Effects of NaCl concentration on anode microbes in microbial fuel cells

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Abstract

Understanding of how operational parameters affect the composition of exoelectrogenic microbes is an important step in the development of efficient microbial fuel cells (MFCs). In the present study, single-chamber MFCs were inoculated with rice paddy-field soil and continuously supplied with an acetate medium containing different concentrations of NaCl (0–1.8 M). Polarization analyses showed that power output increased as the NaCl concentration increased to 0.1 M, while it was markedly diminished over 0.3 M. The increase in power output was associated with an increased abundance of anode microbes as assessed by protein assays. Notably, the power increase was also accompanied by an increase in the abundance ratio of Geobacter bacteria to total anode bacteria as assessed by pyrosequencing of 16S rRNA gene amplicons and specific quantitative PCR. Although most Geobacter species are known to exhibit high growth rates in freshwater media without NaCl, the present study shows that 0.1 M NaCl facilitates the growth of Geobacter in MFC anode biofilms. This result suggests that the optimum salt concentration in MFC is determined by the balance of two factors, namely, the solution conductivity and salt tolerance of exoelectrogens.

Keywords: Microbial electrochemical cell, Ionic strength, Real-time PCR, 16S rRNA gene, Phylogenetic analysis, Exoelectrogen

Introduction

Microbial fuel cells (MFCs) are devices that use living microbes for the generation of electricity coupled to the decomposition of organic matter (Logan et al. 2006; Watanabe 2008; Pant et al. 2010). Owing to the great diversity of microbial metabolic capacities, MFCs are capable of generating electricity from a wide range of organic and inorganic compounds. Furthermore, MFCs can generate electricity from biomass waste and pollutants in wastewater by exploiting naturally occurring microbial communities as self-organizing anode catalysts (Rozendal et al. 2008; Lefebvre et al. 2011). Due to these advantageous properties, extensive efforts are being made to develop MFCs as energy-saving and cost-efficient options for wastewater treatment (Du et al. 2007; Lefebvre et al. 2011).

For MFCs to be practically applied to renewable energy generation, several factors need to be improved, particularly power outputs. Power outputs from MFCs are affected by numerous factors, including cell configuration, electrode materials, microbial inocula, substrates, and electrolyte compositions (Kim et al. 2007; Rinaldi et al. 2008). Among these factors, the composition of electrolyte has been shown to critically affect various aspects of MFC performance. For instance, proton carriers, such as phosphate and carbonate ions, improve the kinetics of proton transfer, resulting in enhanced power output (Fan et al. 2007). Electrolyte salt concentrations (correlated with ionic strength) have also been shown to affect MFC power output (Liu et al. 2005; Heilmann and Logan 2006; Mohan and Das 2009; Lefebvre et al. 2012; Rousseau et al. 2013). The findings from these studies are useful for the development of MFCs for wastewater treatment, as wastewater salinity varies markedly depending on the geographical region (Lefebvre and Moletta 2006; Lefebvre et al. 2012). In addition, the salt concentration of the aqueous phase may influence microbial metabolic activities (McCarty and McKinney 1961). Although the potential effects of salt concentration on microbes, including exoelectrogens, in MFCs have previously been
discussed (Lefebvre et al. 2012), no studies have examined the effects of salt concentration on anode microbes in MFCs.

The present study was undertaken to examine the effects of different concentrations of NaCl on anode microbes and power generation of MFCs. MFCs were inoculated with rice paddy-field soil and operated at NaCl concentrations ranging from 0 to 1.8 M for examining potential interdependencies among NaCl concentration, power output, and anode microbes.

