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Original scientific paper

Synergistic of yeast *Saccharomyces cerevisiae* and glucose oxidase enzyme as co-biocatalyst of enzymatic microbial fuel cell (EMFC) in converting sugarcane bagasse extract into electricity

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Abstract

The microbial fuel cell (MFC) is an ecologically friendly alternative energy source. Due to the typically limited electron transfer in MFC systems, co-biocatalysts are necessary to enhance their performance. Enzymes are used as co-biocatalysts due to their superior ability to generate energy, and the system is known as an enzymatic microbial fuel cell (EMFC). One of the substrates that may be used is bagasse waste extracted from sugarcane. Saccharomyces cerevisiae and the enzyme glucose oxidase (GOx) serve as co-biocatalysts in the breakdown of sugarcane bagasse waste in this study, which uses single-chamber EMFCs. In EMFC using sugarcane bagasse waste extract employing S. cerevisiae biocatalyst and glucose oxidase enzyme co-biocatalyst, the open circuit voltage was 0.56 V and the maximum power density was 146.65 mW m^{-2}, an increase of 10.4 times to MFCs that solely employed only yeast biocatalyst. In addition, the chemical oxygen demand (COD) reduction achieved by this technology is 75 %. In addition, the pH of sugarcane bagasse waste extract samples treated with Saccharomyces cerevisiae yeast and GOx enzyme decreased from 4.6 to 4.2. This research demonstrates that adding the co-biocatalyst GOx enzyme may boost the performance of the traditional yeast MFC.

Keywords

Bioelectricity; fuel cells; enzymes; sustainable energy

Introduction

Indonesia is now struggling to deal with its energy sources. Unfortunately, most energy sources rely on nonrenewable fossil fuels. Numerous nations are attempting to reduce their reliance on

fossil fuels because of their detrimental impact on the environment and humans [1]. In an attempt to adopt the usage of new and renewable energy, a number of nations are accelerating their infrastructure construction. The usage of biomass and fuel cell technology are only two examples. Microbial fuel cell (MFC) technology, which mixes biomass with fuel cell technology, is one kind of sustainable power technology. MFC techniques may lessen the electrical strain on fossil fuels, but they cannot replace electrical energy since the electrical power generated is still rather low. MFC is used as a source of electrical energy for nighttime lighting and as a power source for microcomputers, for instance.

MFC is an electrochemical technology that uses the catalytic activity of live microorganisms to transfer chemical energy from organic components into electrical energy [2]. Utilizing organic waste components, MFC is an alternative energy-generating technology that is ecologically beneficial and capable of reducing environmental pollution. The microbial fuel cell technique offers a number of special benefits, including its applicability to treatment *via* a low-concentration substrate at temperatures below 20 °C [3]. In addition, the quantity of electrical energy generated by MFC is small, hindering its use on a broad scale [4]. The selectivity of microorganisms to the substrate is also one of the limitations of MFC, which is impacted by the kind of waste used as the substrate [5].

Sugarcane is a plant with considerable economic value and the primary raw material for sugar-producing industries [6]. Around 35-40 % of the weight of milled sugarcane may be extracted as bagasse from a sugar refinery. Indonesian sugarcane production reached 33.7 million tons in 2013 [7]. Therefore, it can be determined that the average quantity of bagasse generated throughout that time period was 11 million tons. This amount is estimated to rise since sugar production in Indonesia is projected to rise. As a waste, bagasse may be harmful to the environment and must be properly managed. Bagasse may be used as fuel in the sugar industry, as well as in the production of composites, textiles, paper, and animal feed [8]. However, its use has not been optimal, thus the rise in bagasse's economic worth has not been realized, and there are still many great deals of bagasse that have not been employed. Typically, unused bagasse waste is stacked around the mill or on the factory scale in the form of cube-shaped bagasse, generating an offensive odor issue that disturbs people [9,10]. Because the effect of sugarcane bagasse waste management is unfavorable, a new approach is required. MFC is one answer to the issue. Bagasse's glucose content may be used as a substrate for the MFC system.

