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Marcelinus Christwardana ✉; Satrio Kuntolaksono

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Immobilization of *Saccharomyces cerevisiae* in Agar Polymer Matrix to Improves The Performance of A Yeast Microbial Fuel Cell

Marcelinus Christwardana[a)] and Satrio Kuntolaksono[b)]

Department of Chemical Engineering, Institut Teknologi Indonesia, Jl. Raya Puspiptek Serpong, South Tangerang, Banten, Indonesia 15320

[a)]Corresponding author: marcelinus@iti.ac.id
[b)]satrio.k@iti.ac.id

Abstract. Encapsulation of yeast *Saccharomyces cerevisiae* in agar and its electrochemical characteristic of single cell with immobilized yeast *S. cerevisiae* were examined in this study. Yeast entrapped on agar matrix at varied concentrations was manufactured using a conventional method. The potential of yeast *S. cerevisiae* to produce and transmit extracellular electrons without the need of an external mediator has important implications for the construction of microbial fuel cells. With an agar concentration of 11.7 mg/mL, the current density reached 7 mA/m2 in 23 hours, resulting in an MPD of 0.91 mW/m^2. The result of Km was 9.15 1.01 mg/mL, while sensitivity and J_{max} were 0,39 ± 0,03 $(mA/m^2)/(mg/mL)$ and 3,53 ± 0,16 mA/m^2, respectively. This demonstrates that yeast immobilization on agar as a matrix has high potential and is beneficial to MFC growth in terms of sustainability.

INTRODUCTION

Microbial Fuel Cell (MFC) is a device that can transform chemical energy into electrical energy using microorganisms as biocatalysts [1]. MFC is a bioelectrochemical system that can be used in the waste management industry, both as generators of energy and as biosensors. There are many problems confronting the implementation of MFCs in the waste management market, such as poor contact between microorganisms and electrodes, as well as the viability of the use of microorganisms themselves as biocatalysts in the destruction of waste into electrical energy [2]. All of these aspects make the transfer of electron run slower so that electricity output is interrupted. Many waste micro-organisms will impact the viability of the use of MFC in the sewage processing system.

Electron transfer is recorded to take place between the microorganism cell and the surface of the electrode, where the conductivity of the electrodes and the presence of a monolayer biofilm play a key role in this process [3]. In general, the transfer of electrons between microorganisms and electrodes takes place in two ways: Direct Electron Transfer (DET) and Mediated Electrons Transfer (MET) [4]. In DET, the conductivity of the biofilm produced is correlated with the presence of pili in each microorganism where the pili is connected directly to the electrode surface such that the electrons can pass directly from the microorganism cell to the electrode surface [5]. In MET, an internal or external mediator is required for the electron transport process, where this mediator is experiencing a redox reaction [6].

Microorganisms are immobilized in a suitable matrix to solve the problems and respond to the challenges above [7]. Polymers, for instance, would be a clear answer. Microorganisms in the biopolymer matrix may be immobilized and have multiple advantages, including an attempt to regulate the growth of microorganisms and stop microorganisms from escaping the environment while they are captured in the matrix. The immobilization of bacteria in biopolymer is likely to lead to an increasing electron transfer in a three-dimensional structural environment [8].

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Agar is a biopolymer such as jelly, and is synthesized by many red algae as the major components of their cell walls [9]. Agar is a combination of gel-forming polysaccharides composed of D and L-galactose, consisting of many elements, including agarose, agaroids, agarans and agaropectin [10]. Agarose is a neutral polysaccharide with a linear structure composed of D-galactose units and 3,6-anhydrous-L-galactose units bound by β-1,3-and 5-007-1,4-glycosidic bonds, respectively [11]. Commercial use is made of agar in the cosmetics, dairy, medicinal, pharmaceutical and biotechnology sectors [12-15].

A number of researchers have previously carried out numerous experiments on the immobility of microorganisms in polymers. In order to improve the efficacy of electron transfer, Shewanella oneidensis MR-1 has been encapsulated into redox polymer redox hydrogels by Lin et al. [16]. While Ghach et al. encapsulated Pseudomonas fluorescens as an electron mediator to facilitate electron transport in sol gel containing the bovine heart cytochrome c [17]. Eun et al. studied Escherichia coli embodiments for cell analysis and insulation in agarose microparticles and not as MFC biocatalysts [18]. While Hadiyanto et al., tried to immobilized yeast in chitosan matrix for the microalgae-microbial fuel cells (MMFCs) system [19].

