



## A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor

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### Abstract

Mediator-less microbial fuel cells (MFC) enriched with oligotrophic microbes were optimized through enhancement of cathode reaction and lowering O<sub>2</sub> diffusion into the anode compartment as a low BOD sensor. The optimization of the MFC has greatly improved the maximum current and coulomb yield. The oligotroph-type MFC could be used as a low BOD sensor with high operational stability, good repeatability and reproducibility.

### Introduction

Biochemical oxygen demand (BOD) is widely used in the evaluation of water and wastewaters. Since the conventional method takes at least 5 d and requires skilled manpower, it is not suitable for *in situ* measurement or on-line monitoring. Since the first report on a microbial BOD sensor by Karube *et al.* (1977), numerous BOD sensors have been developed based on monitoring the dissolved oxygen (DO) consumption by immobilized microorganisms (Kulys & Kadziuskiene 1980, Riedel *et al.* 1988, Marty *et al.* 1997, Chee *et al.* 1999). However, such BOD sensors using a membrane and an O<sub>2</sub> probe have many disadvantages such as membrane fouling, short-term stability, and calibration drift.

A mediator-less microbial fuel cell (MFC) has been used as a BOD sensor (Kim *et al.* 2003). This device measures BOD more accurately than BOD sensors based on a pure cultures since a naturally-enriched, electrochemically active, microbial consortium is employed metabolizing a wide range of organic contaminants as suggested previously (Chee *et al.* 1999).

Almost all BOB sensors have been developed to determine high BOD values in industrial wastewaters

and have not been applied to measure low BOD values. Surface water and secondary effluents usually contain low concentrations of biodegradable organic compounds, such as humic acid, lignin, tannic acid, gum arabic and surfactants (Chee *et al.* 2000). The microorganisms present in environments with low levels of nutrients grow under apparent optimal conditions. They are known as 'oligotrophs' in contrast to 'copiotrophs', which are common in environments with greater nutritional opportunities. The ability of oligotrophic microorganisms to grow in this way has a number of important biotechnological, medical and environmental implications.

In this laboratory, studies are being made to develop an MFC using oligotrophs obtained from surface waters for a rapid determination of low BOD values. The MFC showed low coulomb yield of around 1% with graphite as the cathode. This low yield was believed due to low O<sub>2</sub>-reducing activity of the cathode and to fuel consumption through aerobic respiration in the anode compartment using O<sub>2</sub> diffused through the membrane (T.H. Pham *et al.* unpublished work). In this study, a novel MFC was developed as low BOD sensor by enhancing cathode reaction, and lowering O<sub>2</sub> diffusion into the anode compartment. To

the authors' knowledge this is the first report on the electrochemically active oligotroph.

## Materials and methods

### Wastewater

Surface water used as a fuel in this study was collected locally. The chemical oxygen demand (COD) was  $5 \pm 1.2 \text{ mg l}^{-1}$ . Also used was artificial wastewater containing glucose and glutamate (AWW) in some experiments (Eaton *et al.* 1995). The fuels were autoclaved at 121 °C for 15 min and purged with O<sub>2</sub>-free nitrogen gas before use. The bottles containing them were connected to nitrogen gas-tight bag to avoid air diffusion into the bottle due to the negative pressure developed during the use.

### Microbial fuel cell and enrichment

Two types of fuel cell were used. They are a sensor-type described earlier (Chang *et al.* 2003) and oligotroph-type designed in this study. The fuel cells were constructed using transparent polyacrylic material. The sensor-type was modified to construct the oligotroph-type fuel cell reducing the size of cation-specific membrane ( $10 \times 50 \text{ mm}^2$ , Nafion450, Dupont Co., USA). Graphite felt (GF series, GEE Graphite Ltd., Dewsbury, West Yorkshire, UK) and platinum-coated graphite felt were used as the anode and cathode, respectively. They were of the same size,  $10 \times 40 \times 5 \text{ mm}^3$ , and connected with platinum wire (0.5 mm diam.) through a resistance of 500  $\Omega$  and a digital voltmeter (Model 2000, Keithley Instruments, Inc., Cleveland, OH). The void volumes of anode and cathode compartments were 20 ml each (Figure 1). MFC system has been described in details in previous publications (Gil *et al.* 2003, Kim *et al.* 2003). The MFC was placed in a temperature-controlled chamber (33 °C). The flow rates to the anode and cathode compartments were adjusted depending on each experimental purpose.

