Performance comparison of microbial fuel cells equipped with different membrane electrode assemblies

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Abstract. It is important for practical use of microbial fuel cells (MFCs) to not only develop new materials including electrodes and proton exchange membranes but also to understand the bacterial community structure related to electricity generation. Here, four kinds of novel membrane electrode assemblies (MEAs) were made. Four lactate fed MFCs equipped with the membranes were characterized by electrochemical, molecular-dependent and molecular-independent methods. MFC1 equipped with Nafion 117-type MEA (18\ \mu m thickness) exhibited the highest performance. Although the other MEAs with different configurations of three kinds of polymers; poly (diallyldimethylammonium chloride), polyallylamine hydrochloride and poly (2-acrylamino-2-methyl -1-propanesulfonic acid) had thicknesses of about 0.3 \mu m (MEA 2 and 3) and 1.0 \mu m (MEA4), their power densities were lower. Denaturing gradient gel electrophoresis (DGGE) and phylogenetic analyses showed that anaerobic bacteria dominated in anode biofilms of MFC1. A bacterium completely corresponding to nucleotide sequence of one of the DGGE bands was isolated from the anode biofilm in MFC1. Interestingly, BLAST search indicated that the bacterium (named strain RO1) belonged to the genus of gram positive bacterium, \textit{Propioniferax}. It was confirmed that strain RO1 was capable of producing electricity and constructing biofilm on the anode surface in pure culture MFC. These results suggested that the property of MEA affects significantly the bacterial community structure, thereby influencing the MFC-performance.

1. Introduction
Microbial fuel cells (MFCs) are devices that exploit microorganisms as “biocatalysts” for generating electric power from organic matter, which have been studied about energy recovery from waste as electrical power [1, 2, 3,4, 5] and generating power from marine aquatic sediments [6, 7], rice paddy field soil [8, 9], wheat straw biomass [10] or cellulose [11].

Although power densities have been improving steadily from a few mW m\textsuperscript{2} to several hundreds of mW m\textsuperscript{2}, there is need for optimization for improved power densities and development of large-scale devises [2]. Increasing the power density is one of the greatest challenges for the practical application of MFCs. Potential loses include the anodic reaction limited by microbial activity, cathodic reaction...
limited by the abiotic electron-accepting reaction, and oxygen intrusion through the cathode and proton exchange membrane [8, 4]. Furthermore, it is generally accepted that thinner membranes are ideal in that they exhibit lower internal resistance which is one of most important parameters [5]. It is important for accomplishment of this challenge not only to develop the materials of electrodes and proton exchange membranes but also to analyze electricity-producing bacteria.

While several membranes have been tested in chemical fuel cells, not much work on membranes has been reported with MFCs. Hence, the aims of this study were to evaluate novel ultrathin membrane electrode assemblies (MEAs) by analyzing the electrochemical properties and bacterial community structures. Four kinds of MEAs were used; Nafion 117-type MEA with a thickness of 18 μm, the other three had different configurations of three kinds of polymers; poly (diallyldimethylammonium chloride) (PDDA), polyallylamine hydrochloride (PAH) and poly (2-acrylamino-2-methyl -1-propanesulfonic acid) (PAMPS). A bacterium with 100% similarity to nucleotide sequence of one of the DGGE bands was isolated from the anode biofilm of MFC1. The electricity-producing properties and phylogenetic position were also analyzed.

2. Materials and Methods

2.1. Membrane electrode assembly

Four kinds of membrane electrode assemblies (MEAs) were made by using a casting method or a layer-by-layer method. For the casting method, 0.4 mL solution of Nafion 117 [5% (w/w) in lower aliphatic alcohols and water, Aldrich] was applied on the surface of carbon paper electrode electroplated with platinum (TGP-H-060, CHEMIX Co., Ltd., Japan. 0.5 mg Pt cm⁻²) and then dried up. Five mL of Nafion 117 solution [5% (w/w) in lower aliphatic alcohols and water, Aldrich] and 5 mL of dH₂O were mixed and then concentrated to around 3 mL at 80°C by evaporating the alcohols in the Nafion solution. 0.6 mL of the concentrated Nafion solution was then coated onto the carbon paper and then dried up at 90°C (denoted Nafion-type MEA). The Nafion 117-type MEA (MEA1) was used on MFC1.