This research focuses on the preparation of commercial agar gel and the encapsulation of *Saccharomyces cerevisiae* yeast in the gel matrix. The agar gel serves not only as a biocompatible cellular matrix for yeast encapsulation, but also as a semi-solid electrolyte medium to facilitate electron transfer between the yeast and the electrode surface. The separation membrane between the anode and the cathode is not introduced to this system, since the MFC follows a membraneless system. The voltage and current density produced by the MFC device with varying agar gel concentrations are analyzed as well as the maximum power density (MPD). Kinetic reactions to glucose in the MFC system are also calculated to validate the rate at which the electron flows or diffuses from the yeast cell to the electrode surface through the agar gel matrix. We assume that this engineered agar gel represents important and substantial advancement in the production of cell-based MFC devices.

METHODOLOGY

Preparation of Encapsulated Yeast on Agar Gel

Various weights (2.73, 4.10, and 5.46 g) of commercial agar powder (PT. Dunia Bintang Walet, Jakarta, Indonesia) were applied to 300 mL of distilled water along with a YPD medium consisting of 4.9 g of D-glucose (Merck, Darmstadt, Germany), 1.75 g of yeast extract (Merck, Darmstadt, Germany) and 0.875 g of peptone (Himedia, Mumbai, India). The mixed solution was softly mixed, followed by heating at a temperature of 100 °C in less than 3 minutes, so that all components become dissolved. Separately, 4.9 g of commercial yeast *S. Cerevisiae* (Lessafre, Marcq-en-Baroeul, France) was introduced to 50 mL of warm distilled water to enable the enzymes within the yeast and then gently stirred. The mixed agar solution + commercial sugar was cooled at room temperature for around 10 minutes, and then the yeast solution was added. The mixture was then stirring for 3 minutes, placed in a mold with a size of 11 x 11 cm and allowed to stand at room temperature to undergo a phase of gelatinization. Agar gel with a composition without commercial sugar and without yeast was also used as a comparison.

Microbial Fuel Cell Configuration

In addition, the commercial copper plate (size 10 x 9 cm) bonded together with the commercial carbon plate (size 9 x 9 cm) acted as the current collector and anode, respectively. The anode and the current collector were then placed into the middle of the agar gel that was produced, so that the anode was not directly exposed to oxygen from the ambient air. Later, the carbon plate cathode was mounted on the top surface of the agar gel, where the underside of the cathode faces the agar gel, and the upper side of the cathode was directly in touch with ambient air. The membrane as a separator was not used in this MFC system. The configuration of the MFC system is seen in Figure 1.

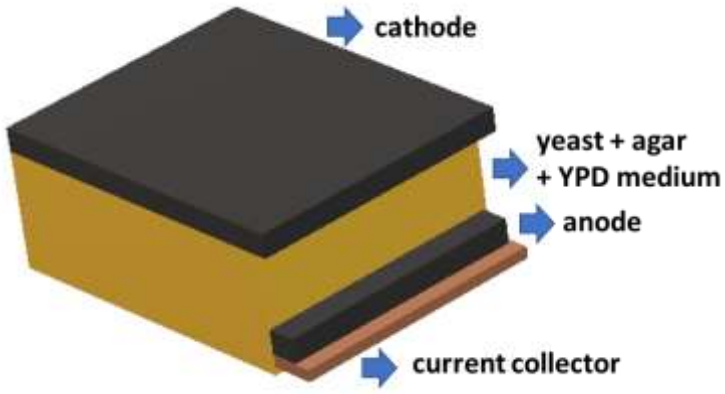

FIGURE 1. Schematic of immobilized yeast MFC configuration

Electrochemical Characterization

The voltage was measured using a KRISBOW KW06-229 multimeter (Jakarta, Indonesia) with a closed-circuit voltage of 1000 Ω as an external resistance [20,21]. Current (I) was obtained by dividing the voltage with added external resistance, while the current density (J) was determined by dividing the current obtained by the total area of the carbon plate anode (81 cm^2), while the power density (P) was calculated by multiplying the potential and current density. The data was obtained every hour and observed for 30 hours. Maximum power density (MPD) was obtained by calculating the potential and current density under various external resistances from 1 MΩ to 10 Ω, produced by polarization and power curves [22]. Each measurement took 30 minutes until the potential was stable.

pH Characterization

During the incubation process, the pH of the yeast agar gel was determined every 6 hours. The agar gel, with or without yeast and YPD medium, was slightly cut and weighed approximately 10 gr, then diluted with 100 mL of distilled water for 5 min. Lutron WA-2015 commercial pH meter (Taipei, Taiwan) was used to measure the pH of the gel.

