



## Short Communication

## A rapid selection strategy for an anodophilic consortium for microbial fuel cells

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## ABSTRACT

A rapid selection method was developed to enrich for a stable and efficient anodophilic consortium (AC) for microbial fuel cells (MFCs). A biofilm sample from a microbial electrolysis cell was serially diluted up to  $10^{-9}$  in anaerobic phosphate buffer solution and incubated in an Fe(III)-acetate medium, and an Fe(III)-reducing AC was obtained for dilutions up to  $10^{-6}$ . The activity of MFC inoculated with the enrichment AC was compared with those inoculated with original biofilm or activated sludge. The power densities and Coulombic efficiencies of the AC (226 mW/m<sup>2</sup>, 34%) were higher than those of the original biofilm (209 mW/m<sup>2</sup>, 23%) and activated sludge (192 mW/m<sup>2</sup>, 19%). The start-up period of the AC (60 h) was also shorter than those obtained with the other inocula (biofilm, 95 h; activated sludge, 300 h). This indicated that such a strategy is highly efficient for obtaining an anodophilic consortium for improving the performance of an MFC.

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## 1. Introduction

Current generation in microbial fuel cells (MFCs) is dependent on the presence of exoelectrogenic bacteria that oxidize organic matter and transfer electrons to the anode (Logan, 2008). Pure exoelectrogenic cultures have been tested as inocula for MFCs, but the power densities generated were usually lower than those obtained using a mixed culture in the same MFC (Ishii et al., 2008), although some pure cultures produced power densities equal to or higher than those of the mixed culture (Nevin et al., 2008).

Rich sources of microorganisms, such as wastewater, activated sludge, and sediments, are often used as inocula for MFCs, and analyses of anode biofilms in these systems have shown great bacterial diversity in anodophilic communities, but did not reveal specific trends in dominant members (Logan, 2008). This finding may be due to the large number of non-exoelectrogens in the anode biofilm. Logan and Regan (2006) reported that an anodophilic community can be sustained in MFCs even when the biofilm is enriched through successive transfer and enrichment. Thus, selective pressure in MFC systems may not be sufficient to exclude non-exoelectrogens. Since many of the exoelectrogens detected in MFCs are dissimilatory metal-reducing bacteria (DMRB) (Nevin et al., 2008), we developed a rapid selection method to obtain an anodophilic consortium (AC) through dilution and regrowth of DMRB using poorly crystalline Fe(III)-oxide.

## 2. Methods

## 2.1. Medium and anodophilic consortium (AC) culture selection

A modified phosphate buffered basal medium was used for the selection of the Fe(III)-reducing exoelectrogenic culture (Nelson and Zeikus, 1974). The medium contained (per liter): (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.45 g; NaCl, 0.90 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.18 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.10 g; NH<sub>4</sub>Cl, 0.50 g; KH<sub>2</sub>PO<sub>4</sub>, 1.50 g; K<sub>2</sub>HPO<sub>4</sub>, 2.19 g; and a trace mineral solution (9 ml) and vitamin solution (5 ml). The pH was adjusted to 6.8–7.0 using NaOH or HCl. Acetate (10 mM) and Fe(III)-oxide (100 mM) (Lovley and Phillips, 1988) were provided as the electron donor and electron acceptor. Standard anaerobic techniques were used throughout all tests (Hungate, 1950). Stock solutions (acetate, Fe(III)-oxide) were prepared and sterilized separately prior to being added into the basal medium.

Biofilm was collected from the anode of an MEC (Liu et al., 2008) and used as the inoculum for the DMRB enrichment procedure. A portion of the carbon paper anode (10 cm<sup>2</sup>) from the MEC was transferred to a glass serum bottle containing 80 ml of the anaerobic phosphate buffer solution (PBS, 50 mM, pH 7.0) and glass beads. The bottle was shaken vigorously to produce a cell suspension from the biofilm. After measuring the cell concentration (*ca.*  $5 \times 10^9$  cells/ml), the suspension was serially diluted from  $10^{-1}$  to  $10^{-9}$  (in triplicate) with buffer (50 mM anaerobic PBS) into 100 ml-anaerobic serum bottles containing Fe(III)-acetate medium (60 ml). The bottles were incubated at 28 °C.

