Journal of Power Sources 196 (2011) 6036–6041

Contents lists available at ScienceDirect

Journal of Power Sources

journal homepage: www.elsevier.com/locate/jpowsour

Improved performance of microbial fuel cell using combination biocathode of graphite fiber brush and graphite granules

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ARTICLE INFO

Article history:
Received 15 February 2011
Received in revised form 27 March 2011
Accepted 29 March 2011
Available online 8 April 2011

Keywords:
Microbial fuel cells
Biocathode
Graphite fiber brush
Graphite granules
Cell performance
Microbial community

ABSTRACT

The efficiency and sustainability of microbial fuel cell (MFC) are heavily dependent on the cathode performance. We show here that the use of graphite fiber brush (GBF) together with graphite granules (GGs) as a basal material for biocathode (MFC reactor type R1) significantly improve the performance of a MFC compared with MFCs using GGs (MFC reactor type R2) or GFB (MFC reactor type R3) individually. Compared with R3, the use of the combination biocathode (R1) can shorten the start-up time by 53.75%, improve coulombic efficiencies (CEs) by 21.0 ± 2.7% at external resistance (R_{EX}) of 500 Ω, and increase maximum power densities by 38.2 ± 12.6%. Though the start-up time and open circuit voltage (OCV) of the reactor R2 are similar to R1, the CE (R_{EX} = 500 Ω) and maximum power density of R2 are 21.4 ± 1.7% and 38.2 ± 15.6% lower than that of R1. Fluorescence in situ hybridization (FISH) analyses indicate the bacteria on cathodes of R1 and R2 are richer than that of R3. Molecular taxonomic analyses reveal that the biofilm formed on the biocathode surface is dominated by strains belonging to *Nitrobacter*, *Achromobacter*, *Acinetobacter*, and *Bacteroidetes*. Combination of GFB and GGs as biocathode material in MFC is more efficient and can achieve sustainable electricity recovery from organic substances, which substantially increases the viability and sustainability of MFCs.

1. Introduction

Among electrochemical cells, microbial fuel cells (MFCs) are special types of biofuel cells, producing electric power by utilizing microorganisms to assist redox reactions [1]. A MFC consists of the anode and cathode separated by a proton exchange membrane (PEM) or both electrodes are positioned in a single chamber without a PEM [2]. MFCs can be operated with an abiotic cathode or biocathode. In MFCs with biocathode both anodic and cathodic reactions are catalyzed by electrochemically active microorganisms [3].

In MFCs with abiotic cathodes, platinum is the most commonly used catalyst for oxygen reduction but expensive and unsustainable as it suffers from poisoning compounds produced from bacterial metabolisms. Other transition metals, such as pyrolyzed iron (II) phthalocyanine (FePc) and cobalt tetramethylphenylporphyrin (CoTMPP) have been tested as cathodic catalysts, and appear to be promising [4,5]. These electrocatalysts, however, are often time-consuming to prepare, and sensitive to poisonous compounds too. Furthermore, a recent study showed MFC with an activated carbon fiber felt without any metal catalysts also is a good cathode material [6]. In addition, a number of chemicals, such as potassium ferricyanide [7,8], potassium permanganate [9] and potassium dichromate [10] have successfully been used as cathodic electron acceptor/sink, but these cathodic systems are not considered to be sustainable since, they generally require periodical compensation for depletion.

Recently, studies with biological cathodes have gained interests for several reasons [3]: (i) the cost of construction and operation of MFCs may be reduced, because microorganisms can function as catalysts to assist the electron transfer instead of metals or chemicals. In addition, when alternative electron acceptors such as nitrate or proton are used, the aeration cost can be omitted. (ii) Biocathodes may improve MFC sustainability because of their resistance against poisonous compounds. (iii) The microbial metabolism in biocathodes may be utilized to produce useful products or to remove unwanted compounds, for example, heavy metals from wastewater (see below) [11], hydrogen and methane producing [12,13] or nitrogen removal from wastewater [14] among others. Feasibility studies showed that the maximum power outputs achieved by MFCs are lower than that by anaerobic digestion processes at the

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doi:10.1016/j.jpowsour.2011.03.096

same investment costs [15,16]. Recently, more emphases are given to biocathodes to improve the overall performance of MFCs, which could provide a road to commercialization of this new technology.

