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Enlargement of anode for enhanced simultaneous azo dye decolorization and power output in air-cathode microbial fuel cell

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Abstract Air-cathode, microbial fuel cells (MFC) with different anode surface areas were evaluated for simultaneous decolorization of Congo Red and bio-electricity production. Doubling the anode area from 18 to 36 cm^2 increased net power by 150 % (0.16–0.4 mW), normalized power (per anode surface area) by 22 % (88–107 mW m^{-2}) and Congo Red decolorization by 163 % (1.6–4.2 mg l^{-1} h^{-1}). Qua-drupling the original anode area induced an additional 5 % increase (up to 4.2 mW) in net power and 174 % increase (up to 11.5 mg l^{-1} h^{-1}) in Congo Red decolorization; however, normalized power decreased by 85 % (down to 58 mW m^{-2}). Increased bacterial attachment could account for both the enhanced power and Congo Red decolorization in larger anode MFCs. The limited effect on power output likely arises from cathode limitation or inefficient utilization of anodes.

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Introduction

As a novel bioelectrochemical device, the microbial fuel cell (MFC) is currently being explored for its ability to eliminate recalcitrant industrial and agricul-tural wastes while simultaneously producing electric-ity (Zheng and Nirmalakhandan 2010; Luo et al. 2009, 2010; Li et al. 2010). Recently, an azo dye, a representative recalcitrant pollutant present in the effluent from dye manufacturing, was degraded at the MFC anode during bioelectricity generation (Sun et al. 2009a; Chen et al. 2010; Li et al. 2011).

Microbial fuel cells performance depends largely on the anode efficiency, since electricity generation is directly related to the interaction between biofilm and

anode surface. Increased power output of an MFC appears to be positively correlated with higher anode surface area (Pham et al. 2009), provided that the MFC is not limited by a high internal resistance (Oh and Logan 2006). The air-cathode, single-chamber MFC represents a simple design with a substantially low internal resistance. Omitting the air cathode membrane or enlarging its pore size can further reduce the internal resistance (Liu and Logan 2004; Sun et al. 2009b).

From a waste-treatment viewpoint, MFC performance also depends on the presence of specific types of bacteria which proliferate in certain contaminants (Luo et al. 2010; Cheng et al. 2011; Li et al. 2010). These unique species interact with electrochemically active bacteria in the MFC anode, enhancing degradation of contaminant while simultaneously generating bioelectricity. In such a system, the surface area of the anode could primarily affect the activity of both contaminant-degrading bacteria and electrochemically active bacteria, since both types readily establish as biofilms on anodic surfaces. To date, the possibility of manipulating the anodic electrode to increase MFC performance during simultaneous waste removal and bioelectricity generation has been little explored.

Our previous research demonstrated that simultaneous azo dye decolorization and electricity generation in MFCs depends on the mixed-culture biofilm formed on the anode surface, rather than on the suspended biomass (Sun et al. 2009a). The anode biofilm comprises a diverse microbial community containing azo dye-degrading bacteria and electrochemically active bacteria (Hou et al. 2011). To better understand how the anode area contributes to simultaneous azo dye decolorization and bioelectricity generation, we developed several air-cathode single-chamber MFCs with different anode surface areas and evaluated their performance in terms of power output, anode impedance and dye decolorization rate. Carbon paper was used as the anode due to its easily calculated projected surface area and high conductivity.

Materials and methods

To examine the effect of anode surface area on MFC performance, anodes of surface areas 18, 36 and 72 cm^2 were applied to three individual air-cathode, single-chamber MFCs. The air-cathode, single-chamber MFC was constructed as reported by Sun et al. (2009a), with a slight modification. Total MFC volume was decreased to 512 ml (8 × 8 × 8 cm, with an operating volume of approx. 450 ml plus a 62 ml headspace). The anode was composed of non-wet-proofed plain porous carbon papers (E-Tek, 0.26 mm thickness) and was used without further treatment. The cathode, with a projected surface area of 5 × 5 cm (one side), was prepared by coating 0.5 mg Pt cm^{-2} onto a wet-proofed carbon paper as described by Liu and Logan (2004). The microfiltration membrane (pore size 0.22 μm) was applied directly to the water-facing side of the cathode. The anode was fixed at 2.5 cm from the cathode. For the 72 cm^2 anode, two 36 cm^2 anodes were connected in parallel by titanium wire with a gap of approx. 0.5 cm between them.

