



Short Communication

In situ measurements of dissolved oxygen, pH and redox potential of biocathode microenvironments using microelectrodes

Zejie Wang, Huan Deng, Lihui Chen, Yong Xiao, Feng Zhao*

Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen, Fujian Province 361021, China

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ABSTRACT

Biofilms are the core component of bioelectrochemical systems (BESs). To understand the polarization effects on biocathode performance of BES, dissolved oxygen concentrations, pHs and oxidation–reduction potentials of biofilm microenvironments were determined *in situ*. The results showed that lower polarization potentials resulted in the generation of larger currents and higher pH values, as well as the consumption of more oxygen. Oxidation–reduction potentials of biofilms were mainly affected by polarization potentials of the electrode rather than the concentration of dissolved oxygen or pH value, and its changes in the potentials corresponded to the electric field distribution of the electrode surface. The results demonstrated that a sufficient supply of dissolved oxygen and pH control of the biocathode are necessary to obtain optimal performance of BESs; a lower polarization potential endowed microorganisms with a higher electrochemical activity.

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1. Introduction

Bioelectrochemical systems (BESs) can recover green energy during the process of decomposing pollutants in wastes with the aid of electrochemically active microorganisms (Logan and Rabaey, 2012). In addition to contaminant removal (Desloover et al., 2011; Aulenta et al., 2011; Zhao et al., 2009) BESs can also generate value-added products e.g. organic compounds (Nevin et al., 2011).

Biofilm microenvironments are physical and chemical conditions within the biofilm that play very important roles in wastewater treatment. Using micro-sensors in combination with fluorescence *in situ* hybridization and confocal laser scanning microscopy, Schramm et al. (2000) determined that nitrifying bacteria in a biofilm was dependent on the gradients of oxygen, nitrite and nitrate. Mass transfer of air within a biofilm is affected by the film's thickness (Rasmussen and Lewandowski, 1998), which leads to stratification into aerobic and anaerobic zones (Terada et al., 2007). The stratification of the biofilm shapes the structure of the microbial community and makes it possible for different processes to occur simultaneously (e.g. nitrification and denitrification) (Hibiya et al., 2003). Bissett et al. (2008) investigated the effects of pH and temperature on cyanobacterial photosynthesis-induced calcification by determining the gradient of Ca²⁺ in the biofilm.

Biofilms in BESs are somewhat different from those of conventional bio-treatment processes as bio-electrochemical reactions are involved in current and potential generation. For biocathode

processes, biofilms adopt the surface of cathode as electron donor to reduce electron acceptors e.g. oxygen or contaminants. Biocathodes using oxygen as an electron acceptor were designed to eliminate the need for expensive chemical catalysts. Oxygen is reduced by the biocatalysts following one or more of the reactions below:



The reduction of oxygen is accompanied by the consumption of protons in solution or through electrolysis of water. Sufficient protons promote oxygen reduction reaction based on Reactions (1) and/or (2). When the proton concentration is low, oxygen is reduced through Reaction (3). The reactions increase the pH of the solution due to the accumulation of OH[−]. An increase in the pH of biocathode surface can not only influence the microbial electro-activity, but also increase the oxygen reduction over-potential. Both of them inhibit the performance of a biocathode.

While the electron transfer mechanism of *Shewanella* has been studied by determining the gradients of pH and redox-potential within an anodic biofilm (Babauta et al., 2011), biocathode microenvironments have not been investigated. Therefore, the objective of the present study was to determine the dissolved oxygen (DO), pH as well as oxidation–reduction potential (ORP) of the biofilm of an aerobic biocathode *in situ*, using microelectrodes to learn more about the limiting factors for the optimization of BES operations.

* Corresponding author. Tel./fax: +86 592 6190766.

E-mail address: fzhao@iue.ac.cn (F. Zhao).

2. Methods

2.1. Electrochemical cell

The electrochemical cell with a volume of 125 cm³ (5 × 5 × 5 cm) was constructed using Plexi-glass (Fig. 1). An Ag/AgCl (3 M KCl, CHI) was used as a reference electrode. Carbon cloth (Haoshi Carbon Fiber Co., Ltd., China) served as working electrode and counter electrode with surface areas of 4 cm² (2 × 2 cm) and 16 cm² (4 × 4 cm), respectively. The working electrode was soaked in a culture of aerobic microorganisms collected from the Jimai wastewater treatment plant (Xiamen, China) to allow formation of a biofilm. The electrochemical cell was filled with 100 mL of a Na₂HPO₄/NaH₂PO₄ (0.2 M/0.3 M, pH 6.8) buffer solution. The upper surface of the working electrode was located 1 cm below the solution surface.