Also, electrode material is known to play an important role in MFC. A wide variety of carbon-based anode materials have been examined in MFCs, including carbon paper [17], plain graphite [18], carbon cloth [19], carbon felt [20], or graphite granules (GGs) [21,22], reticulated vitreous carbon (RVC) [7,8] and graphite fiber brush (GFB) [23]. However, compared with anode materials, there are few reports on improving performance of MFC through cathode material selection or optimization, especially aerobic biocathode materials.

In this study, we operated three MFCs with different aerobic biocathode materials, GGs, GFB, and combination of GGs and GFB, to assess the performances of these materials. The goals of this study were 2-fold: (i) to compare the start-up procedure, attainable electricity and power production of MFCs with different biocathode materials, and (ii) to investigate microbial community on the biocathode.

2. Materials and methods

2.1. Microbial fuel cells construction

Three MFCs with dual-compartment were constructed to evaluate the performance of combination cathode of GGs and GFB (MFC reactor type R1), GGs cathode (MFC reactor type R2), or GFB cathode (MFC reactor type R3). These MFC reactors made of Plexiglas material had the same rectangular-shaped anode and, cathode compartments with the volume of 108 mL (70 mm × 70 mm × 22 mm). A PEM (Nafion 117, Dupont) with a sectional area of 7.0 × 7.0 = 49 cm^2 was used to separate the anode and the cathode compartments. Preparations of PEM and electrodes were done as described by Bond and Lovley [24]. All cathodic materials in the three MFCs had the same solid volume (57 ± 2 cm^3). The GFB were made of carbon fibers (STS40 24 K, 650 ± 17 $m^2\ m^{-3}$, average fiber diameter of 7.0 μm, Toho Tenax) which were cut to a set length and twisted by two titanium wires. GFB embedded in GGs (diameter of 1–5 mm, 55 $m^2\ m^{-3}$, Jiuxin Carbon Goods Company, Jilin Province, China) was used as the cathode of MFC reactor type R1, while only a large GFB was inserted into the compartment as the cathode of R3. The cathode of MFC reactor type R2 and all anodes of these MFCs were filled with GGs as electrodes, and wet volumes of them were 51 mL. The porosity of the graphite bed was 0.53, and the density of the granules was 1.81 $kg\ L^{-1}$. All cathodes were aerated at the rate of 300 $mL\ min^{-1}$ using fish pumps. Prior to use, both GG and GFB were soaked in 0.1 N HCl and NaOH solution for 18 h in turn, and subsequently in de-ionized water for 24 h. Graphite rods (8 mm diameter and 100 mm length) were inserted into the granule matrix, and standard copper wires sealed with epoxy resin (2 M) were used to connect the anodes and the cathodes to form a closed circuit, in which a fixed resistance (R_{EX} = 500 Ω) was applied as an external load.

2.2. Operational conditions

The anodes of MFCs reactors were inoculated with activated sludge collected from the thickening tank of Wenchang wastewater treatment plant (WWTP), Harbin, China, and operated in a batch-fed mode. The anodic medium (17.1 $g\ L^{-1}$ $Na_2HPO_4{\cdot}12H_2O$, 3.0 $g\ L^{-1}$ KH_2PO_4, 0.3 $g\ L^{-1}$ NaCl, 0.494 $g\ L^{-1}$ $MgSO_4{\cdot}7H_2O$, 0.0111 $g\ L^{-1}$ $CaCl_2$, and trace elements) as previously described [25] was supplemented with 2 g of glucose per liter.

