

Performance evaluations of yeast based microbial fuel cells improved by the optimization of dead zone inside carbon felt electrode

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Abstract—The performance of yeast-based microbial fuel cells (MFCs) and the growth pattern of yeast were evaluated with the optimization of dead zone within carbon felt (CF) electrode. Yeast cells were grown onto the different CFs that have 1 to 4 mm thicknesses, while optical and electrochemical evaluations were implemented to determine the optimal growth pattern of yeast and to elucidate a relationship between the growth pattern of yeast and the performance of MFC. According to the evaluations, biofilm consisting of high-density yeast cells is formed in the upper 1 mm height of CF electrode. As the height goes down, density of yeast cells is reduced to less than half of the upper biofilm, and by calculating the growth rate of yeast cells per CF volume, it is recognized that the coverage of biocatalyst including yeast cell increases from 0.191 to 0.406 $\mu\text{mol}/\text{cm}^3$ with decreasing CF thickness. Then, the performance of MFCs using biocatalysts including yeast cells grown on different thick CFs is measured to investigate how the growth pattern of yeast cells affects the performance of MFCs. Results show their maximum power density (MPD) increases linearly as the area that yeast cells are filled increases, and when CF thickness is 1 mm, MPD reaches 417.13 W/m^3 .

Keywords: Yeast, Biofilm, Carbon Felt, Growth Pattern of Yeast, Microbial Fuel Cells

INTRODUCTION

Microbial fuel cells (MFCs) are devices that use microorganisms as catalysts and convert chemical energy produced by the metabolism process of microorganisms into electrical energy [1-4]. This energy conversion indicates the decomposition of organic substrate by microorganisms, and electricity is produced while decomposing organic substances, such as carbohydrates, alcohols, and amino acids, in soil or wastewater. Many studies have been conducted to use the MFCs for both the device for this energy conversion and environmental purification device [4-9].

Furthermore, since MFC is a kind of fuel cell, this requires related components, such as electrodes, separators, and collector plates. Among the components, optimizing the electrode is important because the microorganisms grow and react with substrate, and the substrate containing microorganisms is placed onto the electrode, while the microbial growth determines the condition of electrode, followed by the performance of MFCs [10-15].

With microbial growth, biofilm is usually formed, which plays a crucial role in keeping the microorganisms attached to the medium and supplying nutrients to the microorganisms. This occurs even in MFCs. Namely, the biofilm grown onto electrode surface is used as a fuel for oxidation reaction occurring in anode, and how well

the biofilm adhere to electrode surface can decide the reactivity of oxidation reaction and the performance of MFC [16-20]. Therefore, how to obtain the well grown biofilm is important to achieve a better performance of MFC, and there have been some associated studies.

For example, Kimberley and Kwon et al. grew gold nanoparticles on a carbon felt (CF) electrode, and the best conditions for yeast cell growth were determined to enhance microbial adhesion and biofilm formation. Through this, maximum power density (MPD) of 2,771.25 mW/m^2 was recorded [21]. Dominico and Kwon et al. used an inexpensive material called as graphite-cement composite (GC70S) as electrode, and silica nanoflowers that helped the microorganisms to grow well were doped on the electrode. As a result, MPD of 329.8 mW/m^2 were recorded [22]. Zhao et al. increased a roughness of surface structure of anode to promote the growth of microorganisms. Here, the microorganisms were chemically treated by $\text{HNO}_3/30\% \text{H}_2\text{O}_2$ on the electrode. With that, the growth rate of desirable biofilms increased, and the MPD of MFCs using them could reach 758.2 mW/m^2 [23]. Feng et al. showed similar biofilm growth pattern to Zhao group. They used the electrode consisting of carbon fiber brush, and the surface of electrode was modified using ammonium peroxydisulfate and H_2SO_4 . Through this process, the MPD of MFCs was 1,370 mW/m^2 [24].