Materials and methods

Reactor configuration and operation

The MFCs used in the present study are shown in Figure 1. Three MFC units were housed in a single MFC box but were operated independently. Each unit was equipped with a cassette electrode (Shimoyama et al. 2008) which consisted of two sets of air cathodes (Cheng et al. 2006), separators and graphite felt anodes (5-mm thickness; Sohgo Carbon, Yokohama, Japan) and were prepared as previously (Miyahara et al. 2013). The anode and cathode had projected areas of 68 and 65 cm², respectively. The liquid capacity of each unit was approximately 300 mL. The liquid surface was covered with a poly styrene board, and the reactor boxes were placed in a water bath at 30°C during operation. MFCs were continuously supplied with an acetate medium (pH 7.0) containing (per liter) 820 mg sodium acetate (10 mM), 50 mg BBL yeast extract, 175 mg NH₄Cl, 5.26 mg KH₂PO₄, 22.05 mg CaCl₂·2H₂O, 0.43 mg MgSO₄·7H₂O, 21.3 mg KCl, 8.76 mg NaHCO₃, and 1 mL of trace element solution (DSMZ 663; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). The operation of MFCs was initiated by inoculating each reactor with 1 g rice paddy-field soil (collected from Noda, Chiba, Japan) and supplying the acetate medium at a flow rate of 300 ml day⁻¹, corresponding to a hydraulic retention time of 1 day, using peristaltic pumps (SJ1220, Atto, Tokyo, Japan). The anodes and cathodes of each unit were connected via an external resistor (R_{ext} [Ω]), and the voltage across the resistor (E [V]) was monitored using a data logger (GL820, Graphitec, Yokohama, Japan). Current (I [A]) was calculated from R_{ext} and E according to the Ohm’s law (I = E/R_{ext}).

Chemical analyses and evaluation of MFC performance

Polarization curves were measured by linear sweep voltammetry (LSV) using a potentiostat (HZ-5000, Hokuto Denko, Tokyo, Japan) at a scan rate of 0.5 mV s⁻¹, and power curves were generated based on the polarization curves (Logan et al. 2006). In these analyses, current and power densities (I [A m⁻²] and P [W m⁻²], respectively) were calculated based on the projected anode area. Open-circuit voltage (E_{oc} [V]), maximum power density (P_{max}), and internal resistance (R_{int} [Ω]) were then determined from the polarization and power curves. Acetate was measured using a high performance liquid chromatograph (1100 series; Agilent Technologies, Tokyo, Japan) equipped with a Zorbax SB-Aq column (Agilent Technologies) as described elsewhere (Newton et al. 2009).

Analyses of anode microbiomes

Pieces of graphite felt were cut from anodes on both sides of the cassette electrode on day 82 of MFC operation and were stored at −20°C. To determine the total protein content of anode to estimate the total microbial biomass, proteins were extracted from the anode pieces (0.5 cm²) using B-PERII reagent (Pierce, Rockford, IL, USA) and were quantified using a BCA protein kit (Pierce) as described previously (Shimoyama et al. 2009).

DNA was extracted from the pieces of graphite-felt anodes (0.5 cm × 0.5 cm) using a Fast DNA Spin Kit for Soil (Q-Bio, Carlsbad, CA, USA) according to the manufacturer’s instruction and was finally dissolved in 50 μl of the DES solution supplied in the kit. For sequence analyses, PCR amplification of 16S rRNA gene fragments (V1–V3 region) was performed using primers ad-tag-8F (5’-CGTATGCGCTCCTCGCCGATCGGAGTTAGATCTAGAGAGG-3’) and ad-533R (5’-CCTATGCGCTTCCTCGGGCCTGCTGCGAC-3’) (Watanabe et al. 2004), in which the underlined sequences are adaptors added for pyrosequencing and XXXXXX represents an arbitrary tag.
sequence for sample identification (Dowd et al. 2008). The PCR conditions were as described elsewhere (Miyahara et al. 2013), and amplicons were purified using a QIAquick PCR Purification Kit (Qiagen K. K., Tokyo, Japan). Amplicons from different samples were mixed at the same concentration (1 ng μl−1 each) and then subjected to pyrosequencing using a Genome Sequencer FLX system (Roche Applied Science, Tokyo, Japan). Phylogenetic analyses were conducted using the Silva rRNA database (http://www.arb-silva.de/), and a tree was constructed by the neighbor-joining method using MEGA5 (Tamura et al. 2011). Nucleotide sequences determined in the present study were deposited into the DDBJ Sequence Read Archive Database (accession numbers: DRX025202 to DRX025213 and DRR027607 to DRR027618).