The other MEAs (MEA2, 3 and 4) were made by the layer-by-layer method as described previously [12, 13]. These MEAs had different configurations of three kinds of polymers, PDDA, PAH and PAMPS. MEA2 consisted of five layers of (PDDA/PAMPS)/(PAH/PAMPS)₄ stacked on a carbon paper electrode electroplated with platinum (0.5 mg Pt cm⁻²) and was used on MFC2. MEA3 consisted of five layers of (PDDA/PAMPS)/(PAH/PAMPS)₄ stacked on a carbon paper electrode electroplated with platinum (2.0 mg Pt cm⁻²) and was used on MFC3. MEA4 consisted of ten layers of (PDDA/PAMPS)/(PAH/PAMPS)₉ stacked on a carbon paper electrode electroplated with platinum (2.0 mg Pt cm⁻²) and was used on MFC4. The thicknesses of MEA1, 2, 3, and 4 were approximately 18 μm, 0.3 μm, 0.3 μm, and 1.0 μm, respectively.

2.2. MFC configuration and operation

Mediator less air-cathode MFCs were constructed to evaluate novel MEAs by electrochemical and microbiological analyses. Each MEA was used as both proton exchange membrane on one side and cathode electrode on the other side, providing a total projected cathode surface area (on one side) of 16 cm². 153 pieces of cubic (125 mm³) graphite felts (SOHGOH-C Co., Ltd. Yokohama, Japan) were packed into the anode chamber (36 mL in capacity) and the total area of anode surface was 0.02425 m². 20 of these pieces were directly connected to platinum wires (0.3 mm; AlfaAesar).
Figure 1. Graphite packing in the anode (A) and the MFC design used in this study (B)

A chemically defined anoxic medium designated BE was used for cultivation. The BE medium was modified from previously reported media [14, 15, 16], and contained 0.5 g of KH$_2$PO$_4$, 0.20 g of MgSO$_4$·7H$_2$O, 0.15 g of CaCl$_2$·2H$_2$O, 0.5 g of NH$_4$Cl, 2.5 g of NaHCO$_3$, 1.0 mL of Se/W solution [17], 1.0 mL of trace elements SL8 solution [18], and 1.0 mL of vitamin solution PV1 [19] per liter. 0.4 g of paddy field soil was inoculated into each MFC containing 20 mM lactate (DL-lactic acid, Sigma) and the electrodes were then connected with an external resistor (10 Ω) 5 days after inoculation. The MFCs were enriched by recirculation of BE medium at a rate of 25 mL h$^{-1}$. Lactate was added to be 20 mM in the anode chamber whenever the cell voltage dropped to baseline levels. Another MFC that was also constructed using the same materials was run under open circuit conditions as a control (denoted control MFC).

2.3. Electrochemical Measurements and operation

The voltage (V) across a 10 Ω resistor was measured every 5 min using a data logger GL200A (GRAPHTEC, co. Ltd., Yokohama, Japan) with a data acquisition system connected to a personal computer. Power (P) was calculated according to $P = VI$, and maximum power densities were calculated using the maximum power values that remained constant for approximately 4 hours (corresponding to each substrate feeding) divided by the total apparent surface area of the electrode (0.02025 m$^2$). Polarization curves were obtained by imposing a linear potential decrease of 1 mV s$^{-1}$ from the open circuit voltage potential to a cell potential of 0 mV followed by a linear voltage increase of 1 mV s$^{-1}$ of the cell potential by a potentiostat HSV-110 (Hokuto Denko, Tokyo, Japan). Coulombic efficiency was obtained by calculating the ratio of total recovered coulombs by integrating the current over time to the theoretical amount of coulombs that can be produced from lactate [20].

2.4. HPLC and organic acids

Organic acids were measured using a high-pressure liquid chromatograph (GL-7400 series, GL Science Inc., Shinjuku-ku, Tokyo, Japan) equipped with a Shodex RSpak KC-811 column (300 x 8.0 mm) (SHOWA DENKO Co. Ltd., Kanagawa, Japan) and UV detector. Column oven was set at 50°C. Samples were eluted with 0.1% H$_3$PO$_4$ solution and elutes were monitored at 210 nm. Formate, pyruvate, lactate, butyrate and acetate were identified according to the retention time and the concentration was determined by comparing the peak area with that of its respective standard sample.

