Influence of growth curve phase on electricity performance of microbial fuel cell by Escherichia coli

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ABSTRACT

The microbial fuel cell of Escherichia coli can convert microorganism biochemistry energy into electrical energy. To realize the influence of the growth curve phase with respect to different culture times on electricity performance, three kinds of E. coli (BCRC No. 10322, 10675, 51534) are selected, and it is both required and important to improve the performance of the microbial fuel cell (MFC). Results show that the BCRC No. 51534 of E. coli would be a better choice because a larger open-circuit voltage of 0.88 V and a limiting current of 10.1 mA possessed by it would result in an excellent power density of 547 mW/m². In addition, the selection of culture timing set as at the middle of the logarithmic phase and phase transition from logarithmic to stationary is suggested because the growth curve is suitable for electricity generation of the MFC. These observations would be useful for the improvement of the MFC.

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1. Introduction

The exhaustion of petroleum outlets and the impending energy crisis will result in serious destruction of the environment. Seeking a clean and renewable energy to replace traditional petrochemical energy is required and necessary to help prevent this grim problem. Green energy, whose sources include solar energy, wind power, biomass and bio-energy, is worth noting.

The working principle of a microbial fuel cell (MFC), that is similar to a traditional fuel cell, is that it can directly convert chemical energy into electrical energy. But the main difference is that the microorganisms in an MFC can carry out energy conversion during the metabolic process. The materials within fuel often indicate that hydrogen, methyl alcohol, ethyl alcohol, natural gas, and other hydrocarbons are present. Hence, a fuel cell whose product is heat and water could be seen as one kind of clean and renewable energy.

In this study, Escherichia coli could be used as the substrate and glucose could act as the fuel for an MFC. The reaction equations at the anode and cathode are addressed as follows:

At the anode:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{e}^- + 24\text{H}^+ \] (1)

At the cathode:

\[ 6\text{O}_2 + 24\text{e}^- + 24\text{H}^+ \rightarrow 12\text{H}_2\text{O} \] (2)

The glucose will lose the electrons in the anode when oxidizing and producing hydrogen ions. The electrons could transfer to the cathode via an external circuit and the hydrogen ions will go through the proton exchange membrane to the cathode at the same time. A complete
reaction of the MFC system would be achieved and electricity and water would be produced [1-4].

Two kinds of electron transfer methods would be classified as direct and indirect microbial fuel cells. The electrons in a direct microbial fuel cell will transfer directly to the electrode. Conversely, an electron mediator would be required for electron transmission in an indirect microbial fuel cell [5]. The E. coli microbial fuel cell used in this study is one of the indirect microbial fuel cells [6].

As for the electron mediator, some characteristics possessed would be noted as follows: (1) passing through the cell wall easily; (2) easily obtaining the electron from the cell membrane; (3) fast electrode reaction; (4) high solubility and stability; (5) without poisoning microorganisms; (6) unab sorbed by the microorganism. Therefore, thionine, Fe (III) EDTA and neutral red would be often used as electron mediators in the MFC [7,8].

Results indicated that the neutral red acting as the electron mediator in the MFC of E. coli could produce a potential of 0.68 V and a current of 4.5 mA [9]. The Proteus vulgaris microbial fuel cell, using HNQ as an electron mediator, could produce a potential of 0.5 V and a current of 0.5 mA [10]. Conversely, using thionine as an electron mediator in a P. vulgaris microbial fuel cell would produce a potential of 0.3 V and a current of 1.25 mA [11]. Furthermore, using a new methylene blue as the electron mediator in the E. coli microbial fuel cell would produce a maximum power density of 116 mW/m² at the potential of 0.76 V and 1.108 mA, but a maximum power of 89 mW/m² would be executed under the condition of 0.806 V and 0.85 mA when using neutral red as the electron mediator [12]. When the cathode uses potassium ferricyanide as the reducing agent in the MFC, the cathode reaction equation would be shown as follows [13]:

\[
4\text{Fe(CN)}_{6}^{3-} + 4e^{-} \rightarrow 4\text{Fe(CN)}_{6}^{4-} \quad (3)
\]

\[
\text{Fe(CN)}_{6}^{4-} + 4H^{+} + O_{2} \rightarrow 4\text{Fe(CN)}_{6}^{3-} + 2H_{2}O \quad (4)
\]