Topsoils known to be rich in hydrogen-oxidizing bacteria were used to enrich hydrogen-oxidizing bacteria [26,27]. And the previous studies have shown that hydrogen-oxidizing bacteria are predominant on aerobic biocathodes of MFC [28–30]. Thus, in our study, the cathodes were inoculated with topsoil obtained from the turf at Harbin Institute of Technology. The cathodic medium contained (per liter of deionised water): 1.0 $g\ L^{-1}$ NH_4Cl, 1.2 $g\ L^{-1}$ K_2HPO_4, 0.5 $g\ L^{-1}$ $MgSO_4$, 0.5 $g\ L^{-1}$ KCl, 0.14 $g\ L^{-1}$ KH_2PO_4, 0.01 $g\ L^{-1}$ $Fe_2(SO_4)_3{\cdot}H_2O$ and 0.02 $g\ L^{-1}$ yeast extract. The same trace elements as the anode were also added to the cathodic medium.

2.3. Analyses and computations

Voltage was recorded directly every 1 min by using a 32-channel data acquisition system (PISO-813, ICP DAS, Co., Ltd.) connected to a personal computer via PCI interface [31]. The potentials of the cathodic and anodic electrodes were monitored with Ag/AgCl reference electrodes (assumed to be +0.195 V vs SHE) (model RE-5B, BASi). Current (I) and power ($P = IV$) were calculated as previously described [32]. CE was calculated for a fed-batch system as [33]

$$CE = \frac{M_s \int_0^{t_b} I\ dt}{F b_{es} \nu_{An} \Delta c} \quad (1)$$

where Δc is the substrate concentration change over the batch cycle over a time = t_b, M_s is the molecular weight of the substrate, F is the Faraday's constant, ν_{An} is the volume of liquid in the anode compartment, I is the temporary current, and b_{es} is the number of electrons exchanged per mole of substrate. The volumetric power density was normalized by the anode compartment void volume. Polarization curves were obtained by measuring the stable voltage generated at various external resistances (for 30 min at each resistance) and then used to evaluate the maximum power density [33]. Internal resistance (R_{int}) was determined by the slope of polarization curves [4]. All analyses were made at least 3 times and the average values with standard deviations are presented.

2.4. FISH

FISH is a powerful tool for the detection and quantification of specific microorganisms in microbial communities [34]. Therefore, FISH was applied to investigate the quantity and modality of microorganisms on the biocathodes in our study. FISH analysis was based on the protocols described previously [35,36]. The biofilm (wet weight about 0.2 g) was completely separated from the biocathode of R1 (0.1 g sample from the GFB and 0.1 g sample from GGs were fully mixed), R2, and R3, and suspended in phosphate-buffered saline solution (PBS, pH 7.4) consisting of 8 $g\ L^{-1}$ NaCl, 0.2 $g\ L^{-1}$ KCl, 1.44 $g\ L^{-1}$ Na_2HPO_4 and 0.24 $g\ L^{-1}$ K_2HPO_4 in distilled water. Then the samples were fixed with 4% paraformaldehyde (in PBS) at 4 °C for 12 h. The fixed samples were washed twice with PBS, and suspended in a solution of 50% PBS and 50% ethanol and stored at −20 °C. For FISH, 10 μL of the fixed sample was applied on a gelatin-coated well, dried for 1 h at 46 °C, and subsequently dehydrated in solutions of 50%, 80% and 96% ethanol (v/v, in 10 mM Tris–HCl, pH 7.5) for 3 min each. To start hybridization, 9 μL of hybridization buffer and 1 μL of fluorescently labelled probe (50 $ng\ mL^{-1}$) were added to a well. The hybridization was conducted for 2 h at 46 °C in a humidified chamber. Following hybridization, a stringent washing step was performed for 10 min at 48 °C in a buffer with the appropriate NaCl concentration. The oligonucleotide probes used for the FISH procedure was EUB338 (5′-GCTGCCTCCCGTAGGAGT-3′) labelled with CY_3 (TaKaRa, Dalian, China). Microscopy was performed on an Olympus BX50 microscope, equipped with filters HQ-CY3 (Analysentechnik AG, Tübingen, Germany). For each sample from the biocathode of

R1, R2 and R3, a batch of wells (including 24 wells) were hybridized and examined under fluorescence microscope.