RESULTS AND DISCUSSION

Encapsulated Yeast

Yeast is spontaneously encapsulated in a three-dimensional gel matrix after a warm agar solution comprising the YPD medium has been combined with a yeast solution. The formation of yeast-containing gel occurs under warm conditions at room temperature, thereby providing excellent conditions for the process of dispersed yeast encapsulation, as seen in Figure 2. The distribution of yeast on agar gel can increase the potential for electron transfer, as yeast can optimally consume the substrate at a certain point without competition. It is noted that, in this case, agar also acts as a semi-solid electrolyte with several functions, including (i) delivering protons formed by the yeast to the surface of the cathode where there will be an oxygen-reduction (ORR) process, and (ii) delivering electrons from the surface of the yeast cell to the surface of the anode (and also the current collector), then move to the cathode via the external circuit.

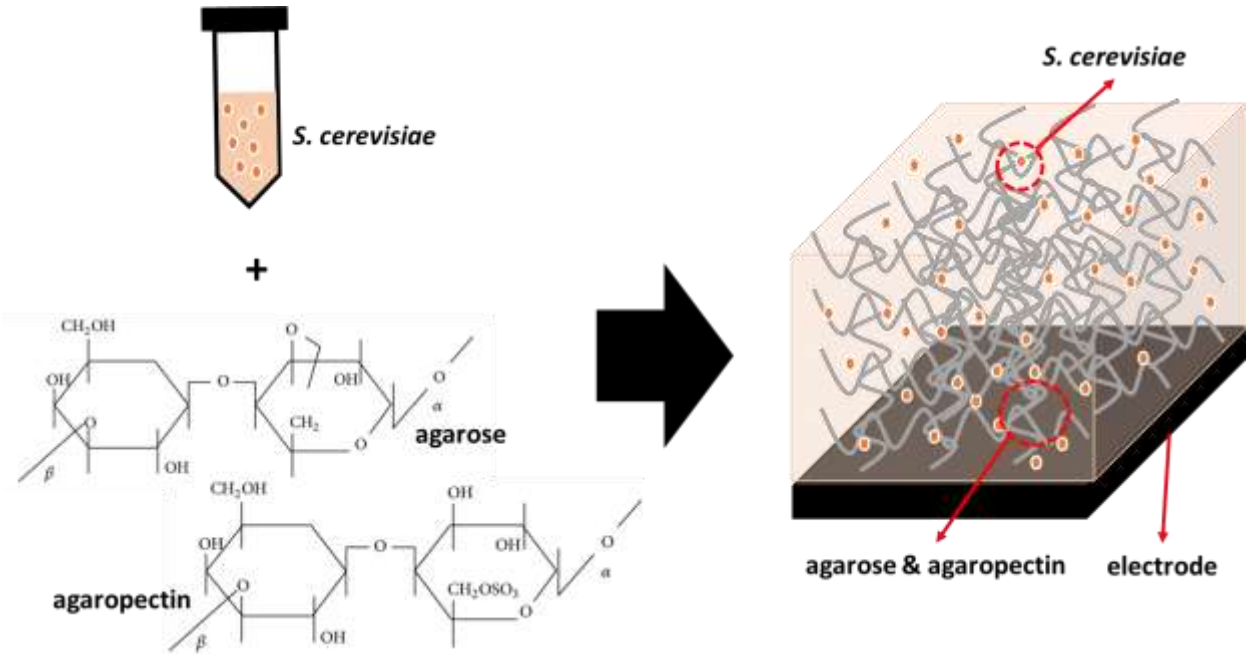

FIGURE 2. Schematic representation of agar formation mechanism and design of agar/yeast hybrid anode catalyst structure

