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ENHANCEMENT OF TOLUENE DEGRADATION AND ELECTRICITY GENERATION USING SPIRULINA MICROALGAE IN A MICROBIAL FUEL CELL

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ABSTRACT

The microbial fuel cell (MFC) is a new type of green technology, combining pollution treatment and renewable energy. In a MFC system, the energy released from the degraation of organic contaminants is harnessed as electricity. One can increase the MFC's power production efficiency by using oxygen, which has a high oxidation-reduction potential, as the system's terminal electron acceptor. In our present study, bacteria cultured under microaerobic conditions to decompose BTEX (benzene, toluene, ethybenzene, and xylene) were introduced into the anode chamber of a cube-type MFC. Toluene was added to the anode chamber, and the subsequent biodegradation of toluene by bacteria released both electrons and protons, which then migrated to the cathode (electrons via the wire, and protons through the proton exchange membrane) and reacted with the oxygen produced by the microalgae Spirulina via photosynthesis to form water. Our experimental results show that a sufficient concentration of dissolved oxygen (DO) must be maintained within the cathode in order for the MFC system to generate electricity. Furthermore, we found that when Spirulina was added to the cathode, DO levels stayed high enough to sustain continuous toluene degradation and power generation. We conclude from these results that the microalgae Spirulina is suitable as an oxygen source in the MFC cathode with its ability to maintain DO concentrations, thus stabilizing the MFC's power output and extending the system's effective lifespan.

Keywords: Microbial fuel cell, toluene, microalgae, photosynthesis, electricity generation, dissolved oxygen.

1. INTRODUCTION

A traditional microbial fuel cell (MFC) system is composed of anode and cathode chambers separated by a proton exchange membrane (PEM). Bacteria present in the anode chamber decompose the organic matter introduced into the chamber, releasing protons and electrons as byproducts. The protons pass through the PEM and, when the two electrodes are connected in a circuit, the electrons travel through the wire from anode to cathode chamber, creating a current; once both are present in the cathode, they react with a terminal electron acceptor (e.g. oxygen) to produce water as the product (Moqsud *et al*. 2013; Tanikkul and Pisutpaisal, 2017). In practice, bacteria in the MFC require organic matter as fuel to produce current, and studies have shown that different substrates result in different currents. Thus far several wastewater sources have been tested, including brewery wastewater (Liu *et al*. 2017; Lu *et al*. 2017), swine wastewater (Ma *et al*. 2016a; Ma *et al*. 2016b), dye wastewater (Solanki *et al*. 2013; Zou and Wang 2017), and industrial wastewater (Abbasi *et al*. 2016; Zhang *et al*. 2010). With regards to industrial wastewater, Luo *et al*. (2009) through multiple experiments demonstrated that the biodegradation of phenol could produce an electric current; the following year, Zhang *et al*. (2010) discovered that benzene and toluene could also serve as suitable substrates.

Under normal conditions, bacteria can utilize the dissolved oxygen (DO) in their surroundings as terminal electron acceptors with decomposing organic substrates via cellular respiration. Predominantly DO is inversely related to the depth; as one goes deeper, DO gradually approaches microaerobic (< 1.0 mg L-1) levels (White *et al*. 1990). Bioremediation has been proven to be much more efficient in aerobic environments than anaerobic ones (Salminen *et al*. 2004). However, aerobic bioremediation requires that DO levels be maintained high enough for the microbes to continually consume, increasing the total cost of treatment and power production. From these results, groundwater can be classified as a microaerobic environment and is thus difficult to treat via aerobic bioremediation. However, according to published literature, oxygen can act as the terminal electron acceptor for respiration even when separated from the bacteria (Rhoads *et al*. 2005). In other words, it is possible for respiration to proceed under anoxic conditions as long as the electrons are able to eventually react with a terminal electron acceptor. Thus, by initiating cellular respiration under anaerobic or microaerobic conditions,

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one can increase the amount of electrons harvested as electrical energy before they complete respiration by reducing a terminal electron acceptor. Using the techniques discussed above, the MFC system could be considered as a viable option to simultaneously degrade organic contaminants while generating power under microaerobic conditions.

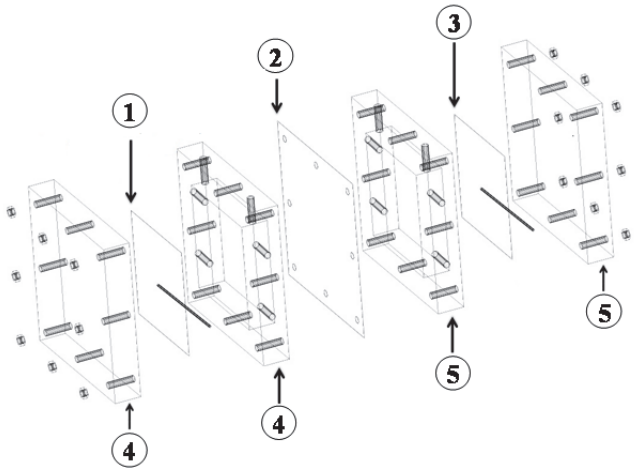
When using a MFC, one must consider that, in addition to the type of fuel and microorganisms used in the anode, the terminal electron acceptor selected for use in the cathode will also affect power-production efficiency. Some common electron acceptors include nitrates, sulfates, ferricyanide (Liu *et al*. 2014), and permanganate (You *et al*. 2006). However, when these salts are used in the system, they are reduced, releasing unwanted byproducts. The treatment of these byproducts, combined with the fact that the salts must be continuously replenished to maintain electricity production renders them practically not sustainable. Oxygen, on the other hand, is easy to acquire, has a relatively high oxidation-reduction potential, and does not produce dangerous side-products after reduction. The only challenge of using oxygen as the terminal electron acceptor is maintaining sufficient concentrations of it in the cathode chamber; according to published literature, the concentration of DO in water under atmospheric conditions is insufficient for the MFC system to function efficiently (Jang *et al*. 2004). Thus, a suitable method is needed to maintain a high DO to improve biodegradation and power generation efficiency.