As described so far, many researchers have focused on growing the desirable microorganisms by handling the electrode. However, the above approaches have typical problems. In general, MFC is driven in water, and if there is a portion of electrode that is not

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surrounded by biofilm (a portion where microorganisms do not grow) that is called a dead zone; the dead zone reacts with other undesirable substances that are present in electrolyte, and the operation of MFC is interrupted and its performance is then degraded. Therefore, it is needed to investigate how the dead zone of microorganisms affects the performance of MFCs.

In this study, to investigate the effect of dead zone on the performance of MFCs, the dead zone produced on CF surface was estimated. For the purpose, MFCs using biocatalysts containing yeast cells that the four different thicknesses of CF electrode (1, 2, 3 and 4 mm) were prepared were fabricated, thereby the dead zone where the growth of yeast cells was depleted was examined, and how the dead zone affected the performance of MFCs was then evaluated. For this process, *Saccharomyces cerevisiae* (Baker's yeast) was used as a main biocatalyst because this yeast is grown very quickly among various fungi and has excellent electron transfer [2,25-27]. The biofilm grown by the difference in thickness of CF electrode was identified through scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS), and the portion of biofilm produced was evaluated by its coverage calculation.

EXPERIMENTAL

1. Materials

Commercial CF (XF-30A) was obtained from Toyobo (Osaka, Japan). Commercial dried yeast originating from *Saccharomyces cerevisiae*, yeast extract, 0.1 M phosphate buffer solution (PBS, pH 7.4), and D-glucose were purchased from Sigma Aldrich (St. Louis, USA). Peptone was purchased from Duksan Pure Chemicals Co. (Gyeonggi-do, Republic of Korea).

2. Configuration of MFC Kits for Half-cell and Full-cell Characterization

A comparison of yeast biofilm grown in CF electrodes of differ-

ent thicknesses was performed through cyclic voltammetry (CV) measurement, while a three-electrode half-cell configuration was used for the CV measurements (Fig. 1(a)). The cell consisted of Pt wire, saturated calomel electrode (SCE), and modified carbon felt whose active area was 1.13 cm^2 with different thicknesses such as 4, 3, 2 and 1 mm, as a counter, reference, and working electrode, respectively [28-30]. The supporting electrolyte solution for half-cell tests was prepared by yeast extract/peptone/D-glucose (YPD) medium dissolved in 0.1 M PBS (pH 7.4) with yeast, and its volume was of 150 mL. Regarding the composition of YPD medium, previously optimized composition was used [15,31]. Based on that, the YPD medium consisted of 5 mg/mL yeast extract, 2.5 mg/mL peptone, yeast, and 13.18 mg/mL D-glucose.

For MFC full-cell tests, in house MFC full cell reactor was used. The cell kit for MFC reactor was designed as shown in Fig. 1(b). Here, anode was placed in electrolyte to form a biofilm on the electrode that was stemmed from the growth of yeast cells contained in electrolyte, and a cathode was installed on the surface of the electrolyte to react with oxygen contained in air. Meanwhile, the distance between anode and cathode was set as 2 cm, and four different thicknesses of electrodes (4, 3, 2 and 1 mm) were prepared for the experiments. All the electrodes prepared for both anode and cathode of MFC were in disk shape designed as the geometric surface area of 7 cm^2 , while the electrodes for three electrode tests were prepared in small disk shape that had an active area of 1.13 cm^2 . 150 mL YPD medium was fed to MFC full-cell reactor, while this volume of YPD medium was the same as that of YPD medium used for half-cell tests.

MFCs were operated with YPD medium of 150 mL under 0.1 M PBS (pH 7.4). Yeast cells were cultured for three days (72 h) in constant temperature box ($26 \pm 2^\circ \text{C}$) under semi-anaerobic state. Polarization and power curves were measured until open circuit voltage (OCV) of MFC reached 0 V at a scan rate of 10 mV/s.