2.5. Microbial community analysis

Equal amount of cathodic biofilm samples from MFC reactors type R1, R2, and R3 were mixed, and total DNA was extracted from the biofilm samples using the Bacteria DNA Mini Kit (Watson Biotechnologies, Shanghai, China) according to the manufacturer's instructions to use as the template for polymerase chain reaction (PCR). The DNA concentration was measured with a UV/Vis-spectrophotometer (UNICAM HELIOS, UK). PCR amplification was performed in a 50 μL reaction mixture containing approximately 25 ng of template DNA, 25 μL of PCR Mastermix (Qiagen), 0.5 mM (each) primer, and distilled water. PCR primers used were 08f with GC clamp and 534r [37]. The sample was amplified in a GenAmp PCR System 9700 (Perkin-Elmer Applied Biosystems, CA, USA) programmed as follows: initial denaturation of DNA for 5 min at 94 °C; 30 cycles of 30 s at 94 °C, 20 s at 60 °C, and 30 s at 72 °C; decreasing 0.1 °C per cycle to 57 °C, and extension for 7 min at 72 °C. Agarose gel electrophoresis was used to detect and estimate the concentrations of PCR amplicons.

Denaturing gradient gel electrophoresis (DGGE) was performed as previously described [37] except the linear gradient of the denaturants was from 40 to 60% instead of 40 to 65%. Prominent DGGE bands were excised for nucleotide sequence determination. For each band selected, only the middle portion was excised with a sterile pipette tip. The gel was crushed in 20 μL of TE buffer [2 mM Tris–HCl, 1 mM EDTA (pH 8.0)] and allowed to equilibrate overnight at 4 °C. One microliter of supernatant was used to reamplify the band. The PCR products were cloned in *Escherichia coli* JM109 using the pMD18-T plasmid vector system (TaKaRa, Dalian, China) in accordance with the manufacturer's instructions. The cloned PCR fragments were sequenced using an ABI-Prism model 3730 automatic sequencer (Perkin-Elmer, Foster City, CA, USA) using a M13-47 primer. All sequences were compared with GenBank database using BLAST, and the closest matched sequences were obtained and included in the downstream analysis. These partial 16S rRNA sequences, their closest relatives, and appropriate type strain sequences were aligned using ClustalW. Phylogenetic trees were constructed by the neighbor-joining method made by the algorithm in MEGA 4 for Windows, including bootstrap analysis. The nucleotide sequences generated in this study were deposited to GenBank under the accession numbers HM153429 through HM153440.

3. Results and discussion

3.1. Start-up procedure

During the start-up period with a 500 Ω external resistor, the cathodic potentials of the three reactors changed in a similar trend of initial decrease followed by gradual increase (Fig. 1). The potentials decreased for 40 h, 34 h, and 116 h in R1, R2 and R3, respectively, before began to increase at different rates. When the cathode potential reached higher than +0.400 V versus SHE, the cathode was considered fully enriched [16]. Reactors R1 and R2 needed the start-up period of 186 h and 200 h to become fully active upon inoculation, whereas the R3 showed a similar activity after 402 h.

Though GFB and GG with high specific surface area and porosity are considered as excellent anode materials for MFCs [8,23], their performance as biocathode was different. Reactors R1 and R2 started up successfully in a shorter period of time than R3, probably due to better growth of microorganisms with electrocatalytic