Glucose oxidase (GOx) is an enzyme that has been extensively developed for fuel cell applications due to its electrochemical catalytic activity under physiological settings [11,12]. GOx has the capacity to convert glucose into gluconolactone and electrical energy, making it an excellent candidate as a co-biocatalyst in a biological fuel cell for energy production. Christwardana *et al*. [13] researched the use of glucose oxidase and catalase as co-biocatalysts for the anode of an enzymatic fuel cell (EFC). Christwardana *et al*. [14] investigated glucose oxidase and peroxidase as co-biocathode catalysts in EBC. In biological fuel cells, the use of GOx as a co-biocatalyst with microbes is now a distinct option Kovačević *et al*. [15] explored the production of fructose and gluconic acid from sucrose using yeast cell walls expressing GOx. As fuel cell biocatalysts, Bahartan *et al.* [16] combined GOx and glucoamylase enzymes shown on the yeast cell surface and compared them to unmodified yeast or pure enzymes. Bahartan *et al*. [17] encapsulated yeast with GOx on its surface in graphene oxide hydrogel as a co-biocatalyst fuel cell. Co-biocatalyst yeast with GOx has been studied for its electrochemical properties, but it has never been used in wastewater treatment. This may be investigated further so that enzymes and microorganisms can be integrated as co-biocatalysts to

minimize COD in the wastewater treatment process and turn them into electrical energy utilizing a biological fuel cell system.

In this study, the yeast *Saccharomyces cerevisiae* was paired with the enzyme glucose oxidase as a co-biocatalyst in the enzymatic microbial fuel cell (EMFC) system for treating sugarcane bagasse waste extract. The inclusion of the glucose oxidase enzyme may facilitate the conversion of glucose in sugarcane bagasse waste extract into protons and electrons, hence enhancing the efficiency of glucose conversion into energy. The employment of enzymes as co-biocatalysts in EMFC for waste or wastewater treatment applications has never been done previously, hence it may be considered a novelty in this study. The goals of this research are to (1) establish the function and impact of adding glucose oxidase enzyme to the yeast MFC to form an EMFC system in degrading sugarcane bagasse waste extract into electrical energy and (2) assess the value of lowering COD waste that can be achieved using enzymatic microbial fuel cells.

Experimental

Materials

Bagasse is purchased from a seller of sugarcane ice in Serpong, Tangerang, Indonesia. Bagasse is composed of around 33 % lignin, 32 % hemicellulose, and 35 % cellulose [18]. As a separating membrane, Nafion 212 (DuPont, USA) was employed. Lesaffre produced the yeast *Saccharomyces cerevisiae* (Lessafre, Marcq-en-Baroeul, France), and commercial glucose oxidase enzyme extracted from *Penicillium notatum* (light yellow powder; activity 2800 unit/g) was bought from Xi'an Taicheng Chem (China).

Bagasse waste extract preparation

Bagasse waste is cut into smaller pieces before being sun-dried for typically three days. This treatment may be utilized as raw material for the subsequent waste processing step. 15 g dry bagasse waste is mixed in 150 ml of distilled water [18] using a blender Phillips HR2056 for 60 seconds. This procedure tries to dissolve glucose or other sugars from the tissue matrix of sugarcane into distilled water. The processed bagasse waste slurry is subsequently filtered using a cheesecloth to remove the cellulose component, while the permeate fraction may be used as an EMFC substrate. Analyzed using a glucose sensor Gluco Dr AGM-2100 (Gyeonggi-do, South Korea), the sugarcane bagasse extract had 11900 ppm of glucose and 9975 ppm of COD.

Structure and operation of the EMFC reactor

In this experiment, three identical plexiglass cubic single-chamber reactors with an active volume of 28 mL were utilized, with plain carbon electrodes with a projected surface area of 7 cm^2. In addition, Nafion 117 (3 wt.% H_2O_2, 0.5M H_2SO_4, and DI water) was utilized as a proton diffusion area separator between the two electrodes [19,20]. The sugarcane bagasse extract was mixed with 14 mg/mL of commercial baker's yeast *Saccharomyces cerevisiae*, used as a bio-catalyst, and incubated semi-aerobically in a system known as MFC [21]. Copper wire was used to complete the circuit, after which 1000 Ω of external resistance was applied. Voltage and current density were measured during the incubation period while the system was run in batch mode at 27 °C for three days (72 hours) every cycle for three cycles. At each cycle, fresh substrates and biocatalysts were introduced into the system. For EFC and EMFC systems, the biocatalyst is replaced by the GOx enzyme and GOx-yeast combination, respectively. The schematic diagram of MFC, EFC, and EMFC system can be shown in Figure 1.