When lactic acid and graphite plates were used as the substrate and the electrode respectively in the E. coli microbial fuel cell it would produce a current of 2.6 mA and a power density of 91 mW/m² [14,15]. Contrarily, using glucose as the substrate, and using glass carbon as the electrode in a P. vulgaris microbial fuel cell, a current of 0.8 mA and a power density of 4.5 mW/m² would be produced [16]. In addition, using glucose as the substrate and using graphite fiber as the electrode in an Erwinia dissolvens microbial fuel cell could produce a current of 0.7 mA and a power density of 0.27 mW/m² [17]. When lactic acid and graphite fiber were used as the substrate and the electrode respectively in a Shewanella putrefaciens microbial fuel cell, a current of 0.04 mA and a power density of 0.00032 mW/m² would be produced [18]. Using the glucose as the substrate and using the graphite plates as the electrode in a Pseudomonas aeruginosa microbial fuel cell would produce a current of 0.1 mA and a power density of 88 mW/m² [19]. These results show that different kinds of substrate and electrode material would affect the electricity performance of a microbial fuel cell. As for the subject of the influence of the growth phase on the electricity performance of a microbial fuel cell, it was rarely addressed but important to improve the performance of an MFC.

2. Materials and methods

2.1. Bacterial culture

In this study the E. coli would be selected as an experimental microorganism because it is less harmful to humans and easy to grow. Three kinds of E. coli, named as No. 10322, No. 10675 and No. 51534, and originating from the Bioresource Collection and Research Center (BCRC), were used. The Celsius temperature of the E. coli culture was maintained at 37°C and the pH value was kept at 7.0. These culture conditions for the three kinds of E. coli were applied and are shown in Table 1.

The gene type of the BCRC No. 10322 E. coli is thr-1 leuB6 thy-1 galT1 xyl-7 malA1 ara-13 tonA2 supE44 and the culture medium used for the BCRC No. 10322 E. coli is nutrient agar. The culture medium included 3 g of beef extract, 5 g of Peptone, 15 g of agar, and 1 L of de-ionized water.

The BCRC No. 10675 E. coli is of the B126 variety and originates from the urea. This E. coli is pathogenic to chickens. The other collection of BCRC No. 10675 E. coli is ATCC 11775; CCM 5172; CIP 54.8T; DSM 30083; IAM 12119; JCM 1649; NCDO 1989; NCIMB 11943; NCTC 9001 and the culture medium of is nutrient agar. The culture medium included 3 g of beef extract, 5 g of peptone, 15 g of agar, and 1 L of de-ionized water.

The gene type of the BCRC No. 51534 E. coli is F-supE44 lacY1 ara-14 galK2 xyl-5 mtl-1 leuB6 Δ(mcrC-mrr) recA13 rpsL20 thi-1 Δ( gpt-proA)62 hsdSB20 Δ- and the culture medium of BCRC No. 51534 E. coli is a LURIA-BERTANI (LB) medium. The culture

### Table 1 – The culture conditions for three kinds of Escherichia coli.

<table>
<thead>
<tr>
<th>BCRC Number</th>
<th>Organism</th>
<th>Growth conditions</th>
<th>Medium conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>10322</td>
<td>E. coli (Migula) Castellani and Chalmers</td>
<td>37°C, pH 7.0, Nutrient broth (NB) Medium</td>
<td>Beef extract 3.0 g, Peptone 5.0 g</td>
</tr>
<tr>
<td>10675</td>
<td>E. coli (Migula) Castellani and Chalmers</td>
<td>37°C, pH 7.0, Nutrient broth (NB) Medium</td>
<td>Beef extract 3.0 g, Peptone 5.0 g, De-ionized water 1.0 L</td>
</tr>
<tr>
<td>51534</td>
<td>E. coli HB101</td>
<td>37°C, pH 7.0, LURIA-BERTANI (LB) Medium</td>
<td>Yeast extract 5.0 g, Tryptone 10.0 g, NaCl 10.0 g, De-ionized water 1.0 L</td>
</tr>
</tbody>
</table>
medium includes 5 g of extracted yeast, 10 g of the protein Tryptone, 10 g of sodium chloride (NaCl), and 1 L of de-ionized water.