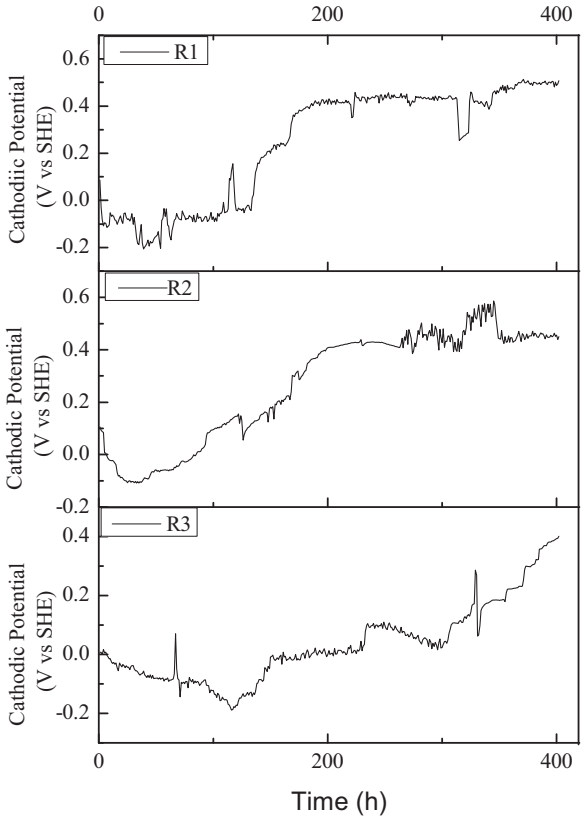

Fig. 1. Potential time profiles of MFC R1 with combination biocathode of GFB and GGs, MFC R2 with GGs biocathode and MFC R3 with graphite fiber brush biocathode during start-up at constant external resistance (R_{EX} = 500 Ω).

oxygen reducing activity on cathodes of R1 and R2. It seems most likely that the rough surface of graphite granules is responsible for the fast development of electrochemically active microbial biofilm onto the electrode.

3.2. Electrochemical characterization

When microbial fuel cells generated current stably, it was believed that electrochemically active microbial biofilms were fully developed both on the anode and cathode surfaces. After the MFCs had been fully enriched, they were subjected to experiments to obtain the polarization and power density curves. As shown in Fig. 2A, the OCV of R2 was 0.829 ± 0.007 V, which was higher than R1 (0.826 ± 0.005 V) and R3 (0.796 ± 0.012 V). The cell voltage decreased less steeply in R1 than others with the increase in current density. This suggests that R1 with the combined cathode material had a lower ohmic resistance than others. The maximum power densities were 99.83 ± 3.72 W m^{-3}, 72.84 ± 5.54 W m^{-3}, 72.35 ± 3.66 W m^{-3} at current densities of 240.62 ± 14.60 A m^{-3}, 175.74 ± 8.76 A m^{-3}, and 181.80 ± 17.58 A m^{-3} in R1, R2, and R3, respectively (Fig. 2B). The maximum power output from the combined cathode (R1) was 26.73 ± 8.23% and 27.29 ± 6.38% greater than that obtained with GG (R2) or GFB (R3) cathode.

It has been well documented that the efficiency and effectiveness of a MFC are determined by physical factors (ohmic resistance) not by biological factors [38]. Though R2 showed the highest OCV among three reactors, its maximum power production was lowest. This demonstrates that GG provides good conditions for microbial growth, but it has higher ohmic resistance than R1 as shown in the polarization curve (Fig. 2A), probably due to high contact resistance and clogging. In addition, GG in the cathode compartment of R2 was packed loosely for good aeration, which might increase the inter-

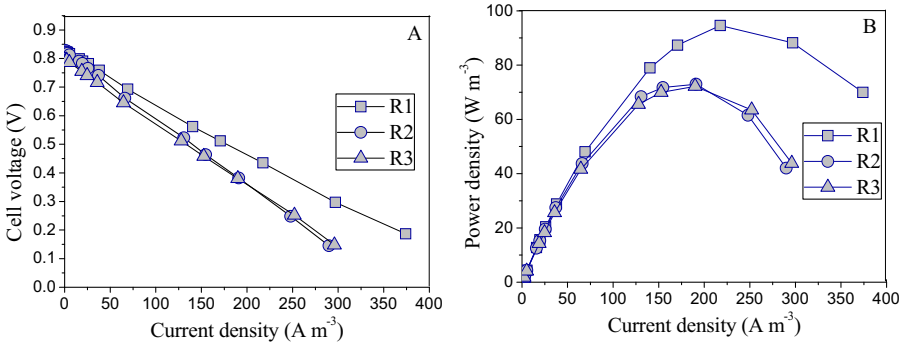

Fig. 2. Polarization curves (current vs cell voltage (A) and power (B)) of R1, R2, and R3-type MFC after the MFCs stably generated current.