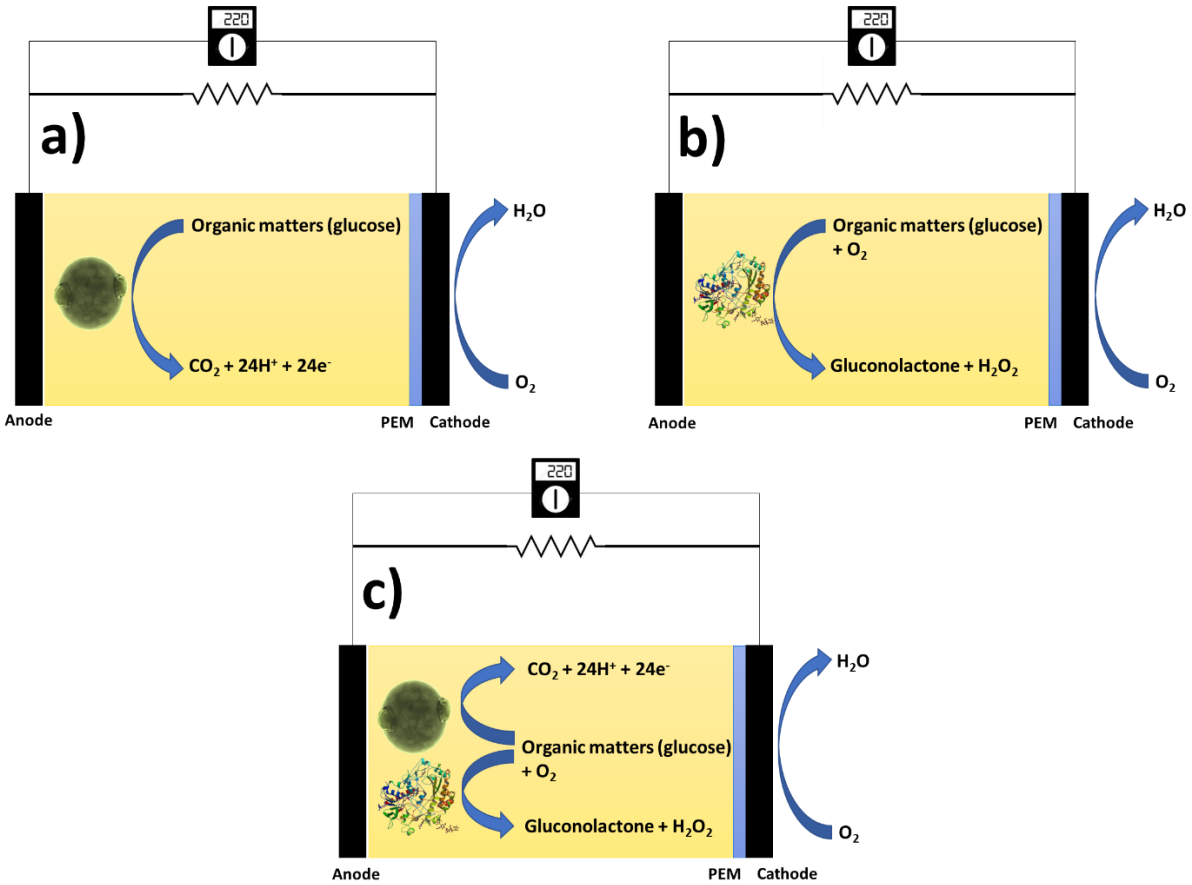

Figure 1. *Schematic illustration of a) MFC, b) EFC, and c) EMFC system for wastewater treatment*

Electrochemical and COD analysis

The output current and voltage density throughout the incubation procedure (3×72 hours) are the primary parameters used to assess MFC performance. This required the collection of closed-circuit voltage (CCV) with an external load set to 1000 Ω. Voltage and current are measured using a digital multimeter UNI-T UT61E, followed by the calculation of current density, mA m^{-2}, dividing the produced current by anodic surface area, m^2. In addition, manual polarization was done to analyze MFC behavior, using a resistor configuration of Elenco RS-500 that was set between 5 MΩ and 100 Ω. The relative decrease in cell potential (RDCP) was used to determine the maximum sustainable power produced during operation, inspired by the relative decrease in anode potential (RDAP). When doing polarization analysis, RDCP is evaluated using a graph between RDCP content *vs*. external resistance value, where the content of RDCP, %, is determined by the formula $(V_{OCV}/V_n)\times100$, in which V_{OCV} is the voltage at an open circuit, and V_n is the voltage when external resistance is applied. Analyzing the COD and pH of anolyte using the Lutron WA-2015, the performance in terms of treatment efficiency was measured.

Results and discussion

Mechanism of biocatalyst activity in MFC, EFC, and EMFC systems

Saccharomyces cerevisiae uses glucose from sugarcane bagasse waste extract as a carbon source for its development, producing protons and electrons in the process. In MFC, yeast absorbs sugarcane bagasse waste extract and converts it to pyruvate *via* glycolysis [22]. Pyruvate is subsequently utilized in the tricarboxylic acid (TCA) cycle, which involves numerous enzymes [23]. Then the electron transfer chain (ETC) process takes place, during which the redox reaction of NAD^+

and FAD to NADH and $FADH_2$ occurs [24]. The protons and electrons that have been generated then leave the cell. However, yeast has a complex ETC, requiring more time for electron transport. The reaction that happens in MFC with yeast biocatalyst is shown in equation (1):

$$C_6H_{12}O_6 + 6H_2O \xrightarrow{\text{yeast}} 6CO_2 + 14H^+ + 24e^- \quad (1)$$