The abundance ratio of Geobacteraceae bacteria to total bacteria in the anode microbes was evaluated by quantitative real-time PCR (qPCR), as described previously (Kato et al. 2010). Briefly, real-time PCR was performed using a LightCycler system and LightCycler DNA Master SYBR Green I kit (Roche Applied Science) according to the manufacturer’s instructions. 16S rRNA genes of Geobacteraceae bacteria were amplified using the primer pair Geo494F and Geo825R (Holmes et al. 2002), while those of total bacteria were amplified using the primer pair 341f and 534r (Watanabe et al. 2001). Standard curves for Geobacteraceae and total bacteria were generated using serially diluted genomic DNA extracted from Geobacter sulfurreducens (10 pg μl−1 to 100 ng μl−1). The abundance ratio was calculated by dividing the 16S rRNA gene copy number of Geobacteraceae bacteria by that for total bacteria.

**Results**

**Effects of NaCl concentration on power output**

We examined the power outputs from MFCs supplied with the acetate medium containing NaCl at concentrations of 0, 0.05, 0.1, 0.3, 0.6 and 1.8 M (0M-MFC, 0.05M-MFC, 0.1M-MFC, 0.3M-MFC, 0.6M-MFC and 1.8M-MFC, respectively). These NaCl concentrations were selected to mimic freshwater (0 M), brackish water (0.05–0.3 M), seawater (0.6 M) and hyper-saline lakes (1.8 M). Since concentrations of other electrolyte ions were low (sodium acetate [10 mM] was the highest), NaCl was the major determinant of ionic strength in the electrolyte.

The operation of MFCs was initiated with $R_{\text{ext}}$ of 10,000 Ω, and it was decreased when $E$ exceeded 600 mV (Figure 2a, d). In 0M-, 0.05M- and 0.1M-MFCs, $E$ relatively rapidly increased (Figure 2b) and $R_{\text{ext}}$ was finally maintained at 300 Ω (Figure 2a). In contrast, $E$ slowly increased in 0.3M-MFC (Figure 2e), but $I$ only reached 0.04 mA during the 100-day operation (Figure 2f). Furthermore, $E$ only slightly increased in 0.6M- and 1.8M-MFCs and did not exceed 100 mV (Figure 2e). In all of the MFCs, the acetate concentration in the reactor effluent was between 1 and 5 mM; it is noteworthy

![Figure 2](image-url)
that the removal of organics was partially attributable to oxygen respiration in air–cathode MFCs (Shimoyama et al. 2008). Taken together, these results indicate that the NaCl concentration largely influenced the MFC performance and should be below 0.1 M for electricity generation in MFCs inoculated with paddy-field soil.

Polarization analyses were conducted once electric output of these MFCs became stable (after day 60), and representative data are presented in Figure 3. Mean polarization parameters estimated for these MFCs during day 60–100 are summarized in Table 1. Although typical polarization and power curves were obtained for the 0M-, 0.05M- and 0.1M-MFCs (Figure 3a), those for the other MFCs operated at higher NaCl concentrations were atypical (Figure 3b). In addition, $E_{\text{op}}$ values of 0.3M-, 0.6M- and 1.8M-MFCs were low, suggesting that these MFCs operated poorly as fuel cells. The polarization data (Table 1) show that the MFC performance of 0M-, 0.05M to 0.1M-MFC increased with increasing NaCl concentration.