2.5. Microbial analysis

2.5.1. DNA extraction. Aliquots of anaerobic culture (1 mL) from MFCs were centrifuged for 10 min at 4°C and 20,000 xg, and cells were collected. Anode samples (2 pieces) from MFCs were taken out
and washed thoroughly in 10 mM sodium phosphate buffer (pH 7.0) and were crushed using a sterile pipette tip. DNA was extracted according to the conventional method described previously [14].

2.5.2. PCR-DGGE. To analyze the total bacterial community structure, the variable V3 region of bacterial 16S rRNA genes (corresponding to positions 341 to 534 in the *Escherichia coli* sequence) was amplified using primers P2 and P3 (containing a 40-bp GC clump) [21] and a thermal cycler (ASTEC PC320, Astec Co., Ltd., Fukuoka, Japan) as described previously [21]. A DCode DGGE system (Bio-Rad, CA, USA) was used for electrophoresis following the manufacturer’s instructions. A total of 10 μL of a PCR-amplified mixture was subjected to electrophoresis in a 10% (wt/vol) polyacrylamide gel at 200 V for 3.5 h. The gel gradients used for separation, which were applied in parallel to the electrophoresis direction, were 40 to 60%. After electrophoresis, the gel was stained with SYBR Green I (Molecular Probes) for 30 min according to the manufacturer’s instructions. The nucleotide sequences of DGGE bands were determined as described previously [22].

2.5.3. Isolation of bacteria. BE medium was used for isolation of anaerobic bacteria. Lactate (final concentration 20 mM) was used as an electron donor and gellan gum was added to a final concentration of 2.0% w/v. Either Na₂SO₄ or Fe(III) EDTA was used as a sole electron acceptor at a final concentration of 20 mM and 5 mM, respectively. The diluted culture suspension was directly spread onto a solid plate containing either medium under anaerobic conditions. The plate was placed in a nylon bag (Mitsubishi Gas Chemical Company, Tokyo, Japan) with a catalyst sachet (AnaeroPouch-Anaero; Mitsubishi Gas Chemical Company) and was then quickly sealed with a clip. One-tenth strength Trypticase soy broth (TSB) medium (1/10TSB) was also used for isolation of aerobic bacteria. After the plates were incubated at 25°C for three weeks, colonies formed were picked and spread onto the same medium to confirm their purity.

2.5.4. Sequencing of 16S rRNA gene. Purified bacterium, named strain RO1, was incubated in an anaerobic BE medium containing lactate (20 mM) and Na₂SO₄ (20 mM) at 25°C for 10 days. The DNA was extracted from cells using a method described previously [14]. The quality and quantity of the extracted DNA was checked by measuring the absorbance at 260 and 280 nm. Almost-full-length fragment of 16S rRNA gene was amplified using the 27f (5’-AGAGTTTGATCCTGGCTCAG-3’, *E. coli* 16S rRNA gene positions 8 to 27) and 1525r (5’-AAGGAGGTGATCCAGCC-3’, positions 1525 to 1542). Amplification was performed with the PC320 thermal cycler (ASTEC) by using a 50 μL reaction volume as described previously [14].

2.5.5. Scanning electron microscopy observation. Anode electrode particles were taken out and were washed three times with 10 mM phosphate buffer. The anodes were fixed in a 2.5% solution of glutaraldehyde and processed through an ethanol dehydration series (i.e.10, 15, 25, 50, 75, 100% v/v ethanol. Each treatment was carried out for 15 min and repeated twice) as described previously [23]. The samples were then coated with gold using a coater device (SC-701AT Quick Auto Coater, Sanyo, Tokyo, Japan) set at 50 Å and observed using a scanning electron microscope (S3000N, HITACH, Japan) at 7 kV.

2.5.6. Accession numbers. The nucleotide sequence data reported here have been deposited in the DDBJ under accession numbers AB693055 to AB693099.
3.1. Characterization of current generation

The electricity producing capabilities were different from each other. Unexpectedly, MFC1 equipped with Nafion 117-type MEA had the highest power density although it had the thickest membrane (Figure 2). MFC3 and MFC4 produced comparatively very low power production.