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## 2.2. MFC systems

The electrochemical activity of different cultures was examined in aqueous-cathode cubic MFCs consisting of two chambers with dimensions of 3.5 cm × 6 cm × 10 cm (210 ml) separated by a proton exchange membrane (PEM, NAFION 117; 24 cm<sup>2</sup>). Plain carbon cloth (30 × 40 mm, without wet proofing; E-Tek Div, PEMEAS, USA) was used for the anode, and carbon paper with 0.35 mg/cm<sup>2</sup> Pt catalyst (E-Tek DIV, PEMEAS, USA) was used for the cathode. The electrode spacing was 4 cm between the anode and the cathode, with a fixed external resistance of 1000 Ω (except as noted). The anode chamber medium contained acetate (1 g/L), NH<sub>4</sub>Cl (0.31 g/L), KCl (0.13 g/L), metal salts (12.5 ml/L) and vitamins (5 ml/L), and buffer (50 mM PBS). The cathode chamber contained only buffer, and was aerated to produce a dissolved oxygen concentration of 8.0 mg/L. All reactors were sterilized using 2% glutaraldehyde (immersing time: 12 h) and UV illumination before inoculation. To eliminate possible carry over of iron precipitates into MFCs, the AC culture was first transferred to LB medium containing (per liter): peptone, 10 g; yeast extract, 5 g; NaCl, 10 g. Cells were then washed by centrifugation (2300g, 7 min) and resuspended into 50 mM anaerobic PBS prior to inoculation into the MFCs.

Voltages (*E*) measured across an external resistance were recorded using a data acquisition system (Model TG0012, Sem-tech Instruments, China). MFCs were operated in fed-batch mode, with complete replacement of the liquid solution after each cycle (voltage <20 mV). To obtain polarization and power density curves, external circuit resistance was varied from 150 to 4000 Ω. The value used for each resistor was based on stable current production for 36 h. After inoculation, all MFCs were operated at 28 °C.

## 2.3. Analytical methods and calculation

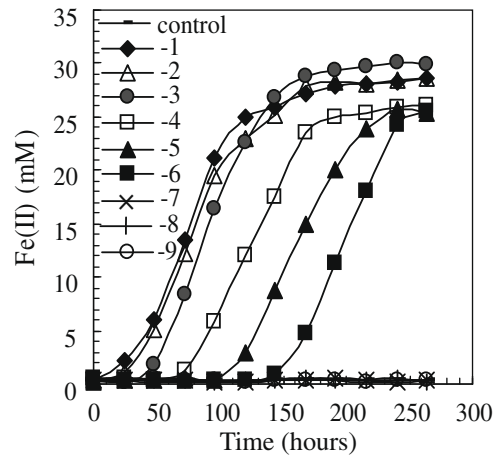
Cell numbers were measured using a Petroff-Hausser counting chamber. Dissolved oxygen concentration was determined by Handheld Multi-Parameter Instruments meter (pH/Oxi 340i, WTW, Germany). The Fe(II) produced was monitored spectrophotometrically with a ferrozine assay of HCl-extractable Fe(II) (Lovley and Phillips, 1988). The total cell protein content of the samples using a modified method of Bradford (Bradford, 1976). Acetate concentrations were determined by high performance liquid chromatography (LC-10AT, Shimadzu, Japan). Current (*i*), current density (*I*), power density (*P*) and Coulombic efficiency (CE) were calculated as described by Logan (2008).

## 3. Results and discussion

### 3.1. Rapid selection of the anodophilic consortium (AC)

After serial dilution and incubation for 11 days, we observed the formation of black precipitates (from initially red colored solutions containing poorly crystalline Fe(III)-oxide) in bottles with 10<sup>-1</sup> to 10<sup>-6</sup> dilutions. No black precipitates formed in higher dilution bottles (10<sup>-7</sup>–10<sup>-9</sup>), although cells were present in the solution. According to Roden and Lovley's work (1993), the black precipitates, mainly magnetite (Fe<sub>3</sub>O<sub>4</sub>) and siderite (FeCO<sub>3</sub>) could be adopted as the positive indicator for Fe(III) reduction.