Meanwhile, the EFC process turns chemical energy into electricity by using the high selectivity of enzyme for glucose substrates. It is responsible for glucose oxidation, oxygen reduction, and $FAD/FADH_2$ redox processes. As glucose becomes accessible, it is oxidized to generate electrons, protons, and gluconolactone. The protons and electrons reacted with FAD to form $FADH_2$, and vice versa, while some protons and electrons will be used for O_2 reduction to H_2O_2 [25]. Without O_2, GOx cannot convert glucose into protons, electrons, and gluconolactone, as GOx is an oxidoreductase enzyme. This GOx enzyme biocatalyst has a high electron transfer rate, therefore it would generate a great deal of energy [26]. In equation (2), glucose undergoes an oxidation process with oxygen and the presence of GOx enzyme biocatalyst to generate gluconolactone and H_2O_2. In equation (3), the redox process at the active site of GOx, $FAD/FADH_2$ as co-enzyme, involves proton and electron activity.

$$\text{glucose} + O_2 \xrightarrow{\text{GOx}} \text{gluconolaceton} + H_2O \quad (2)$$

$$Gox(FAD) + 2H^+ + 2e^- \leftrightarrow Gox(FADH_2) \quad (3)$$

The production of electrons in an EMFC is a joint effort of yeast and enzymes. Yeast first takes in glucose and uses glycolysis to turn it into pyruvate; the pyruvate is then used in the TCA cycle, which concludes the multienzyme process. In turn, this activates NAD^+ and FAD, which pass through the ETC pathway. Electricity is produced as a result of the following generation of protons and electrons. Simultaneously, the GOx enzyme co-biocatalyst oxidizes glucose, transforming it into protons, electrons, and gluconolactone. It is expected that the combination would increase electrical energy production from the EMFC system due to the high electron transfer rate of GOx.

Voltage measurement in MFC, EFC, and EMFC systems

Figure 2 represents the observed voltage in the MFC, EFC, and EMFC systems. At the 75th hour, the EFC produces the maximum voltage with a value of 335 mV, followed by the EMFC at the 81st hour with 295 mV. At the 96th hour, MFC generates the maximum voltage with a value of 80 mV. EMFC produces the greatest average voltage value at 92.3 ± 2.51 mV, followed by EFC at 36.6 ± 2.42 mV and MFC at 27.3 ± 1.19 mV. The yeast biocatalyst required more time per cycle than the enzyme and yeast-enzyme combination to achieve the maximum current density. This is possible since microbes are still in the lag phase of forming biofilms on the anode surface [27]. In contrast, the time needed when using enzyme biocatalysts is less than when using yeast because the GOx enzyme has a faster electron transfer rate [28]. Because the GOx enzyme has a higher electron transfer rate, the yeast-enzyme combination achieves the maximum current density more quickly than yeast alone. In addition, the enzyme biocatalyst required a short time during the second and third cycles to provide the highest current density. Enzymes perform their activities immediately and do not need an adaptation period, unlike yeast, since they may react directly with existing substrates to produce energy supported by co-enzyme $FAD/FADH_2$ activity [29]. In addition, the enzyme dissolves directly on the substrate, which brings the enzyme molecule, substrate, and electrode closer together and accelerates electron transfer [30]. Regarding the usage of yeast-enzyme biocatalyst, the time necessary to generate the greatest current density in the second and third cycles was not too lengthy. This resulted from the formation of biofilm on the electrode surface during the second and third cycles, which increased electron transport to the anode. Moreover, following the second

substrate change (cycle 2), the resultant current density rose. However, after the third substrate change (cycle 3), the resulting current density declined significantly. This is because the biofilm layer is excessively thick, creating a large distance between the biofilm and the electrode, which hinders electron transmission. Although the enzyme biocatalyst produced the maximum current density in the EFC, the yeast-enzyme combination biocatalyst produced the highest average value in the EMFC system. This is due to the fact that the addition of enzyme co-biocatalyst to yeast biocatalyst increases the electromotive force of electrons produced during the substrate conversion process.

