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Energy Harvesting from Sugarcane Bagasse Juice using Yeast Microbial Fuel Cell Technology

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Abstract

This study demonstrates the feasibility of producing bioelectricity utilizing yeast microbial fuel cell (MFC) technology with sugarcane bagasse juice as a substrate. Yeast Saccharomyces cerevisiae was employed as a bio-catalyst in the production of electrical energy. Sugarcane bagasse juice can be used as a substrate in MFC yeast because of its relatively high sugar content. When yeast was used as a biocatalyst, and Yeast Extract, Peptone, D-Glucose (YPD) Medium was used as a substrate in the MFC in the acclimatization process, current density increased over time to reach 171.43 mA/m^2 in closed circuit voltage (CCV), maximum power density (MPD) reached 13.38 mW/m^2 after 21 days of the acclimatization process. When using sugarcane bagasse juice as a substrate, MPD reached 6.44 mW/m^2 with a sugar concentration of about 5230 ppm. Whereas the sensitivity, maximum current density (J_{max}), and apparent Michaelis-Menten constant (K_m^{app}) from the Michaelis-Menten plot were 0.01474 mA/(m^2.ppm), 263.76 mA/m^2, and 13594 ppm, respectively. These results indicate that bioelectricity can be produced from sugarcane bagasse juice by Saccharomyces cerevisiae.

Keywords: *biomass valorization, biofuel cell, acclimatization, maximum power density, Michaelis-Menten constant*

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INTRODUCTION

In the form of electrical energy demands, Indonesia is presently confronted with a difficult challenge that threatens the lives of many people. Rising human growth is increasing demand for electrical energy, but electrical energy resources are running depleted (Geels *et al.*, 2018). The oil crisis encouraged the development of emergent green energy sources to replace the consumption of petroleum, which was the community's primary source of energy (Troccoli *et al.*, 2018). According to the ratio of fossil energy source supply, coal has the highest potential consumption, followed by crude oil and natural gas (Hasan *et al.*, 2012; Shahbaz *et al.*, 2013). As a result, it is critical to conduct more research and produce green energy for the production of electricity. Microbial Fuel Cell (MFC) is a kind of renewable energy that is ecologically friendly and has

the potential to be a future energy source (Rahimnejad *et al.*, 2015).

MFC is an energy conversion system that takes advantage of the capabilities of bacterial metabolism. The MFC theory proposes using a bacterial catalyst to convert the chemical energy present in the bio-convertible substrate into electrical energy. MFC may generate electrical current from many organic substrates by using bacterial metabolism. MFCs may be generated electricity from almost any biodegradable organic product, including volatile acids, carbohydrates, proteins, alcohols, and even somewhat recalcitrant materials like cellulose. Santoro *et al.*, (2017) describe the benefits of MFC, such as its use for the treatment of low concentration substrates at temperatures below 20 °C, which creates several loopholes in the specific application of MFC technology, where it neither interferes with nor complements anaerobic digestion technology. However, in terms of large-scale applications, microbial fuel cells have significant limitations. Investment expenses, high-quality technological obstacles, and performance-limiting constraints, both in terms of anodic and cathodic electron transfers, are all covered by limits (Du *et al.*, 2007). Research to make microbial fuel cell technology more commercially viable and applicable would concentrate on design of reactors, power density and material costs. In addition, the reliability of the open-air cathode is the most dominant barrier to the MFC. Catalysts that selectively reduce oxygen without compromising improper activation must be identified (Rismani-Yazdi *et al.*, 2008). One of the current disadvantages of MFCs is their inability to eliminate the modest electrochemical efficiency of COD.

Sugarcane bagasse is a by-product of the sugarcane milling process that is collected or eliminated from the sugar manufacturing industry, resulting in a substantial volume of fibrous waste materials (Pandey *et al.*, 2000). Aside from being utilized as a fuel for sugarcane bagasse, some sugar factories are attempting to overcome extra bagasse by overburning (Anukam *et al.*, 2016). They can minimize the volume of bagasse this way, but the remaining inadequate combustion gas pollutes the environment. This is a costly option since, if you don't want to waste your bagasse, you may utilize it as electrical energy using the MFC system. Sugarcane bagasse is composed of three major constituents: 32–34% cellulose, 19–24% hemicellulose, 25–32% lignin, 6–12% extractives, and 2–6% ash (Sakdaronnarong and Jonglertjunya, 2012; Rezende *et al.*, 2011; Pandey *et al.*, 2000).