The fuel cells were inoculated with river sediment and fed continuously with surface water at the rate of  $0.15 \text{ ml min}^{-1}$ . In some experiment AWW was used as fuel. Fuel and oxidant were fed at the rate of 0.15 and  $0.55 \text{ ml min}^{-1}$ , respectively during the enrichment process. All experiments were conducted in triplicate, and mean values or typical results were presented.

### Instrumentation and analysis

Signal from the multimeter (Model 2000, Keithley Co., USA) was recorded to a personal computer through a data acquisition system (Testpoint, Capital Equipment Co., Richmond, VA). Chemical Oxygen Demand (COD) was measured by using COD reagent (Ultra-low grade, HACH Co., Loveland, CO).

## Results and discussion

### Enrichment of oligotrophs

Within 8 weeks of continuous fuel feeding, MFC generated a stable current of over 0.01 mA showing that electrochemically active oligotrophs had been enriched. The fuel cells were then used as the low BOD sensor.

### Development of a novel MFC for low BOD values

Our previous studies have shown that the copiotrophic microbial fuel cell gave a coulomb yield over 70% (Chang *et al.* 2003), whilst a low yield of around 1% was obtained from an oligotrophic MFC using sensor-type fuel cells (see below). Considerable amounts of O<sub>2</sub> diffused through the cation specific membrane, and coulomb yield decreased when the anode was aerated (unpublished work). From these results, it was hypothesized that the low coulomb yield in the oligotrophic MFC is due to the fact that O<sub>2</sub> diffusing to the anode is more than the amount of O<sub>2</sub> needed to oxidize the fuel supplied through aerobic respiration.

Calculations were made to determine the minimum membrane size that renders enough proton transfer capacity with decreased O<sub>2</sub> diffusion. A maximum current of 5 mA was generated from a copiotrophic MFC having 26 cm<sup>2</sup> membrane (Lee *et al.* 2003). The current (A) is equivalent to C s<sup>-1</sup>, and 5 mA is 5 mC s<sup>-1</sup>, which is 18 000 mC h<sup>-1</sup>. By dividing this figure with Faraday constant (96 487 C mol<sup>-1</sup>), the electron consumption rate in the cathode compartment can be calculated:

$$18 \text{ C h}^{-1} / 96\,487 \text{ C mol}^{-1} \approx 0.187 \text{ mmol electron equivalent h}^{-1}.$$

Since the cathode reaction consumes the same moles of protons and electrons, the proton transfer rate through the membrane can be expressed as 0.187 mmol h<sup>-1</sup> in the MFC. This figure is divided

