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Procedia Chemistry 17 (2015) 36 – 43

Procedia Chemistry

3rd International Seminar on Chemistry 2014

Preliminary Investigation of Electricity Production Using Dual Chamber Microbial Fuel Cell (DCMFC) with *Saccharomyces cerevisiae* as Biocatalyst and Methylene Blue as an Electron Mediator

Dani Permana[a,c,*], Desi Rosdianti[b], Safri Ishmayana[b], Saadah D. Rachman[b], Herlian Eriska Putra[a,d], Diana Rahayuningwulan[a], Hari Rom Hariyadi[a]

[a]*Research Center for Chemistry, Indonesian Institute of Sciences (LIPI), Kampus LIPI Bandung, Jl. Cisitu – Sangkuriang, Bandung 40135*
[b]*Department of Chemistry, Faculty of Mathematic and Natural Sciences, Universitas Padjadjaran, Jln. Raya Bandung-Sumedang km. 21 Jatinangor 45363, Indonesia*
[c]*M.Sc Student of School of Life Sciences and Technology, Bandung Institute of Technology, Jl.Ganesa 10 Bandung 40132, Indonesia*
[d]*Ph.D Student of Department of Environmental Engineering, Faculty of Civil and Environmental Engineering, Bandung Institute of Technology, Jl. Ganesa No.10, Bandung 40132*

Abstracts

Microbial fuel cell (MFC) is one of promising fuel cell technologies. MFC utilizes biochemical activity of microorganism to convert the substrate to produce electricity through metabolism processes. Although the generation of the electricity is still in mW scale, MFCs has a great potential for the future application. The aim of this study was to investigate the performance of MFCs with and without the methylene blue (MB) as an electron mediator utilizing *Saccharomyces cerevisiae* as biocatalysts and glucose as substrate to generate electricity. Methods performed comprise of the *S. cerevisiae* yeast culture rejuvenation, preparation of the inoculum, preparation of the MFC reactor, preparation of MFC medium with 2% of glucose with and without MB mediator, periodical sampling, determination of growth curve, measurement of current, potential, calculation of power density, energy, glucose consumption, and production of bioethanol. MFC with mediator generated 5.5×10^{-5}A of current, 0.886 V of potential, 4.48×10^{-3} W/m^2 of power density, 4.14×10^{-3} J of energy, 95.0% of glucose consumption and 0.74% (v/v) of bioethanol produced during the MFC process, while MFC without mediator generated 4.5×10^{-5} A of current, 0.689 V of potential, 2.12×10^{-3} W/m^2 of power density, 1.96×10^{-3} J of maximum energy, 96.3% of glucose consumption and 0.74 % (v/v) of bioethanol produced. Power density yields from both type of MFC are still very low and not differ significantly. From the present study, it can be concluded that MB mediator only effected on potential yield in MFC using the condition applied in this study.

Keywords: Dual chamber microbial fuel cell; electricity; methylene blue; *Saccharomyces cerevisiae*

1. Introduction

Microbial fuel cell (MFC) is a bioelectrochemical cell which utilizes electrogenic bacteria to oxidize a variety of substrates including acetate[1], glucose[2], volatile fatty acids[3]. and inorganic substances such as sulfides[4] and nitrite[5], to form electrical current. Through the oxidation process electrons and protons are generated at anode and recombined at the cathode to produce water[6,7].

*Corresponding Author. Tel : +622-22503051 ; Fax :+622-22503240
E-mail address: dani017@lipi.go.id; dani.permana@pnsmail.go.id

Peer-review under responsibility of Department of Chemistry, Faculty of Mathematics and Natural Sciences, Padjadjaran University
doi:10.1016/j.proche.2015.12.123

Generally, MFC consists of two compartments: an anaerobic anode and aerated cathode compartments which are separated by a proton exchange membrane or salt bridge[8]. Membrane or salt bridge allows protons generated in anode chamber to be transferred to cathode chamber[9]. Other parameters including internal resistances, anode and cathode materials, cell configurations and surface area are affecting the performances of MFC[9].