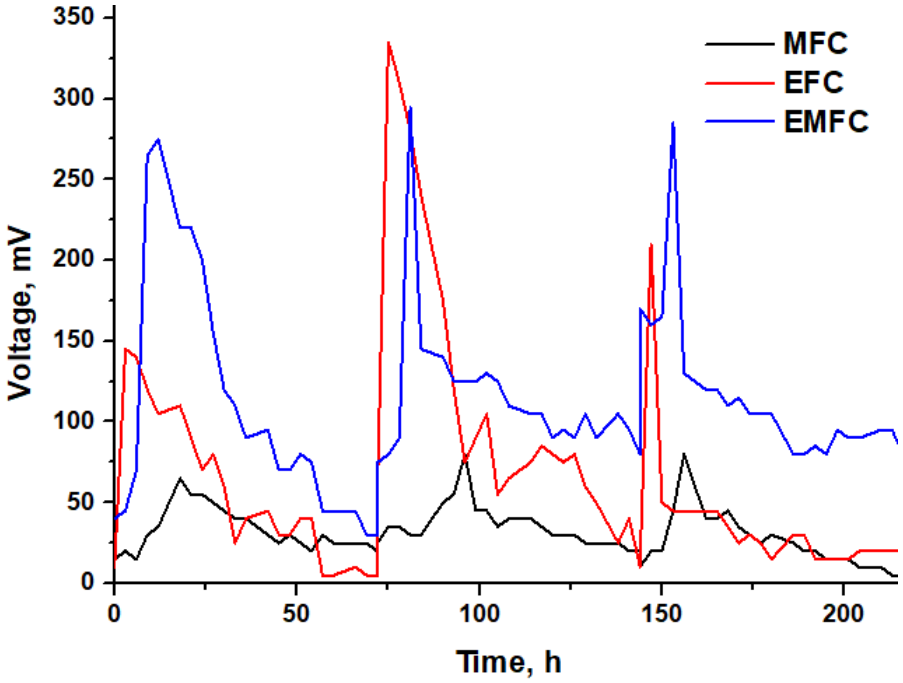

Figure 2. *Voltage of MFC, EFC, and EMFC system during sugarcane bagasse waste extract incubation process of 216 h*

Maximum power density analysis

According to Figure 3a, the open circuit voltage of MFC, EFC, and EMFC is 0.45, 0.53, and 0.56 V, respectively. The interesting thing about this polarization curve is the high activation or reaction rate losses. Since the electrochemical reaction in the MFC is limited by the absorption reaction, the necessary activation energy will be larger (from 0.45 to 0.11 V). While reactions involving GOx in EFC and EMFC are limited by surface reactions, the reaction rate losses are not as significant as MFC, with EFC from 0.53 to 0.26 V and EMFC from 0.56 to 0.28 V. From Figure 3b, the maximum power density generated by EMFC is 145.65 mW m^{-2} at a current density of 645.1 mA m^{-2}. While the MFC generates a maximum power density of 14 mW m^{-2} at a current density of 200 mA m^{-2}, the EFC produces a maximum power density of 73.94 mW m^{-2} at a current density of 459.64 mA m^{-2}. These power density results are consistent with the current density result presented in the previous sections. Several factors influence the value of the electric power density, including the design, type, and surface area of the membrane, the electrodes, the substrate, and the biocatalyst [31,32]. Using a catalyst to transform the substrate into electrical energy generates a certain quantity of electricity, as measured by this power density. Because each catalyst has a unique performance, the employment of various catalysts results in distinct power densities [33]. Additionally, the presence of molecules other than glucose on the substrate will limit the efficacy of the biofuel cell in converting it into electrical energy.

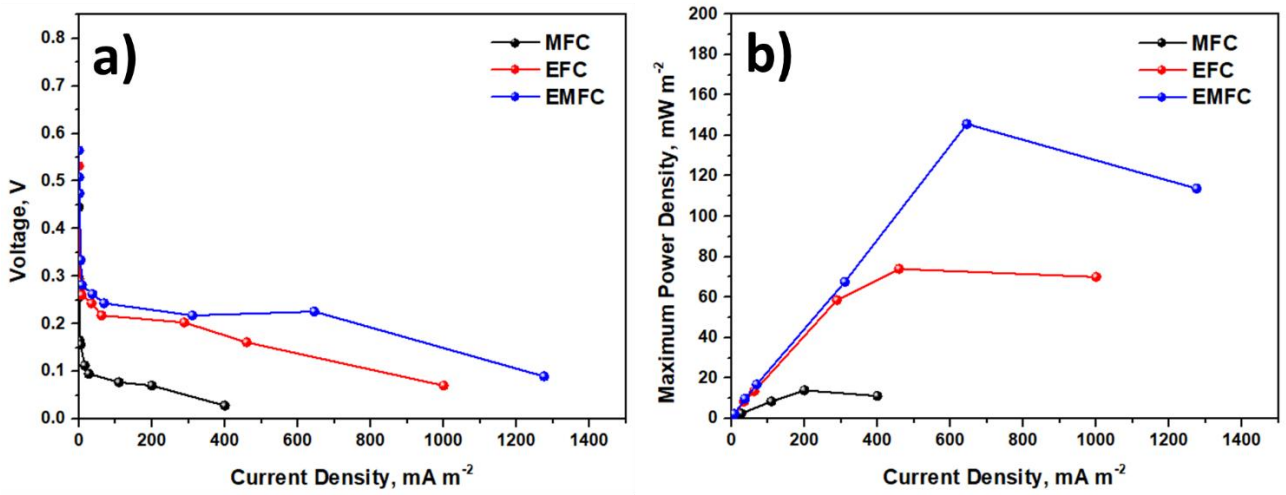

Figure 3. *a) polarization and b) power density curves of MFC, EFC, and EMFC system during sugarcane bagasse waste extract incubation process of 216 h*

