



Effect of Fructose and Butyric Acid Addition in Electricity Production Using Single Chamber Microbial Fuel Cells

Tri Mulyono^{1,a}, Zulfikar¹, Misto², and Wulan Islamintari¹

¹Department of Chemistry, University of Jember, Indonesia

²Department of Physics, University of Jember, Indonesia

^atrimulyono.fmipa@unej.ac.id

Abstract. *The substrate is an important factor in the efficient production of electricity in Microbial Fuel Cells systems. The substrate is an organic compound that promotes the growth of active microbes. The goal of this study was to investigate the effect of substrate type and concentration on the bioelectricity produced by a single-chamber MFC. In this study, fructose and butyric acid were used as substrates. Carbon felt is used as the electrode. Both types and variations in substrate concentration were applied to the soil media used in the MFC. At 3 weeks of incubation, the optimum power density value produced by MFC with 90 g/L fructose substrate was 20.5 mW/m². At 3 weeks of incubation, MFC treated with 800 mg/L butyric acid produced a maximum power density of 19.7 mW/m².*

Keywords: Substrate, fructose, butyric acid, power density, microbial fuel cells

Introduction

In the last few decades, energy consumption has increased around the world. Fossil fuels, renewable sources, and nuclear sources are usually classified as energy sources [1], [2]. The use of fossil fuels has a negative impact because they emit CO₂ into the atmosphere, which can worsen global warming, in addition to depleting fossil fuel supplies. Therefore, it is necessary to have alternative energy sources that are environmentally friendly [3].

Renewable sources of energy such as solar energy, wind energy, and water energy have been gaining popularity around the world as a solution to the energy crisis. As one alternative energy source, microbial fuel cells (MFCs) use biocatalysts in the anode compartment to produce bioelectricity from biological processes in the body [4]. A biocatalyst in the anode oxidizes organic substrates and generates electrons and protons. Protons and electrons react at the cathode by reducing oxygen to water because oxygen in the anode can inhibit electricity production [5]. In Potter [6], electric current generated by microorganisms will be a much more useful device.

Microorganisms assist in the creation of electrical energy through the oxidation of organic materials [7], [8]. Soil is full of microorganisms, especially organic soil that hasn't been contaminated by chemicals. The term "electrogenic bacteria" refers to a variety of bacteria and microorganisms found in soil, such as the *Shewanella* and *Geobacter* species [8]. What is accessible in the soil, such as sugars and tiny nutrients, is consumed by soil bacteria [9]. Microorganisms use various biodegradable materials such as acetate, glucose, cysteine, ethanol, and butyrate [10], [11], [12], [13].



Microorganisms, according to Rahimnejad [4], can generate electricity through metabolic processes in their bodies. Logan [14] demonstrated that the bacterium *Saccharomyces cerevisiae* activity in a glucose substrate produced a current and voltage of 224 A and 196 mV. While the power density produced by various substrates, such as glucose (156.0 mW/m²), acetate (64.3 mW/m²), propionate (58.0 mW/m²), and butyrate (61.4 mW/m²) [15]. This condition allows electrons generated during microorganism metabolism to be used as a source of electrical energy. According to Borisov [16], under anaerobic conditions, electrons originating from microorganism metabolism can flow toward the anode, where they can be converted into electrical energy using an electrochemical cell in the form of a galvanic cell. Since 1991, galvanic cells have been used in MFCs media.

MFC components such as electrodes (anode and cathode), proton exchange membrane (PEM), MFC design, and the number of microorganism cultures on the MFC all affect the ability of the MFC to capture electrons from bacterial metabolism. Several factors influence MFCs performance, one of which is the presence of a substrate in the soil [17]. As a result, research on electricity production in microbial fuel cells using different substrates, such as butyrate and fructose, is required so that microbial fuel cells can later be used as alternative energy in generating electricity by utilizing underutilized soil.