### Effects of NaCl on anode microbes

To examine the effects of NaCl concentration on anode microbes in MFCs, we first analyzed the total protein content of anode samples as a surrogate measure of the abundance of microbes attached to the anodes. We found that microbes were the most abundant on the anodes of the 0.05M- and 0.1M-MFCs, followed by the 0M-MFC (Figure 4), whereas anode microbes in the 0.3M-, 0.6M- and 1.8M MFCs were only one tenth to one-fifth as abundant as those in the 0.1M-MFC (Figure 4). Interestingly, these findings suggest that there is a threshold NaCl concentration between 0.1 and 0.3 M that determines the growth of anode microbes in MFCs.

Analyses of MFC anode-associated microbial communities frequently detect bacteria affiliated with the family Geobacteraceae which includes well-characterized exoelectrogens, such as Geobacter (Logan 2009). In the present study, qPCR was used to examine if Geobacteraceae bacteria were present among anode-associated microbes in the MFCs (Figure 4). Geobacteraceae bacteria were substantially detected in the 0M-, 0.05M-, and

| NaCl (M) | $E_{\text{op}}$ (mV) | $P_{\text{max}}$ (mW m$^{-2}$) | $R_{\text{int}}$ ($\Omega$) |
|----------|----------------|-----------------|----------------|
| 0        | 766 ± 29       | 114 ± 4         | 192 ± 15      |
| 0.05     | 812 ± 22       | 340 ± 21        | 83 ± 14       |
| 0.1      | 816 ± 18       | 504 ± 41        | 43 ± 5        |
| 0.3      | 555 ± 50       | 15.5 ± 1.4      | 1,102 ± 120   |
| 0.6      | 257 ± 15       | 2.4 ± 0.3       | 3,318 ± 200   |
| 1.8      | 247 ± 47       | 1.6 ± 0.3       | 1,866 ± 320   |

Data are mean ± SD (n = 5).

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**Table 1 Polarization parameters after electric outputs became stable**

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**Figure 3** Electrochemical characterizations of the MFCs. Polarization and power curves for the 0M-, 0.05M- and 0.1M-MFCs (red, blue and green symbols, respectively) are shown in a, while those for the 0.3M-, 0.6M- and 1.8M-MFCs (red, blue and green symbols, respectively) are shown in b.

**Figure 4** Total protein contents and abundance ratios of Geobacteraceae bacteria to total bacteria, as determined by qPCR for microbial samples collected from the MFC anodes.
0.1M-MFCs, and their abundance ratio relative to total bacteria increased with increasing NaCl concentration, reaching over 60% in the 0.1M-MFC. However, Geo-
bacteraceae bacteria were not substantially detected in MFCs with NaCl concentrations of 0.3M or higher.

To confirm that the observed effects of NaCl on electricity generation were associated with the growth of anode-associated Geo-
bacteraceae bacteria, the abundance of Geo-
bacteraceae bacteria as expressed by the Geo-
bacteraceae protein content were estimated from the total-protein content and their abundance ratio, and the estimated values are compared with \( P_{\text{max}} \) values at the different NaCl concentrations (Figure 5). A close correlation was clearly detected between these values, confirming that Geo-
bacteraceae bacteria were responsible for the MFC power generation.

To further characterize anode-associated microbes in the MFCs, we conducted pyrosequencing and phy-
genetic analyses of PCR-amplified 16S rRNA gene fragments. Figure 6 presents the abundance ratios of bact-
erial groups classified at the class level in each MFC. As expected, the relative abundance of the class Deltap-
roteobacteria, which includes the family Geo-
bacteraceae, increased as the NaCl concentration increased from 0 to 0.1 M; this class comprised over 60% of the total bact-
eria in the 0.1M-MFC. However, the community structure dramatically differed at NaCl concentrations of 0.3 M and higher; members of the class Gammaproteobacteria were the most abundantly detected at 0.3 and 0.6 M, whereas Bacilli was the most abundant at 1.8 M.