2.2. Microelectrode system

Microelectrodes were purchased from Unisense (Denmark). A stepper motor was used to control the movement of microelectrodes. For pH and ORP measurements, an external Ag/AgCl reference electrode (REF 321, Unisense, Denmark) connected to the microelectrodes was introduced into the electrochemical cell (Fig. 1). The signals were acquired by a Microsensor Multimeter (Unisense, Denmark) interfaced with a computer to record data. Microelectrodes were calibrated with three standard solutions (Fig. S1). To offset the disturbance of the electric field, calibrations of pH and ORP microelectrodes were performed under the same potential as that of the working electrode.

2.3. Operation and measurements

Potentials of the working electrode were controlled by a CHI600D potentiostat at −300, 0, and +100 mV vs. Ag/AgCl. To confirm the contribution of the biofilm, control experiments were performed using an abiotic-cathode (bacteria-free) as a working electrode in terms of current generation and oxygen consumption. The significant difference in current and DO change between

abiotic- and bio-cathode was determined at the level of $P < 0.05$ using independent-sample *T*-tests. Three points for each polarization potential were randomly selected and examined. The interval was set at least 30 min between two measurements so that the working electrode could recover to a stable status prior to the next measurement. The thickness of biofilm was determined using a microscope with scale bars (SMZ800, Nikon, Japan). The temperature was controlled at 26 °C.

3. Results and discussion

Seven days post inoculation, a biofilm about 700 μm thick had formed on the carbon cloth surface (Fig. S2). For the biocathode, the current increased when the potential shifted to negative, e.g. the current increased from 3.82 μA at 0 mV to 42.43 μA at −300 mV (Table 1). These results confirmed the contribution of biofilm to the generation of current at relatively low potentials. The application of an electric field can change the surface properties of microbial cells, increase the activity of enzymes and shorten the doubling time of microorganisms (Luo et al., 2005; Busalmen and De-Sanchez, 2005). An appropriate potential may optimize the physiological process of electroactive microorganisms and thus achieve optimal performance of BESs.

Activity of aerobic microorganisms can be evaluated by the consumption of oxygen. Fig. 2 shows that more negative potential resulted in a larger DO gradient within the biofilm. The DO change at +100 mV was similar to that of the control i.e. 0.49 mg L^{−1}. The decrease in DO concentration was 3.36, 1.25 and 0.57 mg L^{−1} when the biocathode was held at −300, 0, and +100 mV, respectively. In an aerobic biocathode, the generation of current is accompanied by the consumption of oxygen as demonstrated by Reactions (1)–(3). But it should be noted that the consumption of DO in this study is attributable not only to reduction reactions on the electrode, but also diffusion restriction and metabolic consumption by aerobic and facultative bacteria within the biofilm.

The DO gradient as a function of potential may be caused by insufficient DO transfer from the bulk solution into the biofilm. The change of oxygen concentration will shape the structure of the microbial community within the biofilm. For an oxygen-reducing biocathode, mass transfer of DO was considered as a serious limiting factor for the electrode reaction (Ter Heijne et al., 2010). DO will be rapidly consumed at high-current conditions, e.g. achieving 42.43 μA at −300 mV in the present study, leading to insufficient electron acceptors for a biocathode, and further limiting the energy recovery efficiency of BESs. Therefore, it is important for an aerobic biocathode to keep sufficient DO within the biofilm.

Fig. 3 shows the pH as a function of electrode potentials. After stable current at the biocathode was observed in the potentiostatic experiments, the pH values determined by the microelectrode were 7.78, 7.83 and 8.05 at +100, 0 and −300 mV, respectively. In the nearly neutral solution, protons were rapidly depleted according to Reactions (1) and/or (2). To provide protons for oxygen reduction, water was electrolyzed into H⁺ and OH[−]. Since H⁺ was used for oxygen reduction, OH[−] was left and consequently increased the pH. The increase in pH was positively related to the current because the generation of a larger current needed more protons. This result is in good agreement with the conclusion of a mathematical prediction (Torres et al., 2008).