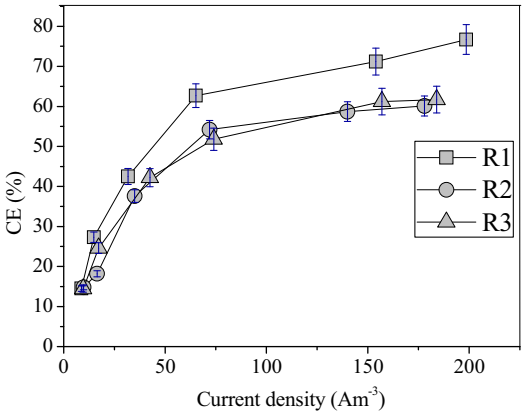

Fig. 3. CE as a function of current density for MFCs (R1–3) measured on a batch-fed basis.

nal resistance of R2 (R_{int} = 47.2 ± 1.7 Ω) with low maximum power density.

Compared with GGs, the surface of GFB is more smooth, less suitable for efficient microbial biofilm formation but easy for air to flow. The low power production from R3 seemed due to increased charge transfer resistance (R_{int} = 43.8 ± 1.2 Ω) with less developed biofilm on the cathode. It is assumed here that the combined biocathode of GFB embedded in GGs in R1 has the strength of both materials. The highest power production was achieved in reactor R1 with the lowest internal resistance (34.2 ± 0.07 Ω).

3.3. Coulombic efficiency

The coulombic efficiency (CE) reflects the ratio between coulombs recovered as current over the total amount of coulombs from the electron donor used (e.g., eight electrons per mole acetate). CE for three MFC reactor types (R1–3) were calculated based on batch-operations under various current densities by changing external resistance over the range of 50–2000 Ω. The results (Fig. 3) showed the better performance of combined cathode MFC compared with those of the other two materials, and this was similar to the results of polarization and power measurements. R2 and R3 showed similar CEs increasing from 14 to 62% as the current density was increased from 9 to 157 A m^{-3} (Fig. 3). These values were lower than that of R1 measured under the same conditions. With the external resistance of 100 Ω, CE of R1 was 75 ± 3%. CEs of all three MFC reactors increased rapidly as current density increased up to75 A m^{-3}. However, when current density exceeded 75 A m^{-3}, the rates of increase in CE significantly reduced. It is well documented that oxygen diffuses through the membrane and reduces CE. Under the low current density the relative amount of electrons consumed to reduce oxygen diffused into the anode chamber is bigger than that under higher current density conditions since the oxygen diffusion rate is independent to the current density. This might be the reason why higher CE increase with the increase in current density.

In some circumstances, MFCs are easy to lose systematic stability at higher current density due to kinetic or mass-transfer limitation, and this may lead to a substantially instable voltage output [39]. According to this rationale, compared with R2 and R3, R1 had excellent systematic stability at higher current density, so that CE for R1 was higher.

3.4. Fluorescence in situ hybridization

Biomass was separated from parts of MFC cathodes quantitatively for fluorescent microscopy for bacterial 16S RNA-based labeling, and the representative results for R1, R2 and R3 were shown in Fig. 4. The results indicated the highest eubacterial population was observed on the biocathode of R2 followed by that of R1, and the cathode from R3 showed the lowest population. These results suggested that the cathode material used in R3, GFB provide less favorable condition for biofilm formation than GGs, resulting in low bacterial population, low electro-catalytic activity and higher charge transfer resistance as discussed earlier. The bacterial population on the biocathode of R1 was not as high as R2, probably because the biocathode of R1 was consisted of GBF and GGs, while the biocathode of R2 was consisted of GG.