Materials and Methods

Preparation of the Substrate

In this study, fructose and butyric acid were used as substrates. As substrates, fructose, and butyric acid are mixed with soil samples. The fructose (C₆H₁₂O₆) used is prepared by dissolving 0.5 grams of fructose in 6 mL of distilled water. Fructose solution 0.3 M made from 0.5 grams of glucose in 10 mL of distilled water. Fructose solution 0.1 M is made by dissolving 0.5 grams of fructose in 30 mL of distilled water. Butyric acid is an organic compound that was used in this study as a variety of substrate types. The concentrations of butyric acid used in this study were 600 mg/L, 800 mg/L, and 1000 mg/L.

Preparation of a Single Chamber Microbial Fuel Cell in Experiment

The single Chamber Microbial Fuel Cell design employed consists of a single vessel (single chamber) with a vessel volume of 500 mL and a lid [18]. The anode and cathode are made of graphite fibers that are connected by a titanium wire, with the green wire connecting to the anode and the orange wire connecting to the cathode. The anode is placed 1 cm above the substrate in the vessel, while the cathode is placed 5 cm above the substrate from the anode. The anode and cathode wires will be connected to the hacker board, which will calculate the power density. The single Chamber Microbial Fuel Cell design is shown in Figure 1.

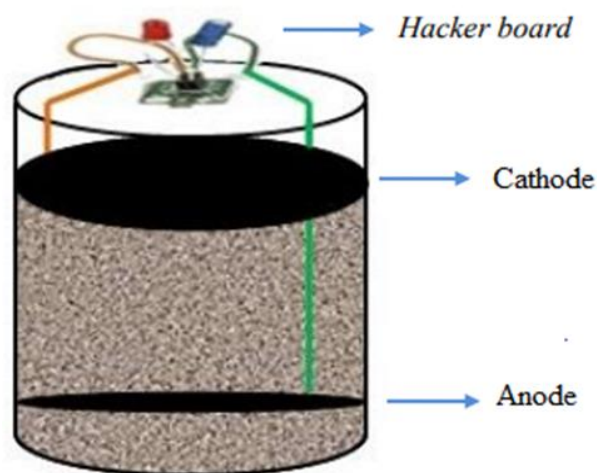


Figure 1. Design of a Single Chamber Microbial Fuel Cell

Variation in Substrate Types and Concentrations

The substrate is an important factor in the efficient production of electricity in a microbial fuel cell system. The substrate is an organic compound that promotes the growth of active microbes [19]. This study used organic soil with a variety of substrate types, including fructose and butyric acid. The fructose concentrations were 0.1 M, 0.3 M, and 0.5 M. Butyric acid concentrations of 600 mg/L, 800 mg/L, and 1,000 mg/L were used as substrate variations, with organic soils treated without the addition of substrate serving as a control.

To 450 mL of a soil sample, 10 mL of 0.1 M fructose solution was added. The fructose substrate was then mechanically stirred until completely mixed. As a variation of the fructose substrate concentration, the fructose substrate will be inserted into the single-chamber microbial fuel cell compartment. The experiment was repeated with fructose concentrations of 0.3 M and 0.5 M.

As a control, organic soil was used without the addition of substrate. This control is used to compare the power density values before and after the addition of the substrate. Organic soil was added to the compartment along with 450 mL of distilled water, but no organic compound substrate was added.

Variation in Substrate Incubation Time

The substrate was incubated for 7 days, 14 days, and 21 days after being inserted into the single-chamber microbial fuel cell compartment. The microbes require approximately 7 days to form biofilms on the anode. From the first day to the twenty-first day, measurements of current, potential difference, and power output were made. The value of current strength and voltage is expected to increase after 7 days, while the measurement value is expected to remain stable after 21 days [9].



Measurements of voltage and current in an MFC system

Using a digital multimeter, the voltage from the MFC system is measured. Meanwhile, the current strength of the MFC system is measured with an analog microampere. The voltage and current strength data are then processed to determine the power density (mW/m^2), or power per unit surface area of the electrode. Equation (1) can be used to calculate power density.

$$\text{Power Density} = \frac{I \times V}{A} \quad (1)$$

Results and Discussion

Results of Voltage and Current Measurements on Substrate Variations

MFCs generate electricity through the activity of microorganisms that oxidize organic compounds, such as fructose, and transfer the electrons produced to an electrode. The voltage generated by an MFCs depends on several factors, including the concentration of the substrate, the type of microorganisms used, the electrode materials, and the operating conditions.