Keeping the cost of production in mind, selecting microalgae to serve as a source of oxygen is theoretically practical, as it eliminates the need to continuously pump oxygen into the system. When microalgae undergo photosynthesis, CO_2 is consumed to produce O_2. This characteristic can be utilized in the cathode chamber to increase DO (Saba *et al*. 2017); in open ponds, DO levels have been recorded to rise as high as 25-40 mg L^{-1} (Weissman *et al*. 1988), much higher than the measured 8.2 mg L^{-1} at Sewage Treatment Plants (Tchobanoglous *et al*. 2014). According to De Schamphelaire and Vertraete (2009), certain species of microalgae are able to produce sufficient amounts of O_2 to keep the MFC system functional. In this study, we selected the fast-growing, high oxygen output microalgae Spirulina platensis (Hoshino *et al*. 1991). By adding toluene to the anode chamber and using Spirulina to produce oxygen in the cathode, we examined the relationship between toluene degradation and Spirulina oxygen output under various light conditions.

2. MATERIAL AND METHODS

2.1 Construction of Microbial Fuel Cell

We constructed a cube-type MFC (Fig. 1) using acrylic sheets with an effective volume of 500 cm^3 (10 cm × 10 cm × 5 cm). The cathode and anode chambers were separated by a PEM made of DuPont™ Nafion®117, with a surface area of 100 cm^2 (10 cm × 10 cm). Inflow and outflow ports were drilled into the sides and top of the cell, and openings were made for connections to external circuits. In each chamber, a square (10 cm × 10 cm) piece of carbon cloth (Hephas Energy, Thickness: 0.65 mm, Taiwan) served as the electrode. The cathode and anode were connected by a copper wire with a 1 kΩ resistor, forming a closed circuit system. The MFC system's voltage and power output efficiency were monitored using a multifunction voltmeter (Model 48R, CHY, Taiwan).



① Carbon cloth (Anode); ② Proton Exchange Membrane;
③ Carbon cloth (Cathode); ④Anode chamber; ⑤Cathode chamber

Fig. 1 Schematic diagram of microbial fuel cell

2.2 Bacteria and Microalgae

The bacteria for this study came from a mixed culture taken from the waste sludge of a petroleum treatment plant in Yunlin (Nan-Ya Plastics Corporation, No. 6 Naphtha Cracking Project, Yunlin County). The culture was grown in a 5-L separatory funnel under microaerobic conditions using a constant influx of N_2. During this period, BTEX (benzene, toluene, ethybenzene, and xylene) was introduced as the sole carbon source, with a set daily inflow of 250 μL BTEX (approximately 55 mg L^{-1} initially), to isolate a mixed-culture capable of degrading BTEX.

The fluid used in the MFC system was phosphate buffer (pH 7.0) with some added minerals; each liter of solution contained 4.27 g K_2HPO_4, 3.48 g KH_2PO_4, 0.95 g NH_4Cl, 0.53 g $MgCl_2$ $MgCl_2 \cdot 6H_2O$ $6H_2O$, 0.018g $CaCl_2 \cdot 2H_2O$, 10 g $NaHCO_3$, 0.01 mg $CuCl_2 \cdot 2H_2O$, 0.2 mg $CoCl_2 \cdot 6H_2O$, 0.047 mg $ZnCl_2$, 0.03 mg $MnCl_2 \cdot 4H_2O$, 0.03 mg $Na_2MoO_4 \cdot 2H_2O$, 0.02 mg $NiCl_2 \cdot 6H_2O$, and 0.972 mg $FeCl_3 \cdot 6H_2O$.

The microalga S. platensis (Taihsi Branch, Fisheries Research Institute, Council of Agriculture) provided a source of terminal electron acceptor (oxygen) in the cathode. It was operated under alternating 12-h light-dark cycles, with a light intensity of 8000 lx (Lux meter, LX-101, Lutron, Taiwan). For this experiment, 20 mL of mixed-culture bacteria (27.7 mg L^{-1}) were added to the anode chamber, and 20 mL of the Spirulina culture (0.4 g L^{-1}) were added into the cathode. Various light intensities and cycles were used to test the effects of light on toluene-degradation and electricity generation efficiencies.

2.3 MFC Power Output Monitoring and Analysis

The MFC system's output voltage was monitored using a digital voltmeter (Model 48R, CHY, Taiwan). Power density ($W\ m^{-2}$) was calculated using the formula $P = IV/A$, where I represent current (A), V represent voltage (Rashid *et al*. 2013), and A is the effective anodal surface area (m^2). Coulombic efficiency (C_E, %) was calculated as $C_E = C_P/C_T$, where C_P is defined as the experimental number of coulombs calculated by integrating the measured current over time, and C_T is the theoretical number of coulombs possible in the added substrate as calculated from chemical oxygen de-

mand measurements. Using toluene as the substrate, C_E was calculated as follows:

$$C_E(\%) = \frac{M \int_0^{t_b} Idt}{F\, b_{es}\, V_{An}(\Delta C \times 100\%)} \quad (1)$$

Where F is the Faraday constant (96,485 C mol^{-1} e^-), b_{es} is a defined number of moles of electrons for a given substrate (b_{es} for toluene is 36), ΔC is the change in toluene concentration (g L^{-1}), V_{an} is the liquid volume in the anode chamber, and M is the molar mass of toluene (92 g mol^{-1}).

2.4 Measurement of MFC Internal Resistance

The MFC system's internal resistance was estimated using both the power density peak and polarization slope methods as described by Feng *et al*. (2008). In the power density peak method, different resistances were applied to the system (50 Ω to 1 MΩ in set intervals), and the voltages produced were measured. The power density curve was then calculated from the recorded voltages using $P = E2R$ (where P is power, E is the system's voltage, and R is resistance). According to equation (2) below (Feng *et al*. 2008), the maximum value for power density is obtained when internal resistance and external resistance are equal:

$$P_{\max} = \frac{OCV^2 \times R_{ext}}{(R_{\text{int}} + R_{ext})^2} \quad (2)$$

where $P_{\max}$ is the maximum power density (W m^{-2}), OCV is the measured open circuit voltage, and R_{int} and R_{ext} (Ω) are the internal and external resistances, respectively. The two methods, polarization slope and power density peak method, are similar to each other except for the fact that the polarization curve is produced by plotting the current density (normalized by the anodal surface area) against the recorded voltages.