Prior to producing the culture of E. coli, the plate culture medium and liquid culture medium would first be prepared. As for the plate culture medium, the nutrient agar would be composed of 0.75 g of beef extract, 1.25 g of peptone, 250 mL of de-ionized water, and 12.5 g of agar that has been mixed slightly. The medium would be put in the autoclave at 121°C for 15 min, and then removed at room temperature for cooling. Finally, 15 mL solutions were taken to coagulate. The nutrient broth for the liquid culture medium were made up of a coagulate mixture whose components were 3 g of beef extract, 5 g of peptone, and 1000 mL of distilled water. The medium would then be left in the autoclave at the temperature of 121°C for 15 min, and then removed at room temperature for cooling and coagulation. The plate culture medium and the liquid culture medium for the LURIA-BERTANI (LB) medium would follow the same procedure with respect to the nutrient agar and the nutrient broth medium.

The culturing process for E. coli was addressed as follows:

1. Stroke the bacteria: Take out the E. coli and medium dishes from the refrigerator, and then stroke the bacteria on the plate medium dishes three times. Finally, put it into incubator at 37°C to rise for 12 h.
2. Select the bacteria: Select a suitable E. coli from the third plate medium dishes and put it into the liquid culture medium for mixing. Finally, put it into the incubator on the condition that the Celsius temperature of 37°C, and a speed of 200 rpm, would be provided and raised for 12 h.
3. A colony culture: Taking a colony culture of E. coli into a 100 mL fresh liquid medium for culture.
4. Measure the optical density value (OD value): Use a spectrophotometer to measure the OD value of the liquid culture medium. The wavelength for measuring E. coli is 600 nm. A large OD value indicates more E. coli in the liquid culture medium. Thus, the OD value would be used in this study for representing the growth of the E. coli.
5. Growth curve: Measure the OD value from the E. coli liquid culture medium per hour and finish after 22 h. Fig. 1 indicates the growth curves of the E. coli with respect to BCRC No. 10322, No. 10675 and No. 51534. Although, three curves of OD for three kinds of E. coli change with the culture time, they appear to be similar. The maximum OD would be produced by No. 51534. This evidence shows that the bacterium of No. 51534 was more active than that of the others. Concerning the growth curves of Fig. 1, four culturing points with respect to the transition timing from the lag phase to the logarithmic phase, the middle timing of the logarithmic phase, the transition timing from logarithmic phase to stationary phase and the middle timing of the stationary phase, respectively, would be selected as four growth curve phases for realizing the influence of growth phase on the electricity performance of the microbial fuel cell. Here, the four culture timings for BCRC No. 10322 were set at 6 h, 9 h, 12 h, and 18 h respectively and 6 h, 8 h, 10 h and 16 h for BCRC No. 10675. In addition, four culture timings were set at 9 h, 13 h, 18 h and 22 h for BCRC No. 51534.

2.2. Microbial fuel cell design

The material of form for an MFC whose dimension is 10 × 6.2 × 5 cm is made of PMMA. The anode and cathode chambers, whose volume is 100 mL for each chamber, was separated by a proton exchange membrane (PEM). Nafion-117 membrane (originating from the Du Pont Company), whose effective surface area is 9 cm² was used for the PEM. The PEM would be first soaked in a 5% hydrogen peroxide solution and heated to about 70–80°C for 1 h before usage. Then the PEM film would be put in de-ionized water to eliminate the residue from the hydrogen peroxide solution. Immediately, the sulfate ions on the proton exchange membrane would be allowed to unify with the hydrogen ions, and hence form a hydrogen proton exchange membrane by soaking in 1 M sulfuric acid whose required Celsius temperature of 70–80°C would be heated and maintained.