In general, the voltage generated by an MFCs increases with the concentration of the substrate up to a certain point, beyond which the voltage may plateau or even decrease due to substrate inhibition or other factors [20]. The relationship between voltage and substrate concentration is often described by a power-law or other empirical models.

Figure 2 in the next shows the voltage value generated in the control soil without substrate (control) increased with increasing incubation time and reached a maximum value of 0.74 volts at 3 weeks of incubation time. The addition of fructose substrate has the effect of increasing the voltage value in this microbial fuel cell system. The microbial fuel cell system with the addition of 0.1 M produces a voltage value of 0.24 volts for 1 week of incubation, 0.39 volts for 2 weeks of incubation, and 0.65 volts for 3 weeks of incubation. Meanwhile, the concentration variation of 0.3 M produces a voltage value of 0.35 volts for 1 week of incubation, 0.73 volts for 3 weeks of incubation, and 0.80 volts for 3 weeks of incubation. Variation of 0.5 M fructose concentration has an optimum voltage value of 0.47 volts for 1 week of incubation, 0.7 volts for 3 weeks of incubation, and 0.8 volts for 3 weeks of incubation. The variation of 0.5 M fructose concentration has a higher value when compared to the other concentration variations.

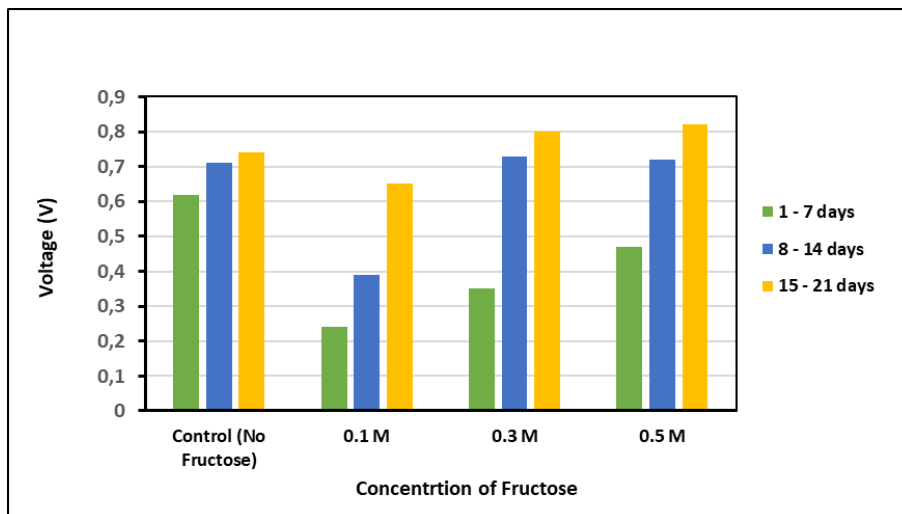


Figure 2. Voltage value generated by MFCs upon addition of various concentrations of fructose substrate

Figure 2 also shows that the voltage generated in the control soil without substrate (control) increased with increasing incubation time, reaching a maximum of 0.74 volts after 3 weeks. The addition of fructose substrate raises the voltage value in this microbial fuel cell system. With the addition of 0.1 M, the microbial fuel cell system produces a voltage of 0.24 volts after one week of incubation, 0.39 volts after two weeks, and 0.65 volts after three weeks. Meanwhile, the concentration variation of 0.3 M produces a voltage value of 0.35 volts for 1 week of incubation, 0.73 volts for 3 weeks of incubation, and 0.80 volts for 3 weeks of incubation. Variation of 0.5 M fructose concentration has an optimum voltage value of 0.47 volts for 1 week of incubation, 0.7 volts for 3 weeks of incubation, and 0.8 volts for 3 weeks of incubation. The variation of 0.5 M fructose concentration has a higher value when compared to the other concentration variations.