Yeast (*Saccharomyces cerevisiae*) is a eukaryotic microorganism that is known as a part of the fungal kingdom. Several strains of yeast have been studied as biocatalysts in MFCs with or without external mediators such as *Saccharomyces cerevisiae*, *Candida melibiosica*, *Hansenula anomala*, *Hansenula polymorpha*, *Arxula adeninvorans*, and *Kluyveromyces marxianus*. Yeast is a well-known biocatalyst for microbial fuel cell applications since most strains are non-pathogenic, can metabolize a variety of substrates, are resilient, and easy to control (Hubenova and Mitov, 2015). The existence of several natural electron transports, mediators such as azurine, ferredoxin, and cytochromes, which may be employed by redox enzymes to transfer electrons from the yeast cells to the surface of the anode, can be connected to the presence of yeast bio-catalytic activity. This is in addition to the electroactive species' high protein levels in yeast cell membranes. Yeast cytochrome is found in mitochondria, and transmembrane protein (tPMET) is present in the cell membrane, which is bounded by cell walls (Sayed and Abdelkareem, 2017). As a result, it has been proposed that in order to elicit an electrochemical reaction from yeast cells, mediators must cross the cell wall and interact with membranes and/or internal redox sites, such as NAD^+/NADH, or that the response is caused by soluble electroactive species exported from the cell (Schaetzle *et al.*, 2008). The use of yeast as biocatalysts in microbial fuel cells has numerous advantages, but many difficulties remain in the profitability of their usage in real applications, and growing conditions must be optimized.

Several previous research on the subject have been conducted. Hasan *et al.* (2019), for example, employed sugarcane molasses as a substrate for MFCs. Umar *et al.*, (2021) utilized sugarcane waste as a substrate for MFC, whereas Elakkiya and Niju (2021) exploited bagasse-based-paper mill effluent. All in all, a double chamber MFC was employed, with a mixed culture acting as a biocatalyst. Christwardana *et al.*, (2021) recently compared bagasse juice with and without fiber as a substrate for yeast MFC, finding that fiberless bagasse juice generated more electricity. This study, on the other hand, focuses on the sensitivity of single chambered yeast MFC to bagasse juice in the generation of electricity. This sensitivity should be investigated as a baseline for the future development of yeast MFCs that use glucose or similar substrates to generate electrical energy. We thus claim that this is an academic innovation for this research.

Bagasse juice with various glucose concentrations was employed as a substrate for this yeast MFC in this study. To assess its sensitivity, current density and maximum power density (MPD) would be measured, and the Michaelis-Menten constant would be established. We hope that this work will set the standard for future MFC yeast research.

MATERIALS AND METHODS

Sugarcane bagasse composition and preparation

The sugarcane bagasse utilized in this study was obtained from sugarcane juice sellers in South Tangerang, Indonesia. Table 1 shows the chemical structure of sugar cane bagasse, which includes pH, total solids, sugar concentration, cellulose, ash, and nitrogen.

Table 1. The physicochemical parameters of sugarcane bagasse

Parameters	Values	Unit
pH	7.3	-
Cellulose	43	%
Total sugar	9.6	%
Ash	12.2	%
Nitrogen	0.3	%
Fiber	15.7	%

Sugar is supposed to dissolve in water since it is made up of sucrose and glucose. The bagasse juice mixture was then separated from its solids, which primarily included cellulose, and the liquid fraction was employed as an MFC substrate.