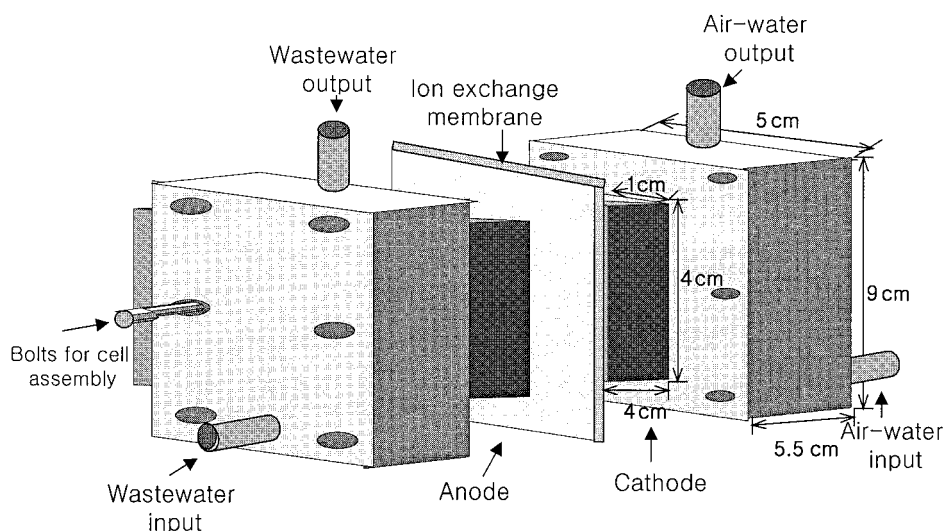


Fig. 1. Schematic diagram of the oligotroph-type microbial fuel cell.

by the membrane size to calculate the specific proton transfer rate as  $7.19 \mu\text{mol cm}^{-2} \text{h}^{-1}$ .

Assuming that an oligotrophic MFC is operated at a maximum fuel loading rate of  $15 \text{ mg h}^{-1}$ , protons generated through the anode reaction can be calculated as:

$$\begin{aligned} 15 \text{ mg l}^{-1} \text{ h}^{-1} / 32 \times 4 \text{ mg mmol}^{-1} &= \\ 1.875 \text{ mmol l}^{-1} \text{ h}^{-1} \times 0.02 \text{ l} &= 37.5 \mu\text{mol h}^{-1}. \end{aligned}$$

The minimum membrane size is calculated as:

$$37.5 \mu\text{mol h}^{-1} / 7.19 \mu\text{mol cm}^{-2} \text{ h}^{-1} \approx 5.2 \text{ cm}^2.$$

From these calculations the oligotroph-type fuel cell was designed with the membrane size of  $5 \text{ cm}^2$ .

The oligotroph-type fuel cell was compared with the sensor-type in terms of  $\text{O}_2$  diffusion rate and the expected coulomb yield. Calculations were made using specific  $\text{O}_2$  diffusion rates at the dissolved  $\text{O}_2$  concentration identical to the critical  $\text{O}_2$  concentration of the graphite ( $6.6 \text{ mg l}^{-1}$ ) and that of the platinum-coated graphite ( $2 \text{ mg l}^{-1}$ ), where the cathode reaction is not  $\text{O}_2$  limited (T.H. Pham, unpublished work).

As shown in Table 1, the  $\text{O}_2$  diffusion rate was calculated as  $135.2$  and  $70.2 \mu\text{g h}^{-1}$  in the sensor-type fuel cell (membrane size of  $26 \text{ cm}^2$ ) with graphite cathode and platinum-coated graphite cathode, respectively. The figures were  $26$  and  $13.5 \mu\text{g h}^{-1}$ , respectively in the oligotroph-type fuel cell (membrane size of  $5 \text{ cm}^2$ ). When the fuel cells are loaded with  $10 \text{ mg fuel l}^{-1}$  at a rate of  $0.15 \text{ ml min}^{-1}$ , the fuel loading rate will be  $90 \mu\text{g h}^{-1}$ . The  $\text{O}_2$  diffusion rate in the

sensor-type fuel cell was calculated as  $135.2 \mu\text{g h}^{-1}$ , higher than needed to consume the fuel loaded at this rate. Under these conditions the sensor-type MFC generated current (Table 2). These results might be due to the anaerobic pockets developed in the anode within the aerobic environment. The  $\text{O}_2$  diffusion rate calculated was  $13.5 \mu\text{g h}^{-1}$  in the oligotroph-type fuel cell. If this amount of  $\text{O}_2$  is completely reduced in the anode, which is added with fuel at  $90 \mu\text{g h}^{-1}$ , the coulomb yield of 85% is expected.

