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IMMOBILIZED CYANOBACTERIA ON THE CATHODE AS OXYGEN SOURCE FOR MICROBIAL FUEL CELL

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ABSTRACT. Oxygen is commonly used as the electron acceptor for the cathode reaction in microbial fuel cells (MFCs). This study demonstrates how to generate oxygen via photosynthesis by means of Synechocystis AICB 51 cyanobacteria immobilized on the cathode. The advantage of using cyanobacteria immobilized on the cathode was demonstrated using two geometries: two-chamber and membrane-less MFCs. The anode chamber was filled with sludge collected from the wastewater treatment plant from Cluj-Napoca, Romania. The oxygen concentration in the cathode space of two-chamber cells rises from about 1mg l^{-1} at 130 lux to 11 mg l^{-1} at 2500 lux of incident illumination. In the case of membrane-less cells, the oxygen concentration varies from 0.2mg l^{-1} to 4.5 mg l^{-1} for the same conditions of illumination. In the case of membrane-less MFCs, the power generated with the immobilized cyanobacteria on the cathode is up to 20 of times greater than the power generated with the standard plain graphite cathode during illumination. In both cases there is a strong correlation between power and dissolved oxygen concentration.

Keywords: *immobilized Synechocystis cyanobacteria, microbial fuel cell, photosynthetic oxygen, dissolved oxygen, power density*

INTRODUCTION

A microbial fuel cell (MFC) provides direct recovery of the chemical energy stored in organic compounds in wastewater, for example, to electrical energy, *via* the chemical reactions catalyzed by microorganisms [1]. A MFC

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consists of an anode and a cathode, separated in most cases by a cation exchange membrane. Microorganisms in the anode chamber oxidize organic materials, generating electrons and protons (anode reaction):

$$n\ CH_2O + n\ H_2O \rightarrow n\ CO_2 + 4n\ e^- + 4n\ H^+$$

The electrons are transferred to the cathode through the external circuit because of the potential difference developed between the reducing environment in the anaerobic anode chamber and the oxidizing environment in the cathode chamber (supplied with oxygen). The protons are transferred to the cathode through the membrane. Electrons and protons are finally consumed in the cathode chamber, commonly reducing oxygen to water (cathode reaction) [1].

$$n\ O_2 + 4n\ e^- + 4n\ H^+ \rightarrow 2n\ H_2O \quad \text{or}$$
$$n\ O_2 + 2n\ e^- + 2n\ H^+ \rightarrow n\ H_2O_2$$

When considering the overall process, the cathodic reaction is, aside from the flux of protons through the membrane, the main bottleneck identified at the moment in increasing the power of MFCs [2].

The cathodic process is determined by the electrode surface, its catalytic properties, the homogeneity in the cathodic compartment and the concentration of the electron acceptor in the bulk liquid [3].

Oxygen is generally used as the electron acceptor for the cathodic reaction in MFCs. The supply with oxygen through sparging is energy demanding, reducing the net energy output of the MFC [4].

The low coulombic efficiency of the MFC is believed to be due to oxygen limitation in the cathode chamber and to oxygen diffusion into the anode chamber through the membrane [5-7].

In order to eliminate the oxygen limitation, the oxygen concentration in the cathode should be kept high, which requires increased power consumption, and results in more oxygen diffusion into the anode [1].

Algae and cyanobacteria have been used as photosynthetic sources of oxygen in the cathode [7-9]. In a two-chamber MFC, algae and/or cyanobacteria could be dispersed in the entire cathode chamber, but when intending to use them in a membraneless (sediment type) MFC, one must immobilize them on the cathode to avoid mixing with the microbes from the anode.

In this paper it has been proposed a method of generating high oxygen concentration near the cathode surface without consuming electrical energy and reducing oxygen diffusion to the anode.

The immobilized cyanobacteria *Synechocystis* sp. AICB 51 on the cathode was investigated as a photosynthetic oxygen supplier in both two-chamber and membraneless MFC.

The *Synechocystis* sp. AICB 51 strain is a mesophilic unicellular cyanobacteria able to use the inorganic carbon added in the growth medium as NaHCO3 (Zarrouk medium), described in [10]. The optimal growth temperature is 30^{o} C in fluorescent light, but they also develop a good growth at a lower temperature [11].