The production of Fe(II) at different dilution levels is shown in Fig. 1. At the higher dilution levels a longer lag in Fe(II) production was observed. After 260 h, Fe(II) concentrations in all positive dilution bottles reached a similar and stable level of 25–30 mM (Fig. 1), which was slightly less than the value of 33 mM expected from stoichiometric reduction of 100 mM poorly crystalline Fe(III)-oxide. The longer lag time in the higher dilution bottles therefore

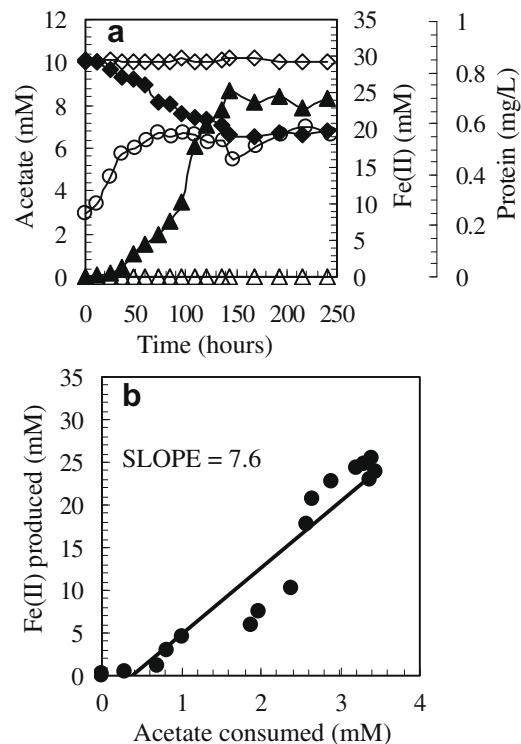


**Fig. 1.** Incubation time course of Fe(II) production in the bottles inoculated with different dilutions of an original anode biofilm (from 10<sup>-1</sup> to 10<sup>-9</sup>) in Fe(III)-acetate medium. The control was not inoculated.

resulted from the smaller number of Fe(III)-reducing bacteria in the inoculum. The cell suspension from the most diluted positive sample (10<sup>-6</sup> dilution), referred to here as the AC, was used as the inoculum for MFC tests.

### 3.2. Fe(III) reduction by anodophilic consortium (AC)

The AC from the original 10<sup>-6</sup> dilution bottle maintained Fe(III)-reducing activity after more than 15 sequential transfers (10% v/v), indicating a stable dissimilatory Fe(III)-reducing consortium. The Fe(II) reached a constant level of ~26 mM (Fig. 2a), and the ratio



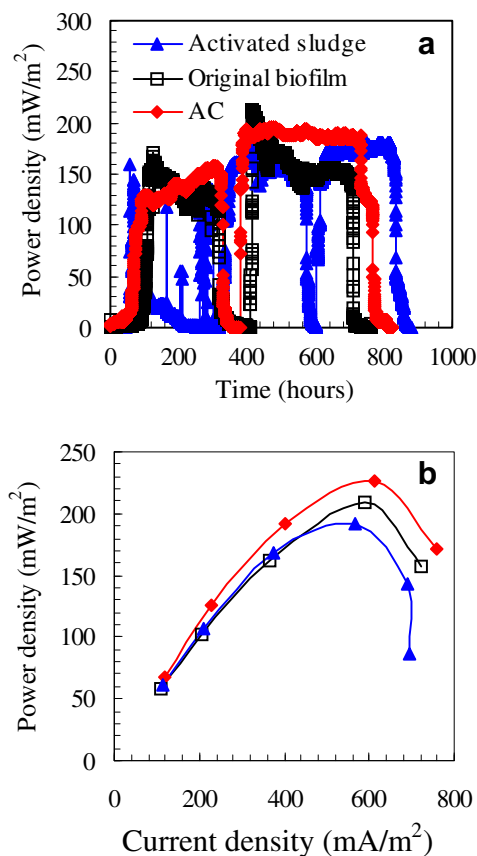
**Fig. 2.** (a) Fe(II) generation (AC, filled triangles; control, open triangles), acetate concentration (AC, filled squares; control, open squares) and biomass (AC, open circles) in Fe(III)-acetate medium inoculated with AC or without microbes (uninoculated control). (b) Fe(II) production versus acetate consumption for data in panel (a).

**Table 1**

Comparison of electricity generation in MFC reactors inoculated with AC, original biofilm and activated sludge.

Inoculum	AC	Original biofilm	Activated sludge
Power density at 1000 $\Omega$ (mW/m <sup>2</sup> )	189 $\pm$ 3	160 $\pm$ 16	174 $\pm$ 3
Max power density at 500 $\Omega$ (mW/m <sup>2</sup> )	226	209	192
CE (%)	34	23	19
Lag time (h)	60	95	300

The standard deviations of power density at 1000  $\Omega$  are calculated with respect to time of operation.