Figure 3 on the next page is the MFC voltage value with the addition of substrate, butyric acid, at a concentration of 600 mg/L, was 0.52 volts after a one-week incubation period, 0.71 volts after a two-week incubation period, and 0.76 volts after a three-week incubation period. This voltage value increased at the start of incubation and remained stable for 2 and 3 weeks, respectively. Meanwhile, the butyric acid concentration at 800 mg/L had an optimum voltage value of 0.82 volts for one week of incubation, 0.80 volts for two weeks, and 0.82 volts for three weeks. The voltage value produced at 1,000 mg/L is lower than at 800 mg/L. The resulting voltage value was 0.76 volts in week one, 0.76 volts in week two, and 0.65 volts in week three.

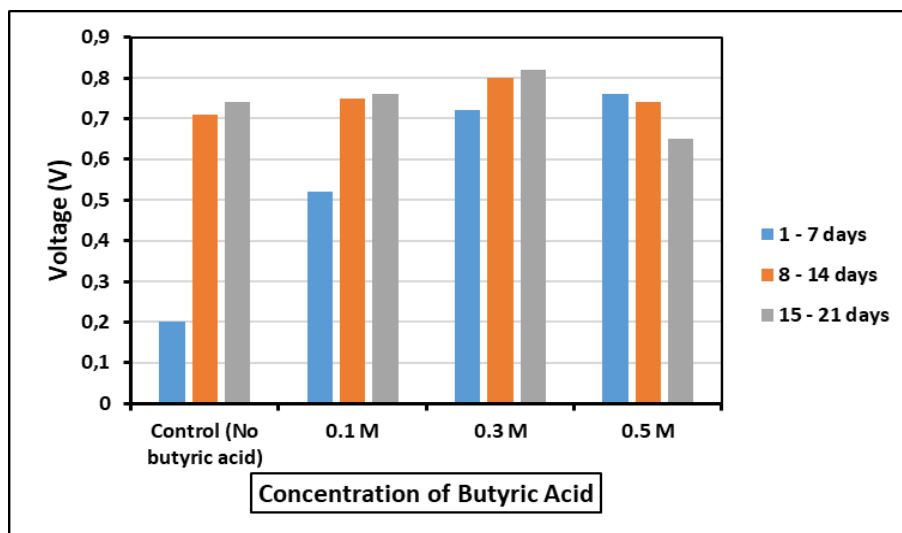


Figure 3. The voltage produced by the MFCs following the addition of various concentrations of butyric acid substrate

Microbes in the anode compartment do metabolism with fructose and butyric acid as a source of carbon. Carbon dioxide, protons, and electrons will be produced as a result of the conversion of fructose.



The reaction to the equation results in a negative change in the free energy of Gibbs (G). ΔG represents the ease of a chemical reaction thermodynamically. ΔG with a negative value will become more popular and can occur spontaneously in the production of electricity. The increase in the voltage value produced by the increase in the number of organic compounds that can be consumed by microbes causes a sharp increase in microbial metabolism. Microbes on the anode's surface multiply so quickly that the number of microbial cells multiplies as the incubation time of the microbial fuel cell system increases.

A decrease in voltage value can also be caused by the formation of biofilms on the anode's surface as a result of microbial activity. This biofilm impedes the transfer of protons from the anode to the cathode [10]. This restrained proton causes pH changes in the anode and can interfere with microbial life, resulting in potential differences.

The results of measuring the electric current on the MFCs with different substrate concentrations

The electric current produced by the fructose substrate at 0.1 M concentration variations during incubation times ranging from 1 week to 3 weeks in a row of 0.157 mA, 0.79 mA, and 0.26 mA. Among other concentration variations, this value represents the optimum current. In the substrate, a 0.3 M concentration variation resulted in currents of 0.0935 mA, 0.196 mA, and 0.192 mA. With a one-week incubation time, the current variation concentration of 0.5 m is 0.112 mA, 0.212 mA with a two-week incubation time, and 0.199 mA with a three-week

incubation time. Figure 4 displays the profile of the electric current produced by MFC after it is applied to a fructose substrate.