2.5 Toluene Concentration Measurement

In this experiment, toluene was added to the anode chamber at three different concentrations (10.4, 17.3, and 34.6 mg L^{-1}). These concentrations were measured by drawing out 1 μL sample from the anodal chamber into a gastight syringe (Hamilton, USA), and injecting them into a gas chromatography- flame ionization detector (GC-14B, Shimadzu, Japan). During chromatography, the oven temperature was maintained at 105°C, the column inlet temperature at 200°C, and the detector oven temperature at 250°C. A combination of hydrogen and air was used as fuel for the detector flame with N_2 as the carrier gas. A calibration curve (R^2 = 0.9993) was created using solutions with known toluene concentrations. Then, the unknown samples were tested and the measured values were interpolated onto the curve to determine concentrations.

2.6 Measuring DO Levels

DO levels were monitored using a multifunction meter (SX751, Major Science, Taiwan). By placing the probe in the anode and cathode chamber, and recording the displayed values, we measured changes in DO.

3. RESULTS AND DISCUSSION

3.1 Effects of Limited Oxygen in the Cathode

In a MFC system, oxygen acts as a terminal electron acceptor in the cathode to react with the protons and electrons released by the biodegradation of organic matter in the anode chamber, forming water as the final product (Oh *et al*. 2009). Once the concentration of DO decreases to the point where there is not enough oxygen to react with the released protons and electrons, the MFC system will cease generating electricity.

In this study, we used phosphate buffer solution with limited amount of oxygen, as the MFC system's terminal electron acceptor to study microbial toluene degradation and electricity generation. Theoretically, the saturation point of dissolved oxygen in deionized water at 101 kPa (760 mm Hg) and 25°C is approximately 8.24 mg L^{-1} (Tchobanoglous *et al*. 2014). In our experiment, we found the DO saturation concentration of the phosphate buffer to be 7.0 ± 1.0 mg L^{-1}. As shown in Fig. 2(a), from t = 0 to t = 5 h the voltage stabilized at approximately 0.41 ± 0.31 mV, showing that no noticeable changes in voltage occur before adding toluene. At t = 5 h, toluene was added to three systems at concentrations of 10.4, 17.3, and 34.6 mg L^{-1}. Once the toluene was added and biodegradation began, the system's voltage steadily increased, until they reached maximum values of 39.6 mV (10.4 mg L^{-1}), 59.3 mV (17.3 mg L^{-1}), and 84.7 mV (34.6 mg L^{-1}) at t = 12.7, 11.2, and 13.8 h, respectively. Biodegradation was completed after 15.3, 18.7, and 25.6 h, after which the voltages decreased, finally returning to their initial values after 19.8, 25.0, and 38.6 h (Table 1). These results show that when toluene concentrations are within the microorganism's tolerance levels (10.4 ~ 34.6 mg L^{-1}), the initial toluene concentration and system's maximum achieved voltage are directly related. After the CE for the three concentrations were calculated to be 1.0, 1.3, and 1.7%, respectively, we concluded that CE is also directly related to initial toluene concentration.

For the three toluene concentrations, the maximum power densities were 0.18, 0.18, and 0.20 mW m^{-2} (average of 0.19 mW m^{-2} across all three systems), while the current densities were 2.09, 2.07, and 2.23 mA m^{-2} (average of 2.13 mA m^{-2}). Using the polarization slope method, the internal resistances of the three MFCs were determined to be 2.0, 2.1, and 1.9 kΩ (average of 2.0 kΩ). These results demonstrate that under stable conditions and constant internal resistance, the initial toluene concentration added to the system has no significant effect on power output density or internal resistance.

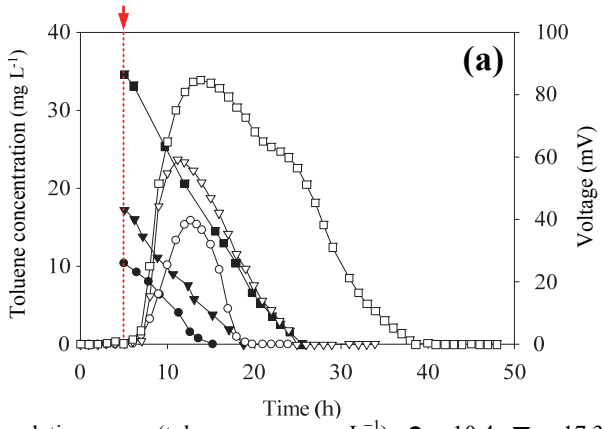
Toluene was added to the anode chamber in successive portions at an initial concentration of 34.6 mg L^{-1} (Fig. 2(b)); after the first addition, it was observed that along with electricity production, the concentration of DO in the cathode decreased from 6.2 to 4.0 mg L^{-1}. Once biodegradations of the added toluene reached completion, oxygen consumption and DO stabilized at 4.0 ± 0.2 mg L^{-1}. These results show that when the MFC system has no organic matter for bacteria to degrade, protons and electrons are not released as byproducts, without which the system cannot proceed with power generation. Thus, even though the cathode contained plenty of terminal electron acceptors, the lack of proton or electron available to reduce the oxygen explained the stabilization of DO at 4.0 ± 0.2 mg L^{-1} after the toluene was completely consumed. At t = 60 h, toluene was added a second time; DO decreased from 4.0 to 1.5 mg L^{-1}, and the maximum voltage was 84.7 mV. Once again, DO stabilized as soon as biodegradation was completed, producing

Table 1 Effects of anodic toluene concentration on biodegradation and electricity-generation efficiencies