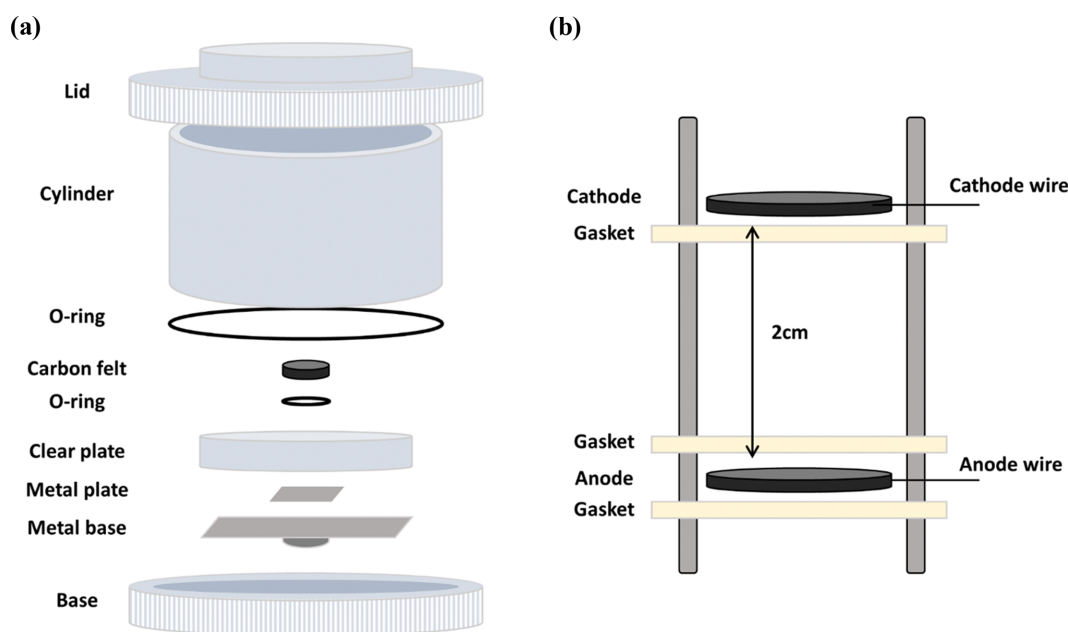


Fig. 1. Labeled segmented schematics of (a) half-cell reactor and (b) full-cell reactor designed for MFC.

3. Optical Characterization

High-resolution SEM (HR-SEM, Hitachi SU8010, Japan) was used to investigate how the CF surface was changed with the growth of yeast biofilm. The characterization was performed using 10.0 kV under high vacuum. EDS analysis was used for observing the cross section of CF. With this analysis, the atomic composition of CF and the growth degree and distribution of yeast were determined.

RESULTS AND DISCUSSION

1. Dead Zone Formation and the Effect of Dead Zone Size on the Performances of MFCs

Yeast cells can float freely or are attached to CF electrode. When they are attached to CF electrode, they can participate in the electron transfer process [2,32]. Thus, it is important to investigate in which condition of CF electrode yeast cells grow well. According to our preliminary tests, the thickness of CF electrode is one of crucial factors to determine the growth rate and growth pattern of yeast cells. To examine the effect of the thickness of CF electrode on the growth rate and growth pattern of yeast cells, four different thicknesses of 4, 3, 2 and 1 mm were considered, and yeast cells were grown onto the different thick CF electrodes for three days [31,33,34], while the growing process of yeast cell was optically evaluated (Fig. 2). Fig. 2(a) represents a bare CF electrode and Fig.

2(b) is an image of CF electrode taken after yeast cells were grown. As shown in Fig. 2, yeast cells were grown in a fixed form onto the CF electrodes that had different thicknesses, while they were well attached to the CF electrodes regardless of its thickness.