MFC operation

In this experiment, a single chamber MFC reactor built of acrylic with an active volume of 28 mL was used to generate electrical energy, as illustrated in Figure 1. During the 21-day acclimation period, the YPD medium (5 mg/mL yeast extract, 2.5 mg/mL peptone, and 14 mg/mL D-glucose) was employed as a medium in MFC (Christwardana *et al.*, 2018c). As a biocatalyst, 14 mg/mL of yeast *Saccharomyces cerevisiae* was put into the anode chamber (Christwardana *et al.*, 2018a; Christwardana *et al.*, 2019). When the current density was less than 50 mA/m^2, fresh medium and yeast were introduced. Following the acclimation period, various concentrations of sugarcane bagasse juice were employed as a carbon source in this experiment. For each substrate concentration, MFCs were incubated for three days with bagasse juice as a substrate (Christwardana *et al.*, 2018b; Christwardana *et al.*, 2018c). MFC acclimation and incubation were performed in closed-circuit conditions with an external resistance of 1000 Ω. MFC voltages have been quantified using multimeters. Both the acclimatization and incubation processes were carried out at semi-aerobic conditions to decrease the possibility of the formation of alcohol, which can destroy yeast. In the presence of oxygen, the glucose has to be oxidized completely to CO_2.

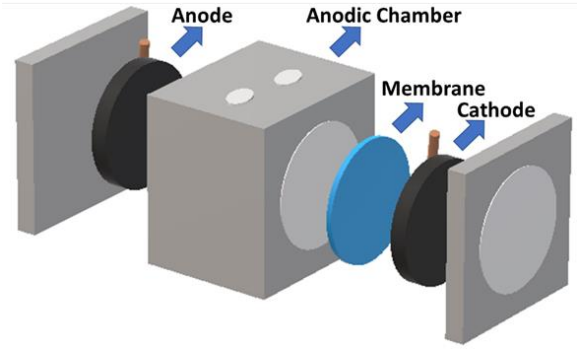


Figure 1. Schematic of MFC reactor

Electrochemical analysis

Current (I) was calculated by dividing the According to the Ohm Law, voltage is proportional to external resistance. The current density (J) was calculated by dividing the current by the electrode's projected surface area (7 cm^2). Calculating the voltage at various external resistance changes yielded the polarization curve. Meanwhile, the power density (P) was calculated by multiplying the current density by the voltage generated in the polarization curve. The slope of the polarization curve was used to calculate the MFC's internal resistance (R_{int}).

