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Research Article
Effect of Concentrations of Bacterial Consortia in Culture Medium from Wastewater in Microbial Fuel Cells

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Abstract

Background and Objective: Microbial fuel cells (MFCs) are technology by which electricity is generated from microbes. Electricity can be generated by MFCs by using wastewater as a substrate. Bacterial consortia from wastewater have been widely used in MFCs. Bacteria degrade substrates, resulting in a difference in potential, which in turn generates electricity. This research aimed to analyze the potential of bacterial consortia and identify the best number of additional bacterial consortia cultured in nutrient broth containing tempeh wastewater as the primary glucose source. **Materials and Methods:** In this study, used wastewater from tempeh production with different concentrations of bacterial consortia in culture medium (0, 0.4, 1 and 10%). Voltage was recorded with a digital multimeter instrument for 50 h. Bacterial growth was assessed every 3 h at 486 nm with a spectrophotometer UV-Vis. The COD of tempeh wastewater was analyzed before and after the experiment with COD kits and heating it on a COD Digester Block for 2 h at 150°C. Statistical analysis method used in this study is monothetic analysis or one-factor-at-a-time method. **Results:** The study indicates that the nutrients present in tempeh wastewater can be used as an effective energy source for MFCs using bacterial consortia as a biocatalyst. **Conclusion:** The optimum voltage and power density generated was up to 291.1 mV and 66.33 mW m^{-2} following 1% culture medium and its coulombic efficiency reached 4.48%. In addition to generating electricity, MFCs can remove chemical oxygen demand (COD). The highest COD removal value reaches 42.97% when 10% of culture was used.

Key words: Microbial fuel cell, bacterial consortium, wastewater, chemical oxygen demand, power density, coulombic efficiency

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Membrane-less microbial fuel cells (MFCs) use bacteria to break down organic matter without a proton exchange membrane[1]. Substrates are degraded by bacteria and directly produce electrons that flow to the anode. Electrons from the anode flow to the cathode via an external circuit and generate an electric current.

Pure bacterial cultures have been widely used to generate electricity. Bacteria that can produce electricity are called electrochemically active bacteria. In addition, bacterial consortia in wastewater can also be used to generate electricity[2].

Previous studies have used sucrose[3], glucose and starch[4] as carbon substrates, which are used by bacteria such as *Desulfovibrio desulfuricans*, *Clostridium butyricum* and *Lactobacillus plantarum*[1]. Additionally, wastewater has been widely used in MFC research. The common types of wastewater used includes chocolate factory effluents, domestic wastewater, brewery wastewater[5] and tempeh wastewater. When wastewater is used as a substrate, MFC can reduce the chemical oxygen demand (COD) of the wastewater[6].

The mechanism of electron transfer to the anode can be explained as follows: (1) Direct electron transfer through the bacterial membrane to which cytochrome c is bound[7] or bacteria with nanowires[8] and (2) Direct electron transfer via extracellular electron transporters (e.g., manganese and iron)[9,10].

Electrons are transferred from bacteria to anodes derived from byproducts of metabolism reaction of bacteria: Nicotinamide Adenine Dinucleotide (NADH) and $FADH_2$ (Flavin Adenine Dinucleotide). Bacterial metabolic pathways include glycolysis, oxidative phosphorylation, Krebs cycle and electron transport chain. In glycolysis, NADH is formed when 2-glyceraldehyde 3-phosphate molecules undergo oxidation due to the addition of phosphate then reducing NAD^+.

This reaction leads to the formation of two NADH molecules while carrying 4 electrons to the cell membrane. Furthermore, this reaction leads to oxidative decarboxylation reactions.

In oxidative phosphorylation, NADH is formed by the oxidation of pyruvic acid, which binds to coenzyme A and the resultant NADH carries 4 electrons to the cell membrane. Furthermore, the reaction enters Krebs cycle. In the Krebs cycle, NADH is formed by the oxidation of isocitric acid, alpha-ketoglutaric acid and malic acid. Meanwhile, $FADH_2$ is formed by the oxidation of succinic acid. This reaction also occurs twice to form 6 NADH and 2 $FADH_2$, which carries 16 electrons to the cell membrane. Furthermore, NADH and $FADH_2$ formed from all the reactions enter the electron transport chain. Each molecule of NADH and $FADH_2$ passes through electron transport chain and if oxygen is not present at the end of the electron transport chain, electrons are transferred to the anode by bacteria due to the positive value of the anode. For every mole of glucose utilized by bacteria, 24 electrons are transferred to the anode[10].