**Fig. 3.** (a) Power densities as a function of time for the AC, original biofilm, and activated sludge in three MFC reactors (triplicate reactors, all at 1000  $\Omega$ ). (b) Power density as a function of current density (normalized to the anode area) obtained by varying the external circuit resistance (150–4000  $\Omega$ ) for the AC, original biofilm and activated sludge.

of Fe(II) to acetate consumed was 7.6 (Fig. 2b). This ratio compared well to a ratio of 8.0 expected from the complete oxidation of acetate with Fe(III) reduction. Thus, approximately 95% (7.6 mol/mol) of the acetate was consumed for Fe(III) reduction, with the balance used for cell synthesis or other processes.

### 3.3. Power density and current efficiency of AC

Three inocula (AC, original biofilm, activated sludge) were tested in MFCs to compare their electrochemical activities (Table 1). Since the bacteria in the AC in the Fe(III)-acetate medium were likely attached to the iron precipitates, and therefore could not be

separated by a centrifugation/washing procedure, the cell suspension was transferred into LB medium and grown, thus avoiding subsequent transfer of iron precipitates into the MFC reactor.

Electricity was rapidly generated with a stable power density after 96 h of 139  $\pm$  11 mW/m<sup>2</sup> (1 g/L acetate, 1000  $\Omega$ ) (Fig. 3a). A slightly higher power density of 189  $\pm$  3 mW/m<sup>2</sup> was obtained in the second cycle II (400–800 h) (Fig. 3a). The MFC inoculated with the original biofilm exhibited a lag of  $\sim$ 95 h and produced similar power density in the first cycle (135  $\pm$  12 mW/m<sup>2</sup>) but a lower power density compared to the AC in the second cycle (160  $\pm$  16 mW/m<sup>2</sup>) (Fig. 3a). The MFC inoculated with activated sludge did not produce a constant level of power in the first cycle, but reached 174  $\pm$  3 mW/m<sup>2</sup> by the third cycle (Fig. 3a). In the latter cycles, power production by each MFC became reproducible in terms of stable maximum voltages. There was no electricity produced in a sterile MFC.

Power density and polarization curves (Fig. 3b) show that inoculation with the AC produced a maximum power density of 226 mW/m<sup>2</sup> (613 mA/m<sup>2</sup> at 500  $\Omega$ ), which is 8% higher than that of the original biofilm (585 mA/m<sup>2</sup>) and 18% higher than that obtained by activated sludge (565 mA/m<sup>2</sup>). The AC also produced a higher Coulombic efficiency (34%) than the original biofilm (23%) or activated sludge (19%).

## 4. Conclusions

A serial dilution enrichment method using solid phase iron produced a stable anodophilic consortium that was superior to that of the initial inoculum or an activated sludge inoculum in terms of maximum power production. The AC reduced the lag time for power generation and achieved a higher CE than the original biofilm or activated sludge inoculum. The AC can easily be preserved and used as an inoculum in future MFC studies.

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## References

- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Hungate, R.E., 1950. The anaerobic mesophilic cellulolytic bacteria. *Bact. Rev.* 14, 1–49.
- Ishii, S., Watanabe, K., Yabuki, S., Logan, B.E., Sekiguchi, Y., 2008. Comparison of electrode reduction activities of *Geobacter sulfurreducens* and an enriched consortium in an air-cathode microbial fuel cell. *Appl. Environ. Microbiol.* 74, 7348–7355.
- Liu, W., Wang, A., Ren, N., Zhao, X., Liu, L., Yu, Z., Lee, D., 2008. Electrochemically assisted biohydrogen production from acetate. *Energy Fuels* 22, 159–163.
- Logan, B.E., Regan, J.M., 2006. Electricity-producing bacterial communities in microbial fuel cells. *Trends Microbiol.* 14, 512–518.
- Logan, B.E., 2008. *Microbial Fuel Cells*. John Wiley & Sons.
- Lovley, D.R., Phillips, E.J.P., 1988. Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiology* 6, 145–150.
- Nelson, D.R., Zeikus, J.G., 1974. Rapid method for the radioisotopic analysis of gaseous end products of anaerobic metabolism. *Appl. Microbiol.* 28, 258–261.
- Nevin, K.P., Richter, H., Covalla, S.F., Johnson, J.P., Woodard, T.L., Orloff, A.L., Jia, H., Zhang, M., Lovley, D.R., 2008. Power output and coulombic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. *Environ. Microbiol.* 10, 2505–2514.
- Roden, E.E., Lovley, D.R., 1993. Dissimilatory Fe(III) reduction by the marine microorganism *Desulfuromonas acetoxidans*. *Appl. Environ. Microbiol.* 59, 734–742.