A novel mediator–polymer-modified anode for microbial fuel cells†

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A high-performance anode system based on a combination of mediator–polymer-modified graphite felt and bacteria capable of reducing extracellular materials shows significant potential for practical use in microbial fuel cells (MFCs).

The microbial fuel cell (MFC) is a promising technology for treatment of waste biomass. The minimum system components of a MFC are an anode, a cathode, and microorganisms.[1] Four types of MFC have been reported thus far, including a mediatorless MFC[2] using metal-reducing bacteria[3] (MRB) capable of reducing extracellular materials (type 1); a mediator-modified MFC[4] which uses a mediator (Med) to support electron transfer from the bacteria to an anode (type 2); and an *E. coli*-based hydrogen oxidation system[5] using biologically produced hydrogen as an electron donor (type 3). The type 1 MFC can provide stable power generation using various types of biomass, but has a low power output ($\sim$8 mW m^{-2}), as the system is limited by the performance of the anode, which is much lower than that of the cathode.[6] Type 2 can also operate with various types of biomass, and gives a higher power output ($\sim$200 mW m^{-2}), but it is unsuitable for continuous biomass treatment. Type 3 has the greatest power output of the three types (6000 mW m^{-2}), but can use only sugar as an electron donor, with quite low efficiency.

Another type of MFC, which features a mediator immobilized on the anode (type 4), has potential for continuous biomass treatment and improved electric output.[7]

We evaluated the anode properties of the type 4 MFC using a Med–polymer-modified anode and MRB. Our strategy was to immobilize a bioactive redox compound as a Med on the anode surface with a thin layer of a functional polymer. Previous studies have shown that it is difficult to immobilize a large amount of Med directly on an anode surface,[7] and that Med adsorbed on an anode showed little efficiency because it was easily removed from the anode surface.[8] However, it has been reported that a large amount of enzymes or chemical compounds immobilized with a thin polymer layer is stable. The polymer-modification was applied to immobilization of Med.[9]

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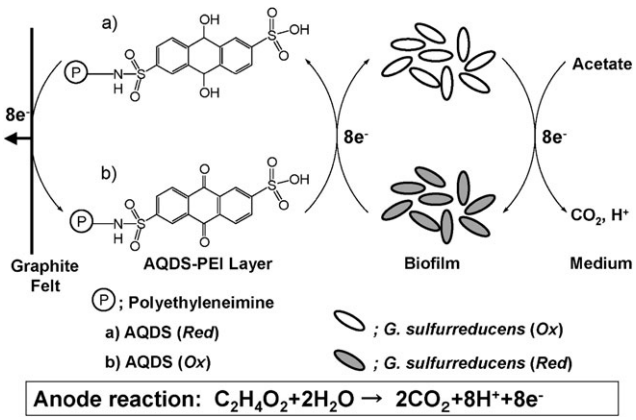
The performance of the modified anode is dependent on the bioavailability and redox potential of the Med. In order to maximize the power of the MFC, the redox potential of the Med should be as low as possible while being higher than the redox potential of NADH[4] ($E_0' = -0.325$ V *vs.* normal hydrogen electrode (NHE)) in a bacterial cell. In practice, electrons from NADH are passed through an electron transfer chain to a terminal membrane enzyme, then exocellularly released. Therefore some degree of potential difference should be considered between NADH and Med.[10] In this work, a derivative of 9,10-anthraquinone-2,6-disulfate[11] (AQDS) is used as the Med. The novel system is composed of a Med-bonded polyethyleneimine (PEI)-modified graphite felt (GF) anode, with the MRB *Geobacter sulfurreducens*[3] as a bioelectrocatalyst (Scheme 1). We demonstrate that the performance of this anode system is more than 100 times greater than those of previously reported systems.

The standard redox potential (E_0') of AQDS is -0.184 V *vs.* NHE. Therefore, electrons from NADH in a bacterial cell can be transferred to AQDS, which is known to serve as a bioactive electron acceptor.[12] Osa *et al.* reported the use of a TEMPO-modified polyacrylic acid-coated GF electrode for electrocatalytic and enzyme-catalytic organic macro-synthesis.[13] A modification of this method was used for the synthesis of our anode.

In order to bind the Med to PEI, AQDS (Aldrich) was converted to its disulfonyl chloride (80–90% yield) using phosphoric chloride in tetrahydrothiophene-1,1-dioxide for 4 h at 90 °C.[14] PEI (Nippon Shokubai Co., Ltd., EPOMIN® P-1000, M_W = 320 000) contains a large amount of primary and secondary amines, which can be reacted with



Scheme 1 Proposed mechanism of electron transfer from acetate to graphite felt electrode *via* both *G. sulfurreducens* and AQDS.

9,10-anthraquinone-2,6-disulfonyl chloride (AQDS chloride) to give sulfonamide bonds or with 1,5-pentanedioic acid (PA) to give amide bonds using a dehydration–condensation agent.