MFC1 and MFC2 had a similar trend of an initially high power production followed by a lower, stable and reproducible phase (Figure 2 and 3). Power production occurred in cycles depending on degradation of lactate and concentration of resultant organic acids. The power generation of MFC1 started on day 12 and the power density was about 4 mW m\(^{-2}\) during day 20 to day 40. The power density slightly decreased later, and was stable at around 1 mW m\(^{-2}\). Correspondingly, the coulombic efficiency of MFC1 changed from 35 ± 4\% (during day 20 to day 40) to 16 ± 2\% (after day 50). This result indicated that the electron flow changed dramatically in the anode of MFC1. The power density of MFC2 equipped with MEA2 was as high as 0.08 mW m\(^{-2}\) during day 25 to day 50, and finally became stable at less than 0.02 mW m\(^{-2}\). The coulombic efficiencies of MFC2 were slightly less than
5%. These results suggested that bacterial community succession occurred and their community structures were different from each other. Polarization curve analyses showed that the electricity-producing properties of MFC1 changed (Table 1). Polarization curve analyses of MFC2, 3 and 4 could not be performed due to their low power production. In the initial stage until day 38, MFC1 maximum power and current densities were 5.8 mW m$^{-2}$ and 95 mA m$^{-2}$, respectively. However these values decreased to 1.2 mW m$^{-2}$ and 37 mA m$^{-2}$ in the final stage from day 158 to day 197.

Table 1. MFC1 Linear Sweep Voltammetry showing electricity producing properties

| Day | $P_{\text{max}}$ (mW/m$^2$) | $I_{\text{max}}$ (mA/m$^2$) | $R_{\text{int}}$ (ohms) | OCV (mV) |
|-----|-----------------|-----------------|-----------------|---------|
| 18  | 6.2             | 90              | 90              | 205     |
| 38  | 5.3             | 100             | 80              | 198     |
| 158 | 1.0             | 34.5            | 187             | 124     |
| 170 | 1.0             | 34.3            | 175             | 126     |
| 197 | 1.3             | 39.8            | 146             | 131     |

Along with these changes, internal resistance values increased from 90 Ω to 170 Ω. Since the MEAs were completely covered by a biofilm when the experiment was terminated, it was inferred that the fouling of MEA caused the decrease of proton permeability, resulting in changes in electrochemical properties. It has also been reported that the fouling of the cathode results in a reduction of coulombic efficiency due to both utilization of organic acids and a decrease in the active cathode surface area by the cathode biofilm [24].

3.2. Bacterial community structure

Bacterial community structures of MFCs were analyzed by DGGE technique and were then analyzed phylogenetically (Table 2). Since the power densities of MFC1 and MFC2 were significantly different from each other, it was predicted that their bacterial community structures would be also different from each other. Interestingly, dominant anolyte-bacteria and biofilm-bacteria from MFC1 and MFC2 did not make distinct clusters based on MFC of origin. The bacteria from both MFCs were present in almost all clusters, suggesting that members of these bacterial communities were almost similar (Table 2). It therefore appears relative abundances of the members or other factors like oxygen intrusion into the MFCs may be responsible for the differences in power production.

Almost half of DGGE bands sequenced from MFC1 and MFC2, which were mostly from anode biofilm, belonged to *Firmicutes* containing the genera of *Pelosinus* and *Psychrosinus*. *Firmicutes* are generally known as fermenters and it has been reported that *Pelosinus fermentans* strains are capable of fermenting lactate and coupling its oxidation to Fe(III) and metal reduction [25, 26]. *Psychrosinus fermentans* FCF9 is reported as an obligately anaerobic lactate fermenter [27]. These results suggest that these bacterial community structures adapted to transfer of electrons from lactate to anode electrode.
# Table 2. DGGE band sequence analysis

