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The Use of Boron-doped Diamond Electrode on Yeast-based Microbial Fuel Cell for Electricity Production

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Abstract. The dependency of fossil energy in Indonesia caused the crude oil production to be drastically decreased since 2001, while energy consumption increased. In addition, the use of fossil energy can cause several environmental problems. Therefore, we need an alternative environment-friendly energy as solution for these problems. A microbial fuel cell is one of the prospective alternative source of an environment-friendly energy source to be developed. In this study, Boron-doped diamond electrode was used as working electrode and Candida fukayamaensis as biocatalyst in microbial fuel cell. Different pH of anode compartment (pH 6.5-7.5) and mediator concentration (10-100 μM) was used to produce an optimal electricity. MFC was operated for 3 hours. During operation, the current and voltage density was measured with potensiotstat. The maximum power and current density are 425,82 mW/m² and 440 mA/m², respectively, for MFC using pH 7.5 at anode compartment without addition of methylene blue. The addition of redox mediator is lowering the produced electricity because of its anti microbial properties that can kill the microbe.

Keywords : Microbial Fuel Cell, Candida fukayamaensis, Boron-doped Diamond electrode

1. Introduction

The Dependence on fossil energy as source of energy is increased continuously while fossil fuel is decreasing slowly. Not only lack of source, it is become serious problem for environment recently because it causes air pollution, depletion of ozone layer, acid rain, and global warming. Therefore, alternative environment-friendly energy is needed to solve this problems.

Microbial Fuel Cell (MFC) is a device that can produce electricity by using anaerobic microbe as biocatalyst and organic or inorganic matter as a fuel that usually used for energy recovery from waste. With this ability, it can be promising alternative energy to overcome both environment and energy problems. However, the performance that generated from this device is relatively low so that further study is needed to improve its the performance.

Some microbes are used as biocatalyst in a microbial fuel cell such as Arxula adeninivorans⁷, Candida melibiosica⁸, Saccharomyces cerevisiae¹⁴,¹⁷,²⁰, E. coli, Geobacter sulfurreducens², Shewanella sp¹⁹, and Rhodoferax ferrireductus¹⁸ had been studied. Moreover, The use of redox mediator has been studied to improve the MFC performance. Lohar et al¹⁰ reported that the use of neutral red and methylene blue as mediator on MFC could increase power density approximately as much ± 2 mW/cm². Permana et al¹⁴ reported that the use of methylene blue as mediator could increase electricity on yeast based MFC with maximum energy was 4.14 J. The other mediator such as thionin and fericyanide also used to improve MFC performance.
The use of various electrode also has been studied to improve the MFC performance. Schroder et al\textsuperscript{22} reported Pt electrode modified with polyaniline increase MFC performance drastically. The maximum power density produced by this electrode approximately 6000 mW/m\textsuperscript{2}. Other modified electrode such as PTFE/graphite\textsuperscript{25} showed high performance on MFC approximately 760 mW/m\textsuperscript{2}.

Boron-doped diamond (BDD) electrode is material that usually used as an electrode in electrochemistry process. BDD has a good stability, biocompatibility, low background current, and specific to oxygen. However, this electrode hasn’t been used in MFC. In this work, Boron-doped diamond electrode and \textit{Candida fukuyamaensis} are used in MFC with the expectation to improve its performance.

2. **Methods**

2.1. **Materials**

Pepton, yeast extract, malt extract, agar, glucose, H\textsubscript{2}O\textsubscript{2} 3%, potassium ferricyanide, methylene blue, H\textsubscript{2}SO\textsubscript{4} 1 M and aquademin.

2.2. **Growth medium**

\textit{Candida fukuyamaensis} UICC Y-247 isolate was obtained from UICC (Univeristas Indonesia Culture Collection). \textit{Candida fukuyamaensis} was cultured using YMA (Yeast Malt Agar) and YMB (Yeast Malt Brooth) medium. YMA was prepared by solving 3 g/L malt extract, 5 g/L pepton, 10 g/L glucose, 3 g/L yeast extract and 15 g/L agar with aquademin. Then, it was stirred and heated until it dissolved completely. YMB was prepared by using same method as YMA without agar. Yeast was regenerated from the isolated culture then transferred aseptically to YMA medium (working culture). Working culture was incubated in an incubator at the temperature of 30\textdegree C for 48 hours.

2.3. **Preparation of yeast suspension**

Working culture was transferred aseptically to 50 ml YMB medium. After that, The medium was incubated at the temperature of 30\textdegree C for 24 hours. This suspension was ready to use in the experiment.

2.4. **Preparation of proton exchange membrane.**

Membran Nafion 117 which had underwent pretreatment before being used in MFC. The membrane was immersed in aquadest and then heated. After that, The membrane was boiled with H\textsubscript{2}O\textsubscript{2} 3 % for 1 hour and then, washed with aquadest. After that, it was immersed in 1 M sulfat acid solution and heated until boiled. Then, it was washed three times with aquadest and the membrane is immersed in aquadest until it ready to used in MFC.

