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The Influence of Various Substrates on Power Generation in The Operation of Yeast Microbial Fuel Cells

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Abstract

Microbial fuel cells (MFCs) are biochemically-catalyzed systems that generate energy by oxidizing biodegradable organic substances in the presence of yeast as a biocatalyst. Several carbon substrates, including commercial glucose, pro analysis glucose, commercial sugar, and yeast extract - peptone - d glucose (YPD) medium, were tested in an effort to increase the efficiency of the single chamber yeast MFC. Using baker's yeast Saccharomyces cerevisiae, the energy production of several electron donors was analyzed. The substrate use pattern was determined by the production of voltage and power density. In addition, an electrochemical analysis of the anodic biofilm was conducted. S. cervisiae consumes YPD medium via anode respiration with a power density of 18.40±1.98 mW/m^2, followed by pro analysis glucose (9.41±1.15 mW/m^2), commercial glucose (1.30±0.10 mW/m^2), and commercial sugar (0.040.01 mW/m^2). In addition, a definite link has been discovered between the power density producing rate and voltage output. MFC yielded voltages of 0.16±0.02 V for YPD medium, 0.13±0.03 V for pro analysis glucose, 0.03±0.01 V for commercial glucose, and 0.01±0.00 V for commercial sugar. According to the weight of the biofilm, yeast attachment was substantially more prevalent in YPD medium than in other MFC-operated media. This research found that the substrate type in the anodic compartment controls biofilm production.

Keywords: *substrate; glucose; energy generation; yeast metabolism*

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INTRODUCTION

The Microbial Fuel Cell (MFC) is a device that generates electrical energy by oxidizing organic substrates with the assistance of microorganisms (Bajracharya et al. 2016). MFC involves reduction and oxidation processes, which necessitate the use of an oxidizing agent in the process, where electrons are generated by bacteria by consuming the substrate (Fitriani, et al. 2017). Thereafter, a conducting substance transports electrons from the anode to the cathode (Slate et al., 2019). Several substrates, including glucose, acetate, butyrate, lactate, ethanol, and cellulose, may be used to extract energy.

MFC is not only ecologically beneficial, but it is also a highly promising technology that can compete with other technologies in renewable energy generation, as well as the cost of developing reactors and sources of materials that can compete with fossil fuels (Sari and Suyati 2016). According to Palanisamy et al. (2019), MFC provides more advantages than traditional fuel cells since it can create electricity from organic waste and renewable

biomass. Microorganisms may become biocatalysts if they are able to adapt to diverse organic chemicals present in environmental waste and create electrons. Using platinum catalysts in fuel cells is a costly investment. In MFC, however, pricey metal catalysts may be replaced by microorganisms. Despite the advantages of MFC, there are a few drawbacks to consider, such as limited electricity production, unstable current, high internal resistance, and low electron transfer rate. (Thulasinathan et al., 2022).

The substrate plays a crucial role in MFC since the amount of electrical energy produced by the MFC system is dependent on the metabolic rate of microorganisms. Throughout the metabolic process, *Saccharomyces cerevisiae* uses a glucose-based substrate or something similar as a carbon source. When glucose is consumed during the yeast metabolic process, adenosine triphosphate (ATP) is produced (Liu, et al. 2017). The Yeast extract, Peptone, D-Glucose (YPD) medium is still the best substrate for yeast growth, for a variety of reasons, including: (i) yeast extract also serves as an external mediator that aids in electron transport, (ii) peptone is a necessary ingredient as a nitrogen source for yeast development, and (iii) glucose as a carbon source is a good, exact, and specific substrate that yeast can consume to produce electrical energy. According to research, *Saccharomyces cerevisiae* digests pure glucose faster because to its simpler structure.

Variations in the substrate type for MFC have been investigated by a number of researchers in the past. Putra et al. (2012) compared the substrates of MFC by using rice washing wastewater and tofu wastewater in their study. Meanwhile, Sinaga (2014) compared tofu whey and glucose as the substrate for yeast MFC. In their research, Ainun et al. (2017) evaluated electrical power on several substrate types, comparing the electrical power produced by fructose, lactose, and starch substrates. In these research, the most of their substrates are pro-analytical materials, hence the operating expenses will be quite high. Consequently, inexpensive and readily accessible substrates are required to lower operational expenses.