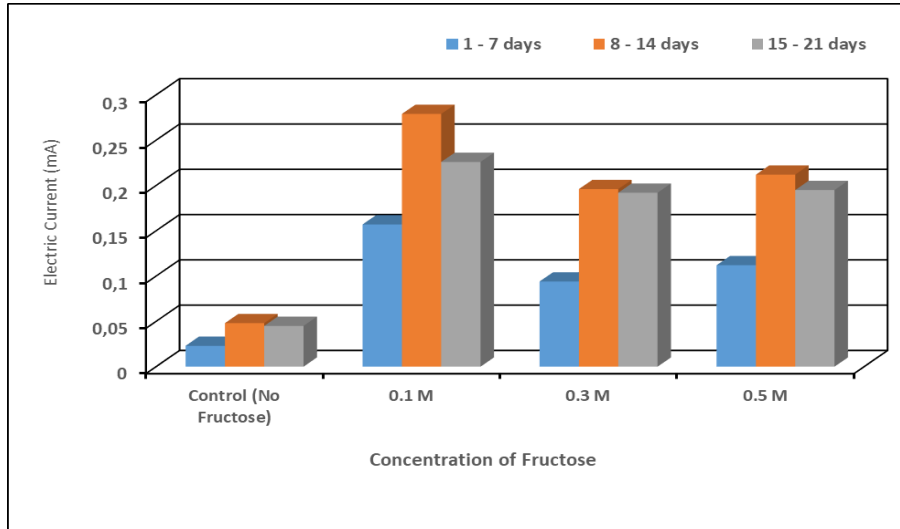


Figure 4. Electric current produced by MFCs with fructose substrate at various concentrations

Figure 5 is the MFCs power density value with a grain acid substrate reaching an optimum value of 19.7 mW/m² after 3 weeks of incubation at a concentration of 800 mg/L. The power density value produced by MFCs at a concentration of 600 mg/L is 17.5 mW/m² after 2 weeks of incubation and 13.3 mW/m² after 1 week of incubation at a concentration of 1000 mg/l.

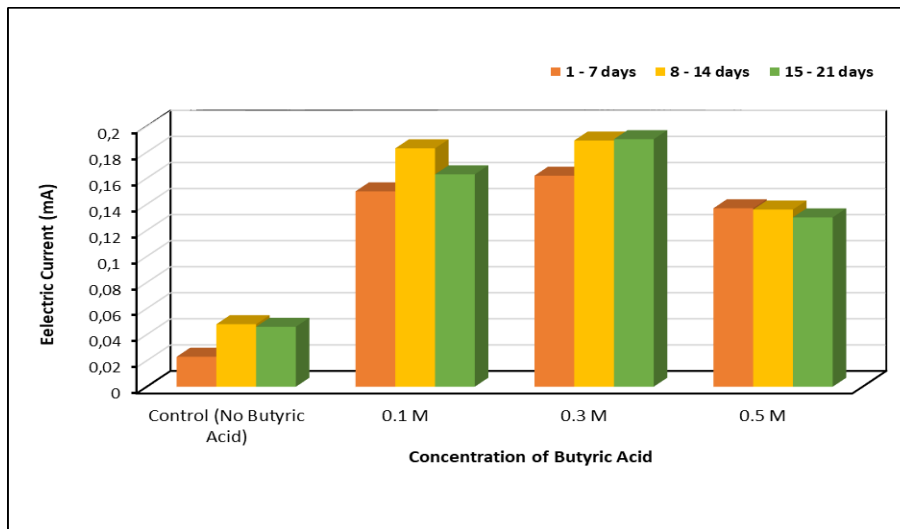


Figure 5. Power Density Value in MFCs with the addition of a grain acid substrate at various concentrations

Power density decreases occur as a result of bacterial activity on the anode's surface, which can eventually form a biofilm [21]. The formation of this biofilm can lead to an increase in

resistance on the anode's surface and a decrease in the power density value. The efficiency of electron transfer from microbes to anodes is proportional to the number of bacteria that come into contact with electrodes [22] if biofilm adheres to the electrode's surface, the number of electrons transferred to the electrode is reduced, resulting in a decrease in power density.