Table 1. *Comparison of EMFC performance with others taken from the literature*

Anode	Cathode	Biocatalyst	Substrate	MPD, mWm^{-2}	Ref.
Carbon paper	Carbon paper	*Saccharomyces cerevisiae*	Glucose	3	[34]
Ammonia-treated carbon cloth	Carbon fiber	*Enterobacter cloacae*	Pure cellulose	5.4	[35]
Bow-shaped Carbon fibers	Circular stain-less-steel wire	*Saccharomyces cerevisiae*	Glucose	52	[36]
Carbon paper	Pt@carbon paper	*Brevibacillus borstelensis*	Sugarcane molasses	188.5	[37]
Carbon felt	Carbon felt	*Rhizobium anhuiense*	Glucose	4.93	[38]
Carbon felt	Carbon felt	*S. cerevisiae* and Enzim GOx	Sugarcane bagasse extract	145.65	*This work*

Table 1 contains a variety of data on the maximum power density taken from some previously published studies. In comparison to prior study findings, it can be stated that this yeast-enzyme biocatalyst delivers an excellent maximum power density. The yeast-enzyme combination in the EMFC system increases the conversion of organic matter in the substrate into electricity when two distinct biocatalysts are simultaneously converting the substrate. Since EMFCs can only generate a small quantity of electricity compared to conventional fuel cells, they will likely only be used as backup power for smaller units in industries, such as nighttime lighting for streets and buildings. A promising method, the EMFC system, can be applied downstream of the sugar industry process that uses sugar cane as a raw material, *i.e.,* before the bagasse waste is disposed of.

A relative decrease in cell potential (RDCP), a novel method for evaluating the maximum sustainability of MFC based on the capacity to produce electricity, is a function of external resistance. As can be seen in Figure 4a-b, the RDCP for the MFC, EFC, and EMFC systems were all around the same value, *i.e.,* 500, 154, and 438 kΩ, respectively. Furthermore, the findings show that the enzyme may lower RDCP resistance, demonstrating the enzyme's superiority to yeast cells in terms of conductivity. In both systems, the anode conditions have a voltage proportional to resistance, while the anode's increased inclination for oxidation offers more energy from the biocatalyst. This is notably linked to the biocatalyst activity, especially when the electron release is quicker, which adds to increased electricity and energy output.