The role of YPD Medium and Yeast in Electricity Production

It is important to confirm and explain the role of yeast as a biocatalyst and the role of the YPD medium as a substrate and growing agent associated with the production of electricity in the MFC system. Figure 3 displays the current density obtained from the MFC system, which adopted various components (yeast, YPD medium and yeast + YPD medium) on agar gel as a semi-solid electrolyte. Three points can be explained in Figure 3. First, there was no rise in current density for 30 hours in the MFC system which adopted only the YPD medium, with an average current density of 0.142 mA/m^2. A slight uptick in current density is due to the activity of the ion in the YPD medium portion, which creates a potential difference in anode and cathode, not due to the activity of the microorganism because the yeast was not seen here. Second, the current density of MFCs which adopted only yeast, decreased in the first 2 hours, followed by an increase to its highest at the 18th hour with a current density of 2.53 mA/m^2. The decrease in current density at the beginning of the incubation is due to the process of adaptation of the yeast in a new environment, namely the agar polymer gel matrix. While the raise in current density is associated with the logarithmic phase of the growth of microorganism cells, it has begun to consume substrate, which results in the output of protons and electrons as a result of metabolism [23]. In this case, the substrate absorbed by the yeast was actually agar gel, of which agar is a polysaccharide made up of glucose-like monomers. The method of consuming agar by yeast takes place slowly, provided that yeast no longer has the ability to cut the polymer chain into its monomers, so there was no noticeable improvement in current density. Decreased current density happens steadily after 18 hours of incubation, which is due to yeast cell death because it cannot absorb enough substrate. Third, the MFC that adopted yeast and the YPD medium had a similar pattern to the previous example, but some factors set it apart. Current density decreased in the first hour, which refers to the mechanism of adaptation of yeast in a new environment. Subsequently, there was a substantial rise which hit its height at the 24th hour (6.91 mA/m2), which was longer than the prior scenario (without YPD medium). This is consistent with the logarithmic phase in which the yeast absorbed glucose as a substrate, as well as the yeast extract and peptone as a growing agent. Yeast cells grew more and more and used glucose to convert to protons, electrons, CO_2, H_2O, and a little bit of acetic acid and lactic acid due to metabolism [24]. The decline in current density is attributed to a lack of agar gel substrate such that the metabolism of yeast cells has been interrupted and death has occurred.

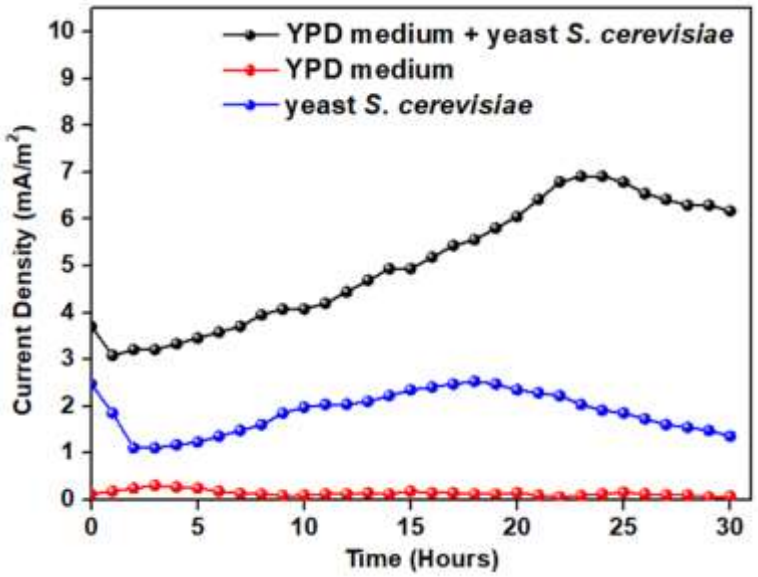

FIGURE 3. Voltage of yeast MFC adopting YPD medium

The effect of agar concentration as a trapping agent and as a semi-solid electrolyte should be further studied. The concentration of agar definitely influences the ability of the yeast cells to consume substrate, which is also dissolved in the gel matrix of agar, as well as the movement of electrons from the surface of the yeast cell to the surface of the anode and the transfer of protons from the surface of the yeast cell to the surface of the cathode. Figure 5a shows the pattern in current density production during MFC incubation with different concentrations of agar gel. Two things can be represented in Figure 4a. First, the current density decreased in the first hour in all variables at which period the yeast was in the lag phase/adaptation time. Second, the acceleration in current density was faster when the gel concentration was low (7.8 g/mL) and the current density reached a limit of 15 hours of incubation time. This time was quicker compared to MFC which used gelatin at concentrations of 11.7 and 15.6 mg/mL. At a gel concentration of 11.7 mg/mL, the current density reached its maximum within 23 hours, while at a concentration of 15.6 mg/mL, the current density had not yet reached its maximum as the incubation time limit was 30 hours. After 30 hours, the current density may increase or decrease due to disruption of yeast metabolism caused by too high a gelatin gel polymer matrix. The smaller the concentration of agar, the more accessible the yeast cells to absorb the gel-dissolved substrate, so that the current density has risen considerably. However, as a result, the current density will also drop dramatically after reaching its absolute limit, which is caused by the depletion of the substrate as a food supply for the yeast cells [25]. On the other hand, the yeast cells have trouble absorbing substrates when the concentration of gel is higher. The high agar content directly encroached on the polymer matrix by way of the substrate molecules making it impossible to disperse. The consumption of substrate is difficult, but the consumption rhythm of yeast cells may retain.