Based on these findings, the utilization of several types of glucose-based substrates as a source of MFC yeast carbon was investigated in this work in order to generate electricity. This research employed use of four different substrates: YPD medium, commercial sugar, commercial glucose, and pro-analyzed glucose. It is anticipated that commercial glucose and/or sugar would replace pro-analytical substrates in order to lower operating expenses. The resulting voltage, current, and power density will be used to assess the yeast MFC's performance. The link between the biofilm formed and substrate type will be evaluated, along with a techno-economic study. This has become a novelty in the academic community.

MATERIALS AND METHOD

MFC Reactor Preparation

The polyacrylic single-chamber cubic reactor was designed with a total reactor capacity of 28 mL as shown in Figure 1. The anode and cathode were made of carbon felt with a projected area of 7 cm^2, and Nafion 117 served as a membrane separator between the anode and cathode (Nevin, et al. 2008). YPD medium was made with the following ingredients: yeast extract 5 mg/mL, peptone 2.5 mg/mL, D-glucose and yeast 13.18 mg/mL (Christwardana et al., 2019). The MFC reactor was then filled with YPD medium, and a tiny magnetic stirrer was installed in the anode chamber to keep the solution homogenous. Other substrates (commercial sugar, commercial glucose, and PA glucose) were produced in the same way as before at a concentration of 13.18 mg/mL.

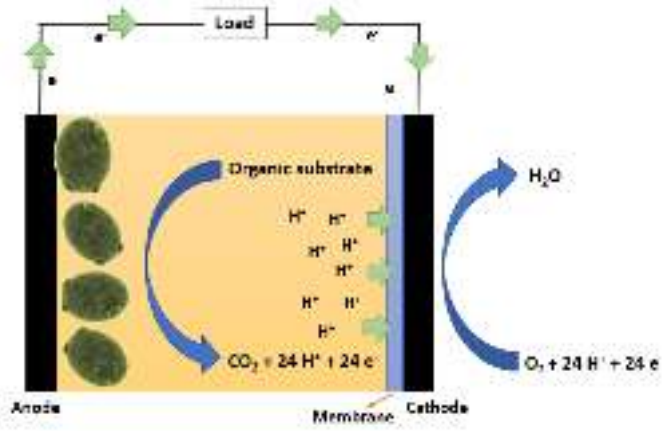

Figure 1. Schematic of Yeast Microbial Fuel Cell system

Full Cell Analysis

Full cell quality was evaluated by connecting the MFC to a multimeter and measuring voltage and current with an external load. Yeast MFC is run for 72 hours in closed circuit voltage (CCV) mode with an external resistance of 1000 Ω (Christwardana et al., 2020; Christwardana et al., 2021). The 72-hour incubation period was chosen because the yeast was in the stationary phase at the time. After 72 hours of incubation, the polarization and power curves were measured by progressively changing the sequential external resistor values from 10000, 5000, 1000, 100, 50, and 10 Ω.

Biofilm Analysis

Subtracting the dry anode weight before incubation from the dry anode weight after incubation yielded the biofilm's weight. The variation in anode weight is due to the yeast biofilm's dry weight clinging to the anode.

RESULTS AND DISCUSSION

Voltage Analysis

Figure 2a-d illustrates the results of voltage measurement during the MFC incubation process,

with the graph displaying the change in electrical voltage over a 72-hour period. The microbial consortia established a biofilm to breakdown organic molecules during the incubation process (Riedl et al., 2019). When consuming the YPD medium substrate, the maximum voltage obtained on the MFC is 0.16±0.02 V, which is utilized as a benchmark for comparison with other substrates. MFC using PA glucose generated a voltage of 0.13±0.03 V, commercial glucose produced a voltage of 0.03±0.01 V, and commercial sugar produced a voltage of 0.01±0.00 V.