RESULTS AND DISCUSSION

Two-Chamber MFC

Any effects of lighting cycles on the microbial flora in the anode chamber were excluded by wrapping the anode chambers in aluminum foil (constant darkness). This also prevents any oxygenic photosynthetic organisms that may be present in the anode sludge from generating oxygen and draining electrons from the outer circuit [12]. Therefore, the evolution of the power density is only influenced by the cathode lighting conditions.

The generated power density (PD) and the dissolved oxygen (DO) at the A1 cell cathode are represented in Figure 1. It is clear that the DO closely follows the light/dark cycles, proving that it is produced via photosynthesis by the immobilized cyanobacteria.

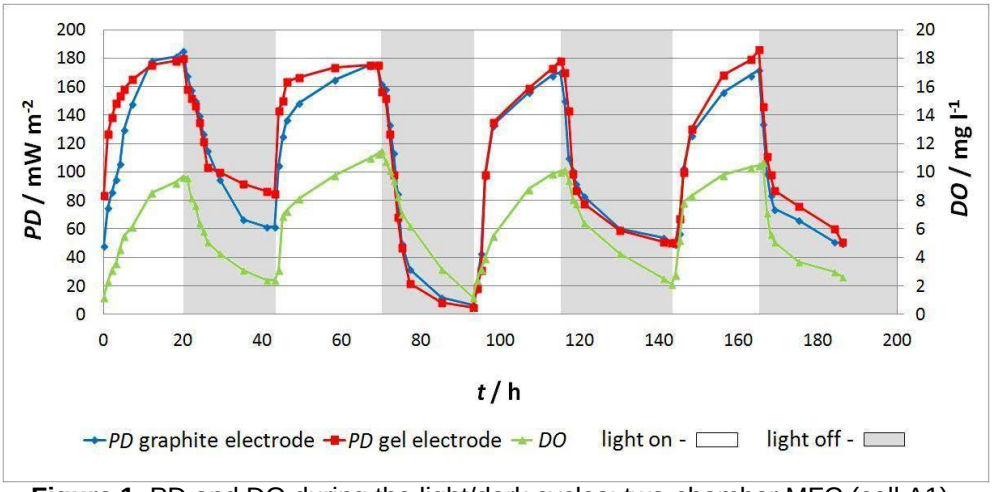


Figure 1. PD and DO during the light/dark cycles: two-chamber MFC (cell A1). Two electrodes were present in the cathode chamber: a graphite electrode and an electrode coated in cyanobacteria immobilized in gel

DO increases from 1 mg l^{-1} when dark to 11 mg l^{-1} when illuminated. The power density, in turn, closely follows the DO concentration. The power densities have comparable values for the immobilized *Synechocystis* cathode and the reference cathode.

In the control experiment represented in Figure 2, the DO reached 7.4 mg l^{-1} and the PD oscillated slightly around 130 mW m^{-2}. In the cell A1 cathode chamber, the DO concentration varied between 0 and 11.5 mg l^{-1}, under the influence of the light cycles. Whenever the DO in A1 reached 7 mg l^{-1} (like in the control experiment), the power output was also similar to that of the control cell (140-160 mW m^{-2} compared to the 130 for the control cell). However, at the end of the light on period, the PD was 30% higher and the DO was 57% higher than the corresponding values in the control experiment.