Microorganisms are used in MFC to convert organic and inorganic compounds into bioelectricity[10.11]. Pure or mixed culture of microorganisms can be used as biocatalyst in anaerobic anode chamber[12]. Active biocatalysts in anode chamber of the MFC produced electrons and protons via anaerobic respiration[13]. The produced electrons travel through an external circuit connected to the cathode surface. The electrons interact with protons which have passed through the ion-exchange membrane on cathode and then react with dissolved oxygen, as a result water molecules are formed[6,9,14]. MFC research has seen a spike in interest since 2002[15,16,17,18,19]. MFC are in turn divided into two types, a fuel cell depending on a mediator to transfer electrons and a fuel cell with direct electron transfer[20]. Electron transfer efficiencies in MFC can be improved using a suitable electron mediator. Most biological fuel cells use electron mediator component to improve the power output of the cell. It has been reported that mediators such as methylene blue (MB), neutral red (NR), thionin, ferricyanide, humic acid or methyl viologenare artificially added to anode chamber. The presence of artificial electron mediators is essential to improve the performance of MFC[9,21,22]. The yeast *Saccharomyces cerevisiae* requires mediators, such as MB, NR, and thionine, to transfer electrons[23], while other microorganism such as *Shewanella oneidensis* and *Geobacte rsulfurreducens* can generate electricity without mediator in a MFC system.

S. cerevisiae can grow either at aerobic or anaerobic condition. It is easily accessible and the metabolism pathway is well understood. The growth of *S. cerevisiae* is optimum at the ambient temperature which is around 30^{o}C. These factors prompted us to choose *S. cerevisiae* for this study.

The main objective of the present study was to evaluate of methylene blue as electron mediator to the performance of Dual Chamber MFCs with *S. cerevisiae* as biocatalysts.

2. Materials and Methods

2.1. Maintenance of pure culture of S. cerevisiae

S. cerevisiae grown on agar slopes of sterile yeast extract peptone dextrose (YEPD) medium containing (% w/v) 0.5 yeast extract (Becto and Dickinson), 0.5 bacteriological peptone (Becto and Dickinson), 0.3 ammonium sulfate (Merck), 0.3 potassium dihydrogen phosphate (Merck), 2 glucose (Sigma-Aldrich), and 1.5 agar (Becto and Dickinson). The agar slopes was streaked with *S. cerevisiae*and incubated at 37°C for 48 hours (Mettler Toledo). Slopes were stored at 4°C and sub-cultured every 6 months.

2.2. Construction of the fuel cell

The fuel cell chamber was constructed from glass with internal diameter of 10 cm. The volume of each chamber is 1000 mL. The two chambers were separated by a Sulphonated Poliether Ether Ketone (SPEEK)[24,25]. as Proton Exchange Membrane (PEM) where the membrane was held by a coupling between the chambers. Each experiment was performed with a new membrane to avoid any interferences and/or contaminations from previous experiments. The electrodes used were Copper electrode (Cu) with 0.008 m^2 of surface area (0.1 m of length and 0.04 m of width, 2 side each). Anode connected with negative pole and cathode with positive pole of Avometer (Sanwa CD800). MB, as electron mediator, was added, to give final concentration of 5 mM in the anolyte solution[26]. While a 200 ppm potassium permanganate solution was used as catholyte and electron acceptors, with pH adjusted to3.6 to 3.8 [27]. Figure 1 showed the reactor of MFCs used in the present study.