The peptone concentration in the YPD medium substrate causes the voltage generated by MFC to be greater than that of other substrates. The yeast extract content, which functions as an external mediator to conduct electricity (Lee et al., 2015), and PA glucose, which has been demonstrated to have an influence on excellent substrate performance, offer ideal sustenance for bacteria to grow. Microorganisms' life stages, which include the lag, exponential, stationary, and death phases, are connected with voltage variations throughout time. When the incubation time was compared to bacterial growth, it was observed that the microorganisms passed through a lag phase, resulting in a voltage that was very low and inconsequential. The microorganisms will adapt to the current substrate environment during the lag period, resulting in a metabolism that is less than ideal. Because the bacteria had entered the exponential phase, the voltage rose dramatically during the experiment in the following hours (logarithmic phase). Cells participate in active metabolism, which is accompanied by rapid division and the creation of cellular elements, during this phase. More protons and electrons are created via metabolic processes as a result of the increased number of microbe cells, resulting in higher power output. The voltage in the measurement then dropped slightly before rising and remained rather constant, suggesting that the bacteria had reached the stationary stage.

The voltage value is greatly affected by time. The substrate's availability is still sufficient to cause a voltage increase, letting the electron transfer process to continue properly. In addition, the substrate's conversion to a carbon source may result in an increase in electrical energy (Rabaey et al., 2003). MFCs employing commercial glucose substrates revealed a faster voltage increase than other substrates due to the presence of the sulfite preservative in the product. Sulfite (SO^{2-}) is a negative ionic that accelerates electron transport, causing the voltage to increase faster than on other substrates. With each passing second, the voltage value of all samples decreases. This occurs because as the number of higher cells grows, the substrate

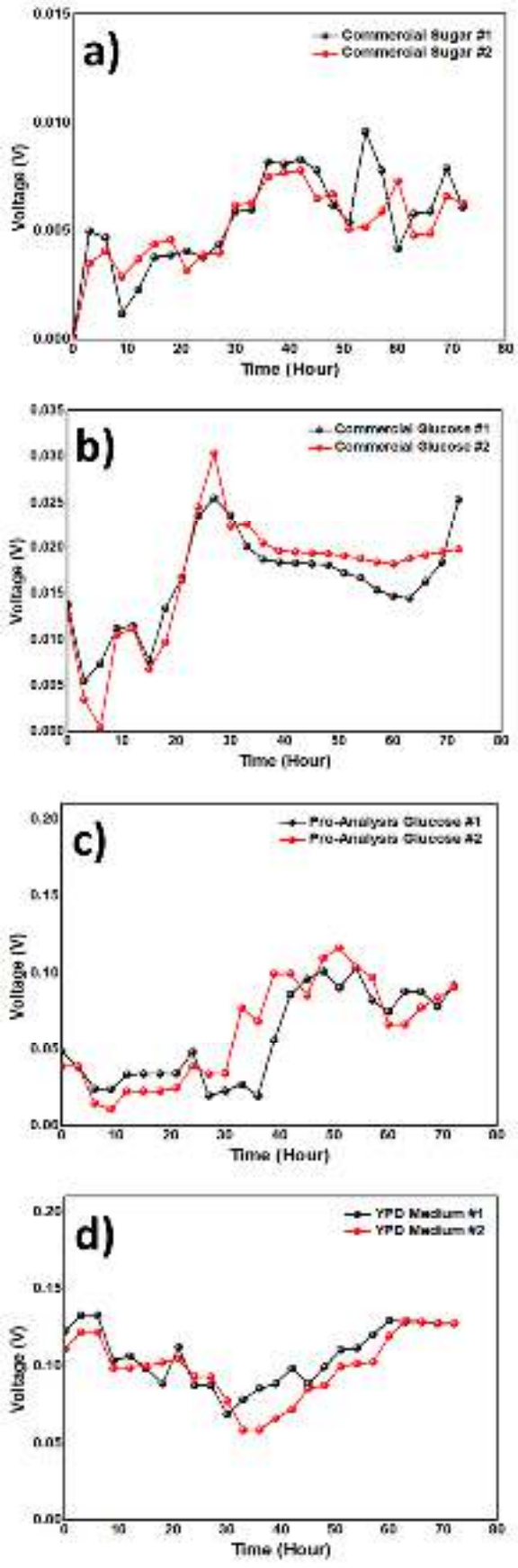

Figure 2. Voltage of MFC during incubation process using a) commercial sugar, b) commercial glucose, c) PA glucose, and d) YPD medium as substrate

concentration drops fast. Yeast gets malnourished and loses its ability to create protons and electrons when there is a shortage of substrate. The flow of electrons from microbe cells to electrodes is connected to glucose levels, according to Lee et al., (2010).