| MFC   | Band | Nearest neighbor                              | Accession number | Similarity |
|-------|------|-----------------------------------------------|------------------|------------|
| MFC1  | 1-1  | *Sphingobacterium multivorum*                 | HE800586         | 95%        |
|       | 1-4  | *Oscillatoria* sp. INT-06                     | AB046728         | 100%       |
|       | 1-6  | *Veillonellaceae bacterium* FCF9B              | DQ767882         | 97%        |
|       | 1-8  | *Delftia* sp. BCA19                           | HE716888         | 95%        |
|       | 1-9  | *Leclercia adecarboxylata* strain MN11SW4      | JX501721         | 100%       |
|       | 1-11 | *Enterobacter hormaechei* strain skg0062      | HQ322394         | 97%        |
|       | 1-12 | *Pelosinus* sp. UFO1                          | EU215386         | 97%        |
|       | 1-14 | *Dechlorosoma* sp. PCC                        | AY126453         | 100%       |
|       | 1-15 | *Propionispora hippei*                        | AI508928         | 99%        |
|       | 1-16 | *Psychrosinus fermentans* strain FCF9         | DQ767881         | 92%        |
|       | 1-17 | *Sphingobacterium multivorum*                 | HE800586         | 100%       |
|       | 1-19 | *Pseudomonas indica* strain NR4_17            | JX843378         | 100%       |
|       | 1-20 | *Veillonellaceae bacterium* FCF9B              | DQ767882         | 97%        |
|       | 1-21 | *Oscillatoria* sp. INT-06                     | AB046728         | 100%       |
| MFC2  | 2-1  | *Azospirillum* sp. C10                        | JX842779         | 100%       |
|       | 2-2  | *Azospirillum* sp. C10                        | JX842779         | 100%       |
|       | 2-3  | *Dechlorosporillum* sp. DB                     | AY530551         | 100%       |
|       | 2-4  | *Pseudomonas stutzeri*                        | FR871674         | 97%        |
| Control | C-1 | *Sphingobacterium multivorum*                 | HE800586         | 100%       |
|       | C-2  | *Chryseobacterium* sp. JC145                  | HE962139         | 97%        |
|       | C-3  | *Delftia* sp. aer2                            | JX501738         | 99%        |
|       | C-4  | *Citrobacter freundii* strain HME8594         | JX426059         | 99%        |
|       | C-5  | *Sporotalea propionica*                       | AM258975         | 97%        |
|       | C-6  | *Acinetobacter* sp. A1060                     | JX266319         | 100%       |
|       | C-7  | *Klebsiella* sp. IARI-ALC-11                  | JX512192         | 100%       |
|       | C-8  | *Klebsiella* sp. IARI-ALC-11                  | JX512192         | 100%       |
|       | C-9  | *Pelosinus* sp. UFO1                          | EU215386         | 96%        |
|       | C-10 | *Delftia* sp. aer2                            | JX501738         | 99%        |
|       | C-11 | *Anaerovibrio burkinabensis* DSM 6283(T)       | AJ010961         | 96%        |
| Biofilm | A1-1 | *Pseudomonas* sp. ISTDF                       | GQ202116         | 92%        |
|       | A1-2 | *Clostridium* sp. SW001                       | HM755724         | 95%        |
|       | A1-3 | *Sporotalea propionica*                       | AM258975         | 97%        |
|       | A1-4 | *Propioniferax* sp. P7                       | EU109728         | 100%       |
|       | A1-5 | *Clostridium quercicolum*                     | AJ010962         | 97%        |
|       | A1-6 | *Desulfovibrio inopinatus*                    | AF177276         | 91%        |
|       | A1-7 | *Pelobacter propionicus* DSM 2379             | CP000482         | 93%        |
|       | A1-8 | *Dechlorosoma* sp. PCC                       | AY126453         | 100%       |
|       | A1-9 | *Veillonellaceae bacterium* FCF9B             | DQ767882         | 96%        |
|       | A2-10| *Macellibacteroides fermentans* strain LIND7H | HQ020488         | 99%        |
|       | A2-11| *Clostridium quercicolum*                     | AJ010962         | 97%        |
|       | A2-12| *Sporotalea propionica*                      | AM258975         | 98%        |
|       | A2-13| *Desulfovibrio desulfuricans* strain E062     | JX267091         | 99%        |
|       | A14  | *Macellibacteroides fermentans* strain LIND7H | HQ020488         | 99%        |
|       | A15  | *Klebsiella* sp. IARI-ALC-11                  | JX512192         | 100%       |
Other dominant biofilm-bacteria of MFC1 and MFC2 belonged to several classes, α-, β-, γ-, δ-Proteobacteria, Bacteriodetes, and Actinobacteria (Table 2). A bacterium with 100% similarity to the DGGE band A1-4 was isolated in this study. The strain was named Propioniferax sp. strain RO1 due to its 98.2% similarity to Gram-positive Propioniferax sp. P7 (Table 2). To investigate whether the stain RO1 was capable of producing electricity, the strain RO1 was inoculated into an air-cathode MFC equipped with normal Nafion 117 membrane (170 μm thickness) and lactate as an electron donor. The electrochemical analysis showed that the strain RO1 was capable of producing electricity (Table 3). This is the first report of electricity production for the genus of Propioniferax. We do not know the exact mechanism for electron transfer by this bacterium.