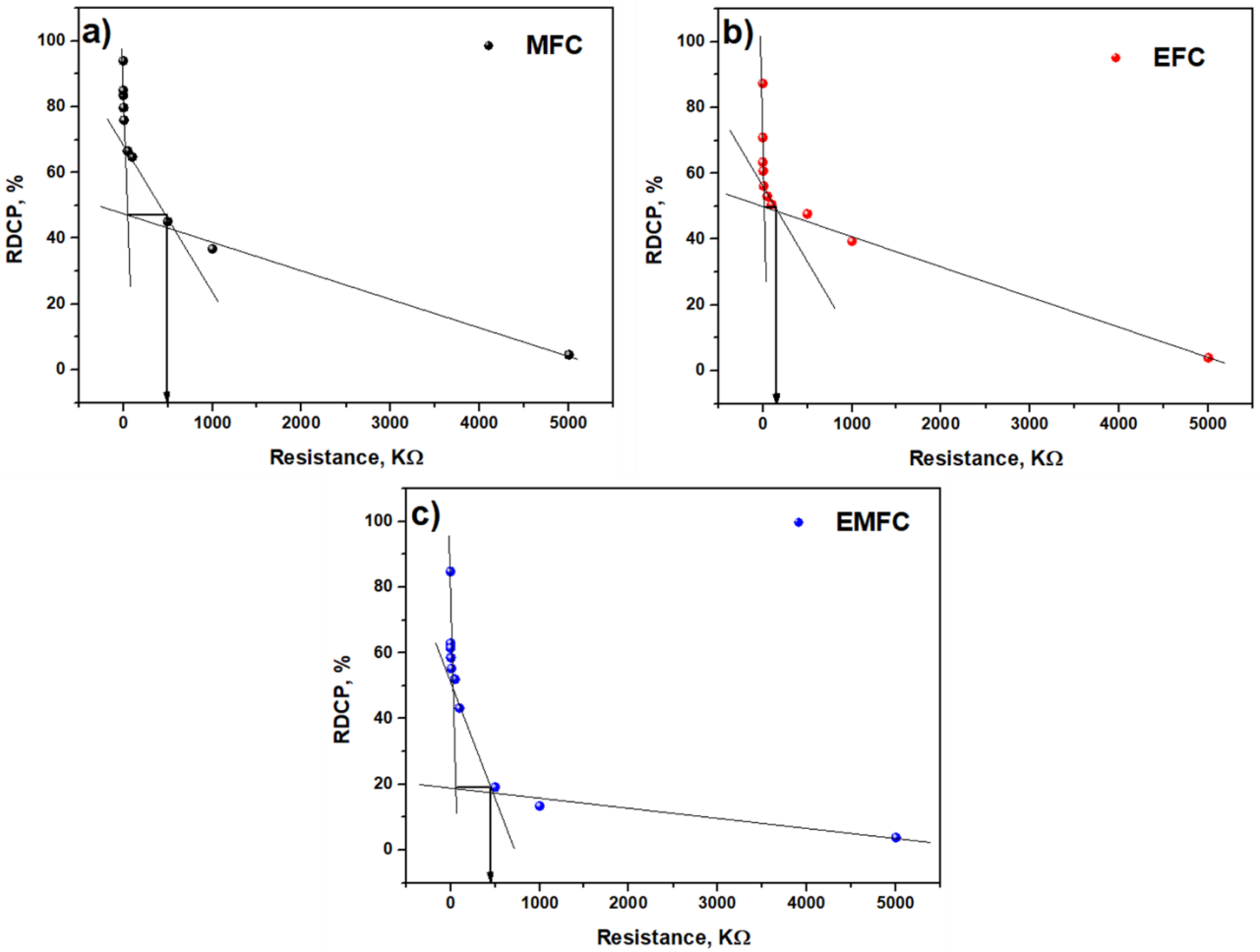

Figure 4. *Relative decrease in cell potential (RDCP) of: a) MFC, b) EFC, and c) EMFC system*

COD removal analysis

Figure 5 demonstrates that the MFC's COD decreased by 27.95 % in the first cycle, 41.32 % cycle, and 19.19 % in the third cycle. The COD removal in the first, second, and third cycles of the EFC experiment was 21.92, 69.34 and 25.01 %, respectively. And for the EMFC system, the COD reduction values for the three consecutive cycles were 74.08, 75.04 and 72.46 %. Using a yeast-enzyme combination biocatalyst in the second cycle resulted in the maximum reduction of COD or elimination of COD by 75.04 %, as shown by these data. Due to poor co-biocatalyst stability, the third cycle of EMFC had a lower COD reduction than the second cycle. Due to the synergistic effect of two kinds of biocatalysts that breakdown organic matter in the substrate of sugarcane bagasse extract, the COD reduction in EMFC was higher than in MFC and EFC. The yeast-enzyme combination allows the decomposition of organic matter by yeast through the metabolism of microorganisms and enzymes via their catalytic activity simultaneously, hence increasing the quantity of organic matter that may be degraded and transformed into electrical energy. As the amount of organic matter is degraded, the COD drop is likewise increasing. This study demonstrated a 75.04 % drop in COD reduction, which is more than the 36.36 % seen in earlier research [18]. There is a correlation between the drop in COD and the generation of power, such that the larger the decrease in COD, the greater the generation of electricity [39].

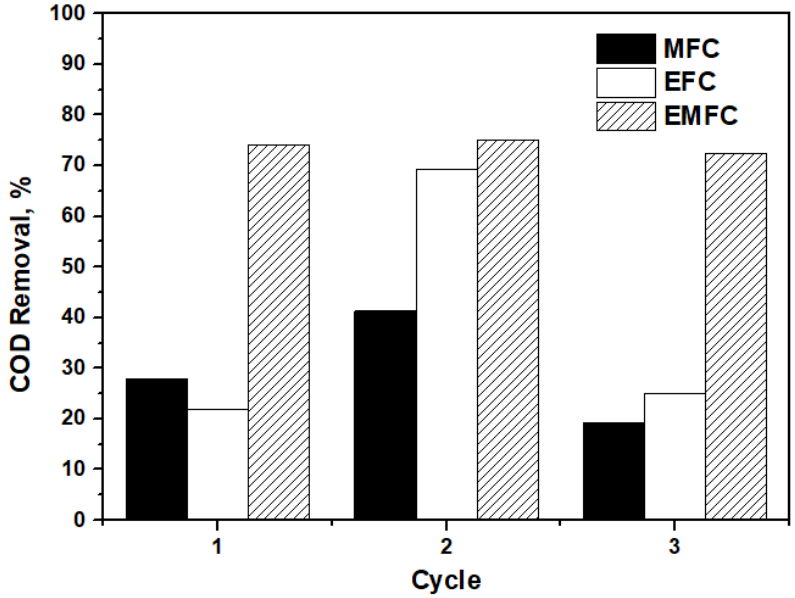

Figure 5. *COD removal of sugarcane bagasse waste extract using MFC, EFC, and EMFC system in every incubation cycle*