Items	Microaerobic			Limited dissolved oxygen			Darkness			Light			Alternating Light-Dark		
Toluene concentration (mg L^{-1})	10.4	17.3	34.6	10.4	17.3	34.6	10.4	17.3	34.6	10.4	17.3	34.6	10.4	17.3	34.6
Biodegradation time (h)	39.7	47.6	66.0	15.3	18.7	25.6	16.4	19.6	27.2	14.9	19.3	26.8	15.7	19.0	26.2
Maximum voltage (mV)	-	-	-	39.6	59.3	84.7	9.8	16.2	24.4	37.8	57.4	84.6	39.5	60.9	86.5
Time at appearance of maximum voltage (h)	-	-	-	12.7	11.2	13.8	15.6	24.5	33.7	14.4	19.1	9.3	12.0	12.9	9.7
Initial voltage (mV)	-	-	-	19.8	25.0	38.6	22.3	30.5	40.2	19.0	24.3	36.8	19.8	29.2	40.5
Coulombic efficiency (%)	-	-	-	1.0	1.3	1.7	0.3	0.7	0.7	1.1	1.6	2.4	1.6	2.6	2.5
Maximum power density (mW m^{-2})	-	-	-	0.2	0.2	0.2	-	-	-	0.3	0.3	0.3	0.8	0.8	0.8
Internal resistance (kΩ)	-	-	-	2.0	2.1	1.9	-	-	-	3.0	3.0	3.0	3.0	3.0	3.0
Open circuit voltage (mV)	-	-	-	296	290	308	-	-	-	364	364	367	464	464	465

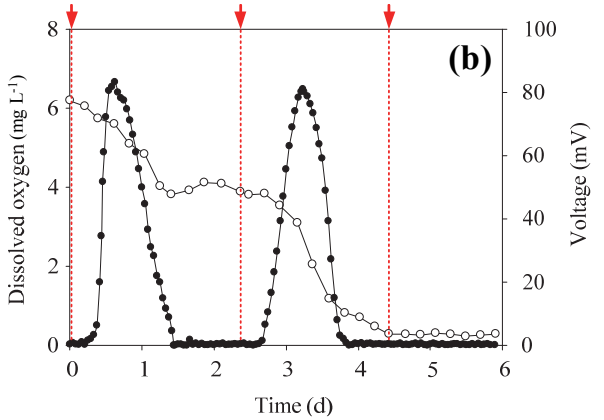
"-" Data were not recorded.

results similar to those observed after the first addition. Finally, at t = 108 h one last toluene addition was made. This time the DO levels decreased to 0 mg L^{-1}, and because the cathode was no longer able to provide enough electron acceptors to react with the protons and electrons from the anode, power generation was interrupted. Taking all of the above into consideration, we concluded that for efficient electricity generation, in addition to considering the type of organic matter used as fuel, one must also ensure that cathodic DO is high enough to react with all of the protons and electrons from the anode (Oh *et al*. 2004).



Degradation curve (toluene conc., mg L^{-1}): ● − 10.4, ▼ − 17.3, ■ − 34.6, Voltage (toluene conc., mg L^{-1}): ○ − 10.4, ▽ − 17.3, □ − 34.6, ➔ Added toluene

(a) Degradation and voltage output



Dissolved oxygen (○), voltage (●), Added toluene (➔)

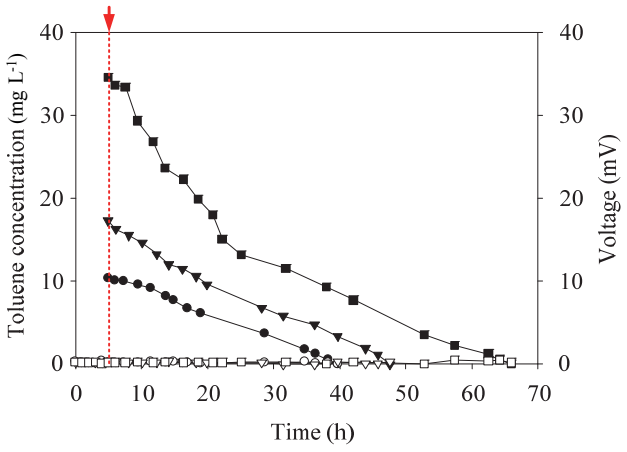
(b) Dissolved oxygen and voltage output

Fig. 2 Performance of MFC under limited oxygen conditions

3.2 Effects of Cathodic Hypoxia on Toluene Biodegradation and Electricity Generation

In this study, nitrogen gas was pumped into the MFC to remove oxygen from the system, creating a microaerobic environment. Under these conditions, when different concentrations of toluene were added to the anode chamber, the bacteria required different amounts of time to degrade the toluene (Fig. 3). At the three concentrations of toluene (10.4, 17.3, and 34.6 mg L^{-1}), complete biodegradation took 34.7, 42.6, and 61.0 h, respectively. Compared to the aerobic systems, which required 15.3, 18.7, and 25.6 h for biodegradation of toluene under microaerobic conditions, it required more than double the time to complete, demonstrating that when the cathode is unable to provide sufficient amounts of oxygen, an "open circuit" phenomenon will occur in the system, where the protons and electrons produced from toluene degradation are unable to travel to the cathode. Without the continuous flow of electrons from the anode to the cathode, electric power is not generated (Zhou *et al*. 2012).

Generally speaking, a MFC uses cellular respiration to decompose organic contaminants, and the protons and electrons released in the process can be converted into electrical power (Vicari *et al*. 2017). While respiration will continue regardless of whether oxygen is present, the presence of a terminal electron acceptor affects the rate at which the process continues. In an open circuit MFC system, there is no way for the electrons and protons in the



Degradation curve (toluene conc., mg L^{-1}): ● − 10.4, ▼ − 17.3, ■ − 34.6, Voltage (toluene conc., mg L^{-1}): ○ − 10.4, ▽ − 17.3, □ − 34.6, ➔ Added toluene

Fig. 3 Degradation and voltage output of MFC under microaerobic conditions

anode to react with the terminal electron acceptor in the cathode. Instead, the reaction is forced to proceed using the ions present in the anode chamber (*e.g.,* nitrate and sulfate ions); without adequate amounts of electron acceptors, the rate of degradation decreases, reducing the system's efficiency (Huang *et al*. 2011). However, when the system's cathode and anode are connected to form a closed circuit, the protons and electrons are able to travel between chambers and complete the reaction in the cathode. This shows that biodegradation of organic matter and power generation can proceed efficiently even in anaerobic conditions, as long as a terminal electron acceptor is present in sufficient concentrations to complete the reaction (Cheng *et al*. 2015; Morris and Jin 2009).