The maximum power density (MPD) of MFC after the incubation time (30 hours) is shown in Figure 4b. Figure 4b indicates that the MPD generated by MFC with agar concentrations of 7.8, 11.7, 15.6 mg/mL are 0.19, 0.91 and 0.52 mW/m^2, respectively. The trend observed at MPD is identical to the pattern seen in Figure 5a at the 30th hour. The outcomes of the MPD were relatively lower compared to the received sources. There are a number of reasons why the MPDs were poor. Second, the MFC system did not use a separating membrane, so the potential for mixed potential is very high. Third, the type of electrode used was only a carbon plate without modification, as opposed to some of the references listed above which manipulated the electrodes, so that they have a higher conductivity to enhance the rate of proton or electron transfer. Fourth, as the solvent used to dissolve agar, YPD medium, and yeast had purified water, the conductivity of the semi-solid electrolytes formed was shallow. This causes the rate of diffuse proton and electron transfer to the cathode and the anode, respectively, to be sluggish.

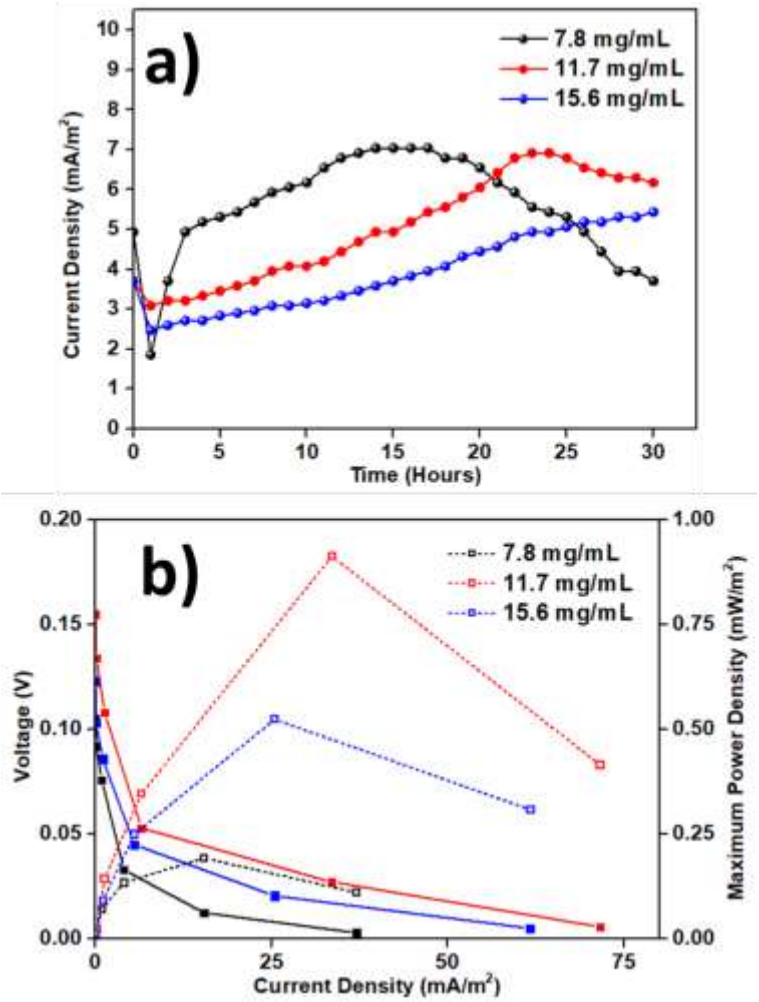

FIGURE 4. a) Voltage of yeast MFC with various agar concentration and b) their polarization and power curves