The Influence of Incubation Time Variations on Power Density Measurement

Differences in variations in concentration in the fructose substrate and butyric acid are very influential on the power density produced by the microbial fuel cell system. Figures 6 and 7 demonstrate this. Another factor that affects the value of power density is the variation in the time of incubation. Based on the data obtained it is clear that the incubation time for 3 weeks in MFCs with the fructose substrate gives a higher power density value than 1 week or 2 weeks. Whereas the butyric acid substrate produces the optimum power density value for 2 weeks of incubation time.

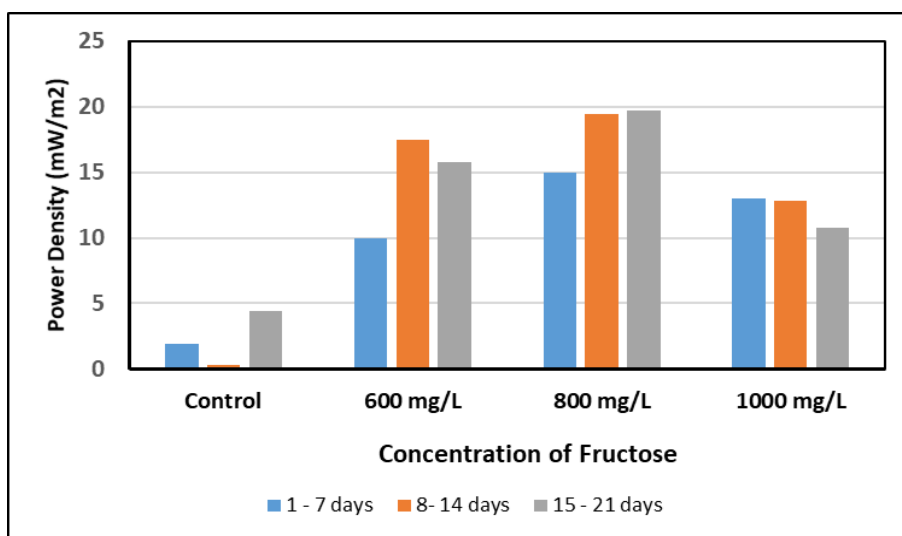


Figure 6. Power density produced by MFCs with fructose substrate at various concentrations

Microbes require time to reproduce and adapt to their surroundings, so forming a stable biofilm takes time. Stable biofilms will aid in the complete degradation of organic compounds so that the amount of electricity produced by microbial metabolism formed at the start of the study is small but tends to increase over time due to the stability of microbes that degrade organic compounds in the substrate. When the incubation time is too long, the organic compounds in the substrate continue to degrade, and if there are no organic compounds left, electricity production decreases. This is due to the fact that there are no more organic compounds to be oxidized. Furthermore, the biofilm that develops over time can cover the electrode and increase internal anode obstacles, resulting in a decrease in power density value [21].