The MFCs were inoculated with a mixture of aerobic and anaerobic sludge (1:1, v/v) collected from Liede municipal wastewater treatment plant of Guangzhou City, China. The sludge was added to the MFC at 2 g volatile suspended solids per reactor volume (g VSS l^{-1}). 300 mg l^{-1} of Congo Red (analytically pure), and anode growth medium [500 mg COD l^{-1} glucose, 50 mM phosphate buffer solution plus nutrients] were added to the MFC (Sun et al. 2009a).

All of the MFCs were tested in fed-batch mode conditions at a fixed external resistance of 500 Ω. Solutions were mixed using a small magnetic stirrer to enhance mass transfer. The anode solution was refreshed when the voltage dropped below 20 mV. All tests were performed at least twice in a temperature-controlled room (30 ± 1 °C) and the average value recorded.

Analytics and calculations

The decolorization of Congo Red was assayed by decrease in absorbance at 496 nm. The voltage difference between anode and cathode (V) was recorded every 11 min using a multimeter and a data acquisition system (Model 2700, Keithley Instruments, USA). Polarization curves and electrochemical impedance spectroscopy (EIS) of the anode were collected at an electrochemical workstation (Model 2273, Princeton Applied Research). Polarization curves were obtained by recording the current response to an imposed linear potential decrease at 1 mV s^{-1}. The potential and current values were then converted to power density

with or without normalization by the projected anode surface area. Anode EIS analysis was performed using the anode as the working electrode, the cathode as the counter electrode and an Ag/AgCl electrode as the reference. Alternating current signals, 10 mV and frequencies ranging from 10 kHz to 5 mHz, were applied. The obtained data were fitted to a predetermined equivalent electrical circuit. The bacterial morphologies on the anode surface were investigated under an environmental scanning electron microscope (ESEM) (XL-30, Philips, Holland). Samples were prepared following standard SEM protocols.

Results

Electricity generation with enlarged anode surface area

Figure 1 shows that, up to a certain anode surface area, voltage output of the MFCs is enhanced as the area is increased. The voltage output was increased by 23 % (from 0.37 to 0.48 V) when the anode surface area was doubled from 18 to 36 cm^2. Quadrupling the anode surface area to 72 cm^2, however, induced a mere 4 % increase in voltage output (up to 0.5 V).

To determine the effect of anode area on net and relative power output of the MFC, the power density was normalized by the projected anode surface area, and compared to the non-normalized power output. Fig. 2a illustrates that the larger the anode, the greater the net power output of the MFC. The net power output was increased by 150 % when the anode
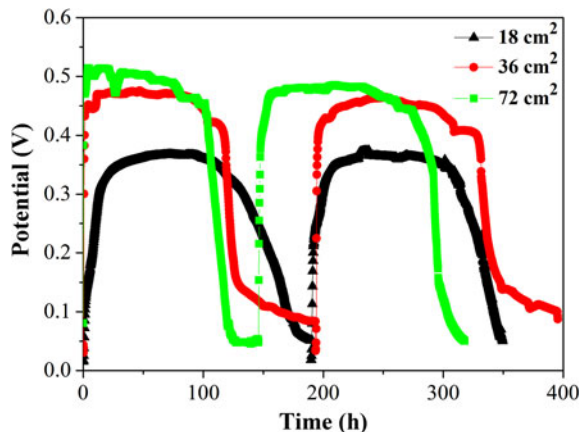