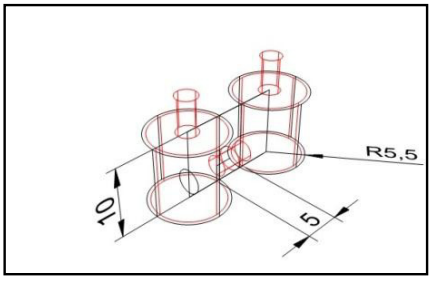

Fig. 1. Design of Dual Chamber MFCs

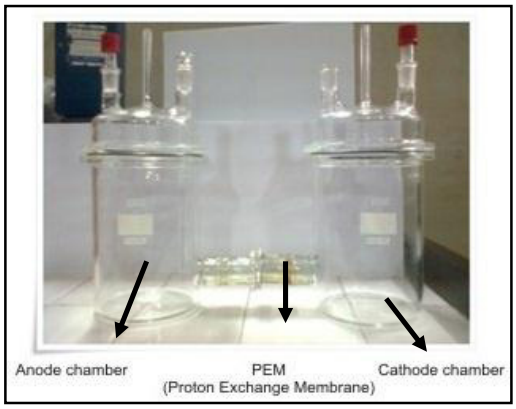

Fig. 2. Reactor of Dual chamber MFCs

2.3. Growth medium and culture conditions

YEPD media without agar, as described earlier, was used as the growth media. The media was autoclaved at 121°C and 15 psi for 15 minutes (Hirayama HL36 AE, Japan)[3].

Starter culture was prepared by inoculating a 25 mL YEPD media from slopes and grown overnight (~18 hours) at 30°C and 150 rpm in a shaking incubator (Certomat B Braun). The ratio of Erlenmeyer size to the volume of the culture volume was maintained at 4:1 to maintain the availability of dissolved oxygen. The entire starter culture was transferred to the MFCs medium and therefore the amount of starter culture added to the MFC medium was 2.5% of the total media (1000 mL).

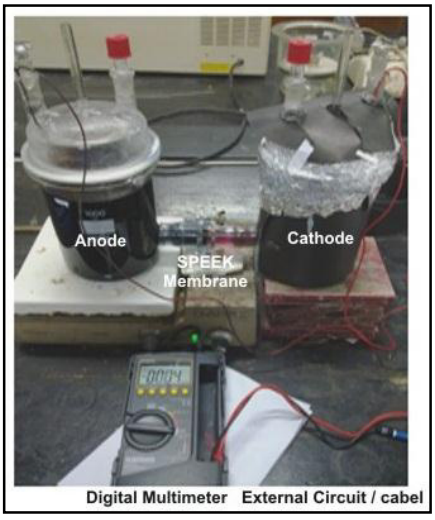

Fig. 3. DCMFC reactor used in this research based on design suggested by You *et. al* (2006). The volume of each chamber is 1000 mL. Anode filled with anolyte that consists of YEPD, MB, and *S. cerevisiae*. Cathode filled with potassium permanganate as catholyte.

2.4. Operation of MFCs and Sampling Conditions

Fermentation carried out for 48 hours. Anolyte solution sample at 4 hours interval were taken aseptically using sterile micropipette (Eppendorf). Examination of samples included measuring growth rate by optical density (OD) at 600 nm (JENWAY6305), electrical current and potential (Sanwa CD800A digital multimeter), and acidity level (Mettler Toledo MP220 pH meter). For OD measurement, centrifuged anolyte solution was used as blank.

2.5. *Power density and energy*

The power generated is calculated using the electrical current and potential measured by a multimeter and calculated using equation (1), the power density is calculated using equation (2), while the energy produced is calculated by equation (3)[4].

$$P = V \times I \quad \textbf{(1)}$$

$$Pd = {}^{P}\!/_{A} \quad \textbf{(2)}$$

$$E = P \times t \quad \textbf{(3)}$$

With:

P = power (W)
V = potential (V)
I = current (A)
Pd = power density (W/m^2)
E = energy (J)
t = fermentation time (seconds)
A = surface area of the anode(m^2)

2.6. *Analysis of glucose and bioethanol concentration*

Concentration of glucose was determined using alkaline potassium ferricyanide method using UV/Vis spectrophotometer at 420 nm[28]. While ethanol produced was determined using enzymatic spectrophotometric micro-method at 340 nm[3].