In order to show what sequences constituted the major class-level taxonomic groups in Figure 6, major sequences (>1% to the total sequence in each library) are listed in Table 2. It is shown that the Deltaproteobacteria detected at 0–0.1 M NaCl are comprised of several major sequences affiliated with the genus Geo-
bacter, the Gam-
maproteobacteria detected at 0.6 and 0.7 M includes major sequences affiliated with Pseudomonas and Aero-
monas, while the Bacilli detected at 1.8 M NaCl was Staphylococcus. The major Geo-
bacter sequences (MFC1 to MFC4) were further analyzed to identify exact phy-
genetic positions (Figure 7); in this figure, Geo-
bacter sequences are divided into three clades according to a previous study (Holmes et al. 2007). This analysis shows that MFC1 affiliated with the G. metallireduces clade.
| NaCl  | Order | No. of read | Percent (%) | Closely related sequence (accession no.) | Taxonomy                        | Description          |
|-------|-------|-------------|-------------|------------------------------------------|---------------------------------|----------------------|
| 0 M   | 1     | 1,328       | 15.6        | Uncultured bacterium Jan1A06 (GU139308) | Geobacter                      | MFC1                 |
|       | 2     | 1,260       | 14.8        | Geobacter sp. Ply1 (EF527233)            | Geobacter                      | MFC2                 |
|       | 3     | 373         | 4.4         | Uncultured bacterium MBR28-36 (EU169844) | Rhodocyclaceae                 |                     |
|       | 4     | 262         | 3.1         | Uncultured bacterium IIB-27 (AJ488087)   | Rikenellaceae                  |                     |
|       | 5     | 258         | 3.0         | Comamonas granuli (AB187586)             | Comamonas                      |                     |
|       | 6     | 249         | 2.9         | Uncultured bacterium R35 (AF407690)      | Alkalophilus                    |                     |
|       | 7     | 193         | 2.3         | Uncultured Geobacter OTU6 (FM204962)     | Geobacter                      | MFC3                 |
|       | 8     | 166         | 2.0         | Uncultured bacterium UB106 (AM4990695)   | Betaproteobacteria              |                     |
|       | 9     | 150         | 1.8         | Uncultured bacterium S1-41 (EU015093)    | Dechloromonas                   |                     |
|       | 10    | 128         | 1.5         | Uncultured bacterium LT-SB-B13 (FJ735757) | Rhodocyclaceae                 |                     |
|       | 11    | 102         | 1.2         | Rhodocyclace sp. HOD 5 (AY691423)        | Rhodocyclaceae                 |                     |
|       | 12    | 95          | 1.1         | Denitrifying bacterium NOB2A10 (FJ802256) |                     |                     |
|       | Others| 3,947       | 46.4        | Others                                   |                                |                     |
| Total |       | 8,511       | 100.0       | **Total**                                |                                | **Total**            |
| 0.05 M| 1     | 4,051       | 38.7        | Geobacter sp. Ply1 (EF527233)            | Geobacter                      | MFC2                 |
|       | 2     | 693         | 6.6         | Uncultured bacterium WCHB1-80 (AF050563) | Leptolinea                     |                     |
|       | 3     | 614         | 5.9         | Uncultured bacterium Kas1658 (EF203202)  | Chlorobiales                   |                     |
|       | 4     | 340         | 3.2         | Uncultured bacterium BS055 (AB240241)    | Geobacter                      | MFC4                 |
|       | 5     | 331         | 3.2         | Uncultured bacterium 613 (FM178812)      | Spirochaetaceae                |                     |
|       | 6     | 247         | 2.4         | Uncultured bacterium TSAC14 (AB186805)   | Chlorobiales                   |                     |
|       | 7     | 241         | 2.3         | Uncultured bacterium EBL49 (GU591537)    | Sphingobacteriales             |                     |