Table 3. Propioniferax strain RO1 pure culture MFC Linear Sweep Voltammetry showing electricity producing properties

| Day | P_{max} (mW/m^2) | I_{max} (mA/m^2) | R_{int} (ohms) | OCV (mV) |
|-----|----------------|----------------|---------------|----------|
| 13  | 1.24           | 13.8           | 17586         | 584      |
| 29  | 1.23           | 21.28          | 5750          | 359      |
| 40  | 1.73           | 34.82          | 3762          | 349      |

SEM observation showed presence of a biofilm of strain RO1 on anode without filamentous structures (Figure. 4), raising the possibility of direct electron transfer. Direct electron transfer to an insoluble electron acceptor was once considered incompatible with a Gram-positive bacterial envelope [28]. However, it was demonstrated that Gram-positive Thermincola potens strain JR has extracellular electron transfer mechanism using c-type cytochromes [29].

Figure 4. SEM images of Propioniferax strain RO1 pure culture attached to graphite anode (A) and a close up image (B)
Part of our future work will be to investigate how the strain RO1 produces electricity, i.e., analyses of c-type cytochromes or mediator-like compounds.

Most of DGGE bands from anolytic cultures of MFC1 and MFC2 were closely related to genera of Sphingobacterium, Pseudomonas, Enterobacter, and Azospirillum (Table 2), which are facultative anaerobes. These results suggested that these bacteria were not only lactate-fermenters but also oxygen-scavengers. Since some strains belonging to these genera are known as exoelectrogens [30, 11], it was suggested that anolytic bacteria also play a role in electricity production. SEM observation revealed that there were chasms and pores in MEA2, MEA3, and MEA4 (data not shown), suggesting oxygen-intrusion into the anode chamber. MEA1 had no chasms and pores, resulting in higher electricity production by MFC1 compared to the other MFCs. It was demonstrated that temporary oxygen intrusion into anode chamber is not necessarily irreparable damage to MFC performance, because bacterial communities adapted swiftly from semi-aerobic to anaerobic conditions [31, 32]. However, it was indicated that MFC performance is damaged seriously by continuous oxygen intrusion. Thicker membranes have been shown to give a lower rate crossover but tend to have higher resistances, reducing the cell performance. Therefore, there exists an optimal thickness for the membranes, which gives the best performance [33].

Table 4. Cell densities on the anode surface on day 112

| Electron acceptor | MFC 1          | MFC2           | Control        |
|-------------------|----------------|----------------|----------------|
|                   | (cfu/cm² anode) | (cfu/cm² anode) | (cfu/cm² anode) |
| FE(III) EDTA      | 3.9±2.4 × 10⁵  | 7.3±0.2 × 10⁴  | 5.5±3.7 × 10⁴  |
| Na₂SO₄            | 1.7±5.0 × 10⁵  | 2.5±4.2 × 10⁵  | 6.2±3.1 × 10⁴  |
| O₂                | 4.5±0.9 × 10⁷  | 1.7±0.1 × 10⁷  | 5.0±0.1 × 10⁵  |

The differences of oxygen-permeability affected the population densities. Significantly, Fe (III)-reducing bacterial population of MFC1 was approximately 5-fold higher than that of MFC2 (Table 4), which may be one of the reason why the current density of MFC1 was higher than that of MFC2.

4. Conclusion
It was demonstrated that bacterial community structure is significantly affected by the device feature. The gram-positive Propioniferax sp. RO1 was isolated from the anode of MFC1, and may have played a role in electricity generation in the MFC. Since the layer-by-layer method is capable of making a very thin layer, an effective MEA might be made using an extremely flat cathode. It is important to evaluate MFC performance equipped with new PEM by electrochemical and microbiological analyses. This subject is currently under investigation.