3.3 Effects of Spirulina under Full Light on Toluene Biodegradation and Electricity Generation

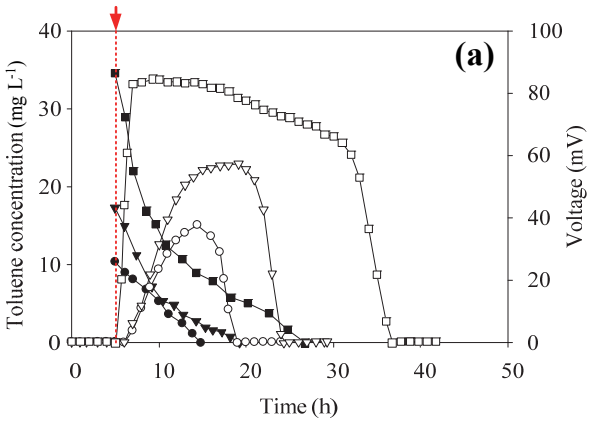
When using oxygen as the terminal electron acceptor in the MFC system's cathode chamber, it is continuously consumed as bacteria degrade organic contaminants in the anode. Since biodegradation, and subsequently power output, is directly affected by the amount of oxygen available for reduction, cathodic DO concentration should be considered as one of the key factors in determining MFC power generation efficiency (Rago *et al*. 2017). While it is possible to directly pump oxygen into the cathode, doing so would significantly increase the cost of production. When placed in the cathode, microalgae are able to consume inorganic carbon in solution (given as $NaHCO_3$) and produce carbohydrates and oxygen through photosynthesis, the latter of which then acts as a terminal electron acceptor for the proton and electron byproducts from the anode chamber. Thus, to reduce the cost of production, microalgae can act as an oxygen source via photosynthesis (Powell *et al*. 2009; Strik *et al*. 2010).

In this study, we tried using the microalga S. plantesis to replenish the amount of oxygen in the cathode and consequently stabilize the MFC system's power output. Given the initial toluene concentrations of 10.4, 17.3, and 34.6 mg L^{-1} (Table 1 and Fig. 4(a)), the systems took 14.1, 19.1, and 9.3 h, respectively, to achieve maximum voltages of 37.8, 57.4, and 84.6 mV.

Biodegradation of the added toluene was completed after 14.9, 19.3, and 26.8 h, and the recorded voltages returned to their initial values after 19.0, 24.3, and 36.8 h. The CEs of the systems were calculated to be 1.1%, 1.6%, and 2.4%; finally, using the polarization slope and power density peak methods, the maximum power density and internal resistance were calculated to be 0.3 mW m^{-2} and 3.1 kΩ, respectively.

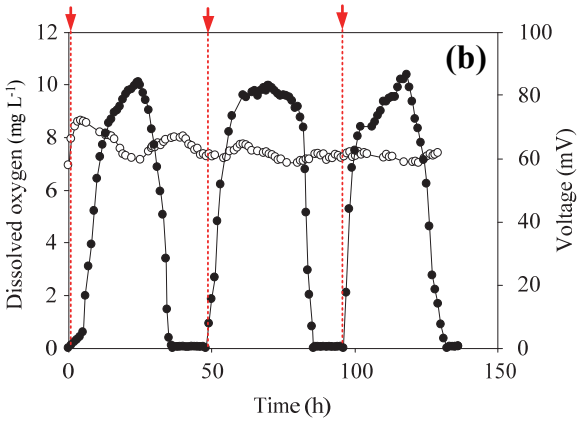
Using Spirulina as the system's oxygen source and an initial toluene concentration of 34.6 mg L^{-1} (Fig. 4(b)), we observed that voltage was related to toluene concentration; gradually increasing after each addition (at 0, 48, and 96 h) before returning to their initial values after the toluene was completely degraded. These results show that the system is able to treat organic contaminants through biodegradation, and that the amount of oxygen produced by the microalgae is sufficient to serve as terminal electron acceptors for the byproducts of toluene degradation. Also, we observed from changes in DO that, excluding the initial 24 h before the system reached a stable state, the microalgae were able to maintain a stable DO (average of 7.5 ± 0.5 mg L^{-1}).

However, when the cathode maintains such a high concentration of oxygen, some of it passes through the PEM via diffusion, turning the anode's anaerobic environment into a microaerobic one with a DO of 0.3 mg L^{-1} (measured over a 120-h period); this



Degradation curve (toluene conc., mg L^{-1}): ● − 10.4, ▼ − 17.3, ■ − 34.6, Voltage (toluene conc., mg L^{-1}): ○ − 10.4, ▽ − 17.3, □ − 34.6, ➔ Added toluene

(a) Degradation and voltage output



Dissolved oxygen (○), voltage (●), Added toluene (➔)

(b) Dissolved oxygen and voltage output

Fig. 4 MFC performance under full light conditions

results in some of the electrons reducing oxygen directly in the anode and subsequently lowering the MFC system's power output. Yet, while the diffusion of oxygen occurs continuously, due to the presence of facultative anaerobes in the mixed culture, the oxygen is consumed soon after crossing the PEM barrier, keeping the DO concentration in the anode chamber well under 0.3 mg L^{-1} and reducing the negative effect oxygen would otherwise have on the system's power generation efficiency.