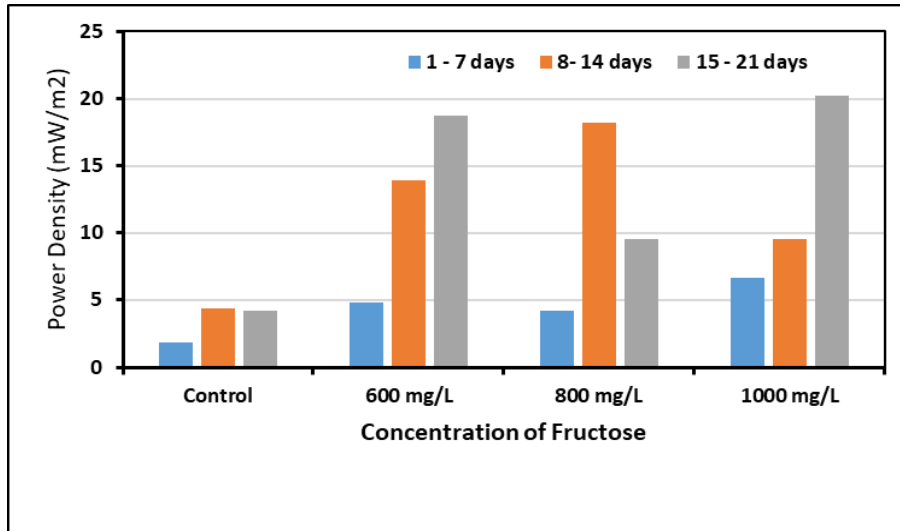


Figure 7. The influence of incubation time on MFCs power density

Conclusions

At a concentration of 0.5 M, the addition of variations in the fructose concession to the Microbial Fuel Cell Single Chamber System results in a maximum power density value of 20.25 mW/m². When the concentration of the Microbial Fuel Cell Single Chamber System is varied, it produces a maximum power density value of 19.70 mW/m² at a concentration of 800 mg/L. The MFCs power density value with fructose reaches an optimum after 3 weeks of incubation, while MFCs with a grain acid substrate reaches an optimum after 2 weeks of incubation.

ACKNOWLEDGEMENTS

The author would like to thank all related parties who helped with the process of this research, especially to the LP2M Jember University who have awarded research grants year 2023 under research groups, Renewable Energy Research Group, Jember University.

References

- [1] F. Tetteh et al., "An overview of plant microbial fuel cells (PMFCs): Con fi gurations and applications," *Renew. Sustain. Energy Rev.*, vol. 110, no. May, pp. 402–414, 2019, doi: 10.1016/j.rser.2019.05.016.
- [2] V. Chaturvedi and P. Verma, "Microbial fuel cell: a green approach for the utilization of waste for the generation of bioelectricity," *Bioresour. Bioprocess.*, vol. 3, no. 1, 2016, doi: 10.1186/s40643-016-0116-6.
- [3] M. Rahimnejad, "Microbial fuel cell as new technol ogy for bioelectricity generation: A review," *Alexandria Engineering Journal*, vol. 54, no. 3. pp. 745–756, 2015. doi: 10.1016/j.aej.2015.03.031.