|       | 8     | 214         | 2.0         | Uncultured bacterium WCHB1-40 (AF050549) | Spirochaetaceae                |                     |
|       | 9     | 133         | 1.3         | Azospirillum sp. BS10 (AP010946)         | Azospirillum                    |                     |
|       | 10    | 125         | 1.2         | Uncultured bacterium WB100 (EU184876)    | Rhizobium                      |                     |
|       | 11    | 116         | 1.1         | Uncultured bacterium SJA-87 (AJ009478)   | Holophaga                      |                     |
|       | 12    | 105         | 1.0         | Uncultured bacterium S5c (FJ462089)      | Chlorobiales                   |                     |
| Others|       | 3,265       | 31.2        | Others                                   |                                |                     |
| Total |       | 10,475      | 100.0       | **Total**                                |                                | **Total**            |
| 0.1 M | 1     | 8,719       | 60.1        | Geobacter sp. Ply1 (EF527233)            | Geobacter                      | MFC2                 |
|       | 2     | 588         | 4.1         | Uncultured bacterium WCHB1-80 (AF050563) | Leptolinea                     |                     |
|       | 3     | 452         | 3.1         | Uncultured bacterium BS055 (AB240241)    | Geobacter                      | MFC4                 |
|       | 4     | 281         | 1.9         | Uncultured bacterium WBB100 (EU184876)   | Rhizobium                      |                     |
| Others|       | 4,460       | 30.8        | Others                                   |                                |                     |
| Total |       | 14,500      | 100.0       | **Total**                                |                                | **Total**            |
| 0.3 M | 1     | 2,433       | 16.3        | Aeromonas media NFB-5 (GU810523)         | Aeromonas                      |                     |
|       | 2     | 2,417       | 16.2        | Aeromonas hydrophila (X87271)            | Aeromonas                      |                     |
|       | 3     | 1,917       | 12.8        | Uncultured bacterium AKAU090 (DQ125857)  | Rhodococcus                    |                     |
|       | 4     | 300         | 2.0         | Ochrobactrum sp. B2 8RT46 (DQ337583)     | Ochrobactrum                   |                     |
|       | 5     | 290         | 1.9         | Uncultured bacterium 4A3B3C1 (GU451204)  | Bacteria                       |                     |
|       | 6     | 256         | 1.7         | Uncultured Bacteroidetes QED1N0DHO5 (CU927327) | Parabacteroides               |                     |
|       | 7     | 252         | 1.7         | Uncultured bacterium G3DCM-82 (EU037335) | Rikenellaceae                  |                     |
|       | 8     | 236         | 1.6         | Uncultured Bacteroidetes RsStar205 (AB522124) | Dysgonomonas                  |                     |
|       | 9     | 234         | 1.6         | Uncultured bacterium SK8E (AY753402)     | Alkalophilus                   |                     |
|       | 10    | 228         | 1.5         | Uncultured anaerobic bacterium B-4C (AY953243) | Dysgonomonas                  |                     |
|       | 11    | 156         | 1.0         | Azospirillum sp. YM 274 (GU396258)       | Azospirillum                    |                     |
| Others|       | 6,238       | 41.7        | Others                                   |                                |                     |
| Total |       | 14,957      | 100.0       | **Total**                                |                                | **Total**            |
was seen only at 0 M NaCl, while MFC2, the most abundant sequence at 0.05 M and 0.1 M NaCl, is affiliated with the subsurface clade 2. It is suggested that the high power density observed in 0.1M-MFC was attributed to the preferential growth of subsurface clade 2 Geobacter at 0.1 M NaCl.
Discussion