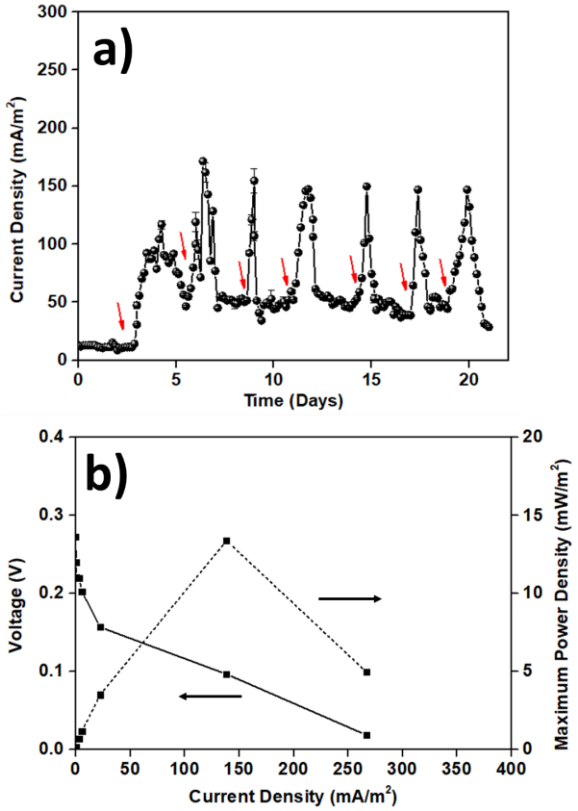
RESULTS AND DISCUSSION

Current and power generation of MFC during the acclimatization process

Figure 2a represents the current density throughout a 21-day period during the acclimatization cycle. The aim of this acclimatization process is to build a yeast biofilm on the electrode surface, which will be utilized subsequently to convert bagasse juice as a substrate into electricity. This phenomenon can be illustrated by a variety of factors. In the first three days, there was no discernible increase in current density. When new media containing yeast was injected on the third day, there was a considerable rise, with an increase to 15.24 mA/m^2 on the fifth day, accompanied by a fall in current density due to a drop in media concentration for yeast consumption. On the sixth day, with the addition of fresh medium and yeast, the current density increased considerably to 116.95 mA/m^2, following by a decline due to substrate decay. On days 9, 11, 14, 17, and 19, fresh medium and yeast were fed into the MFC, causing current density to rise again. Maximum current density values are more or less equivalent with a range of 154.61-171.43 mA/m^2, indicating that stability has been established and that the next action may be taken.

The rise in current density due to the new substrate injection procedure showed that the yeast culture was in an adaptation phase, which was followed by a logarithmic phase. As a result of the oxidation of bagasse juice, yeast produced a large number of protons and electrons. When the substrate is depleted, the yeast enters the stationary phase and continues to die. Unfortunately, the stationary phase is so short that a fast death phase is detected. During this death phase, yeast does not generate protons or electrons. The low pH of the medium also has an impact on the life cycle of yeast (Delgado *et al.*, 2021).

Figure 2b illustrates the polarization (I-V) and power (I-P) curves after the acclimatization period. With decreasing cell voltage, the current density and power density of the cells rose. This behavior is caused by polarization losses, activation losses, and ohm losses. The measured maximum power density (MPD) was 13.38 mW/m^2, with a power density of 138.26 mA/m^2 and an internal resistance of 807 Ω. and cathode potentials, and the voltage drops as external resistance increases.



Arrows in Figure 2a indicate the media replacement time

Figure 2. a) Current density of MFC during the acclimatization process for 21 days under closed circuit voltage condition, and b) its polarization and power curve after the acclimatization process.

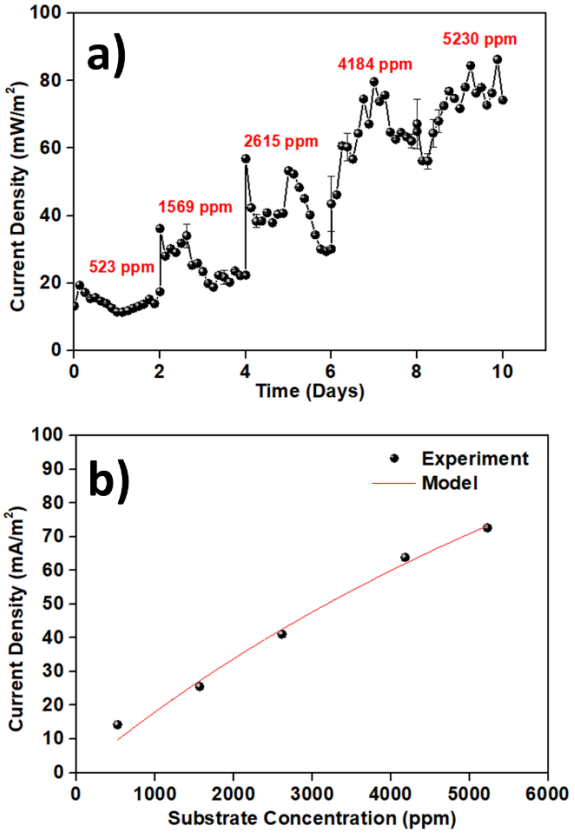


Figure 3. a) Current density with a various glucose concentration of bagasse juice as substrate, and b) its Michaelis-Menten plot between substrate concentration vs. current density. All experiments conducted under closed circuit voltage

The corresponding OCV appears to have been around 0.27 V, as measured by the difference between the anode.