- [4] M. Rahimnejad, "Power generation from organic substrate in batch and continuous flow microbial fuel cell operations," *Appl. Energy*, vol. 88, no. 11, pp. 3999–4004, 2011, doi: 10.1016/j.apenergy.2011.04.017.
- [5] K. Stamatelidou, G. Antonopoulou, A. Tremouli, and G. Lyberatos, "Production of gaseous biofuels and electricity from cheese whey," *Ind. Eng. Chem. Res.*, vol. 50, no. 2, pp. 639–644, 2011, doi: 10.1021/IE1002262.
- [6] M. C. Potter and P. R. S. L. B., "Electrical Effects Accompanying the Decomposition of Organic Compounds," *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character.*, vol. 84, no. 571, pp. 260–276, 1911, doi: 10.1098/rspb.1911.0073.
- [7] A. D. Tharali, N. Sain, and W. J. Osborne, "Microbial fuel cells in bioelectricity production," *Front. Life Sci.*, vol. 9, no. 4, pp. 252–266, 2016, doi: 10.1080/21553769.2016.1230787.
- [8] B. E. Logan, "Exoelectrogenic bacteria that power microbial fuel cells". *Nature Reviews Microbiology.*, vol. 7, no. 5. pp. 375-81, 2009, doi:10.1038/nrmicro2113.
- [9] S. . R. T. Lohner, "How Do Bacteria Produce Power in a Microbial Fuel Cell? | Science Project," Jun. 03, 2021. https://www.sciencebuddies.org/science-fair-projects/project-ideas/MicroBio_p032/microbiology/bacteria-microbial-fuel-cell (accessed Feb. 28, 2023).
- [10] W. He, X. Zhang, J. Liu, X. Zhu, Y. Feng, and B. E. Logan, "Microbial fuel cells with an integrated spacer and separate anode and cathode modules," *Environ. Sci. Water Res. Technol.*, vol. 2, no. 1, pp. 186–195, 2016, doi: 10.1039/c5ew00223k.
- [11] A. E. Franks, K. P. Nevin, H. Jia, M. Izallalen, T. L. Woodard, and D. R. Lovley, "Novel strategy for three-dimensional real-time imaging of microbial fuel cell communities: Monitoring the inhibitory effects of proton accumulation within the anode biofilm," *Energy Environ. Sci.*, vol. 2, no. 1, pp. 113–119, 2009, doi: 10.1039/b816445b.
- [12] S. Kondaveeti, R. Kakarla, H. S. Kim, B. goon Kim, and B. Min, "The performance and long-term stability of low-cost separators in single-chamber bottle-type microbial fuel cells," *Environ. Technol. (United Kingdom)*, vol. 39, no. 3, pp. 288–297, 2018, doi: 10.1080/09593330.2017.1299223.
- [13] B. E. Logan, C. Murano, K. Scott, N. D. Gray, and I. M. Head, "Electricity generation from cysteine in a microbial fuel cell," *Water Res.*, vol. 39, no. 5, pp. 942–952, 2005, doi: 10.1016/j.watres.2004.11.019.
- [14] B. E. Logan, "Exoelectrogenic bacteria that power microbial fuel cells," *Nat. Rev. Microbiol.*, vol. 7, no. 5, pp. 375–381, 2009, doi: 10.1038/nrmicro2113.
- [15] B. Min and B. Logan, "Continuous Electricity Generation from Domestic Wastewater and Organic Substrates in a Flat Plate Microbial Fuel Cell file:///Users/aman/Downloads/apa.csl," *Environ. Sci. Technol.*, vol. 38, no. 21, pp. 5809–5814, 2004, doi: <https://doi.org/10.1021/es0491026>.
- [16] V. B. Borisov and M. I. Verkhovsky, "Oxygen as Acceptor.," *EcoSal Plus*, vol. 6, no. 2, 2015, doi: 10.1128/ecosalplus.ESP-0012-2015.
- [17] T. Mulyono, Misto, Busroni, Siswanto, "Bioelectricity Generation From Single-Chamber Microbial Fuel Cells With Various Local Soil Media and Green Bean



- Sprouts as Nutrient”, *Int. Journal of Renewable Energy Development* 9 (3) 2020 : 423-429, 2020, doi: 10.14710/ijred.2020.30145.
- [18] T. Mulyono., Misto, M., Busroni, B., & Siswanto, S. "Bioelectricity Generation From Single-Chamber Microbial Fuel Cells With Various Local Soil Media and Green Bean Sprouts as Nutrient," *International Journal of Renewable Energy Development*, vol. 9, no. 3, pp. 423–429, 2020, doi: 10.14710/ijred.2020.30145.
- [19] M. Kästner, A. Miltner, S. Thiele-Bruhn, and C. Liang, “Microbial Necromass in Soils—Linking Microbes to Soil Processes and Carbon Turnover,” *Front. Environ. Sci.*, vol. 9, p. 597, 2021, doi: 10.3389/FENV.S.2021.756378/BIBTEX.
- [20] T. Mulyono, Misto, B. E. Cahyono, and N. H. Fahmidia, “The impact of adding vegetable waste on the functioning of microbial fuel cell,” *AIP Conf. Proc.*, vol. 2663, no. 1, p. 20008, Sep. 2022, doi: 10.1063/5.0108950.
- [21] S. M. Daud et al., “Comparison of performance and ionic concentration gradient of two-chamber microbial fuel cell using ceramic membrane (CM) and cation exchange membrane (CEM) as separators,” *Electrochim. Acta*, vol. 259, pp. 365–376, 2018, doi: 10.1016/J.ELECTACTA.2017.10.118.
- [22] D. R. Lovley, “The microbe electric: conversion of organic matter to electricity,” *Curr. Opin. Biotechnol.*, vol. 19, no. 6, pp. 564–571, 2008, doi: <https://doi.org/10.1016/j.copbio.2008.10.005>.