Fig. 1 Voltage output of MFC anodes with different anode surface area (external resistor 500 Ω)
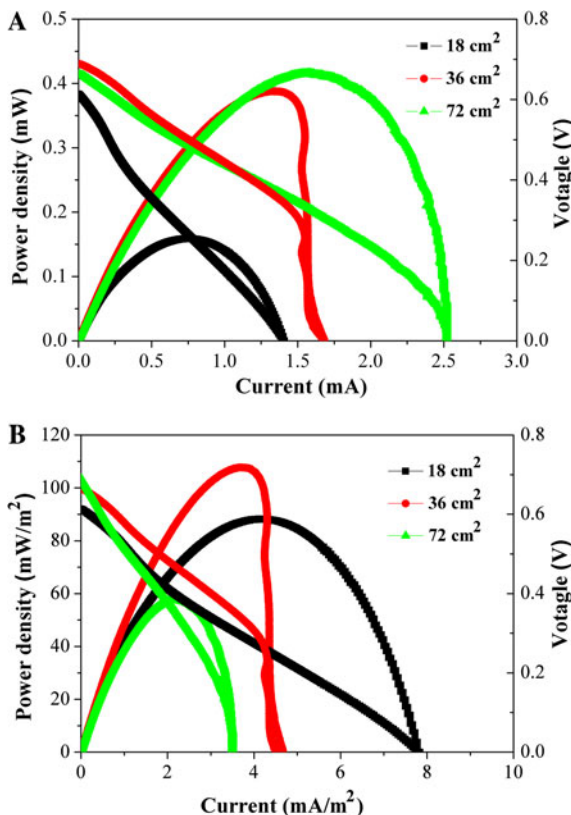
Fig. 2 Power density and polarization curves for MFCs with different anode surface area **a** non-normalized and **b** normalized by the anode surface area. The parabolic curves represent the power density variation, while the decreasing curves show the voltage trends, (or vice versa) as current is increased

surface area was doubled from 18 to 36 cm^2 but quadrupling the surface area to 72 cm^2 induced a mere 5 % power increase. The highest normalized power output was achieved with an anode surface area of 36 cm^2 (107 mW/m^2), which yielded 22 and 85 % more power than the 18 cm^2 (88 mW/m^2) and 72 cm^2 (58 mW/m^2) anodes, respectively (Fig. 2b).

To assess the effect of anode area on simultaneous electricity generation and Congo Red decolorization, impedance spectroscopy measurements were performed on anodes of different area. By fitting the experimental data to an equivalent electrical circuit, we obtained the anode Nyquist plots (Fig. 3). As shown in Fig. 3, the charge-transfer resistance (Rct) overwhelms the anode impedance and the solution resistances (Rs) appear to be insignificant. The Rct decreases with increasing anode surface area, consistent with the power curve results. The anode 18 cm^2 yielded a substantially high Rct of 370 Ω. The Rct
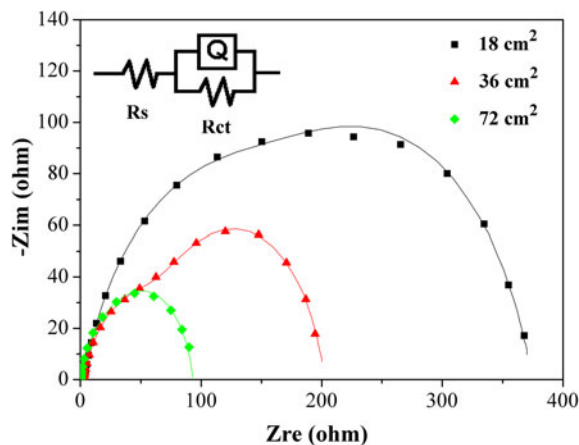
Fig. 3 Nyquist plots for MFC anodes with different surface area. *Symbols* represent the experimental data, *lines* represent the fit of these data to the equivalent circuit (*inset*, where *Rs*, *Q* and *Rct* represent solution resistance, constant phase element, and Rct, respectively). *X*-axis represents real and *Y*-axis represents imaginary components of the complex impedance. Intersections between the *semicircle* and *X*-axis at high frequencies signify Rs and the *semicircular arc* diameters signify Rct. The Nyquist plots are *semicircles* not followed by a *straight line*, which is characteristic of an electrode reaction controlled by the charge-transfer step

decreased by 46 % to 200 Ω when the anode was doubled to 36 cm^2. Quadrupling the anode to 72 cm^2 decreased the Rct by a further 110 %, to 95 Ω.

Decolorization performance with enlarged anode surface area

Increasing the anode surface area accelerated the Congo Red decolorization (Fig. 4). With anode surface area 18 cm^2, the MFC required more than 168 h to completely decolorize. At 36 cm^2 anode surface area, complete decolorization was achieved by 72 h. Quadrupling the anode surface area to 72 cm^2 further hastened the decolorization; the color of the Congo Red was completely removed within 26 h.