The Sensitivity of Encapsulated Yeast on Agar Gel to Glucose

The apparent Michaelis–Menten constant (K_m), the maximum current density (J_{max}) and the sensitivity of the catalysts were evaluated to investigate the catalytic activity of the yeast trapped agar catalysts in the MFC system [23]. For this reason, chronoamperometric (CA) measurements were performed under closed-circuit voltage with an applied external resistance of 1000 Ω and concurrent glucose addition of 0 to 28 mg/mL under aerobic conditions (Fig. 5a and 5b). From these figures, as glucose was steadily added, the current density of all catalysts increased. The result was 9.15 ± 1.01 mg/mL when it came to K_m. This involves the absorption of glucose by the yeast cells which is consumed in the agar gel matrix and created more protons and electrons. The low value of K_m demonstrates reactivity to the reactions needed. Sensitivity and J_{max} were 0,39 ± 0,03 (mA/m^2)/(mg/mL) and 3,53 ± 0,16 mA/m^2, respectively, for yeast-entrapped agar gel in the MFC systems.

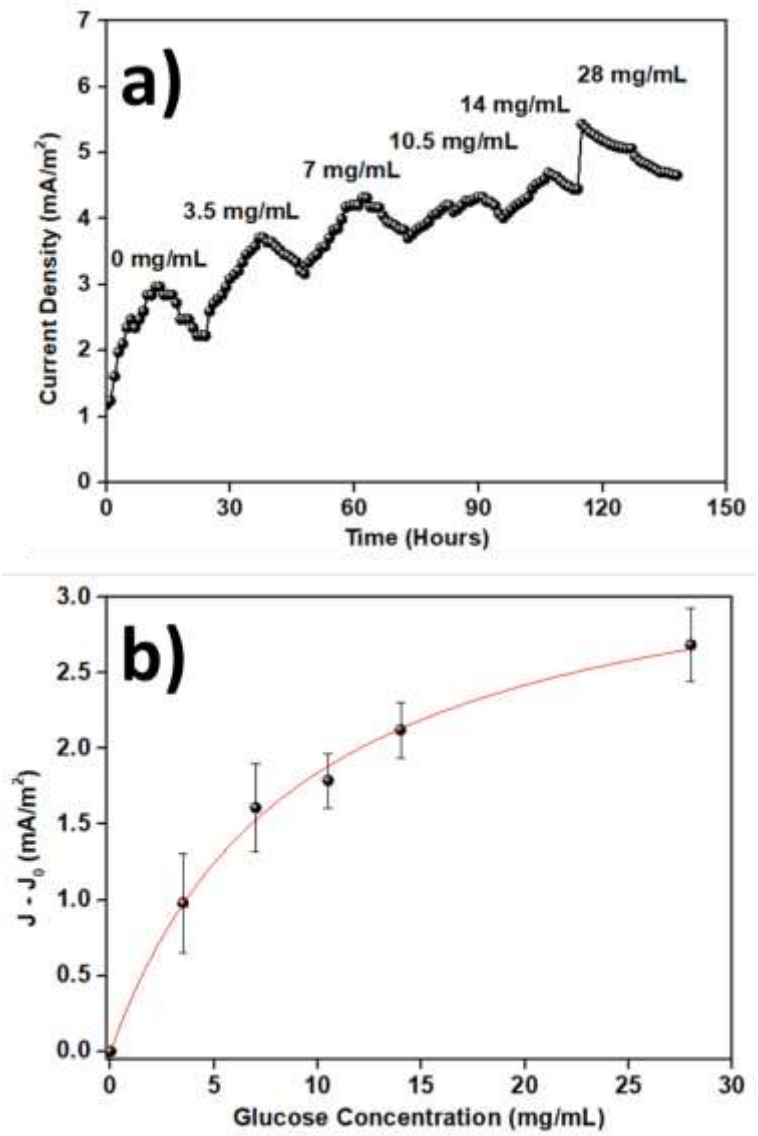

FIGURE 5. a) Current density of yeast MFC with various glucose concentration and b) its Michaelis-Menten plot

The Trend of pH Changes in Agar Gel Containing Yeast During the MFC Incubation Process

The pH-change pattern is very interesting to examine during MFC incubation because it is related to the potential in semi-solid electrolytes. Figure 6 indicates a drop in the pH of the yeast gel between 6.6 and 6.1. The pH of agar gel did not decrease and its value was around 6,7 in the absence of yeast. This shows the presence of acetic acid that is a result of the yeast *S. cerevisiae* metabolism after the ingestion of the substrate. In the gel matrix of gelatin, this drop in pH also is correlated to accumulation of protons (H^+), in which case protons must combat the gravitational power in order to reach the cathode surface. This condition makes for a mixed potential where the anode's potential moves more in a positive way, and the cathode's potential moves in a more negative direction, thereby ensuring that the two meet and create a small net current or closed to zero.