3. Results and Discussions

3.1. *Growth of S. cerevisiae*

Growth curve of *S. cerevisiae* was determined and the result is presented in Figure 4. Growth curve can be used to determine whether MB present in the media affecting the growth of the cell. It was observed that both yeast grown in media with or without mediator had their lag phase from 0 to 4 hours, followed by exponential phase from 4 to 12 hours. After that the growth curve relatively flattened, especially for yeast grown in media without MB. As for cell grown in media with MB, the growth curves still slightly increase, but not as high as the exponential phase. This result indicate that MB present in the media may affecting the cell growth as shown by the slight differences of the OD value at the last 24 hours of the experiment, even though the effect is not too big.

3.2. *Glucose consumption and ethanol production*

Initial glucose concentration used in this study was 2% (w/v). We used this concentration based on previously published study which used the same glucose concentration in their study[24]. At this concentration, glucose runs out in about two days. Therefore, the experiment was conducted for 48 hours and the residue of glucose was measured during the 48 hours experiment at every four hours interval.

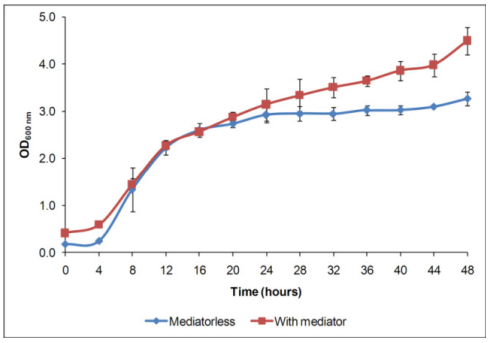

Fig. 4. Growth curve of *S. cerevisiae* with and without mediator added to the medium. Data presented are means of two independent experiments and error bars indicate standard error of means (SEM).

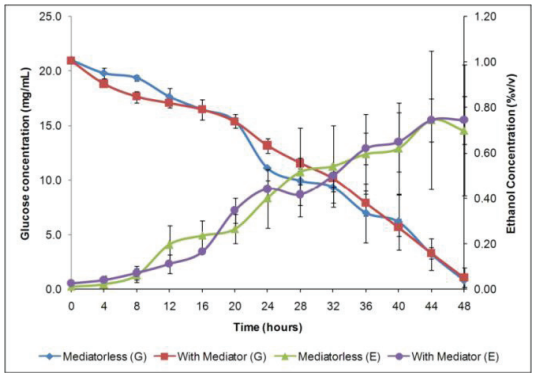

Fig. 5. Glucose consumption (G) and ethanol production (E) in MFC without and without MB as mediator. Data presented are means of two independent experiments and error bars indicate SEM.

The results of the present study showed that glucose concentration was decreased during 48 hours of experiment. Glucose was used by *S. cerevisiae* as carbon source for growth and formation of ethanol with electrons produced as another output for electricity generation. This result also confirmed that 2% (w/v) glucose concentration was sufficient for *S. cerevisiae*-mediated MFC. The glucose consumed by the cells was 96.3 ± 4.2% and 95.0 ± 6% for cell grown without and with MB, respectively. This indicate that MB present in the media did not affecting the ability of the cell to utilize glucose as their carbon source.

In this study, *S. cerevisiae* produced ethanol as the primary product. *S. cerevisiae* is one of yeast that known have high substrate conversion. Therefore we choose this yeast for our study. No substantial differences were observed in ethanol production for MB mediator and mediator less MFC. This finding indicate that MB, like glucose consumption, also does not affecting the ability of the yeast to produce ethanol. Figure 5 showed the glucose consumption and ethanol production during MFC.