However, Fig. 2 only shows the image of yeast cells grown onto the surface of CF electrode. To investigate the growth pattern of yeast cells inside CF electrode, the four different CFs were cut in half, and their cross-sectional images were optically estimated and mapped by SEM with thickness unit of 1 mm.

Usually, yeast cells consist of carbohydrate and protein, and thus, if yeast cells grow inside CF electrode, the amount of oxygen and nitrogen elements increases [35,36]. Figs. 3(a) and (f) are SEM mapping image of oxygen element that is grown onto bare CF of 4 mm thick. According to the images, the amount of oxygen element grown onto bare CF of 4 mm thick was very low (Table 1). Figs. 3(b)-(e) represents SEM mapping images of 4, 3, 2 and 1 mm thick CFs, while Figs. 3(g)-(j) are their elemental mapping images. In all the mapping images, a high-density biofilm was only formed in the top layer of CF that corresponds to 1 mm. The amount of biofilm produced was quantified based on the oxygen content (Table 1). There are some noticeable points about Fig. 3. First, when CF thickness was 4 mm, 33.3% of biofilm formed in the entire electrode was present in the P4 region (top floor), while the portion of biofilm formed was significantly reduced to 18.6, 20.6, and 27.5 %

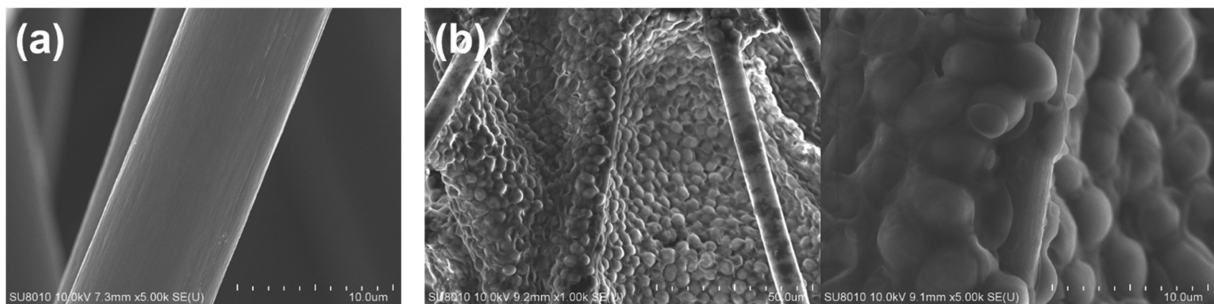


Fig. 2. SEM images of (a) the surface of bare CF electrode and (b) the well-grown yeast.