In addition to reducing the membrane size, the coulomb yield can be improved using a cathode with better affinity for  $\text{O}_2$  to run MFC with low dissolved  $\text{O}_2$  concentration in the cathode compartment since the  $\text{O}_2$  diffusion is dependent on the  $\text{O}_2$  gradient across the membrane. For this reason the oligotroph-type fuel cell employed a platinum-coated graphite that has the critical  $\text{O}_2$  concentration of  $2 \text{ mg l}^{-1}$ , much lower than that of graphite ( $6.6 \text{ mg l}^{-1}$ ) (T.H. Pham *et al.* unpublished work).

#### Effect of resistance

The oligotroph-type MFC was continuously operated with different resistance using AWW as the fuel at a feeding rate of  $0.21 \text{ ml min}^{-1}$  and air-saturated tap water as the oxidant at the feeding rate of  $0.55 \text{ ml min}^{-1}$  to establish the relationship between the resistance and potential, and current. The ratio of current generated at different resistance to that with the resistance of  $10 \Omega$  was calculated, and the ratios were compared between the copiotrophic and oligotrophic MFC. As

Table 1. O<sub>2</sub> diffusion rate in sensor-type and oligotroph-type MFC with different cathode.

Type of MFC	Membrane size (cm <sup>2</sup> )	Cathode	Critical O <sub>2</sub> concentration <sup>a</sup> (mg l <sup>-1</sup> )	Specific O <sub>2</sub> diffusion rate (μg cm <sup>-2</sup> h <sup>-1</sup> )	O <sub>2</sub> diffusion rate (μg h <sup>-1</sup> )	Ratio of fuel needed to consume O <sub>2</sub> <sup>b</sup> (%)
Sensor	26	Graphite	6.6	5.2	135.2	>100
		Platinum-coated	2	2.7	70.2	78
Oligotroph	5	Graphite	6.6	5.2	26	29
		Platinum-coated	2	2.7	13.5	15

<sup>a</sup>Critical O<sub>2</sub> concentration is defined as the catholite DO concentration below which the current generation of the MFC is O<sub>2</sub>-limited.

<sup>b</sup>The ratio was calculated with the fuel loading rate of 90 μg h<sup>-1</sup>.

Table 2. Comparison of current and coulomb yield between sensor-type and oligotroph-type MFC.

MFC	Initial COD (mg l <sup>-1</sup> )	Final COD (mg l <sup>-1</sup> )	Experimental current (mA)	Experimental coulomb (mC)	Theoretical coulomb <sup>a</sup> (mC)	Coulomb yield (%)
Sensor-type	6 ± 0.51	2 ± 0.53	0.0048 ± 0.03	8.6 ± 0.04	720 ± 0.03	1.2 ± 0.51
Oligotroph-type	6 ± 0.52	3 ± 0.44	0.02 ± 0.05	36.0 ± 0.07	180 ± 0.04	20.0 ± 0.34

<sup>a</sup>Theoretical coulomb is defined as the coulomb that is theoretically obtained from Faraday's law assuming the supplied fuel is completely converted into electricity.

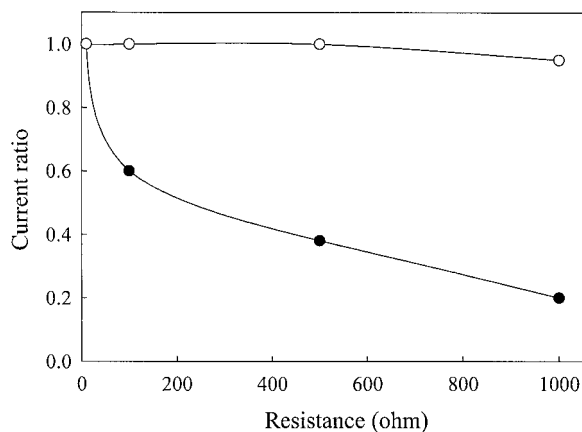


Fig. 2. Ratio of current generated at different resistance to that at 10 ohm in copiotrophic and oligotrophic MFC. Closed circle and open circle represent copiotrophic and oligotrophic MFC, respectively.