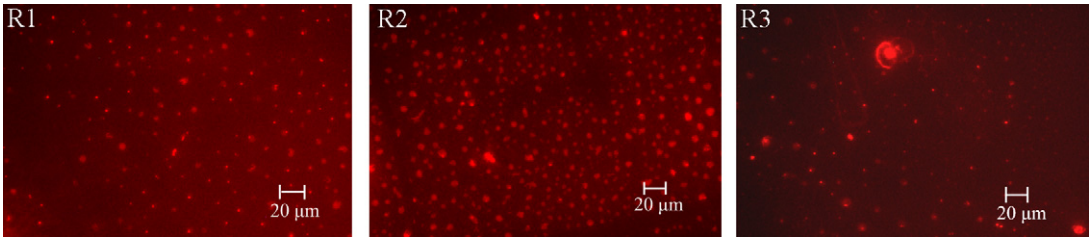

Fig. 4. Fluorescence in situ hybridization of biofilms from biocathodes of R1, R2 and R3 type MFCs with CY3-labelled EUB338 probe.

Table 1
Closest identities of clones from cathode compartment of microbial fuel cell with a biocathode.

Band no	Accession no	GenBank closest match (accession number)	*E*-value	Similarity (%)
Band 1	HM153429	*Nitrobacter winogradskyi* strain R1.30 (AM292292)	7e−146	95
Band 2	HM153430	*Stenotrophomonas* sp. INBio3083D (GU827545)	0	99
Band 3	HM153431	*Achromobacter xylosoxidans* (AB547225)	0	99
Band 4	HM153432	*Nitrobacter* sp. 311 (AM292300)	0	99
Band 5	HM153433	*Agrobacterium* sp. culture clone ECC2-23 (GU056300)	0	95
Band 6	HM153434	Uncultured *Achromobacter* sp. Clone (GU569153)	0	99
Band 7	HM153435	*Acinetobacter johnsonii* (DQ911549)	0	99
Band 8	HM153436	*Acinetobacter* sp. Dui-5 (EF031061)	0	98
Band 9	HM153437	Uncultured *Bacteroidetes* bacterium (CU926896)	0	100
Band 10	HM153438	Uncultured *Methylobacter* sp. (GQ390214)	0	99
Band 11	HM153439	Uncultured gamma *proteobacterium* (FJ516904)	0	98
Band 12	HM153440	Uncultured *Bacteroidetes* bacterium (CU925272)	0	97

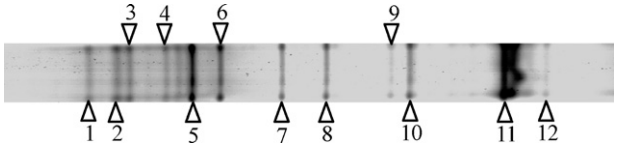

Fig. 5. Denaturing gradient gel electrophoresis profiles of PCR-amplified V1–V3 region of 16S rDNA in the microbial communities from the mixed biofilm samples on the biocathode of type reactors R1, R2 and R3. Signs (▽ and △) indicate the DGGE bands selected for cloning and sequencing.

3.5. Microbial community analysis of biocathode in the microbial fuel cell

Bacterial community profiles of the biofilm on the biocathode of three MFCs were fingerprinted by DGGE (Fig. 5), and a total of twelve prominent bands were excised from DGGE, amplified, cloned and sequenced. The phylogenetic affiliations of the representative DGGE band sequences are shown in Fig. 6, and the closest identities of clones from biocathode were shown in Table 1. The DNA sequences of the 12 bands fell into various species, and the dominant operational taxonomic units (OTU) could be divided into four groups; *Gammaproteobacteria* (bands 2, 7, 8, 10 and 11), *Alphaproteobacteria* (bands 1, 4 and 5), *Betaproteobacteria* (bands 3 and 6) and *Bacteroidetes* (bands 9 and 12). These populations are affiliated with *Nitrobacter* sp. (bands 1 and 4), *Achromobacter* sp. (bands 3 and 6), *Acinetobacter* sp. (bands 7 and 8), and uncultured *Bacteroidetes* bacterium (bands 9 and 12). *Acinetobacter* sp. and *Bacteroidetes* have been reported to be the key members of the biocathodic microbial community [28,29]. *Sphingobacterium* sp. (belonging to *Bacteroidetes*) and *Acinetobacter* sp. were isolated using H_2/O_2 mixtures from the cathode of MFC, and the pure cultures showed an increase in the power output of up to 3-fold compared to a non-inoculated control [28]. The sequences (bands 1, 4 and 5) derived from the biocathodes belong to the same order (*Rhizobiales*), and *species of the order Rhizobiales* were reported to be dominant in a MFC biocathode [30]. Furthermore, the sequence (band 2) is close to *Stenotrophomonas* sp. INBio3083D (GU827545) which belongs to the family *Xanthomonadaceae*, and some strains of the family *Xanthomonadaceae* are found to be leading members of the cathodic microbial community [30]. Strains produced bands 3 and 6, which are affiliated with *Achromobacter xylosoxidans*