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References

[1] Feng Y, Wang X, Logan B E and Lee H 2008 Appl. Microbiol. Biotechnol. 78 873-880
[2] Logan B E, Hamelers B, Rozendal R, Schroder U, Keller J, Freguia S, Aelterman P, Verstraete
W and Rabaey, K 2006 Environ. Sci. Technol. 40 5181-519.
[3] Logan B E and Regan J M 2006 Trends. Microbiol. 14 512-518
[4] Rabaey K and Verstraete W 2005 Trends Biotechnol. 23 291-298
[5] Shimoyama T, Komukai S, Yamazawa A, Ueno Y, Logan B E and Watanabe K 2008 Appl. Microbial. Biotechnol. 80 325-330
[6] Lovley D R 2006 Curr. Opin. Biotechnol. 17 327-332
[7] Tender L M Reimers C E, Stecher III H A, Holmes D E, Bond D R, Lowy D A, Pilobello K, Fertig S J and Lovley D R 2002 Nat. Biotechnol. 20 821-825
[8] Ishii S, Shimoyama T, Hotta Y and Watanabe K 2008 BMC Microbiol. 8, 6 2008, 8:6 doi:10.1186/1471-2180-8-6 (This article is available from: http://www.biomedcentral.com/1471-2180/8/6)
[9] Kaku N, Yonezawa N, Kodama Y and Watanabe K 2008 Appl. Microbial. Biotechnol. 79 43-49
[10] Liu H and Logan B E 2004 Environ. Sci. Technol. 38 4040-4046
[11] Mühler G, de Waal E G and Uitterlinden A G 1993 Appl. Environ. Microbiol. 59 695-700
[12] Futamata H, Nagano Y, Watanabe K and Hiraishi A 2007 The ISME J. 1 471-479
[13] Futamata H, Kairya S, Sugawara M and Hiraishi A 2009 Microbes Environ. 24 330-337
[14] Ishii S, Watanabe K, Yabuki S, Logan B E and Sekiguchi Y 2008b Appl. Environ. Microbiol. 74 7348-7355
[15] Löfler F E, Sanfold R A and Tiedje J M 1996 Appl. Environ. Microbiol. 62 3809-3818
[16] Biebl H and Pfenng N 1978 Arch. Microbiol. 117 9-16
[17] Hiraishi A, Yonemitsu Y, Matsushita M, Shin Y K, Kuraishi H and Kawahara K 2002 Arch. Microbiol. 178 45-52
[18] Liu H and Logan B E 2004 Environ. Sci. Technol. 38 4040-4046
[19] Müyzer G, de Waal E G and Uitterlinden A G 1993 Appl. Environ. Microbiol. 59 695-700
[20] Futamata H, Nagano Y, Watanabe K and Hiraishi A 2005 Appl. Environ. Microbiol. 71 904-911
[21] Gorby Y A et al 2006 Proc. Natl. Acad. Sci. USA. 103 11358-11363
[22] Chung K, Fujiuki I and Okabe S 2011 Bioresource Technol. 102 355-360
[23] Shelobolina E S et al 2007 Int. J. Syst. Evol. Microbiol. 57 126-135
[24] Mosher J J et al 2012 Appl. Environ. Microbiol. 78 2082-2091
[25] Matthew S M, Jung D O, Madigan M T 2008 FEMS Microbiol. Lett. 287 121-127
[26] Pham T H et al 2008 Appl. Microbiol. Biotechnol. 77 1119-1129
[27] Wrighton K C et al 2011 Appl. Environ. Microbiol. 77 7633-7639
[28] Wrighton K C et al 2011 Appl. Environ. Microbiol. 77 7633-7639
[29] Rabaey K, Boon N, Sicilliano S D, Verhaege M, Verstraete and W 2004. Appl. Environ. Microbiol. 70 5373-5382
[30] Futamata H, Bretschger O, Cheung A, Kan J, Rubaba O and Nealson K H. 2012 J. Biosci. Bioeng. http://dx.doi.org/10.1016/j.jbiosc.2012.07.016
[31] Oh S E, Kim J R, Joo J H and Logan B E. 2009 Water Sci. Technol. 60 1311-1317.
[32] Liu JG, Zhao T S, Liang Z X and Chen R 2006 J. Power Sources. 153 61-67