2.5. **O-Boron-doped Diamond (BDD) Electrode Fabrication.**

O terminated Boron-doped diamond electrode was fabricated by the electrochemical method. H-BDD electrode was used in the cyclic voltammetry method (CV) 20 times in range -0.75 V to +2.5 V with 0.1 M H\textsubscript{2}SO\textsubscript{4} solution. After that, it was measured with the chronoamperometry method at voltage +3 V for 20 minutes. O-BDD electrode is more stable and sensitive than H-BDD electrode so that it expected to have better performance.

2.6. **MFC experiment**

2.6.1 **Buffer pH variation**
MFC compartment was constructed as showed in Figure 1. Anode compartment was filled with 50 ml yeast suspension, 10 ml glucose 0.1 M, 20 ml aquademin, and 20 ml phosphat buffer 0.1 M at pH 6.5, 7, and 7.5, while cathode compartment was filled with 50 ml potassium ferricyanide 0.1 M and 50 ml phosphat buffer 0.1 M pH 7. After that, BDD elektrode was used as working electrode in anode and Pt spiral electrode as cathode. Proton exchange membrane Nafion 117 was used to separated the compartements. MFC was operated for 3 hours. During operation, the the current and voltage density was measured with potensiotstat.

2.6.2 Mediator concentration variation
Anode compartment was filled with 50 ml yeast suspension, 10 ml glucose 0.1 M, 20 ml aquademin, 10 ml Methylene blue with concentration 10 μM, 25 μM, 50 μM dan 100 μM and 20 ml phosphat buffer 0.1 M at pH 6.5, 7, and 7.5, while cathode compartment was filled with 50 ml potassium ferricyanide 0.1 M and 50 ml phosphat buffer 0.1 M pH 7. After that, BDD electrode was used as working electrode in anode and Pt spiral electrode as cathode. Proton exchange membrane Nafion 117 was used to separated the compartements. MFC was operated for 3 hours. During operation, the the current and voltage density was measured with potensiotstat.

3. Result and discussion

3.1. Effect of buffer pH variation
MFC was tested with pH buffer variation on anode compartment (pH 6.5-7.5) to study the effect of pH on electricity that produced by MFC. Anode compartment with buffer pH 7.5 produces highest power and current density, approximately 425.32 mW/m² and 0.044 mA. (Figure 2 and 3), respectively. Open circuit voltage that produced by variation of buffer pH (pH 6.5, 7 and 7.5) are similar to each other with value of approximately, 1.6 V (vs E° Ag/AgCl).
Figure 2: Voltage produced by variation of buffer pH on anode compartment (vs $E^\circ_{Ag/AgCl}$)

Figure 3: Current density produced by variation of buffer pH on anode compartment

Figure 4: Power density produced by variation of buffer pH on anode compartment
Small change to buffer pH on anode compartment affects the electricity that is produced by MFC. The produced electricity doesn’t use the addition of redox mediator/external mediator so it can be assumed that the electron, which produced by *Candida fukuyamaensis*, is transferred directly to the electrode (direct electron transfer)\(^\text{15}\). The electron is transferred through physical contact between microbe’s cell membrane and electrode without involving redox species in the anode compartment. Therefore, The produced electricity is depend on intracellular electron transfer that may involve redox protein in the cell membrane in the process of electron transfer to an electrode that its charge change because of the effect of pH so it affect electricity that produced. In neutral buffer pH (pH 7), the produced electricity is the lowest because neutral buffer pH may be close to pH isoelectric of redox protein that causes the charge to become null\(^\text{15}\). Moreover, The small change of buffer pH to both acid and base (pH 6.5 and pH 7.5) are changing the charge of redox protein. However, this assumption hasn’t proved so further study is needed.

3.2. Effect of redox mediator

Anode compartment was added with methylene blue as redox mediator with concentration 10μM, 25μM, 50μM and 100μM, respectively. The redox mediator is used as an electron acceptor that will penetrate cell membrane on the oxidation state and react with NAD\(^+\)/NADH redox pair\(^\text{22}\). After that, it is changed into the reduced state and transferred to the electrode (anode). In electrode (anode), redox mediator will change to the oxidized state again and release the electron that transferred to the cathode through the circuit\(^\text{22}\).

![Figure 5: Voltage produced by variation of redox mediator concentration](image1)

![Figure 6: Current density produced by variation of redox mediator concentration](image2)
Figure 7: Power density produced by variation of redox mediator concentration

Power and current density decrease along with the increasing redox mediator concentration (Figure 5 and 6). This is because methylene blue has anti microbial properties that can cause toxicity to the microbe. The use of methylene blue as a mediator should be extremely low to avoid toxicity to the microbe.

In this work, the maximum power density achieved by the MFC with mediator was 396.2 mW/m² while without mediator was 425.32 mW/m². This result showed that the use of BDD electrode on yeast based MFC provide good performance in producing electricity. Modification of the electrode, more pH buffer variation and the use of other mediator needed for further study.

4. Conclusion
Boron-doped diamond (BDD) electrode and Candida fukuyamaensis can be used in MFC system to produce electricity. The produced electricity depends on some factors such as pH and mediator concentration. In this work, the optimum pH is 7.5 with produced current density and power density are 440 mA/m² and 425.32 mW/m², respectively. Redox mediator addition, with high concentration, is lowering the produced electricity because of its anti microbial properties that can kill the microbe.

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