oxygen Demand (BOD)

The decomposition of organic substances is a natural event in which when a body of water is polluted by organic substances, bacteria will utilize dissolved oxygen in water during the biodegradable process, resulting in the death of aquatic biota (Ayuningtyas & Ellyza, 2017). In this research BOD is one of the liquid waste pollution parameters used, BOD is used to determine the number of organic compounds in degraded waste. During the operating time of MFC, the BOD value is seen to decrease. The decrease in BOD value in each bacterial variation and concentration are shown in Figure 7.

During the MFC process, the BOD value at the initial and final measurements tends to decrease in each substrate variation. In pure tofu liquid waste, the BOD content values at 0 and 12 hours were 4,935 mg/L and 3,450 mg/L. In the variation of the addition of Bacillus subtillis bacteria with a concentration of 10% (v/v), at 0 and 12 hours were 3,216 mg/L and 2,470 mg/L. While at the variation of 15% (v/v) concentration, at the 0th and 12th hours respectively were 3,720 mg/L and 3,020 mg/L. In the variation of the addition of Escherichia colli bacteria with a concentration of 10% (v/v), the value of BOD content at 0 and 12 hours was 2,870 mg/L and 1,650 mg/L, while at a concentration variation of 15% (v/v) at 0 and 12 hours were 3,810 mg/L and 2,831 mg/L. The decrease in BOD value in each variable occurs due to the process of decomposing organic matter contained in wastewater, the process decomposes organic matter that is easily decomposed and causes oxygen supply to decrease, resulting in lower BOD values in wastewater (Romayanto & Wiryanto, 2006).

CONCLUSION

The addition of substrate variations with *Bacillus subtilis* bacteria at a concentration of 15% (v/v) has the highest bacterial population value of 16.15 x 10^9 CFU/mL with a power density value of 0.012667 Watt/m2. While the lowest bacterial population value occurred in the substrate variation with *Escherichia coli* bacteria 10% (v/v), which amounted to 10.82 x 10^9 CFU/mL with a power density value of 0.012667 Watt/m2. The pH value in the substrate variation with the addition of *Bacillus subtilis* bacteria ranged from 5-6, while with the addition of *Escherichia coli* bacteria variation ranged from 4-5. In all variations of *Bacillus subtilis* and *Escherichia coli* bacteria with the addition of concentrations of 10% (v/v) and 15% (v/v) experienced a significant decrease in BOD values.

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