The present study shows that interdependencies exist among salt concentration, power outputs, and anode exoelectrogens in MFCs. Clear correlation was detected between the power output and abundance of Geobacteraceae bacteria (Figure 5), suggesting that NaCl affects the physiology and growth of these exoelectrogens.

The abundance of Geobacter bacteria increased as the NaCl concentration increased from 0 to 0.1 M, while these were markedly decreased above 0.3 M NaCl (Figures 5, 6). This trend is consistent with the fact that members of this family have mostly been isolated from freshwater environments and preferentially grow in freshwater media without NaCl (Lovley et al. 2011). In addition, physiological characterization of Geobacter isolates has demonstrated that they tolerate up to 10 g NaCl per liter (0.17 M) (Nevin et al. 2005). Given these features of Geobacter bacteria, the low electric outputs at 0.3 M NaCl and higher are likely attributable to the inability of these exoelectrogens to grow at these NaCl concentrations. In contrast, the finding that anode-associated Geobacteraceae bacteria preferentially grow at 0.1 M NaCl is notable, and this feature may be specific for those growing by anode respiration in MFCs. This clearly demonstrates that a certain level of the ionic strength (corresponding to the solution conductivity) is required for anode respiration by exoelectrogens. Although a salt-tolerant strain of Geobacter that can generate electricity in an MFC at 0.65 M NaCl was recently isolated and characterized (Sun et al. 2014), this strain also preferentially generates electricity at ionic strengths corresponding to 0.1 M NaCl or lower. These observations suggest that the optimum NaCl concentration (0.1 M NaCl in the present study) is determined by the balance of the two factors, namely, the solution conductivity and salt tolerance of exoelectrogens. Future research will examine transcriptomic responses of Geobacter exoelectrogens to different concentrations of NaCl.

At NaCl concentrations of 0.3 M and higher, bacteria affiliated with Gammaproteobacteria and Bacilli were abundantly detected (Figure 6). Major genera in these phyla are Pseudomonas, Aeromonas and Staphylococcus; among these, Pseudomonas (Boon et al. 2008) and Aeromonas (Pham et al. 2003) are known to include exoelectrogens, while direct electricity generation by species of Staphylococcus has not been reported. Although electric outputs at these NaCl concentrations were low, it may be interesting to isolate these bacteria for examining their abilities to generate electricity in MFCs at high salt concentrations.

References

Boon N, De Maeyer K, Hofte M, Rabaey K, Verstraete W (2008) Use of Pseudomonas species producing phenazine-based metabolites in the anodes of microbial fuel cells to improve electricity generation. Appl Microbiol Biotechnol 80:985–993
Cheng S, Liu H, Logan BE (2006) Increased performance of single-channel microbial fuel cells using an improved cathode structure. Electrochem Commun 8:489–494
Dowd SE, Callaway TR, Wolcott RD, Sun Y, McKeenan T, Hagevoort RG et al (2008) Evaluation of the bacterial diversity in the feces of cattle using 16S DNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol 8:125
Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25:464–482
Fan Y, Hu H, Liu H (2007) Sustainable power generation in microbial fuel cells using bicarbonate buffer and proton transfer mechanisms. Environ Technol 31:815–8158
Heilman J, Logan BE (2006) Production of electricity from proteins using a microbial fuel cell. Water Environ Res 78:531–537
Holmes DE, Finneran KT, O’Neil RA, Lovley DR (2002) Enrichment of members of the family Geobacteraceae associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. Appl Environ Microbiol 68:2300–2306
Holmes DE, O’Neil RA, Vronis HA, Ngussaan LA, Ortiz-Bernad L, Larrahondo MJ et al (2007) Subsurface clade of Geobacteraceae that predominates in a diversity of Fe(III)-reducing subsurface environments. ISME J 1:663–677
Kato S, Nakamura R, Kai F, Watanabe K, Hashimoto K (2010) Respiratory interactions of soil bacteria with (semi)conductive iron oxide minerals. Environ Microbiol 12:3114–3123
Kim BH, Chang IS, Gadd GM (2007) Challenges in microbial fuel cell development and operation. Appl Microbiol Biotechnol 76:485–494
Lefebvre O, Moletta R (2006) Treatment of organic pollution in industrial saline wastewater: a literature review. Water Res 40:3671–3682
Lefebvre O, Uzabagia A, Chang I, Kim BH, Ng H (2011) Microbial fuel cells for energy: self-sufficient domestic wastewater treatment—a review and discussion from energetic consideration. Appl Microbiol Biotechnol 89:259–270
Lefebvre O, Tan Z, Kharkwal S, Ng HY (2012) Effect of increasing anodic NaCl concentration on microbial fuel cell performance. Biores Technol 112:336–340
Liu H, Cheng S, Logan BE (2005) Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration. Environ Sci Technol 39:5488–5493
Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 7:375–381
Logan BE, Hamedes B, Rozendal R, Schroder U, Keller J, Freguia S et al (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40:5181–5192