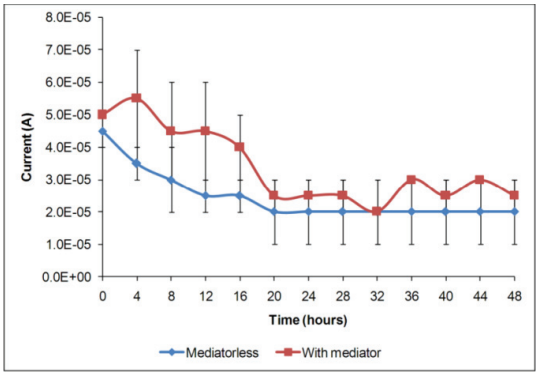

Fig. 6. Profile of current of MFCs with *S. cerevisiae* as biocatalysts source. Data presented are means of two independent experiments and error bars indicate SEM.

3.3. Current profile of S. cerevisiae mediated-MFCs

In the present study, glucose was used as substrate for the MFC. *S. cerevisiae* as biocatalysts in the MFCs used glucose as substrate and carbon source in the anode chamber and generate electrons and protons (H^+). Total of 24 mol electrons and hydrogen ions were generated by oxidation of one mole of glucose in the anaerobic condition. The anodic reaction is taken place at the anode as summarized in equation (4).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad \textbf{(4)}$$

The electron migrates to anode surfaces and protons (H^+) travel from SPEEK Proton Exchange Membrane (PEM) and react with oxygen in cathode. Electron from anode then captured by permanganate ion (MnO_4^-) that play role as electron acceptor. Permanganate ion was used as electron acceptor because it has high oxidization

capacity and also more environmental friendly. Either in acidic and alkaline conditions, permanganate ion accepts three electrons and reduced to manganese dioxide, as shown in equations (5) and (6).

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O, \quad E^\circ = 1.70\ V\ (5)$$

$$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^-, \quad E^\circ = 0.59\ V\ (6)$$

Equations (5) and (6) showed that permanganate ion has higher potential in acidic condition[27]. Therefore in the present study, we used acidic condition for the cathodic electron acceptor expecting to maximize the electric produced by the MFC.

The basic characteristics of yeast-catalyzed mediator and mediator less MFC were examined. The electric current and voltage was measured using digital multimeter. The current profile is presented in Figure 6. There was no strong evidence that MB addition as mediator increase the current produced during the experiment. High variability of the data, as indicated by high error bars in Figure 6, require us to refine and improve the method that we are currently used. The high variability of the data was observed for other parameters recorded in the present study as will be described later.

In the present study, we used 5 mM MB as mediator based on previously published study by Walker and Walker Jr. (2006)[26]. The latest study by Rahimnejad et al. (2011)[23] showed that maximum power and current were obtained when 0.3 mM MB used as mediator and excess MB did not improve power and electrical current. All those study and the present study were not used an exact same MFC construction, and therefore direct comparison of the results becomes problematic. However, it looks like that the concentration of MB used in the present experiment was too high, and therefore future study should be directed to determine the best MB concentration that give the maximum power and current using our system.

3.4. Profile of open circuit voltage (OCV) of S. cerevisiae mediated-MFCs

OCV is one of important parameters of MFC. The results of OCV measurement is presented on Figure 7. Voltage closely related with electrical energy, which is the main product of the MFC system. The maximum OCV value was observed at 36 hours with value of 0.887 and 0.689 V for MFC with mediator and mediator less MFC, respectively.

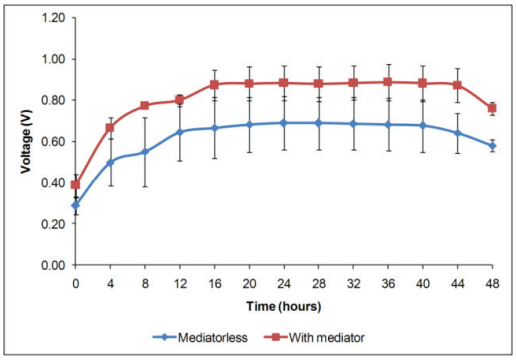

Fig. 7. Profile of OCV measured from *S. cerevisiae* mediated-MFCs. Data presented are means of two independent experiments and error bars indicate SEM.