SEM biofilm characterization

Biofilm samples obtained from different positions on the anode surface were observed by SEM (Fig. 5). A clearly visible homogeneous biofilm has developed on the anode surface after 3 months (Fig. 5a). Most bacteria are globular and arranged in chains while a minority appear as bacilliform (Fig. 5b). These
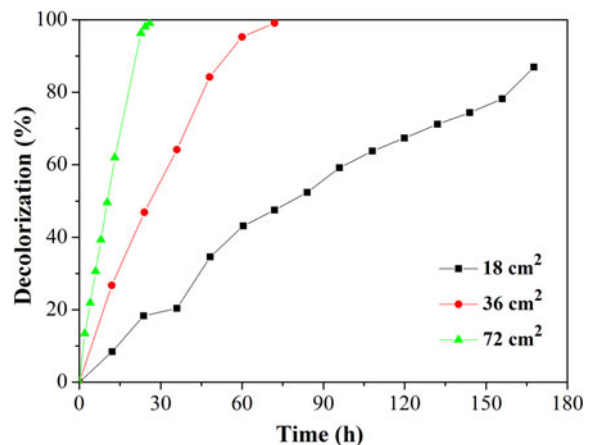

Fig. 4 Decolorization of Congo Red in MFCs with different anode surface area. The percentage of decolorization was calculated from the difference between initial and final absorbance values

uniformly attached bacteria are responsible for both Congo Red decolorization and electricity generation.

Discussion

Increasing the anode surface enhances simultaneous Congo Red decolorization and electricity generation in MFCs (Table 1). Electron supply to the anode can be achieved via cell membrane-bound electron shuttling compounds or soluble electron shuttles produced by the cell (Rabaey and Verstraete 2005). Frequent replacement of anodic medium precluded the accumulation of soluble electron shuttles, thus the electron transfer occurred mainly through direct contact with the anode surface. Increasing anode surface area increases the number of exoelectrogenic bacteria on the anode, thereby providing more reaction sites for anodic reactions in an identical anode compartment. Consequently, the activation over the anode potential is decreased, leading to a decrease in anode potential and increased power production of the MFC. Therefore, decrease of the anode activation potential likely arises from direct electron transfer to the anode by the anodophilic bacteria, and also by increased bacterial coverage on larger outer active anode surfaces.

Increasing the anode surface area relative to the cathode had little effect upon the net power generation, indicating that this quantity is cathode-limited. For platinum based cathodes, the cathode/anode area
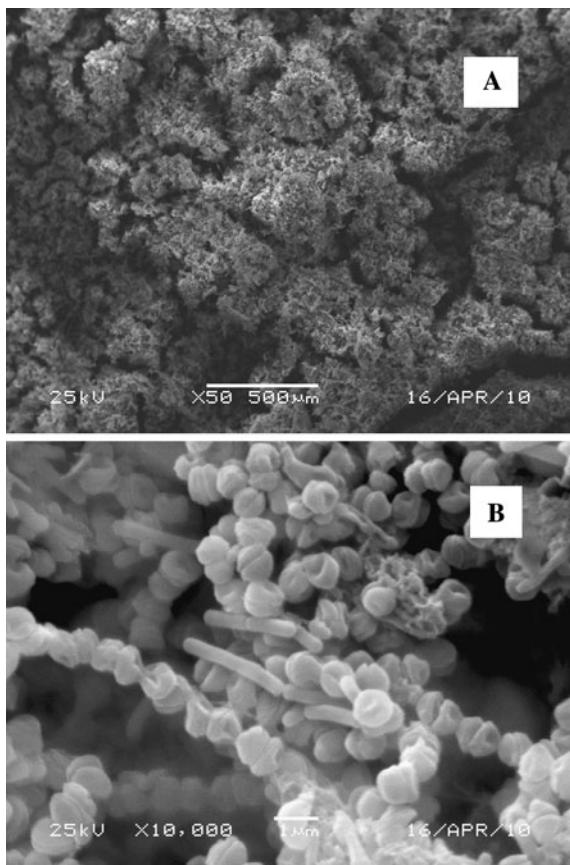
Fig. 5 Biofilm samples were obtained from different locations on the anode surface of the MFC used for Congo Red decolorization, exceeding 3 months of stable operation. **a** and **b** are representative scanning electron microscope images of the established biofilm; for **a** bar = 500 μm, for **b** bar = 1 μm

ratio must exceed 27 before full performance of the anode is guaranteed (Uría et al. 2011). However, the ratios of cathode/anode area in this study are 1.38, 0.69

and 0.35 for anode sizes 18, 36 and 72 cm^2, respectively, considerably lower than those reported in the literature. Thus there is considerable scope for improving the cathode performance (and hence the power output of the MFC), either by using a lager cathode or by optimizing the material preparation. The optimal anode surface area, in terms of power output, is 36 m^2 because it yielded the highest normalized power output of the three anode sizes tested. At higher and lower anode surface areas, normalized power output decreased, probably due to inefficient utilization of the anode by the colonizing bacteria (Logan et al. 2005).