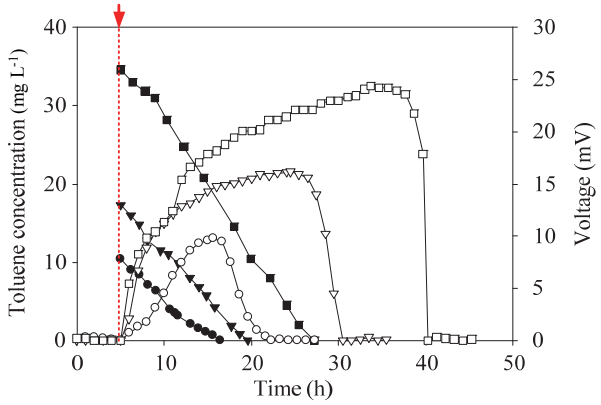
3.4 Effects of Spirulina without the Light on Toluene Biodegradation and Electricity Generation

In this study, Spirulina was added to the MFC cathode and the entire system was run in complete darkness. In the first 24 h before initiating biodegradation, it was observed that the DO levels in the anode chamber decreased from 7.5 to 1.0 mg L^{-1}, showing that under conditions without light not only is the microalgae unable to produce oxygen via photosynthesis, but also it consumes the oxygen present in the water to produce CO_2 via respiration (Siew Moi, 2004). By consuming oxygen, the microalgae decreases the number of terminal electron acceptors available to react with the electrons and protons released from the anode, thus reducing the over-

all efficiency of the MFC system. Given initial toluene concentrations of 10.4, 17.3, and 34.6 mg L^{-1}, the systems took 15.6, 24.5, and 33.7 h (respectively) to reach their maximum voltages of 9.8, 16.2, and 24.4 mV, completed biodegradation of the added toluene at 16.4, 19.6, and 27.2 h, and took 22.3, 30.5, and 40.2 h to return to their initial voltages (Fig. 5 and Table 1). Over this period, the average C_Es were calculated to be 0.3, 0.7, and 0.7%. The fact that the system was able to generate electricity even though DO was below 1.0 mg L^{-1} demonstrates that under microaerobic conditions the protons and electrons from the anode will find and react with any other possible electron acceptors (Feng *et al*. 2008; Kakarla and Min 2014). In this case, the CO_2 ($E^{0'} = -0.24$V) present may be reduced to form CH_4, as seen below in Equation 3 (Rittmann and McCarty 2007):

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \quad (3)$$

The fact that CO_2 has a lower oxidation-reduction potential than O_2 ($E^{0'} = 0.805$V) results in a lower power output, explaining the comparatively lower C_E values in these systems.

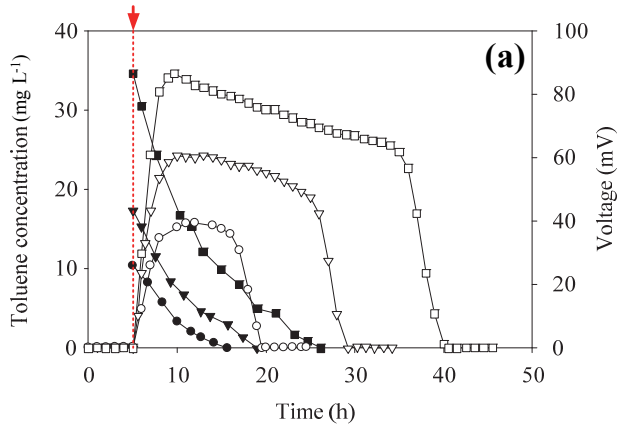


Degradation curve (toluene conc., mg L^{-1}): ● − 10.4, ▼ − 17.3, ■ − 34.6, Voltage (toluene conc., mg L^{-1}): ○ − 10.4, ▽ − 17.3, □ − 34.6, ➔ Added toluene

Fig. 5 Degradation and voltage output of MFC under full dark conditions

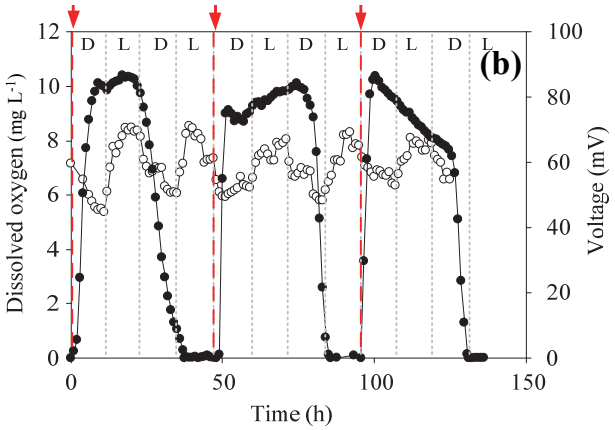
3.5 Effects of Spirulina under Alternating Light on Toluene Biodegradation and Electricity Generation

In this study, Spirulina was added to the system, which was placed under alternating 12-h intervals of light (intensity 8000 lux) and darkness. Using toluene as the organic fuel source, we investigated the effect of alternating light and dark conditions on biodegradation and power output efficiency. When toluene was added to the MFC systems in three concentrations (10.4, 17.3, and 34.6 mg L^{-1}), biodegradation reached completion after 15.7, 19.0, and 26.2 h, respectively (Fig. 6(a)). The systems achieved maximum voltages of 39.5, 60.9, and 86.5 mV after 12.0, 12.9, and 9.7 h before returning to their initial voltages after 19.6, 29.2, and 40.5 h. Using the methods detailed in section 2.3, the C_Es of the systems were calculated to be 1.6, 2.6, and 2.5%. Finally, using the polarization slope and power peak density methods, the maximum power density and internal resistance were found to be 0.8 mW m^{-2} and 3 kΩ, respectively (Table 1). After analyzing the data from all three toluene concentrations, we concluded that the initial toluene concentration did not have a significant effect on the system's final output density.