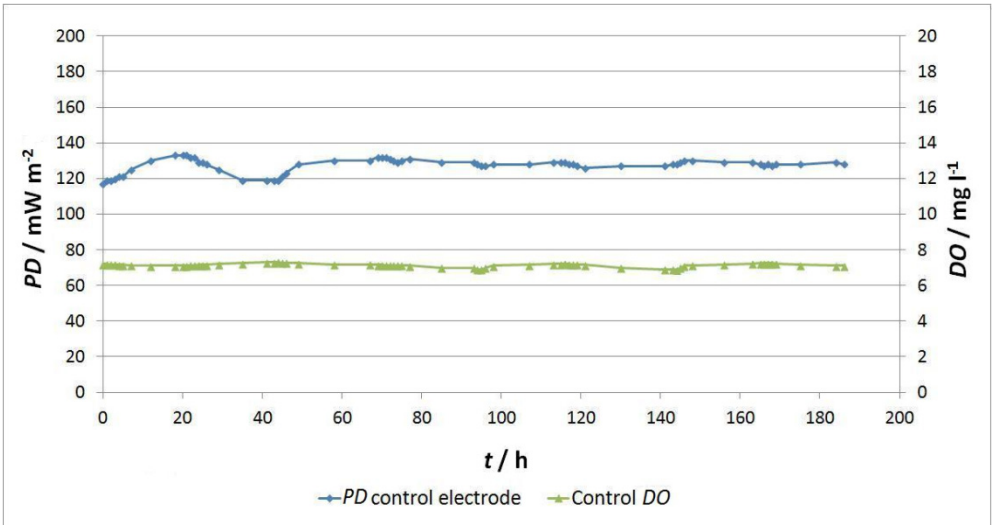


Figure 2. *PD* and *DO* in the control experiment, i.e. oxygen provided by bubbling air at the graphite cathode – two-chamber MFC (cell A0)

Membraneless MFC

It has been observed that the DO and PD dependencies on the light/dark cycles for the membraneless MFC are similar to that of the two-chamber MFC, Figure 3.

The DO produced via photosynthesis varies between 0.2 mg l^{-1} and 4.5 mg l^{-1} and in the control experiment DO reaches 5.5 mg l^{-1}. The PD in the control experiment is 15 mW m^{-2}, greater than that of the graphite electrode at the end of light on period (2 – 8 mW m^{-2}), but far less than the 45 – 60 mW m^{-2} of the immobilized *Synechocystis* cathode, Figure 4.

The immobilized *Synechocystis* had a notable effect on the power density of the gel electrode in the membraneless MFC. During the illumination period, the PD generated by the gel electrode was 8 to 20 times greater than that generated by the graphite electrode.

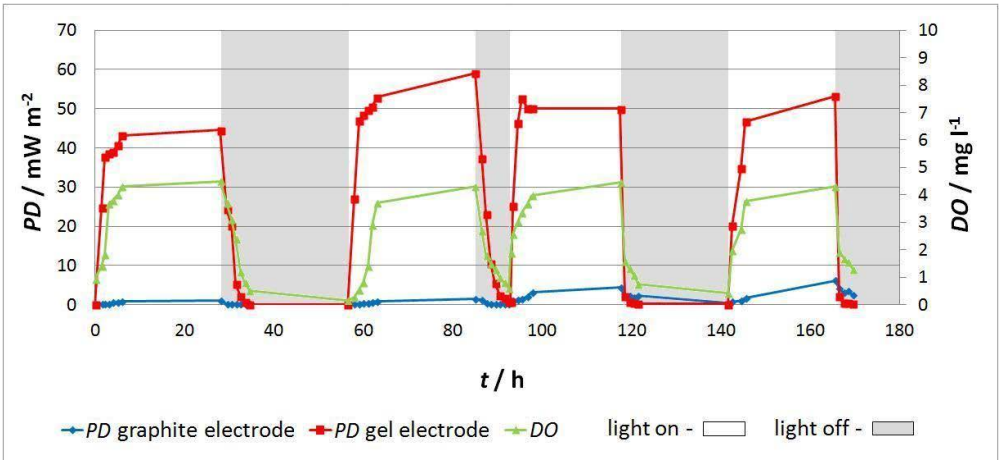


Figure 3. PD and DO during the light/dark cycles – membraneless MFC (cell M1). There are two cathodes: a graphite and an immobilized *Synechocystis* in gel cathode