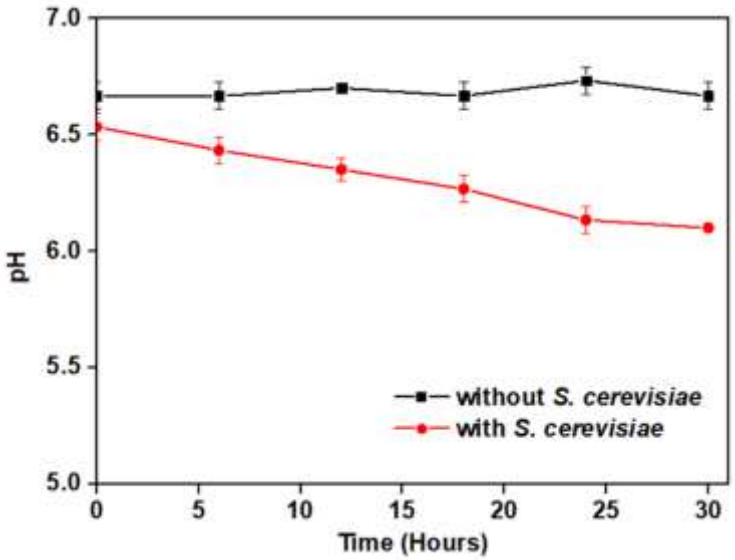

FIGURE 6. pH profile during MFC incubation

Future Prospect of Immobilized Yeast MFC

MFCs have a high potential for use in the wastewater industry. Currently, microbial anodes are critical in electro-microbial processes, prompting many researchers to focus on developing effective anode surfaces rather than cathode components [26]. The yeast in the anode chamber will move, attach to the anode surface, and build biofilm before allowing electron transfer with high energy efficiency. Many strategies are therefore being improved to get the best results possible [27-29], particularly on anode components that are connected with the response of the electron transfer via immobilization of microbial or microorganisms or the use of certain polymers [7]. Both, however, have experienced repercussions. Immobilization of particular microorganisms, which will be anticipated to form a thin polymer layer on the anode for the purpose of trapping of microbes, results in higher electron transfer and energy efficiency than without alteration on the anode surface. Furthermore, the sustainability is significantly superior than that of non-immobilized microorganisms. Encapsulating a specific polymer on the anode chamber, on the other hand, leads in much worse electron transmission and energy efficiency. In the previous four years, there has been significant progress in the field of wastewater MFC, but more research is required before this technology can be successfully used in real-world applications. It should be mentioned that the bulk of research in the MFC sector are being undertaken at the laboratory scale, where controlled conditions are used to assure optimal equipment performance [30]. There is an urgent need for field studies to be conducted before they may be applied in real-world industries. In future study, materials and processing techniques will be critical in MFC development to enable industrial-scale application in terms of scalable production, cost, and stability [31].

CONCLUSION

The design and production of redox agar/microbial biomaterials as yeast MFC anodes are demonstrated in this paper. The agar hydrogel not only serves as a matrix for microbial encapsulation, but it also improves the efficiency of electron transmission. To validate the agar hydrogel's performance, we employed the yeast Saccharomyces cerevisiae as the microbe. The quantity of current and power density produced increased by yeast S. cerevisiae cells enclosed in 11.7 mg/mL agar. Furthermore, the agar polymer creates a friendly and cozy habitat for microorganisms to grow in. We conclude that this new technology has a tremendous deal of promise for use in the production of numerous sorts of devices.