Efficiency of electron transfer from bacteria can be determined as coulombic efficiency, which is calculated using Eq. 3. The coulombic efficiency is used to determine the ratio of the total coulombs transferred from the substrate to the anode to the maximum possible coulombs transferred if all the substrate produces electrons.

In addition to the bacterial efficiency of electron transfer, the anode and cathode are both important factors involved in electron flow. The anode should be chosen according to its purpose of facilitating transfer of bacterial electrons and materials such as graphite, which facilitate easy adherence of bacteria, should be used to facilitate electron transfer from bacteria[11].

This study aims to analyze the potential of bacterial consortia from tempeh wastewater and identify the best number of additional bacterial consortia cultured in nutrient broth containing tempeh wastewater as the primary glucose source. This topic provides the possibility of utilizing tempeh wastewater as a substrate on MFC system.

MATERIALS AND METHODS

This study was conducted in 2016 at Bioprocess Engineering Laboratory, Chemical Engineering Department, Universitas Indonesia.

Bacterial consortium culture: The bacterial consortium from tempeh wastewater was cultured in nutrient broth at room temperature (37°C) on a shaker for 15 h to allow bacterial growth until the mid-exponential phase.

Operating conditions and MFC construction: The MFC was constructed according to a design as follows. A single-chamber reactor with a 2-L active volume was used for the MFC. The anode and cathode chambers were not separated by a proton exchange membrane. A graphite electrode was used in this reactor. The anode has an active surface area of 127.75 cm^2. The cathode is in direct contact with the outside air and is termed an air cathode. The anode and cathode are connected by a copper wire and 100-ohm resistors.

The reactor was filled with artificial tempeh wastewater. Artificial tempeh wastewater was prepared by boiling soybeans with distilled water in a ratio of 3:5 and incubated for 1 week.

The reactor contained 500 mL of nutrient broth containing the bacterial consortium cultured from tempeh wastewater. Different concentrations of bacteria were used, including 0, 0.4, 1 and 10% of the medium. In each variation, the reactor contained culture in 500 mL of tempeh wastewater, 100 mL of 0.1 M phosphate buffer at pH 7 and 500 mL of electrolyte solution (potassium persulfate 0.03 M). Voltage was recorded with a digital multimeter voltage APPA 109N instrument for 50 h. The entire experiment was conducted at room temperature (37°C).

Chemical analysis: Bacterial growth was assessed every 3 h at 486 nm with a single-beam spectrophotometer UV-Vis (Hitachi, Ltd. Tokyo, Japan). In addition, the COD of tempeh wastewater was analyzed before and after the experiment by mixing each sample taken from reactor, with COD kits and heating it on a COD Digester Block for 2 h at 150°C.

Statistical analysis: The multimeter yielded data as voltage (mV or V). Data were processed into current with a resistor value of 100 ohms as follows[12]:

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

Where:

I (A) = Current
V (V) = Voltage
R (ohm) = Resistor

The current data is then used to calculate the power density. The power density can be used to determine the energy generated per square meter of anode surface area as follows[12]:

$$P\left(\frac{mW}{m^2}\right) = \frac{V(volt)\,I(mA)}{A\,(m^2)} \qquad (2)$$

Where:

P ($mW\ m^{-2}$) = Power density
A (m^2) = Anode surface area

The coulombic efficiency equation was used to determine the ratio between the total coulombs transferred from the substrate to the anode with maximum possible coulombs transferred if all the substrate produces electrons. The equation to determine the coulombic efficiency of the MFC is[12]:

$$\varepsilon_{Cb} = \frac{M\int_0^{tb} I dt}{F\, bv_{an}\Delta COD} \qquad (3)$$

Where:

ε_{Cb} = Coloumbic efficiency
M = Molecular weight
I = Current (A)
F = Faraday constants 96.485 C moL^{-1}
b = Number of electrons/mole of the substrate
tb = Time of COD removal
v_{zn} = Volume anode
ΔCOD = COD_{after} (g L^{-1})-COD_{before} (g L^{-1})