A pH gradient was not observed within the biofilm at a same potential. For example, the pH at −300 mV was in the range of 8.04–8.06 (inset of Fig. 3). When phosphate buffer was used, its high ionic strength benefited the OH[−] balance (Zhao et al., 2006) and may have eliminated the pH gradient. An increase in pH leads to a basic environment in the biofilm, which could finally affect

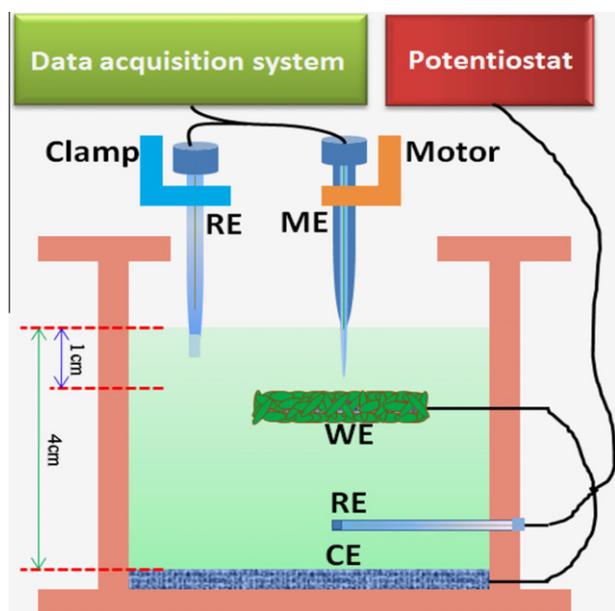


Fig. 1. Schematic of the experiment set-up; WE: working electrode; CE: counter electrode; RE: reference electrode; and ME: microelectrode. All potentials were reported versus Ag/AgCl.

Table 1
Comparison of current and DO between abiotic-cathode and bio-cathode at different potentials.

Potential(mV vs. g/AgCl)	Current (μA)		DO change (mg/L) ^a	
	Abiotic-cathode	Biocathode	Abiotic-cathode ^b	Biocathode
Control	–	–	-0.27 ± 0.23	-0.49 ± 0.06
-300	33.77 ± 3.98	42.43 ± 7.10	$-1.09 \pm 0.28^*$	-3.36 ± 0.20
0	$2.15 \pm 0.70^*$	3.82 ± 0.08	$-0.19 \pm 0.19^*$	-1.25 ± 0.27
+100	2.19 ± 0.36	2.09 ± 0.25	-0.34 ± 0.16	-0.58 ± 0.09

^a Means DO difference between the terminal and the beginning point of each measurement.

^b Error bar means SD based on test in quadruplicate.

* Significant difference between abiotic- and bio-cathode ($P < 0.05$).

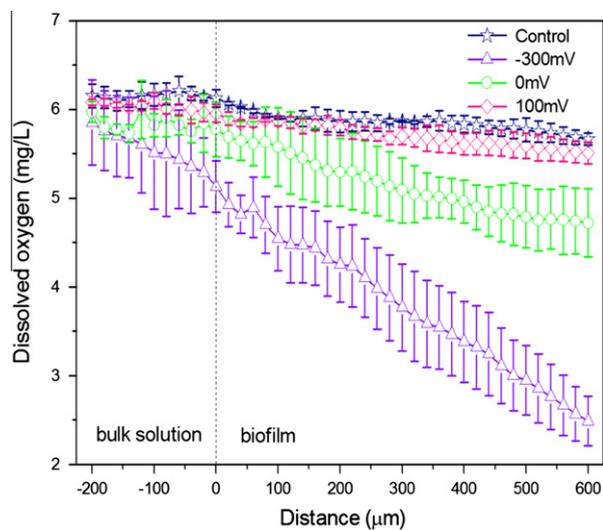


Fig. 2. Profiles of dissolved oxygen concentration with biofilm depth, error bars are based on triplicate measurements.