To prepare the electrodes, GF plates (Nippon Carbon Co., Ltd., Carbolon) with a size of 2.0 × 5.0 × 0.2 cm (geometric area 20 cm^2, bulk density 1.4 g cm^{-3}) were dipped in a 1.32 wt% PEI methanol solution for 1 min at RT and dried at 60 °C in an oven for 12 h. The PEI-coated GF plates were then used as base plates (BP) for the following modifications.

For the preparation of AQDS–PEI-modified GF (E1), a BP was subjected to cross-linking with AQDS chloride as follows: the BP was placed in a 50 ml glass centrifuge tube containing a 20 mM solution of AQDS chloride in a mixture of dimethylformamide, methanol and triethylamine (49/49/2 in volume), and the tube was shaken at 200 rpm for 24 h at 25 °C.

In order to prepare AQDS–PEI–PA-modified GF (E2), cross-linking with PA was carried out as follows: a BP was placed in a glass centrifuge tube containing 100 mM phosphate buffer (pH 6.0), 5 mM PA and 50 mM 1-ethyl-3-(3-diethylaminopropyl)carbodiimide hydrochloride (Tokyo Chemical Industrial Co., Ltd.) and was shaken at 200 rpm for 24 h at 25 °C. The cross-linked BP was rinsed with deionized water for 24 h and then dried for 12 h at 60 °C. The cross-linked BP was treated in the same manner as for E1. These two types of AQDS-modified electrode were prepared in order to compare their electrical characteristics and the amounts of immobilized AQDS contained therein.

For the preparation of PEI–PA-modified GF (E3), which was used as a control to examine the effect of AQDS modification, a BP was reacted with PA using the same technique as for E2. After the cross-linking operations, the three types of modified electrode were rinsed with ethanol for 4 h and then with deionized water for 24 h, and dried at 60 °C for 12 h.

For electrochemical characterization of the three types of electrode—E1, E2 and E3—cyclic voltammetry (CV) was carried out to investigate the redox properties of the modified GFs, and chronocoulometry (CC) was carried out to determine the amount of Med present in the modified GFs. Both these operations were carried out in 100 mM phosphate buffer (pH 7.0, RT). Fig. 1a shows a cyclic voltammogram of E1 at a scan rate of 20 mV s^{-1}. An oxidation peak at −0.10 V and a reduction peak at −0.22 V are clearly observed. The AQDS density per unit geometric area of E1 was determined by CC as 1.2–1.5 μmol cm^{-2}. The cyclic voltammogram of E2 (Fig. 1b) showed a curve similar to that of E1, but the peak current density of E2 was smaller than that of E1, probably due to tight cross-linking in the PEI domain. The density of AQDS was determined as 0.1–0.3 μmol cm^{-2}. The cyclic voltammogram of E3 showed almost no peaks (Fig. 1c), which was considered reasonable due to the absence of an electrochemically active residue; E1 and E2 contain an electroactive site in the AQDS moiety, while E3 does not.

The biological reducing activity of AQDS in the modified GFs was measured in electrogeneration experiments with MFCs. A MFC with E1, the most electrochemically active electrode, as the anode (MFC A) and one with E3, the control electrode, as the anode (MFC B) were tested in the presence of *G. sulfurreducens* as a biocatalyst. The anodes were potentiometrically maintained at 0.04 V *vs.* NHE in order to evaluate the anode performance independently. The electrodes (anode [geometric area 8 cm^2]; cathode, consisting of an untreated GF plate [geometric area 100 cm^2]; and reference electrode [standard calomel electrode, DKK-TOA Corp., HC-205C]) were connected to a potentiostat (Hokuto Co., Ltd., HA-151). The reactor for evaluation was composed of the anode and the reference electrode immersed in an anaerobic medium containing sodium acetate (48 mM) as an organic substrate (pH 7.5, 1.0 l). The cathode was immersed in 100 mM phosphate buffer (pH 7.5, 0.7 l). The liquid in the two compartments was separated by a proton exchange membrane (geometric area 25 cm^2, Dupont Nafion® NX 424).

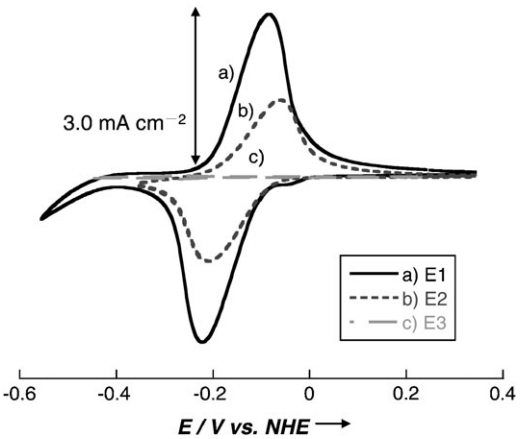


Fig. 1 Cyclic voltammograms of (a) AQDS–PEI-modified GF electrode (E1), (b) AQDS–PEI–PA-modified GF electrode (E2) and (c) PEI–PA-modified GF electrode (E3).