Current generation and sensitivity of MFC in different glucose concentration in bagasse juice

As shown in Figure 3a, current density increased as the glucose concentration of bagasse juice raised. As the glucose content in bagasse juice increased from 523 to 5230 ppm, the MPD in yeast MFC utilizing bagasse juice as fuel increased from 14.27 mA/m^2 to 72.65 mA/m^2. The rise in substrate concentration causes the anode's potential to shift in the negative direction, since the more glucose is oxidized into protons, electrons, and CO_2, the higher the potential of the cells (Logan, 2008). According to Ohm's Law, the value of the cell potential predicts a rise in current density (Gadkari *et al.*, 2019).

The Michaelis-Menten equation, which is useful for calculating the apparent Michaelis-Menten constant (K_m), was used to examine the link between bagasse juice glucose concentration and current density (Christwardana and Kwon, 2017; Cheng *et al.*, 2008; Logan *et al.*, 2005). The non-linear correlation between bagasse juice concentration (x-axis) and current density (y-axis) shown in Figure 3b, shows that the relationship between bagasse juice glucose concentration and maximum current density (J_{max}) follows the Michaelis-Menten curve plot. In MFC, which using bagasse juice as fuel, J_{max} and apparent K_m obtained are 263.76 mA/m^2 and 13594 ppm, respectively. While the sensitivity of this system was 0.01474 mA/(m^2.ppm), obtained from the slope between current density and substrate concentration. All of this takes place in a closed-circuit voltage condition. The R^2 value was 0.9882, implying that the MFC using bagasse juice as fuel has a fairly robust electrical power capability.

The K_m value represents the MFC's sensitivity to the substrate. The smaller the value of K_m, the more sensitive the applied MFC system (Fujii *et al.*, 2021). According to the aforementioned K_m data, the value of K_m is rather high, indicating that the MFC system with bagasse juice substrate still has low sensitivity, resulting in poor substrate conversion efficiency. Electrode modification is one approach for increasing MFC sensitivity. Because conductive electrodes capture electrons generated by microorganisms more quickly, modifying the electrode with other more conductive materials can boost the sensitivity of the MFC system (Li *et al.*, 2017).

Power generation of yeast MFC using bagasse juice as substrate

The study of MFCs with various concentrations of bagasse juice revealed that the increase in power density is directly proportional to the concentration of the substrate. According to our findings, OCV varied between 0.154 to 0.313 V in all samples, with a rise in OCV when substrate concentration was increased. Figure 4 shows the polarization and power curves, which show that using larger substrate concentrations results in higher power density and lower internal cell resistance. This is feasible because high substrate concentrations can accelerate the formation of the biofilm, resulting in improved conductivity.

MFC power density with 523, 1569, 2615, 4184, and 5230 ppm bagasse juice glucose concentrations was 1.01, 2.36, 3.65, 5.59, and 6.44 mW/m^2, with internal resistances of 10559, 10203, 2258, 2197, and 1935 Ω, respectively. These results are comparable to those obtained by the MFC when yeast S. cerevisiae was used as a biocatalyst, as shown in Table 2.

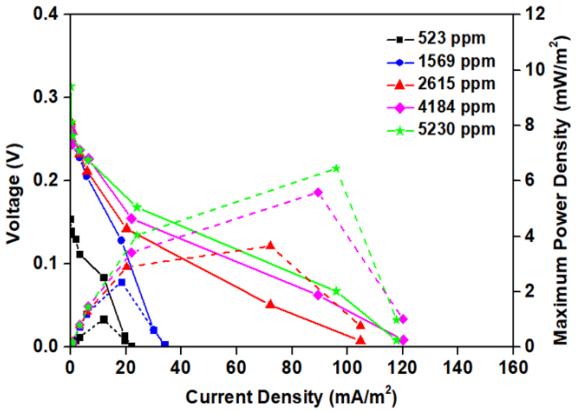


Figure 4. Polarization and power curves of MFC using various substrate concentration. Solid and dash line represent polarization and power curves, respectively

Table 2. Comparison of yeast MFC performance

Substrate	MFC Type	MPD (mW/m^2)	Reference
glucose	Single chamber	3.21	Sayed and Nakagawa, 2018
glucose	two chambers	~4.9	Mardiana *et al.*, 2016
glucose	two chambers	0.95	Powell *et al.*, 2010
glucose	two chambers	6.1	Mardiana *et al.*, 2015
glucose	two chambers	22	Kaneshiro *et al.*, 2014
glucose	single chamber	13.38	*this work*
bagasse juice	single chamber	6.44	*this work*

In comparison to our study, they all employed pure glucose as a substrate, which is much easier to oxidize. Because bagasse juice contains sucrose and glucose, it is more challenging to oxidize. Based on these findings, the yeast MFC that employs bagasse juice as a substrate has a greater chance of becoming an alternative fuel in the future by utilizing the waste-to-energy concept.