The information supplied here is consistent with previous research. When compared to not providing yeast extract, Sayed et al. (2015) discovered that administering S. cerevisiae yeast extract had a significant impact on viability. Peptone may improve yeast production, according to Lavanya et al. (2021). In addition, Sayed and Abdelkareem (2017) observed that using *S. cerevisiae* as a biocatalyst and D-Glucose as a substrate resulted in a greater electric voltage.

Polarization Analysis

To obtain the best MPD, MFC was tried on four different substrates (YPD medium, PA glucose, commercial glucose, and commercial sugar). The greatest MPD values were obtained in MFC with YPD medium of 18.40±1.98 mW/m^2, followed by PA glucose of 9.41±1.15 mW/m^2, commercial glucose of 1.30±0.10 mW/m^2, and commercial sugar of 0.04±0.01 mW/m^2. Microorganisms' ability to create and transmit electrons from their cells to the electrodes is hampered by low substrate levels. The concentration of the substrate reduces more quickly as the cell mass of microorganisms increases. As a consequence, there was a decrease in the quantity of protons and electrons delivered to the electrodes. The growth in MPD was accompanied with a high substrate level, allowing the bacterial metabolic rate to remain constant. Figure 3a-d demonstrates that following MFC with medium YPD, PA glucose generated the second greatest MPD. Apart from that, PA glucose contains just pure glucose, which is a monosaccharide with a simpler structure that S. cerevisiae can simply degrade into food sources. Because it contains sucrose, a kind of disaccharide, commercial sugar has the lowest power when compared to other substrates. Sucrose is formed when two monosaccharides, fructose and glucose, are combined (Stein and Granot, 2019). Commercial sugar is made up of 45 % glucose and the rest is fructose.

Biofilm Analysis

The resulting biofilm findings are directly proportional to the obtained voltage levels (Figure 4). The higher the biofilm produced, the higher the voltage value, which was 58.5±0.49 mg on MFC with commercial sugar, 16.15±0.92 mg with commercial glucose, 43.20±1.27 mg with PA glucose, and 62.75±0.49 mg with YPD Medium. More electrons are created when yeast grows on the anode, enabling it to produce more energy. The biofilm that grows on the electrodes is intricately connected to the activities of the microorganisms that produce energy in the MFC. However, other studies have demonstrated that