RESULTS

Electrical energy generated at four different concentrations (0, 0.4, 1 and 10%) of the bacterial consortium can be seen in Fig. 1. The highest values of voltage, current and power density were obtained when using 1% culture medium (Fig. 1). The highest values of voltage, current and power density were obtained when using 1% culture medium, i.e., 291.1 mV, 2.91 mA and 66.33 mW m^{-2}, respectively. However, the most stable voltage, current and power density after 12 h of the experiment were obtained with 10% culture medium. Bacterial growth by these variations can be seen in Fig. 2 and shows a larger number of bacteria in the reactor.

Figure 3 shows COD removal occured in tempeh wastewater during 50 h process, with 1 and 10% concentrations of bacterial consortia in culture medium. The most efficient COD removal occurred in the 10% culture medium and the COD removal in this case reached 42.97% (Fig. 3).

DISCUSSION

The MFC was tested at four different concentrations (0, 0.4, 1 and 10%) of the bacterial consortium. Electrical energy generated by these variations can be seen in Fig. 1. The highest values of voltage, current and power density were obtained when using 1% culture medium. In the present study, the highest values of voltage, current and power density were 291.1 mV, 2.91 mA and 66.33 mW m^{-2}, respectively.

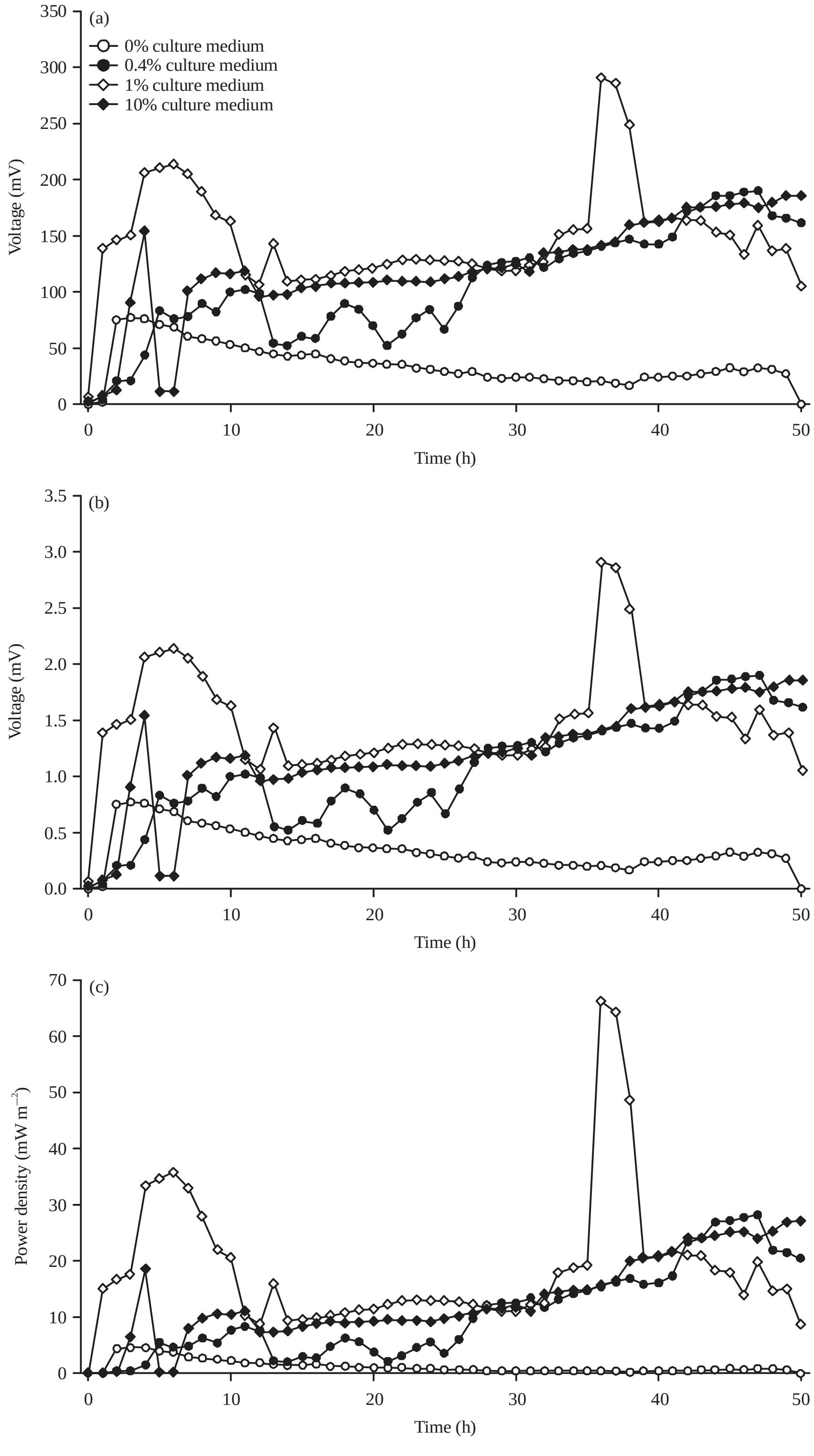

Fig. 1(a-c): Effect of 0, 0.4 and 1% dan 10% of bacterial consortia in culture medium on (a) Voltage, (b) Current and (c) Power density

The most stable voltage, current and power density after 12 h of the experiment were obtained with 10% culture medium. Thereafter, increasing the percentage of culture resulted in higher electrical energy due to increased bacterial activity in the reactor[13].

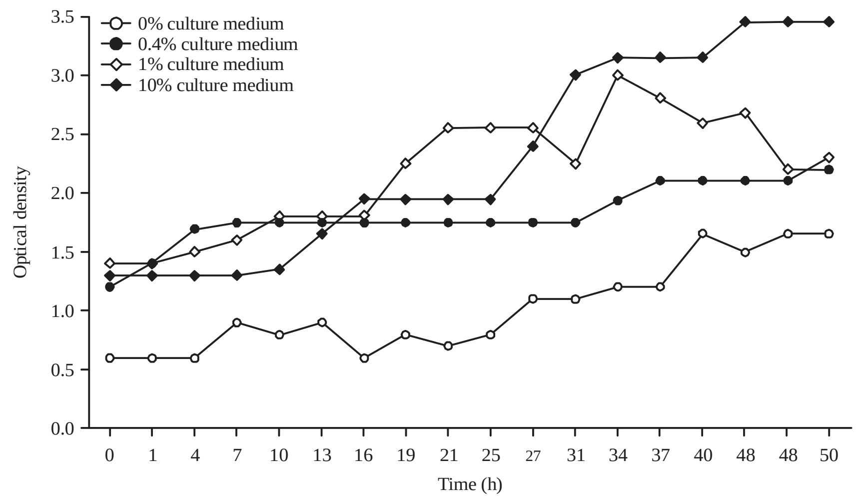

Fig. 2: Growth curve of bacterial consortium inside reactor

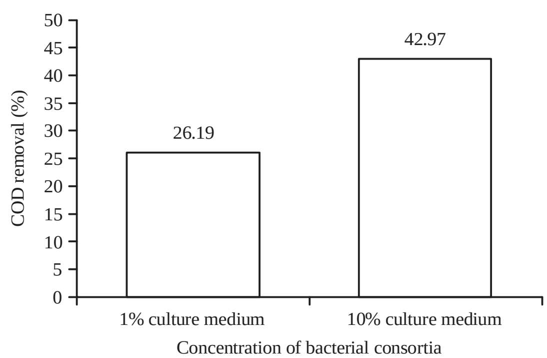

Fig. 3: Effect of concentrations of bacterial consortia in culture medium on COD removal in tempeh wastewater

A larger number of bacteria in the reactor (Fig. 2) correlated with production of more electrons by bacterial metabolism and consequently higher values of voltage, current and power density. This study used tempeh wastewater as a glucose substrate from which bacteria produce electrons as a byproduct of metabolism of NADH and $FADH_2$. The metabolism of glucose through glycolysis, oxidative decarboxylation, Krebs Cycle and electron transport chain produces 10 NADH and 2 $FADH_2$, where each NADH molecule carries two electrons and each $FADH_2$ molecule carries two electrons.