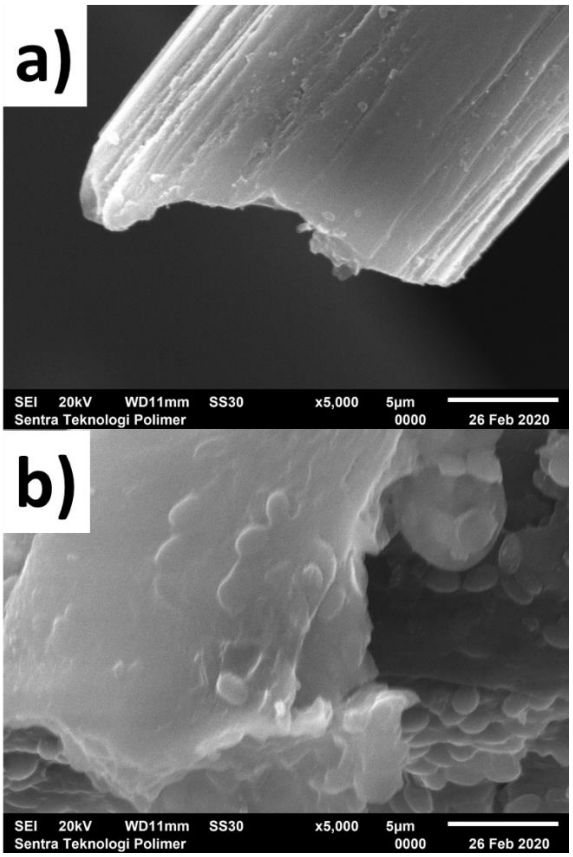


Figure 5. SEM images of carbon felt electrode a) before and b) after incubation process

Biofilm formation in the electrode surface

As illustrated in Figure 5, yeast biofilms grown on electrode surfaces were studied using SEM to acquire in-depth information on the effectiveness of MFCs. Figure 5a depicts the carbon felt before to the formation of yeast biofilms. Carbon fibers are clearly visible in this view, with no microbes on the surface. After incubation (Figure 5b), yeast biofilms formed on the surface of carbon fibers. The yeast cells on biofilms are small and have a thin covering known as exopolysaccharides (EPSs), which yeast secretes to protect microorganisms (Bertsch *et al.*, 2019). Yeast biofilm that grows on the surface of the carbon fiber will give an advantage, as the electrons generated by the yeast will be directly captured by the carbon fiber as anode, however this is also impacted by electrode conductivity.

CONCLUSION

This research reveals that the pure yeast culture of *Saccharomyces cerevisiae* is capable of generating electrical energy directly from the sugar cane bagasse. Sugarcane bagasse produces sugar that serves as a carbon source for the growth of *S. cerevisiae*. In the acclimation period, the current density will exceed 171,43 mA/m^2 with a maximum density of 13,38 mW/m^2 on the 21st day after the acclimatization process. Various glucose concentrations (in bagasse juice) were then used as substrates with concentrations varying from 523 to 5230 ppm. Current density values increase with rising concentration of the substrate.

With respect to MFC yeast sensitivity to bagasse juice, the resulting J_{max} was 263.76 mA/m^2, Km was 13594 ppm and sensitivity was 0.01474 $mA/(m^2.ppm)$. The resulting power density was approximately 6.44 mW/m^2, which is comparable to other studies. These findings suggest that *S. cerevisiae* can generate electricity from sugarcane bagasse juice.

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