pH analysis

Figure 6 demonstrates that the pH declined throughout the fermentation process in cycles one, two, and three and every variation of biocatalyst use. In the MFC system, the starting pH of the substrate was 4.6, while after 72 hours (cycle 1), it fell to 3.38. The pH was then reduced to 3.09 during the subsequent 144 hours (cycle 2) after a change in the substrate where the starting pH was 4. The process was repeated by introducing a new substrate with an initial pH of 4.26 and reducing it to 3.12 at the end of cycle 3.

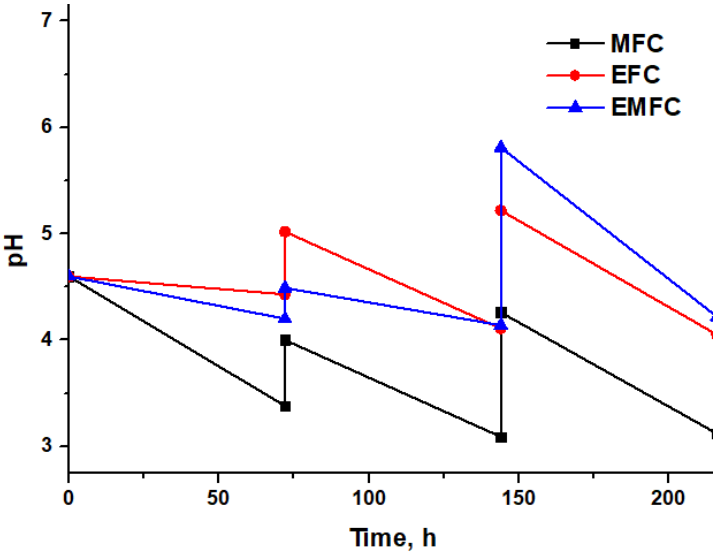

Figure 6. *Decrease in pH of MFC, EFC, and EMFC system during incubation of 216 h*

The initial pH in the EFC was 4.6 and after 72 hours, it dropped to 4.43. pH increased to 5.02 when substrates were introduced but decreased to 4.11 after fermentation. The substrate was switched to a fresh one with a pH of 5.22, and at the end of cycle 3, the pH declined to 4.05. The substrate pH in the first cycle EMFC system was 4.6 at the beginning of the cycle and dropped to 4.2 after 72 hours of fermentation. At the start of the second cycle, the pH was 4.49, and it decreased to 4.14 at the end. The pH of the substrate turnover increased to 5.82 at the beginning of cycle 3 before falling

to 4.2 at the end of fermentation. Based on these results, it can be concluded that the pH continued to decrease during the fermentation process. The MFC has the lowest pH of the three types of biocatalyst use. As a consequence of microbial metabolism, malic acid, tartaric acid, citric acid, lactic acid, acetic acid, butyric acid, and propionic acid are produced during fermentation, resulting in a reduction in pH [40]. In addition, the EFC experiences a decrease in pH because the rate of proton migration from the anode to the cathode across the membrane is diminished, causing an accumulation of protons [41].

Conclusions

This study reveals that the EMFC system combined with the fermentation of sugarcane bagasse to produce electricity directly utilizing *S. cerevisiae* and the enzyme glucose oxidase as co-biocatalyst performs well. With an EMFC system, 145.65 mW m^{-2} of power density was reached, or a larger 940 and 96 % than the MFC and EFC systems, respectively. When compared to MFC and EFC systems, this system achieves a COD elimination rate of 75.04 %, which is 1.8 and 1.1 times higher. Yeast metabolism and/or enzyme activity produced acid throughout the incubation phase, which resulted in a drop in pH after the process. The synergistic effect of GOx and yeast as co-biocatalysts in MFC systems is more impressive than in MFC and EFC systems. The transfer rate of electrons is increased by the presence of GOx in the system, which improves system performance. As a result, the EMFC system has great promise for solving wastewater issues, particularly those associated with sugarcane bagasse extract wastewater. Other forms of wastewater, such as those having high quantities of glucose, may be able to benefit from this approach in the future.

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