The electrical energy generated from the four concentrations of bacterial consortia in culture medium was generally unstable. This condition occurs due to the complexity of electrons transferred from the bacteria to the anode. The synergistic and antagonistic relations of various bacteria greatly influence electron transfer from the bacteria to the anode. Synergies among various bacteria occur in two ways.

First, cells can transfer electrons to another cell through the cell membrane. Bacterial cells transfer electrons through cytochromes on the cell membrane[14]. In addition, electron transfer among various bacteria can occur when using a single-reactor compartment, which makes the substrate and bacteria attach to the cathode and receive electrons from the cathode. As shown in Logan's research[14], bacteria produce differences in potential when they receive electron from cathode.

Apart from using cytochromes, bacteria secrete active reducing molecules called flavin that serve as electron transporters. Flavin takes electrons from the cell and transports them on to a solid electron, the anode. Then, they return to the cell for further processing. *Lactobacillus casei* is one of the bacterial species in tempeh wastewater and this species contains flavin receptors on the cell membrane. Second, the bacterial community can form biofilms by quorum sensing chemicals so electrons can reach the anode through the biofilm[14].

Antagonistic relations occur due to the presence of oxygen, which diffuses through the cathode to the anode chamber. Diffused oxygen is used by tempeh wastewater bacteria, which tend to be facultative aerobic organisms and in the presence of oxygen, oxygen respiration occurs[15]. When oxygen is present in the anode, the electrons from bacteria reduce it and decrease the electricity produced. In addition, the presence of oxygen activates bacteria that do not produce electricity, resulting in substrate competition between these bacteria and electrochemically active bacteria[15].

The MFC system with mixed cultures allowed the competition between exoelectrogen and other microbes (anaerobic or fermentative) to grow on the anode. External resistance that used in this system become advantage for other types of microbial growth and inhibit the growth of exoelectrogen[16,17]. In system with high external resistance, other metabolic processes is much more dominant than exoelectrogenic activity. So that, the total electrons extracted into electrical energy is much smaller than that formed from the degradation of the substrate.

On the condition of systems with high external resistance, most substrates are not utilized to generate current, or it could also be due to the low rate transfer of current, so the total charge that can be extracted into electricity is lower[18].

As shown in Fig. 3, the most efficient COD removal occurred in the 10% culture medium and the COD removal in this case reached 42.97%. This was due to the presence of many biological agents in the consortium, which all contribute to the decomposition of organic matter. However, the coulombic efficiency of the system was low at only approximately 0.803%.

Compared with the study by Liu *et al.*[19] coulombic efficiency obtained in this study are very low. In their study, Liu *et al.*[19] produced coulombic efficiency of 13.2% using 800 mg L^{-1} acetate as substrate. Coulombic efficiency strongly depends on the type of substrate. Acetate is the best substrate in producing much higher coulombic efficiency than other types of substrates, because it is very simple and naturally only selective for exoelectrogen community[20]. So, it is reasonable if it was obtained very high coulombic efficiency in study performed by Liu *et al.*[19].

The decreasing of coulombic efficiency is due to the excessive concentration of the bacterial consortium. This condition will increase the likelihood of an electron transferred by metabolism from one bacteria to other bacteria that can serve as electron acceptors[14].

This research shows the potential of tempeh wastewater as a substrate in the MFC system. This system can be applied to generate electricity as well as wastewater treatment itself. However, further research is needed to improve the performance of this MFC system.

CONCLUSION

The highest electrical energy (291.1 mV, 2.91 mA and 66.33 mW m^{-2}) was obtained when 1% bacterial consortium was used in the medium. The voltage increased by 73.5% when 0% medium was replaced with 1% culture medium. The study findings also indicate that the nutrients present in tempeh wastewater, typical of effluents produced by the tempeh processing industry, can be used as an effective energy source for MFCs using bacterial consortia as a biocatalyst. Electricity production can remove the COD and the optimum value of COD removal of 42.97% was obtained when using 10% bacterial consortium medium.

SIGNIFICANCE STATEMENTS

This study discovers the potential of bacterial consortia from tempeh wastewater that can be beneficial for substrate in microbial fuel cell system. This study will help the researcher to uncover the critical areas of utilization of tempeh wastewater that many researchers were not able to explore. Thus a new approach in tempeh wastewater treatment has been obtained.

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