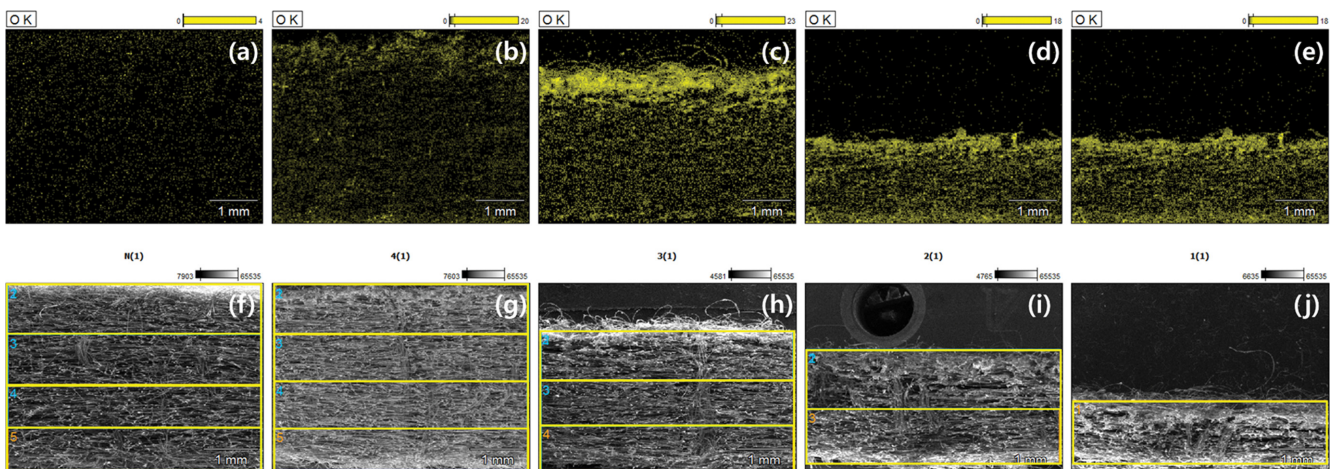


Fig. 3. Cross-sectional images of the corresponding interfaces. EDX mapping of oxygen element measured at (a) fresh CF, and yeast cells grown (b) 4 mm, (c) 3 mm, (d) 2 mm and (e) 1 mm thick CFs, and HR-SEM images of (f) fresh CF, and yeast cells grown (g) 4 mm, (h) 3 mm, (i) 2 mm and (j) 1 mm thick CFs.

Table 1. SEM element mapping of four different thick CFs

| C atom % | | Carbon felt | | Carbon felt with grown yeast | | |
|----------|--------|-------------|-------|------------------------------|-------|-------|
| No. | Area | 4 mm | 4 mm | 3 mm | 2 mm | 1 mm |
| P4 | 4~3 mm | 100 | 56.23 | - | - | - |
| P3 | 3~2 mm | 100 | 69.55 | 57.86 | - | - |
| P2 | 2~1 mm | 100 | 67.08 | 75.26 | 58.85 | - |
| P1 | 1~0 mm | 100 | 61.16 | 68.39 | 68.6 | 58.27 |
| O atom % | | Carbon felt | | Carbon felt with grown yeast | | |
| No. | Area | 4 mm | 4 mm | 3 mm | 2 mm | 1 mm |
| P4 | 4~3 mm | 0 | 28.21 | - | - | - |
| P3 | 3~2 mm | 0 | 15.81 | 27.37 | - | - |
| P2 | 2~1 mm | 0 | 17.46 | 12.36 | 26.54 | - |
| P1 | 1~0 mm | 0 | 23.36 | 18.49 | 17.27 | 27.73 |

in P3, P2, and P1 regions, respectively.

Second, when CF thickness was 3 mm, 47.0% of biofilm was present in P3 region (top floor), 21.2% and 31.7% were present in P2 and P1 regions, while in CF thickness of 2 mm, the portion of biofilm formed was 60.6% in P2 region (top floor) and 39.4% in P1 region. In summary, in all CF electrodes, most of the biofilm was formed on the top region of CF electrode (36.6-64.2%), and as the CF electrode became thicker, the portion of biofilm formed

Table 2. The coverage of biocatalyst (Γ) depending on the four different thickness of CF (4, 3, 2 and 1 mm).

| Thickness | Coverage [$\mu\text{mol}/\text{cm}^3$] |
|-----------|--|
| 4 mm | 0.191 |
| 3 mm | 0.156 |
| 2 mm | 0.216 |
| 1 mm | 0.406 |