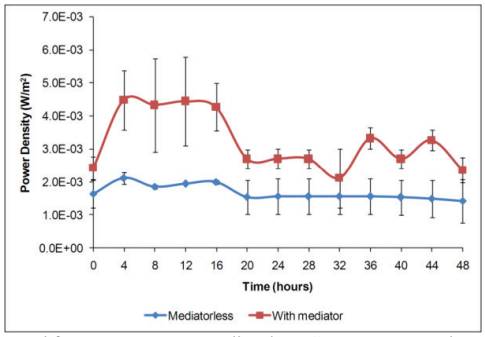

Fig. 8. Profile of power density measured from *S. cerevisiae* mediated-MFCs. Data presented are means of two independent experiments and error bars indicate SEM.

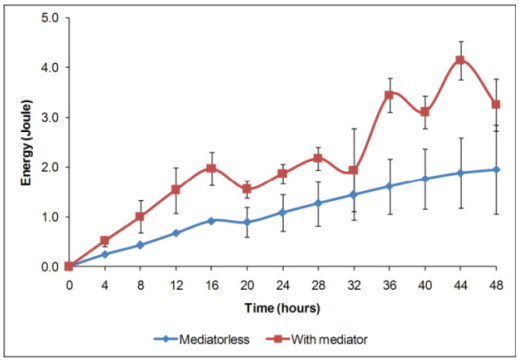

Fig. 9. Profile of energy generated from *S. cerevisiae* mediated-MFCs. Data presented are means of two independent experiments and error bars indicate SEM.

As previously mentioned, high variability also obtained for the OCV data. There was no strong evidence that MFC with MB mediator has higher potential compared to the one without MB. However, there is an indication that MB may improve potential of the cell at the end of experiment by substantially higher voltage value at 44 and 48 hours of experiment. Further investigation is required to confirm this finding.

3.5. Power density and energy

Power density was calculated as ratio of power (calculated from multiplication of the current and potential) to electrode surface area. In this study, copper sheet was used as electrodes with surface area of 0.008 m^2. Power density shows the performance of anode to flow the electrons to cathode. Power density between the two treatments of MFC was found to be not substantially different. The largest power density of MFCs with mediator was 4.48×10^{-3} W/m^2, whereas in mediator less MFCs the maximum power density was 2.12×10^{-3} W/m^2. The value of power density is still relatively small because of small electrical current and big resistance. Figure 8 shown the power density determined in this study.

During the process of metabolism and growth, *S. cerevisiae* released energy through the Gibbs free energy, ΔG_{ox}. MFCs generated energy is determined by the acquisition of power versus time. The energy produced by *S. cerevisiae* in both treatments of MFC increased as the fermentation progressing, but overall energy generated on both the MFC did not differ substantially. The maximum energy achieved by the MFCs with mediators was 4.14J, whereas in MFCs without mediators MB was 1.96J (Figure 9). The energy used to produce the electron was still relatively low because most of the energy is used for cell growth. This is evidenced by Park and Zeikus (2000)[21] which showed that bacterial cells in resting phase can generate more electrons compared to growing bacteria.

Conclusions

Methylene blue used in the present study might affect the cell growth as indicated by substantially higher OD value after 24 hours. However, no strong evidence that MB affecting ethanol production or glucose consumption. As for the electrical properties, there were also no strong evidences that MB with concentration used in the present study improve current or fuel cell potential. There is an indication that MFC with mediator has higher cell potential at the end of experiment, but this result needs further confirmation. The concentration of MB used in the present study maybe too high for our MFC system. Therefore, follow up study by using wider range of MB concentration should be conducted with more replicate to improve the statistical evaluation result.

Acknowledgements

This study was supported by Research Center for Chemistry, Indonesian Institute of Sciences (LIPI) for funding support and Indonesia Endowment Fund for Education (LPDP) for funding my study. Therefore, we would like to acknowledge the support.

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