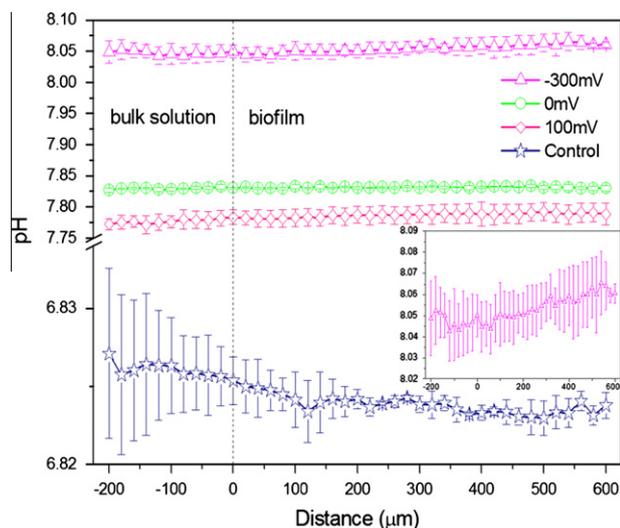


Fig. 3. pH profiles of biofilm at different potentials in the solution at pH 6.83 (inset is pH at -300 mV for clear illustration). Error bars are based on triplicate measurements.

microbial electro-activity. For example, at pH 7.0 and 9.0, the open circuit potentials of the biocathode were 144.4 and 76.3 mV, respectively. For the application of aerobic biocathodes, optimal and stable performance is needed for long-term operation. The result of this study suggests that pH control is necessary for the operation of biocathodes.

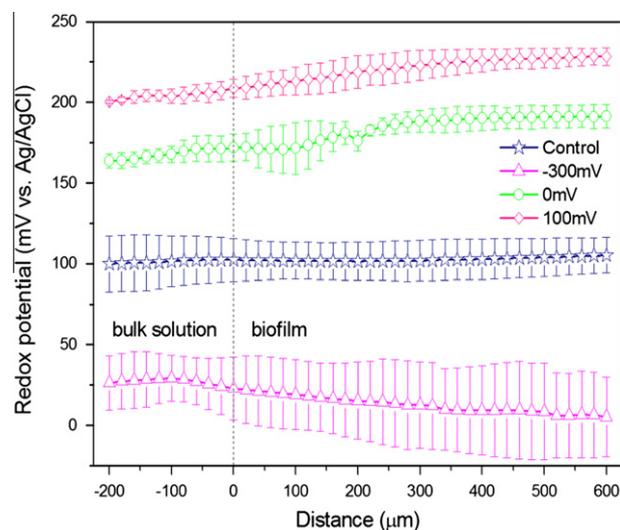


Fig. 4. Oxidation–reduction potential of biofilm determined at different electrode potentials, error bars are based on triplicate measurements except ORP at -300 mV based on duplicate measurements.

ORP is used to access the tendency to donate or receive electrons. It is affected by the concentrations of oxidants and reductants, and the pH of bulk solution according to Nernst equation. Fig. 4 shows the ORP profiles as a function of biofilm thickness. The ORP was positively related to the potential, e.g. +100 mV gave rise to a higher ORP than that of the control, while -300 mV resulted in a lower ORP in comparison to that of the control. The ORP gradients illustrated different tendencies for different potentials. When the potential was held at 0 mV, the ORP increased from 163.67 to 191.19 mV in comparison to that of the control which increased from 99.95 to 105.05 mV. While at -300 mV, the ORP decreased from 26.23 to 5.20 mV within the biofilm.

Based on the observed trends for the ORP, we can conclude that the ORP was mainly affected by the potential of the electrode rather than DO concentration and pH within the biofilm in the present study because the DO decreases and pH remains nearly constant within the biofilm. The trend observed for the ORP is supposed to be related to the decreasing electric field distribution from the electrode surface to the bulk solution. The electric field will affect microbial activity and community in long-term operation.

4. Conclusions

A lower polarization potential of the biocathode resulted in a relatively high microbial electro-activity, consequently generating higher current, and consuming more oxygen and H^+ . Oxidation–reduction potential of the biofilm was mainly affected by the polarization potential rather than DO and pH of the biofilm micro-

environment. Microbial activity and community may change with depth of the biofilm due to electrode reactions. For optimal long-term operation of aerobic biocathode BESs, a sufficient supply of oxygen and control of pH are necessary.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biortech.2012.11.026>.

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