Authors’ contributions

MM carried out the reactor operation and molecular genetic analyses. AK participated in the design of the study and helped to draft the manuscript. KW designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interest

The authors declare that they have no competing interests.

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Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, Flanagan KA et al (2011) Geobacter: the microbes electric’s physiology, ecology, and practical applications. Adv Microb Physiol 59:1–100
McCarty PL, McKinney RE (1961) Salt toxicity in anaerobic digestion. J Wat Poll Cont Fed 33:399–415
Miyahara M, Hashimoto K, Watanabe K (2013) Use of cassette-electrode microbial fuel cell for wastewater treatment. J Biosci Bioeng 115:176–181
Mohan Y, Das D (2009) Effect of ionic strength, cation exchange and inoculum age on the performance of microbial fuel cells. Int J Hydrog Energy 34:7542–7546
Nevin KP, Holmes DE, Woodard TL, Hinlein ES, Ostendorf DW, Lovley DR (2005) Geobacter bemidjiensis sp. nov. and Geobacter psychrophilus sp. nov., two novel Fe(III)-reducing subsurface isolates. Int J Syst Evol Microbiol 55:1667–1674
Newton GJ, Mori S, Nakamura R, Hashimoto K, Watanabe K (2009) Analyses of current-generating mechanisms of Shewanella loihica PV-4 and Shewanella oneidensis MR-1 in microbial fuel cells. Appl Environ Microbiol 75:1667–1674
Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543
Pham CA, Jung SJ, Phung NT, Lee J, Chang IS, Kim BH et al (2003) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. FEMS Microbiol Lett 223:129–134
Rinaldi A, Mechini B, Garavaglia V, Licoccia S, di Nardo P, Traversa E (2008) Engineering and biology to boost performance of microbial fuel cells: a critical review. Energy Environ Sci 1:417–429
Shimoyama T, Komukai S, Yamazawa A, Ueno Y, Logan BE, Watanabe K (2008) Electricity generation from model organic wastewater in a cassette-electrode microbial fuel cell. Appl Microbiol Biotechnol 80:325–330
Shimoyama T, Yamazawa A, Ueno Y, Watanabe K (2009) Phylogenetic analyses of bacterial communities developed in a cassette-electrode microbial fuel cell. Microbes Environ 24:188–192
Sun D, Call D, Wang A, Cheng S, Logan BE (2014) Geobacter sp. SD-1 with enhanced electrochemical activity in high-salt concentration solutions. Environ Microbiol Rep 6:723–729
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGAS: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:7731–2739
Watanabe K (2008) Recent developments in microbial fuel cell technologies for sustainable bioenergy. J Biosci Bioeng 106:528–536
Watanabe K, Kodama Y, Hayarama S (2001) Design and evaluation of PCR primers to amplify bacterial 16S ribosomal DNA fragments used for community fingerprinting. J Microbiol Methods 44:253–262
Watanabe K, Hamamura N, Kaku N (2004) Molecular identification of microbial populations in petroleum-contaminated groundwater. In: Spencer JFT, Spencer AL (eds) Environmental Microbiology; methods and protocols. Humana Press, Totowa, NJ, pp 237–244