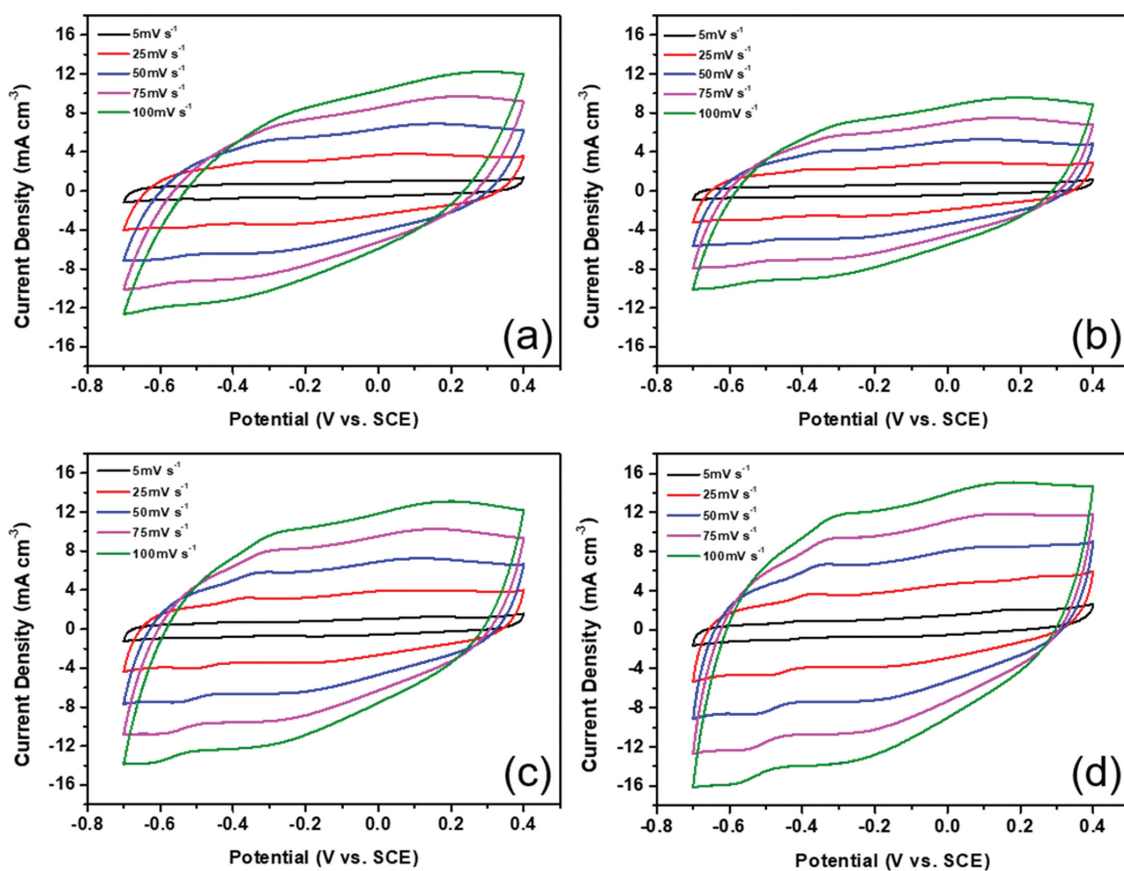


Fig. 4. CV curves showing the relationship between current density and scan rate of CF electrodes with different thickness ((a) 4 mm, (b) 3 mm, (c) 2 mm and (d) 1 mm). Here, scan rate was 5, 25, 50, 75 and 100 mV s^{-1} .

in the middle region was about one-half (45-61%) of the upper region of CF electrode.

To clarify the properties of yeast biofilm of each CF electrode, the coverage of electroactive biocatalyst (Γ) covering CF electrode was calculated using Randles-Sevcik's equation (Eq. (1)). The calculation formula used is as follows [37]:

$$\Gamma = \frac{i_p 4RT}{\nu n^2 F^2 V} \quad (1)$$

Here, ν is the scanning speed (mV/s), i_p is a peak current density in unit volume (mA/cm³), R is the gas constant (J/mol K). T is the temperature, n is the number of electrons, and F is the Faraday constant, V is the volume of the electrode, and finally Γ is the coverage of the electroactive biocatalyst, and the calculated Γ values are shown in Table 2.