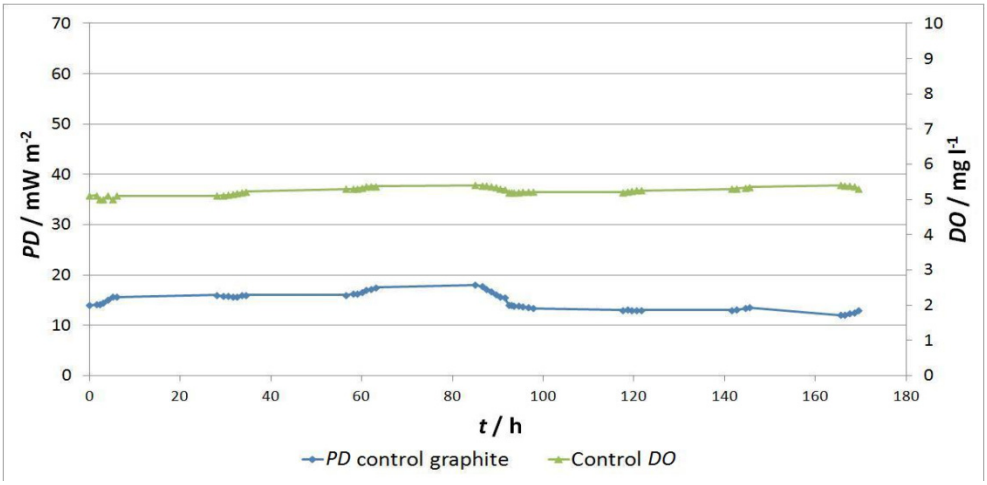


Figure 4. *PD* and *DO* in the control experiment, i.e. oxygen provided by bubbling air at the graphite cathode – membraneless MFC (cell M0)

Despite the decrease in coulombic efficiency determined by the high concentration of DO at the cathode, because of the increase in diffusion of oxygen to the anode [1] [13] [14], the power density will increase along with the DO. It can thus be inferred that in the case of the membraneless MFC, in the gel containing *Synechocystis*, the DO is considerably higher than in the bulk liquid which surrounds the two cathodes. In what regards the two-

chambered MFC, a difference between the power densities produced by the two cathodes cannot be observed. As a result it can be presumed that for the two-chambered MFC the DO is in equilibrium at the gel zone and at the bulk liquid which surrounds the two cathodes (both cathodes present the same concentration of DO).

The DO for the membraneless MFC is higher in the gel than in the bulk liquid which surrounds the two cathodes due to the consumption of oxygen by the anode bacteria.

CONCLUSION

In two MFCs geometries, bubbling air was successfully replaced by photosynthesizing *Synechocystis* immobilized on the cathode. The gel matrix allowed the photosynthesized oxygen to flow in the catholite: in the case of two-chamber cells, the *DO* is at equilibrium between the gel and the catholite, whereas in the case of membraneless MFCs, the *DO* concentration is higher in the gel than in the catholite.

High *PD* requires high *DO*, even if the coulombic efficiency decreases.

In the case of two-chamber MFCs, the power densities have comparable values for the immobilized *Synechocystis* cathode and the reference cathode because both have access to the same *DO* concentration (in equilibrium between gel and catholyte). The *PD* for the immobilized *Synechocystis* cathode, as well for the graphite cathode for A1 MFC is 30% greater than that generated in the control experiment because the *DO* concentration produced by photosynthesis was higher than that obtained through bubbling.

In the case of membraneless MFCs, the power density increases up to 20 times during illumination in the case of the immobilized *Synechocystis* cathode compared to the reference graphite cathode for the M1 MFC. The *PD* for the immobilized *Synechocystis* cathode is 3 to 4 times greater than that generated in the control experiment.

EXPERIMENTAL SECTION

Two-Chamber Cell Design

Two Plexiglas rectangular bottles (working volume of 200 ml each) were separated by a cation exchange membrane (Nafion, 90 μm thick, AlfaAesar), 3.14 cm^2 in surface. Spectroscopically pure rod-shaped graphite electrodes (length = 10 cm, diameter = 0.6 cm) were used for both the anode and the reference cathode. A second cathode covered with immobilized Synechocystis was also present in the cathode chamber, Figure 5A). The distance between anode-cathode was 5 cm.

The anode compartment contained sludge collected from the wastewater plant of Cluj-Napoca, Romania. The cathode compartment contained Zarrouk medium.