Degradation curve (toluene conc., mg L^{-1}): ● − 10.4, ▼ − 17.3, ■ − 34.6, Voltage (toluene conc., mg L^{-1}): ○ − 10.4, ▽ − 17.3, □ − 34.6, ➔ Added toluene

(a) Degradation and voltage output



Dissolved oxygen (○), voltage (●)
Added toluene (➔); D: dark, L: light

(b) Dissolved oxygen and voltage output

Fig. 6 MFC performance under alternating light-dark condition

When comparing values of the microalgal systems against the limited oxygen systems (Table 1), the systems containing Spirulina have a noticeably higher internal resistance. This higher resistance can be explained by the addition of $NaHCO_3$ needed to sustain the Spirulina microalgae which in turn dissociates into bicarbonate and sodium ions with the addition of water. While the bicarbonate ions are consumed the sodium ions are not, leading to their buildup, which prevents H^+ from crossing the PEM barrier as easily due to the electronic repulsion of cations, creating a higher internal resistance (Rozendal *et al*. 2006). Moreover, compared to the limited oxygen system in section 3.1, the light-dark microalgae system produced significantly higher CE. The main difference between the two systems comes from the microalgal system's effect on cathodic pH levels. While undergoing photosynthesis, Spirulina consumes the bicarbonate (HCO_3^-) ions present in solution, subsequently raising the pH and producing higher efficiencies in basic condition as similar to those observed by (He *et al*. 2008; Yuan *et al*. 2011).

Under alternating light-dark conditions, after Spirulina was added the DO concentration displayed a sinusoidal pattern (Fig. 6(b)). Under dark conditions, photosynthesis was interrupted while respiration continued, causing DO to decrease from 8.5 to 5.3 mg L^{-1}; under light conditions photosynthesis resumed, increasing DO

back to 8.5 mg L^{-1}. However, regardless of the large range of concentrations, there was enough oxygen present to continuously serve as terminal electron acceptors to complete the biodegradation and power generation process.

In the last alternating light-dark experiment, toluene was added at t = 0, 48, and 96 h, after which the recorded results were compared to the ones taken from an identical experiment under limited oxygen conditions. It was observed that DO in the light-dark system did not decrease as toluene was consumed, instead stabilizing at 7.0 ± 1.6 mg L^{-1}, eliminating oxygen as the limiting reactant. This gives the microalgal system an advantage over the limited oxygen system, which requires a continuous inflow of oxygen. In conclusion, the alternating light-dark system is a better option for maintaining a stable and sufficient concentration of dissolved oxygen.

4. CONCLUSIONS

Microalgae can be used as a source of oxygen to provide terminal electron acceptors for the MFC system to efficiently degrade organic contaminants and generate electricity. When microalgae were grown under "full light", "full dark", and "alternating light-dark" conditions, all three released sufficient amounts of oxygen to keep the system operating smoothly, with the "alternating light-dark" system producing the best results. In the future, we can use the knowledge gained from this study to treat other pollutants present in water, even under microaerobic conditions, to degrade organic contaminants and produce electricity.

REFERENCES

Abbasi, U., Jin, W., Pervez, A., Bhatti, Z.A., Tariq, M., Shaheen, S., Iqbal, A., and Mahmood, Q. (2016). "Anaerobic microbial fuel cell treating combined industrial wastewater: Correlation of electricity generation with pollutants." *Bioresource Technology*, **200**, 1-7.

Cheng, H.-Y., Liang, B., Mu, Y., Cui, M.-H., Li, K., Wu, W.-M., and Wang, A.-J. (2015). "Stimulation of oxygen to bioanode for energy recovery from recalcitrant organic matter aniline in microbial fuel cells (MFCs)." *Water Research*, **81**, 72-83.

De Schamphelaire, L. and Verstraete, W. (2009). "Revival of the biological sunlight-to-biogas energy conversion system." *Biotechnology Bioengineering*, **103**(2), 296-304.

Feng, Y., Wang, X., Logan, B.E., and Lee, H. (2008). "Brewery wastewater treatment using air-cathode microbial fuel cells." *Applied Microbiology Biotechnology*, **78**(5), 873-80.

He, Z., Huang, Y., Manohar, A.K., and Mansfeld, F. (2008). "Effect of electrolyte pH on the rate of the anodic and cathodic reactions in an air-cathode microbial fuel cell." *Bioelectrochemistry*, **74**(1), 78-82.

Hoshino, K., Hamochi, M., Mitsuhashi, S., and Tanishita, K. (1991). "Measurements of oxygen production rate in flowing Spirulina suspension." *Applied Microbiology and Biotechnology*, **35**(1), 89-93.

Huang, L., Gan, L., Zhao, Q., Logan, B.E., Lu, H., and Chen, G. (2011). "Degradation of pentachlorophenol with the presence of fermentable and non-fermentable co-substrates in a microbial fuel cell." *Bioresource Technology*, **102**(19), 8762-8768.

Jang, J.K., Pham, T.H., Chang, I.S., Kang, K.H., Moon, H., Cho, K.S., and Kim, B.H. (2004). "Construction and operation of a novel mediator- and membrane-less microbial fuel cell." *Process Biochemistry*, **39**(8), 1007-1012.

Kakarla, R. and Min, B. (2014). "Photoautotrophic microalgae Scenedesmus obliquus attached on a cathode as oxygen producers for microbial fuel cell (MFC) operation." *International Journal of Hydrogen Energy*, **39**(19), 10275-10283.

Liu, J., Tian, C., Jia, X., Xiong, J., Dong, S., Wang, L., and Bo, L. (2017). "The brewery wastewater treatment and membrane fouling mitigation strategies in anaerobic baffled anaerobic/aerobic membrane bioreactor." *Biochemical Engineering Journal*, **127**, 53-59.

Liu, X.-W., Li, W.-W., Yu, and H.-Q. (2014). "Cathodic catalysts in bioelectrochemical systems for energy recovery from wastewater." *Chemical Society Reviews*, **43**(22), 7718-7745.

Lu, M., Chen, S., Babanova, S., Phadke, S., Salvacion, M., Mirhosseini, A., Chan, S., Carpenter, K., Cortese, R., and Bretschger, O. (2017). "Long-term performance of a 20-L continuous flow microbial fuel cell for treatment of brewery wastewater." *Journal of Power Sources*, **356**, 274-287.

Luo, H., Liu, G., Zhang, R., and Jin, S. (2009). "Phenol degradation in microbial fuel cells." *Chemical Engineering Journal*, **147**(2), 259-264.

Ma, D., Jiang, Z.-H., Lay, C.-H., and Zhou, D. (2016a). "Electricity generation from swine wastewater in microbial fuel cell: Hydraulic reaction time effect." *International Journal of Hydrogen Energy*, **41**(46), 21820-21826.