Yeast cells grown onto CF showed their catalytic activity at -0.43 V vs AgAgCl as biofilm was formed [38]. When the CV curves were measured in different thick CF electrodes (Fig. 4), there are two noticeable points. First, the non-faradic current was significantly affected by the thickness of CF electrode. As the thickness became thinner, its electrical double layer increased, which means that the biofilm consisting of yeast cells was the densest in 1mm thick CF electrode. Second is the difference in the reaction peak of CF electrodes observed at -0.43 V vs AgAgCl. The peak was an indicator showing the electric reactivity of biocatalysts and in the thinnest CF (1 mm thick CF), its current was highest. Whether the reactivity of biocatalysts is related to the surface coverage, the surface coverage of yeast grown CF electrodes is measured (Fig. 4). According to Fig. 4, the surface coverage of 1 mm thick CF electrode was highest as $0.406 \mu\text{mol}/\text{cm}^3$. This indicates that as the biofilm becomes dense, both the surface coverage of CF electrode and the catalytic activity of yeast-based biocatalyst increase.

2. Performance of MFCs Using Yeast Grown CF Electrodes

Based on the above properties of yeast grown CF electrodes, the performance of MFCs using them was evaluated to determine how the difference in dead zone of yeast grown CF electrode affects the performance of MFCs. Such MFCs were operated without membrane as shown in Fig. 1, while each sample was measured three

times for accurate evaluation (Fig. 5).

Regarding OCV, that of MFCs using 4, 3, 2 and 1 mm thick CF electrodes was 0.707, 0.710, 0.727 and 0.716 V. It can be confirmed that biofilms of the same shape were formed by showing similar OCV at all electrode thicknesses [19]. When the performance of MFC was confirmed, it showed the performance of 191, 234, 311, and 417 W/m³ depending on the thickness of the electrode, which tended to match the surface coverage trend calculated in Table 2. This confirms that as the dead zone size of CF electrode decreases, the performance of MFC using the CF electrode increases.

CONCLUSIONS

The performance of yeast-based MFCs and the growth pattern of yeast were investigated, while the effect of the dead zone of CF electrode on the performance of yeast-based MFCs was elucidated. Yeast cells were grown onto the four different thick CFs (1, 2, 3, and 4 mm thicknesses). According to the evaluations, biofilm consisting of high-density yeast cells was formed in upper 1mm height of CF electrode, and as the height went down the density of yeast cells was decreased less than half of the upper biofilm. Furthermore, when the growth rate of yeast cells per volume of CF was measured, as the thickness of CF became thinner the coverage of yeast cells produced as a form of biofilm increased from 0.191 to $0.406 \mu\text{mol}/\text{cm}^3$, which was measured using Randles-Sevcik's equation. With that, the performance of MFCs using yeast cells grown onto the different thick CFs was measured. As a result, when the CF thickness was 1 mm, MPD of MFC was $417.13 \text{ W}/\text{m}^3$, while the MPD linearly increased as the area that yeast cells were filled increased.

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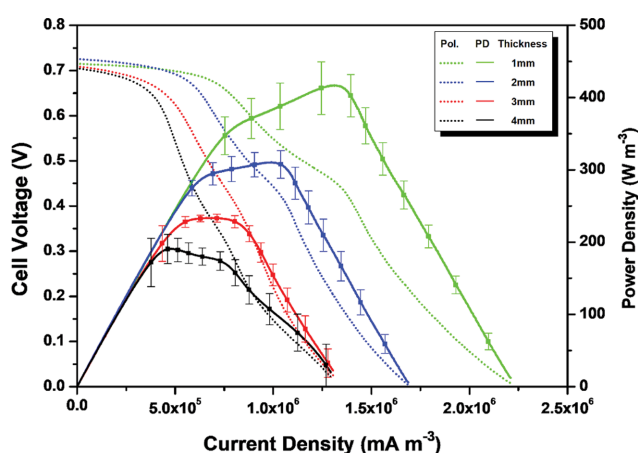


Fig. 5. Polarization and power density curves of yeast-based MFCs using 4, 3, 2 and 1 mm thick CF electrodes.

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