The graphite carbon cloth, whose dimension is 5 × 4 cm wide, was used as an electrode plate for the anode chamber and cathode chamber. In addition, methylene blue was used as an electron mediator because it performs well on electricity production. Here, 100 mL of E. coli solution with the E. coli and cultured medium and 0.37 g of methylene blue would be added in the anode chamber to render safe the E. coli in the MFC. Conversely, the cathode chamber was filled with 100 mL of 0.1 M potassium ferricyanide. The MFC system shown in Fig. 2 would be maintained under conditions in which the Celsius temperature was 23–25°C and the pH value of the E. coli culture was at pH = 7.0.

2.3. Measurement analysis

As for realizing the performance of the MFC, a polarization curve (I–V curve) would be executed by using an electro-chemical workstation (Jiehan 5600, Taiwan) and the power density equation of (5) would also be obtained from I–V curve. The data acquisition system (Jiehan 5020, Taiwan) at
3. Results and discussion

Fig. 3 shows the performance of BCRC No. 10322 E. coli in the MFC. The results of Fig. 3 are addressed as follows. First, an open-circuit voltage (OCV) of 0.375 V, a limiting current of 0.12 mA and a maximum power density of 4.32 mW/m² would appear after a culturing time of 6 h. Similarly, an OCV of 0.61 V, a limiting current of 2.44 mA and a maximum power density of 97.7 mW/m² would be produced after a culturing time of 9 h. As for a culturing time of 12 h, an OCV of 0.36 V, a limiting current of 1.32 mA and a maximum power density of 30.8 mW/m² would be produced. The performance of the microbial fuel cell would yield an OCV of 0.309 V, a limiting current of 1.07 mA and a maximum power density of 21.7 mW/m² after a culturing time of 18 h. From this evidence it can be indicated that a culture time of 9 h would be suggested for BCRC No. 10322 E. coli MFC because of its higher electricity performance.

Concerning the working power density during the external resistance 470 Ω and duration time of 72 h, Fig. 4 shows the variations of the working power density change with different culture times. The results in Fig. 4 show that the MFC system will produce a working power density of 97.9 mW/m² after 9 h, better than other culture times. Therefore a culture time of 9 h, corresponding to the middle of the logarithmic phase in the growth curve of BCRC No. 10322, would be suggested from this study.

As for the performance of the BCRC No. 10675, Fig. 5 shows the I–V curve and I–P curve. The results shown in Fig. 5 indicate that an OCV of 0.54 V, a limiting current of 0.02 mA, and a maximum power density of 1.13 mW/m² would be produced after a culture time of 6 h. Similarly, an OCV of 0.096 V, a limiting current of 0.14 mA, and a maximum power density of 0.71 mW/m² would be obtained after a culture time of 8 h. The microbial fuel cell of BCRC No. 10675 had an OCV of 0.39 V, a limiting current of 0.41 mA and a maximum power density of 11.2 mW/m² after a culture time of 10 h. After a culture time of 16 h, an OCV of 0.47 V, a limiting current of 0.17 mA and a maximum power density of 8.36 mW/m² would be obtained. These results in the figure for BCRC No. 10675, with respect to different culture times, would indicate that the culture time of
10 h seems to be the better choice. In addition, the working power density during the external resistance 470 $\Omega$ and duration time of 72 h was executed and is shown in Fig. 6. Fig. 6 shows that the culture time of 10 h, corresponding to the transition from the logarithmic phase to stationary phase in the growth curve of BCRC No. 10675, would create a greater power density than with the other culture times. Although there is not a big difference in OD values existing between BCRC No. 10322 and 10675, there still appears to be a little difference in genetic makeup between them. Therefore, this feature of cell difference will influence the bacteria metabolism and growth of E. coli. In this study, the electricity performance of BCRC No. 10322 MFC is better than that of BCRC No. 10675.