Ma, J., Ni, H., Su, D., and Meng, X. (2016b). "Bioelectricity generation from pig farm wastewater in microbial fuel cell using carbon brush as electrode." *International Journal of Hydrogen Energy*, **41**(36), 16191-16195.

Moqsud, M.A., Omine, K., Yasufuku, N., Hyodo, M., and Nakata, Y. (2013). "Microbial fuel cell (MFC) for bioelectricity generation from organic wastes." *Waste Management*, **33**(11), 2465-2469.

Morris, J.M. and Jin, S. (2009). "Influence of NO_3 and SO_4 on power generation from microbial fuel cells." *Chemical Engineering Journal*, **153**(1), 127-130.

Oh, S., Min, B., and Logan, B.E. (2004). "Cathode Performance as a Factor in Electricity Generation in Microbial Fuel Cells." *Environmental Science & Technology*, **38**(18), 4900-4904.

Oh, S.E., Kim, J.R., Joo, J.H., and Logan, B.E. (2009). "Effects of applied voltages and dissolved oxygen on sustained power generation by microbial fuel cells." *Water Science Technology*, **60**(5), 1311-1317.

Powell, E.E., Mapiour, M.L., Evitts, R.W., and Hill, G.A. (2009). "Growth kinetics of Chlorella vulgaris and its use as a cathodic half cell." *Bioresource Technology*, **100**(1), 269-74.

Rago, L., Cristiani, P., Villa, F., Zecchin, S., Colombo, A., Cavalca, L., and Schievano, A. (2017). "Influences of dissolved oxygen concentration on biocathodic microbial communities in microbial fuel cells." *Bioelectrochemistry*, **116**, 39-51.

Rashid, N., Cui, Y.-F., Saif Ur Rehman, M., and Han, J.-I. (2013). Enhanced electricity generation by using algae biomass and activated sludge in microbial fuel cell. *Science of The Total Environment*, 456-457, 91-94.

Rhoads, A., Beyenal, H., and Lewandowski, Z. (2005). "Microbial fuel cell using anaerobic respiration as an anodic reaction and biomineralized manganese as a cathodic reactant." *Environmental Science & Technology*, **39**(12), 4666-71.

Rittmann, B.E. and McCarty, P.L. (2007). *Environmental Biotechnology: Principles and Applications*. McGraw-Hill, New York.

Rozendal, R.A., Hamelers, H.V.M., and Buisman, C.J.N. (2006). "Effects of membrane cation transport on pH and microbial fuel cell performance." *Environmental Science & Technology*, **40**(17), 5206-5211.

Saba, B., Christy, A.D., Yu, Z., and Co, A.C. (2017). "Sustainable power generation from bacterio-algal microbial fuel cells (MFCs): An overview." *Renewable and Sustainable Energy Reviews*, **73**, 75-84.

Salminen, J.M., Tuomi, P.M., Suortti, A.-M., and Jørgensen, K.S. (2004). Potential for aerobic and anaerobic biodegradation of petroleum hydrocarbons in boreal subsurface. *Biodegradation*, **15**(1), 29-39.

Siew Moi, P. (2004). Book Review of Handbook of Microalgal Culture. Biotechnology and Applied Phycology. *Journal of Applied Phycology*, **16**(2), 159-160.

Solanki, K., Subramanian, S., and Basu, S. (2013). Microbial fuel cells for azo dye treatment with electricity generation: A review. *Bioresource Technology*, **131**, 564-571.

Strik, D.P.B.T.B., Hamelers, H.V.M., and Buisman, C.J.N. (2010). Solar energy powered microbial fuel cell with a reversible bioelectrode. *Environmental Science & Technology*, 44(1), 532-537.

Tanikkul, P. and Pisutpaisal, N. (2017). Membrane-less MFC based biosensor for monitoring wastewater quality. *International Journal of Hydrogen Energy*, **43**(1), 483-489.

Tchobanoglous, G., Burton, F.L., and Stensel, H.D. (2014). *Wastewater Engineering: Treatment and Reuse*. McGraw-Hill, New York, NY.

Vicari, F., Mateo, S., Fernandez-Morales, F.J., Cañizares, P., Galia, A., Scialdone, O., and Rodrigo, M.A. (2017). Influence of the methodology of inoculation in the performance of air-breathing microbial fuel cells. *Journal of Electroanalytical Chemistry*, 803, 81-88.

Weissman, J.C., Goebel, R.P., and Benemann, J.R. (1988). Photobioreactor design: Mixing, carbon utilization, and oxygen accumulation. *Biotechnology Bioengineering*, **31**(4), 336-44.

White, A.F., Peterson, M.L., and Solbau, R.D. (1990). Measurement and interpretation of low levels of dissolved oxygen in ground water. *Ground Water* **28**(4), 584-590.

You, S., Zhao, Q., Zhang, J., Jiang, J., and Zhao, S. (2006). A microbial fuel cell using permanganate as the cathodic electron acceptor. *Journal of Power Sources*, 162(2), 1409-1415.

Yuan, Y., Zhao, B., Zhou, S., Zhong, S., and Zhuang, L. (2011). Electrocatalytic activity of anodic biofilm responses to pH changes in microbial fuel cells. *Bioresource Technology*, **102**(13), 6887-6891.

Zhang, T., Gannon, S.M., Nevin, K.P., Franks, A.E., and Lovley, D.R. (2010). Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. *Environmental Microbiology*, **12**(4), 1011-20.

Zhou, M., Jin, T., Wu, Z., Chi, M., and Gu, T. (2012). Microbial Fuel Cells for Bioenergy and Bioproducts. in: *Sustainable Bioenergy and Bioproducts: Value Added Engineering Application*s, (Eds.) K. Gopalakrishnan, J. van Leeuwen, R.C. Brown, Springer London. London, pp. 131-171.

Zou, H. and Wang, Y. (2017). Azo dyes wastewater treatment and simultaneous electricity generation in a novel process of electrolysis cell combined with microbial fuel cell. *Bioresource Technology*, **235**, 167-175.