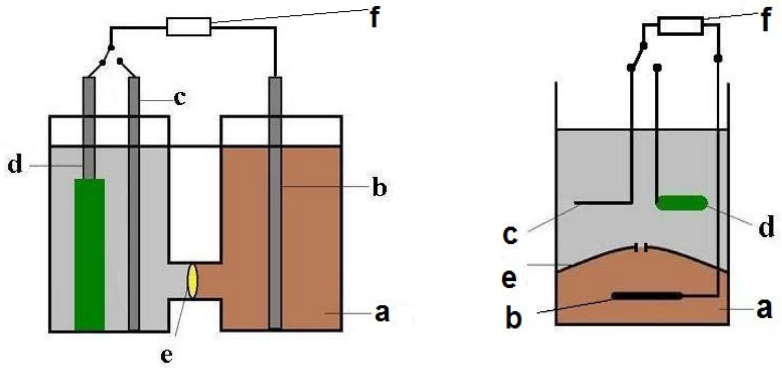
Membraneless Cell Design

A glass cylinder with a diameter of 10 cm, a height of 15 cm and 1000 ml working volume was used for the membraneless MFC. The 4 cm thick sludge layer at the bottom of the cylinder was separated from the clear water above by a conically shaped porous cloth.

The conical porous cloth, with a hole (0.5 cm^2) in the centre, separates the sludge from the clear water and allows the gas bubbles generated to leave the sludge (Figure 5B).

The electrodes were made of 3 mm thick rectangular graphite plates. The surfaces of the electrodes were: 30 cm^2 for the anode, 8 cm^2 for the reference cathode and 11 cm^2 for the cathode covered with immobilized cyanobacteria. The distance between the anode-cathode was 5 cm. When air (oxygen) was supplied to the cathode, an aquarium pump with a flow rate of 6 l h^{-1} was used.

All the MFCs used in this study (two-chamber and membraneless) were enriched for approximately 500 days by periodically (2 days) feeding with 2 ml 1 M sodium acetate. This rhythm of feeding was found to assure a constant power yield. The MFCs were kept at room temperature and continuously loaded with an external resistance of 1 kΩ.



(A) (B)

Figure 5. (A) Schematic of two-chamber MFC: a – sludge, b– graphite anode, c – graphite cathode ,d– immobilized cyanobacteria cathode, e – Nafion membrane, f – load; (B) Schematic of the membraneless MFC: a – sludge, b – graphite anode, c – graphite cathode, d – immobilized cyanobacteria cathode, e – porous cloth, f – load

Cyanobacteria immobilization on the cathode

An ideal immobilization matrix would be functional at ambient temperatures, resist in harsh wastewater conditions and allow the flow of nutrients and oxygen, while at the same time effectively immobilizing the cells within [15].

12 ml of *Synechocystis* culture (≈ $5 \cdot 10^6$ cells ml^{-1}) were mixed with 10 ml nutritive solution containing 1% agar agar dissolved in advance through heating. The cooled mixture was poured into a mould containing the graphite cathode. After curing, the mould was removed (Figure 6). The gel thickness around the cathode was approximately 5 mm.

(A) (B)

Figure 6. Image of the immobilized *Synechocystis* cathode and the control cathode (A) for two-chamber (B) for membraneless MFC.

The Experiment

We built one type of two-chamber cell and one type of membraneless cell, as follows:

A1 – two-chamber cell containing the reference graphite cathode and the immobilized *Synechocystis* cathode in the nutritive solution. The oxygen is generated by photosynthesis.

A0 – two-chamber cell containing the reference graphite cathode in absence of the immobilized photobiocatalyst, but with air bubbling for oxygen supply.

M1 – the membraneless type cell containing the reference graphite cathode and the immobilized *Synechocystis* cathode. The oxygen is generated by photosynthesis.

M0 – the membraneless type containing the reference graphite cathode in absence of the immobilized photobiocatalyst, but with air bubbling as oxygen supplier.

A control experiment has been developed using the A0 and M0 respectively, in order to compare MFC cells with immobilized photobiocatalyst on the cathode as oxygen supplier with standard cells without immobilized photobiocatalyst on the cathode but with air bubbling. The control cells A0 and M0 were in fact the A1 and M1 respectively, where the original cathodes were switched with the control ones.

Measurements of the power density – normalized to the projected surface of the graphite cathode – and of the dissolved oxygen in the bulk liquid at the cathode for light/dark cycles have been made. The luminous flux varied from 2500 lx (light on) to 130 lx (light off).

During the experiment, only the cathode region was subjected to light/dark cycles, whereas the anode region was wrapped in aluminum foil.

The amperage and voltage were measured with the multimeter PeakTech 3340 DMM (PeakTech Prüf- und Messtechnik GmbH Germany), and the DO was measured with Multi 350i (WTW Germany).

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