Membrane structure is one factor affecting power generation in MFCs. When PEM surface area is smaller than that of the electrodes the power output of a two chambered MFC is reduced by high internal resistance (Oh and Logan 2006). However, in our study, the membrane incorporated into the air-cathode single chambered MFC was MFM (pore size 0.22 μm). The relatively large pore sizes of the MFM allow protons and O$_2$ to diffuse readily through the membrane, resulting in a lower internal resistance than when PEM is used.

Increasing anode surface area also greatly improved the decolorization performance of the MFC. As summarized in Table 1, doubling the anode surface area from 18 to 36 cm^2 increased the decolorization rate of Congo Red by 160 % (from 1.6 to 4.2 mg l^{-1} h^{-1}). Quadrupling the anode surface area to 72 cm^2 accelerated the decolorization rate to 11.5 mg l^{-1} h^{-1} (an increase of 600 %). Decolorization of azo dyes by bacteria can occur via adsorption or biodegradation (Pandey et al. 2007). Cellular adsorption contributes little to the Congo Red decolorization

Table 1 Comparison of performance of MFCs with different anode surface area for simultaneous Congo Red decolorization and electricity generation

Anode surface area (cm^2)[a]	Voltage output (V)	Maximum net power output (mW)	Maximum normalized power output (mW m^{-2})[b]	Rct (Ω)	Decolorization rate (mg l^{-1} h^{-1})[c]
18	0.37	0.16	88	370	1.6
36	0.48	0.40	107	200	4.2
72	0.50	0.42	58	92	11.5

Rct charge-transfer resistance

[a] Projected anode surface area

[b] Power density normalized by the projected anode surface area

[c] Decolorization rate (mg l^{-1} h^{-1}) = (300 − C)/T where C is final Congo Red concentration, T is time required for decolorization. Congo Red concentration was read directly from a previously established standard curve (absorbance vs. concentration)

in this study since the adsorption capacity of the biomass becomes exhausted by continuous, long-term (more than 1 month) exposure to Congo Red. In a previous study, we inferred that both azo dye-degrading bacteria and exoelectrogenic bacteria reside in the anodic biofilms of MFCs used for long-term Congo Red decolorization (Hou et al. 2011). Thus, the Congo Red decolorization can be primarily attributed to its degradation. We cannot guarantee that the biofilm will completely cover the anode surface with uniform thickness, but increased attachment of bacteria undoubtedly occurs on lager anode surface area, since SEM clearly revealed that homogenous biofilm had developed at various localities across the anode surface. Therefore, a larger anode surface area which can accommodate much more biomass is responsible for the accelerated decolorization of Congo Red in this study.

There is room for improvement in our study. While investigating the effect of larger anode on MFC performance, the cathode surface area was fixed and thus became the limiting electrode because of its smaller surface area. Carbon paper was used on account of its high conductivity and easily calculated projected surface area. The high conductivity naturally enhances power output. Selecting a more appropriate anode/cathode area ratio and a more effective substrate electrode (such as graphite felt or brush) with lager macro-surface area to which more bacteria could attach would further enhance the performance of the MFC. Despite these limitations, our study highlights a broad range of possibilities for environmental pollutant degradation coupled with electricity generation in MFC systems.

Conclusions

Increasing the anode size is a promising strategy for increasing both power output and decolorization rate of azo dye in air-cathode single-chamber MFCs. Larger anode surface areas result in accelerated Congo Red decolorization but have limited effect on MFC power output. Larger anode surface areas can provide more space for mixed microbial population attachment, which can account for both the accelerated decolorization of Congo Red and the low anode polarization impedance. Larger anode surface area exerts limited influence on power output due to

cathode limitation or inefficient utilization of the anode. Therefore, optimal performance of the system could be achieved by using anode material with lager macro-surface area or by cathode optimization.

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