As for the E. coli MFC performance of BCRC No. 51534, the I–V curve and I–P curve with respect to different culture times is shown in Fig. 7 and addressed as follows. First, an OCV of 0.88 V, a limiting current of 5.42 mA and a maximum power density of 295 mW/m² would be produced after culture time of 9 h. As for the culture time of 13 h, an OCV of 0.82 V, a limiting current of 10.1 mA and a maximum power density of 547 mW/m² could be obtained. After a culture time of 18 h, an OCV of 0.84 V, a limiting current of 8.4 mA and a maximum power density of 448 mW/m² would appear. The E. coli microbial fuel cell of BCRC No. 51534 had an OCV of 0.57 V, a limiting current of 3.7 mA and a maximum power density of 134 mW/m² after a culture time of 22 h. This information would indicate that a good performance of the microbial fuel cell for BCRC No. 51534 would be obtained after a culture time of 13 h. In addition, the working power density under the conditions of an external resistance of 470 $\Omega$ and duration time of 72 h was obtained and shown in Fig. 8. The results in Fig. 8 show that a working power density of 290 mW/m² would be generated after culture time of 18 h. Conversely, the MFC would produce a lower voltage and power with a culture time of 13 h. The middle timing of the logarithmic phase of BCRC No. 51534 E. coli for the culture time of 13 h shows rapid growth, and the individual morphology, chemical composition, and physical characteristics of E. coli are more variable. Therefore, the middle timing of the logarithmic phase would be unstable in a microbial fuel cell system reaction. From evidence shown in Fig. 8, the culture time of 18 h corresponding to the transition from logarithmic phase to stationary phase of the growth curve for BCRC No. 51534, produces a better power performance and can further be suggested.

Comparing the power performance with the studied cases of BCRC No. 10322, 10675 and 51534, the E. coli microbial fuel
cell of BCRC No. 51534 would produce an optimal performance for studied cases. Conversely, the *E. coli* microbial fuel cell of BCRC No. 10675 appears to have the lowest power performance.

Concerning the evidence that the OD value of BCRC No. 51534 seems to be better than No. 10322 and No. 10675, BCRC No. 51534 will have a good bacterial metabolism, and growth and electricity performance in the MFC. In addition, different features of *E. coli* would be affected by the electricity performances of the MFC.

The previous studies showed that *E. coli* microbial fuel cells would produce a current of 2.6 mA and a power density of 91 mW/m² by using lactic acid acting as the substrate and using the graphite plates for the electrode [14,15]. In this study the *E. coli* microbial fuel cell of BCRC No. 51534 will produce a limiting current of 10.1 mA and a maximum power density of 547 mW/m² under the condition of using glucose as the substrate and carbon cloth as the electrode, respectively. From this evidence it is shown that the current and power density of the MFC produced in this study seems to obviously be better than in previous studies [14,15]. In addition, some studies showed that the *E. coli* microbial fuel cell using neutral red as the electron mediator would produce an OCV of 0.68 V [9]. Another study using methylene blue as the electron mediator would produce an OCV of 0.326 V [12]. But, in this study the *E. coli* microbial fuel cell of BCRC No. 51534 using methylene blue would produce an OCV of 0.88 V and the OCV of the MFC in this study would be larger than in other previous studies.
MFC performance produced in this study seems to be better than in previous studies from the viewpoint of the electricity indicators of the OCV, currents and power densities.

During the logarithmic phase of the microorganism there is rapid growth, and the individual morphology, chemical composition, physical characteristics of E. coli are more variable. Conversely, the stationary phase of a microorganism will achieve a maximum growth number of bacteria and reach quasi-equilibrium between the cell sorting and cell death crack speeds. As for the effect of culture time with respect to quasi-equilibrium between the cell sorting and cell death achieve a maximum growth number of bacteria and reach able. Conversely, the stationary phase of a microorganism will appear because the phase would show a stronger growth rate and a higher metabolism. These observations would be useful to improve the performance of MFCs.

4. Conclusion

This study was mainly to find the influence of the growth curve at different culture times for three kinds of E. coli (BCRC No. 10322, 10675, 51534) on the performance of an MFC. Some observations obtained are addressed as follows:

First, the BCRC No. 51534 E. coli possessing an OCV of 0.88 V, and limiting current of 10.1 mA seems to be a better choice because a larger power density of 547 mW/m² could be provided. Second, the culture timing at the middle of the logarithmic phase and phase changing from a logarithmic to stationary phase would be suggested for the growth curve of E. coli because it is suitable for electricity generation of a microbial fuel cell. These observations would be useful to the improvement of a microbial fuel cell.

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