In this study, the inoculum was *G. sulfurreducens* (DSMZ Germany, DSM No.12127^T). The anode chambers of MFC were not sterilized before inoculation of *G. sulfurreducens*. Anaerobic conditions were satisfactorily maintained during the entire measurement process.

Fig. 2 shows the time course of current density for the two MFCs in the presence of *G. sulfurreducens*. In the MFC A cell, the current flow started to increase at 3 days after initiation and reached 1.2 mA cm^{-2} after 35 days. In contrast, the current flow in the MFC B cell was very small and remained as low as 0.02 mA cm^{-2} from the initial day up to day 15. To

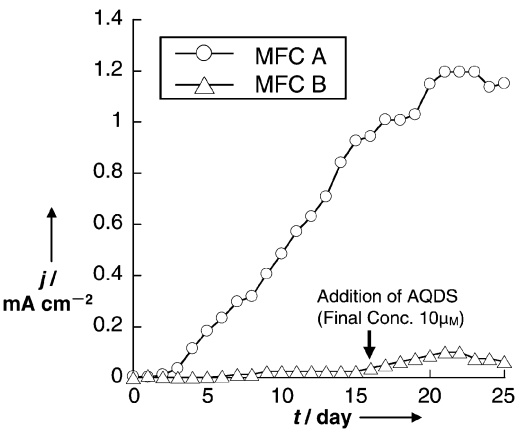


Fig. 2 Time course of current densities of the potentiometrically controlled anodes (0.04 V *vs.* NHE) in the MFC A and MFC B cells.

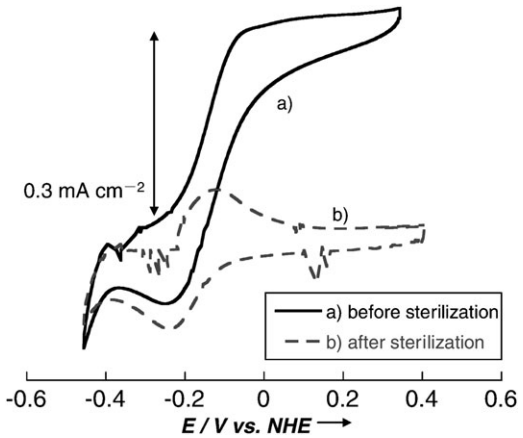


Fig. 3 Cyclic voltammograms of AQDS–PEI-modified GF (E1) after 16 days' incubation: (a) before sterilization, and (b) after sterilization (121 °C, 20 min).

confirm that the effect of AQDS was due to immobilization with PEI, 10 μmol of free AQDS (final conc. 10 μM) were added to the medium. It was found that the current density increased only slightly to 0.08 mA cm^{-2} after 6 days. Thus it was confirmed that AQDS functioned effectively only when immobilized with PEI.

In order to determine the bioactivity of the system, CV measurements of a sample (geometric area 2 cm^2) of E1, taken from another MFC which was incubated under the same conditions as MFC A, were carried out in 48 mM sodium acetate–100 mM phosphate (pH 7.0) solution under anaerobic conditions. A period of more than two weeks is required to incubate the bacteria, because the growth of anaerobic bacteria is usually slow.

Fig. 3a shows a cyclic voltammogram of E1 after 16 days' incubation (scan rate 20 mV s^{-1}). The voltammogram showed a sigmoid curve which began to rise at −0.15 V and reached a peak at −0.04 V, which is slightly more negative than the E_0' value of AQDS. The electrode was then sterilized to remove the incubated bacteria (121 °C, 20 min) and subjected to CV again under the same electrolyte conditions (Fig. 3b). An oxidation peak at −0.15 V and a reduction peak at −0.25 V were observed, indicating the existence of AQDS in the AQDS–PEI-modified GF after incubation. This result shows that the increase in current density in the MFC is due to a combination of the ease of electron transfer *via* immobilized AQDS and the high activity of *G. sulfurreducens* in reducing extracellular material.

Platinum black and CoTMPP cathodes have been reported to show cathodic current densities of over 0.6 mA cm^{-2} at 0.35 V *vs.* NHE.[15] Logan *et al.* demonstrated that the internal resistance of a MFC operating on starch is under 30 Ω.[1] For a MFC consisting of a cathode and a high-performance anode, we should be able to obtain a stable energy output of 200 to 400 μW cm^{-2} (per geometric area of anode). In contrast, the stable energy outputs of previous mediatorless MFCs, calculated on the anode geometric area, were less than 12 μW cm^{-2}.[16]

In conclusion, we have succeeded in constructing a high-performance anode-half-cell system using a polyethyleneimine-coated GF anode modified with AQDS as a mediator in the presence of bacteria (*G. sulfurreducens*). This anode system has been found to be stable for at least four months of operation without decomposition or a decrease in the current. These results suggest that the manufacture of MFCs for practical use may soon become a reality.

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