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REFERENCES

1. M. N. Naseer, A. A. Zaidi, H. Khan, S. Kumar, M. T. bin Owais, J. Jaafar, ... & M. Uzair, Energy Reports **7**, 4126-4138 (2021).
2. M. Rahimnejad, A. Adhami, S. Darvari, A. Zirepour, & S. E. Oh, Alexandria Engineering Journal **54**, 745-756 (2015).
3. S. M. Strycharz-Glaven, R. M. Snider, A. Guiseppi-Elie, & L. M. Tender, Energy & Environmental Science **4**, 4366-4379 (2011).
4. U. Schröder, Physical Chemistry Chemical Physics **9**, 2619-2629 (2007).
5. D. R. Lovley, Current Opinion in Electrochemistry **4**, 190-198 (2017).
6. L. Tao, H. Wang, M. Xie, L. Thia, W. N. Chen, & X. Wang, Chemical communications **51**, 12170-12173 (2015).
7. R. C. Wagner, S. Porter-Gill, & B. E. Logan, AMB express **2**, 1-6 (2012).
8. X. Lin, K. Nishio, T. Konno, & K. Ishihara, Biomaterials **33**, 8221-8227 (2012).
9. W. K. Lee, Y. Y. Lim, A. T. C. Leow, P. Namasivayam, J. O. Abdullah, & C. L. Ho, Carbohydrate polymers **164**, 23-30 (2017).
10. Y. Zhang, X. Fu, D. Duan, J. Xu, & X. Gao, J. Oceanol. Limnol **37**, 815-824 (2019).
11. P. Zucca, R., Fernandez-Lafuente, & E. Sanjust, Molecules **21**, 1577 (2016).
12. F. S. Mostafavi, & D. Zaeim, International journal of biological macromolecules **159**, 1165-1176 (2020).
13. T. Morais, J. Cotas, D. Pacheco, & L. Pereira, Cosmetics **8**, 8 (2021).
14. E. S. de Assis, A. R. Neto, F. G. Silva, L. R. de Lima, P. Dornelles, S. C. V. Filho, ... & E. da Costa Severiano, Australian Journal of Crop Science **13**, 80-87 (2019).
15. S. H. Park, C. R. Lee, & S. K. Hong, Applied microbiology and biotechnology **104**, 2815-2832 (2020).
16. X. Lin, K. Nishio, R. Nakamura, T. Konno, K. Hashimoto, & K. Ishihara, Transactions of the Materials Research Society of Japan **37**, 529-532 (2012).
17. W. Ghach, M. Etienne, V. Urbanova, F. P. Jorand, & A. Walcarius, Electrochemistry communications **38**, 71-74 (2014).
18. Y. J. Eun, A. S. Utada, M. F. Copeland, S. Takeuchi, & D. B. Weibel, ACS chemical biology **6**, 260-266 (2011).
19. H. Hadiyanto, M. Christwardana, W. Z. Pratiwi, P. Purwanto, S., Sudarno, K., Haryani, & A. T. Hoang, Chemosphere **287**. (2021).
20. M. Christwardana, A. S. Handayani, R. Yudianti, & J. Joelianingsih, International Journal of Hydrogen Energy **46**, 6076-6086 (2021).
21. H. Hadiyanto, M. Christwardana, T. Minasheila, & Y. H. Wijaya, International Energy Journal **20**. (2020).
22. M. Christwardana, J. Joelianingsih, & L. A. Yoshi, Bulletin of Chemical Reaction Engineering & Catalysis **16**, 446-458. (2021).
23. M. Christwardana, L. A. Yoshi, & J. Joelianingsih, Reaktor **21**, 52-58. (2021).
24. L. Wang & V. Hatzimanikatis, Metabolic engineering **8**, 142-159. (2006).
25. M. Hampsey, Yeast **13**, 1099-1133. (1997).
26. X. B. Gong, S. J. You, X. H. Wang, J. N. Zhang, Y. Gan, & N. Q. Ren, Biosensors and Bioelectronics **55**, 237-241. (2014).
27. B. Erable, N. Byrne, L. Etcheverry, W. Achouak, & A. Bergel, International Journal of Hydrogen Energy **42**, 26059-26067. (2017).
28. D. Majumder, J. P. Maity, C. Y. Chen, C. C. Chen, T. C. Yang, Y. F. Chang, ... & H. R. Chen, International journal of hydrogen energy **39**, 21215-21222. (2014).
29. H. Zhang, D. Zhu, T. S. Song, P., Ouyang, & J. Xie, International Journal of Hydrogen Energy **40**, 16566-16571. (2015).
30. M. Grattieri & S. D. Minteer, Bioelectrochemistry **120**, 127-137. (2018).

31. B. Erable, R. Lacroix, L. Etcheverry, D. Féron, M. L. Délia, & A. Bergel, Bioresource Technology **142**, 510-516. (2013).