shown in Figure 2, the ratio decreased sharply in the copiotrophic MFC as the resistance increased, but the change was not significant in the oligotrophic MFC. These results show that among various limiting factors affecting the current generation, resistance up to 500 Ω does not limit the current generation from an oligotrophic MFC probably due to the intrinsic low current from the oligotrophic MFC compared with the copiotrophic MFC. For this reason resistance of 500 Ω was used to operate oligotrophic MFC.

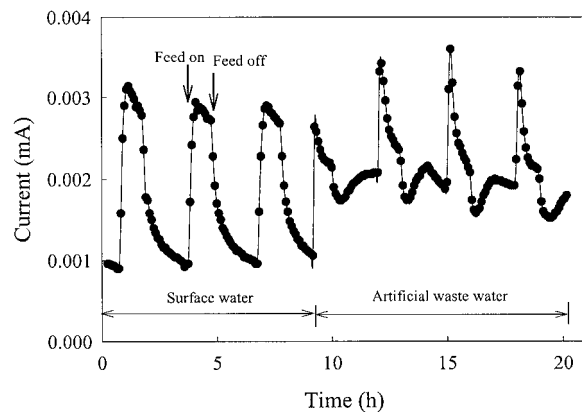


Fig. 3. Current generation from surface water-enriched oligotrophic MFC fed with surface water and AWW. The fuel cells were operated at 33 °C using a resistance of 500 Ω. Surface water and AWW of 5 mg l<sup>-1</sup> were fed as fuel at the rate of 0.55 ml min<sup>-1</sup>. Air-saturated water was fed to the cathode compartment at 1.8 ml min<sup>-1</sup>.

#### Performance comparison between oligotroph-type and sensor-type MFC

The MFC enriched with surface water (6 mg l<sup>-1</sup> BOD) was run in batch mode at the feeding rate of 2.6 ml min<sup>-1</sup> for 30 min followed by stop for 1 h. The cathode was fed with air-saturated tap water continuously at the same rate. The maximum current generated was 0.02 mA with the coulomb yield of 20% (Table 2). These figures are much higher than those of

sensor-type MFC. But the coulomb yield of 20% is much lower than expected from the O<sub>2</sub> diffusion into the anode compartment (Table 1). The low coulomb yield might be due to lower fuel loading than in the calculation to determine the membrane size. In the calculation, the fuel loading rate used was 15 mg h<sup>-1</sup>, whilst the MFC was loaded with less 0.5 mg h<sup>-1</sup> in this experiment. These results show that the performance of the oligotroph-type MFC can be further improved using optimum membrane size at the given fuel loading rate. Even with the low coulomb yield of 20%, the current of 0.02 mA is much higher than reported elsewhere (Chee *et al.* 1999).

#### *Effect of fuel different from that used for enrichment*

MFC enriched and run with surface water for over a year was fed with AWW in a batch mode. Air-saturated tap water as an oxidant was continuously fed at feeding rate of 1.8 ml min<sup>-1</sup>. Figure 3 compares the current generated from surface water with that from AWW. The MFC showed a normal current pattern with surface water, whilst the current pattern was irregular with AWW. This might be due to rapid fermentation of glucose acidifying anode. These results indicate that MFC should be enriched with the fuel similar to that to be analyzed.

**In conclusion**, the performance of an MFC as a low BOD sensor could be improved by reducing O<sub>2</sub> diffusion through cation-specific membrane using a cathode with the enhanced catalytic activity and the membrane with a reduced size. The oligotroph-type MFC can be used as a low BOD sensor with high operational stability and good repeatability. Studies are in progress to increase the sensitivity and signal to noise ratio.

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