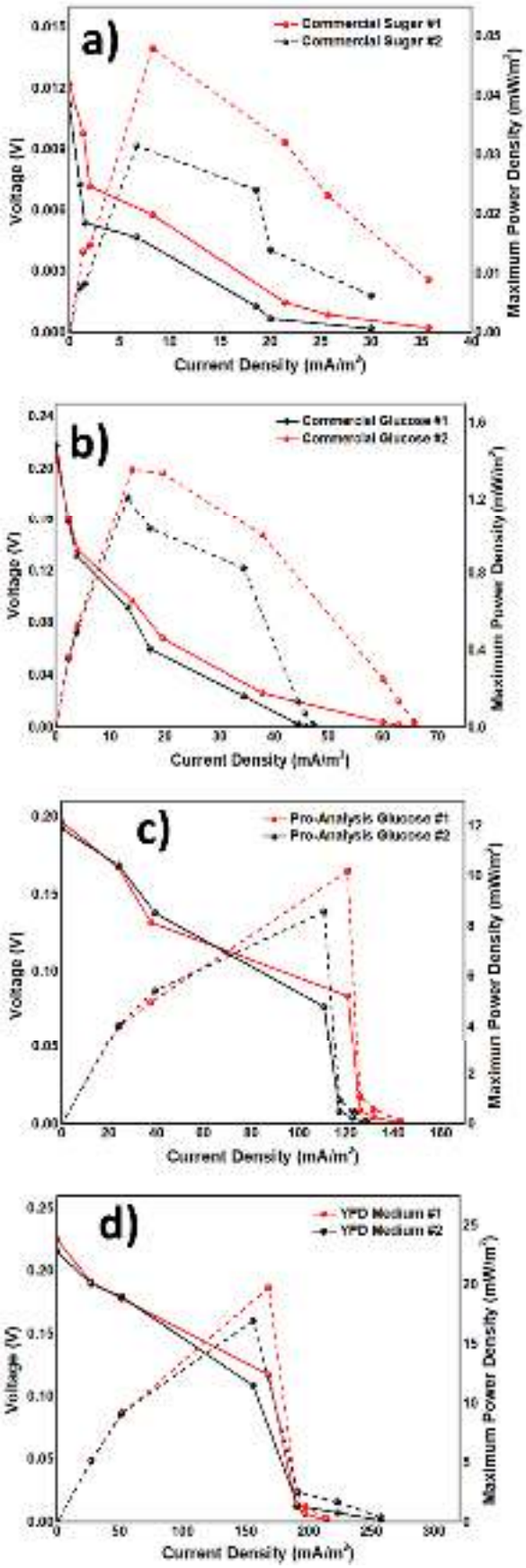

Figure 3. Polarization and power curves of MFC during incubation process using a) commercial sugar, b) commercial glucose, c) PA glucose, and d) YPD medium as substrate

not all of these bacteria make direct contact with the electrode, but rather interact indirectly via other microorganisms (Franks et al., 2010). The magnitude of the ensuing electric potential difference is determined in part by the presence of biofilm on the electrode's surface.

This has to do with the relationship between the density of microorganisms in the biofilm formed on the electrode surface and the MFC's electricality value. The electricality of the MFC generated is related to the thickness of the biofilm developed on the surface of the MFC electrode; the thicker the biofilm at the anode, the greater the electricality of the MFC produced (Baudler et al. 2015).

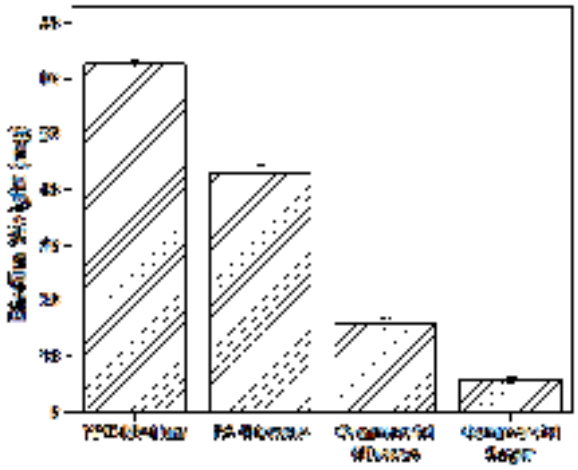

Figure 4. Biofilm growth in the anode surface during incubation process of MFC using various substrate

CONCLUSION

In this study, the effect of substate type on the power output of a single chamber yeast MFC was shown. Several simple substrates were used as electron donors, including commercial glucose, Pro-Analysis glucose, commercial sugar, and YPD medium, with yeast *Saccharomyces cerevisiae* serving as the biocatalyst to create energy. The results of the closed-circuit voltage experiments revealed that YPD medium provided the greatest MFC performance. When comparing several carbon substrates as electron donors, YPD medium had the highest power density (i.e., 18.40±1.98 mW/m^2), followed by PA glucose, commercial glucose, and commercial sugar, with values of 9.41±1.15, 1.30±0.10, and 0.04±0.01 mW/m^2, respectively. The presence of additions such as yeast extract and peptone influences electron transfer since they also serve as electron transfer rate-enhancing mediators. Furthermore, this study found that biofilm growth has a direct relationship with the voltage generated. According to the results obtained, commercial glucose and sugar have not yet been able to compete with YPD medium or pro-analytic glucose as a substrate, despite their lower cost and widespread availability. Furthermore, the data show that substrate inhibition has an influence on MFC performance.

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