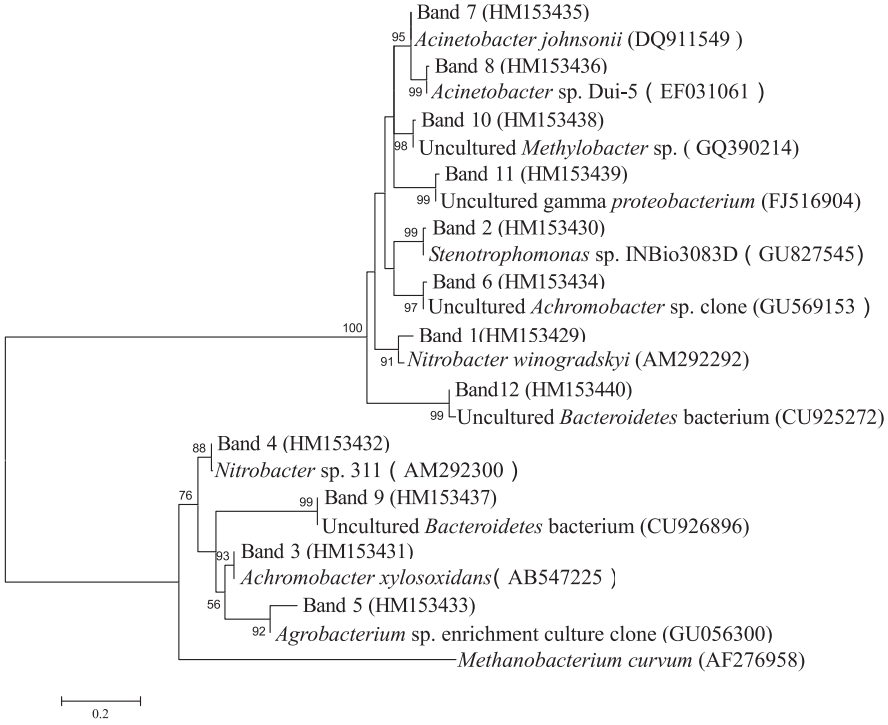

Fig. 6. Phylogenetic tree of 16S rDNA sequences of dominant populations of DGGE profiles. Aligned by Clustal-W using the neighborjoining method and kimura-2-parameter mode replicates receiving >50% bootstrap support. The scale indicates 0.2% sequence divergence. Bootstrap values are shown on or below branches of 1000. The tree was rooted with the *Euryarchaeota*, *Methanobacterium curvum* (AF273958). Numbers in parentheses represent the sequences accession number in GenBank.

and *Achromobacter* sp., might have important roles for electrocatalytic activity of biocathode. *Achromobacter xylosoxidans*, one of the autotrophic hydrogen-oxidizing bacteria, can oxidize H_2 to H_2O under aerobic conditions with one or more hydrogenase genes in these bacteria [26]. As is well known, the hydrogen-oxidizing microbes obtain energy and reducing power by coupling H_2 oxidation with O_2 reduction known as the "Knallgas reaction"

$$2H_2 + O_2 \rightarrow 2H_2O \quad (2)$$

This strongly exergonic reaction ($\Delta G^{0\prime} = -237.2\,\mathrm{kJ\,mol^{-1}}$) is an important energy source in biological systems [40]. Even though the standard redox potential of $H^+/_{1/2}H_2$ is lower than that of pyridine nucleotides, hydrogen oxidation is mainly coupled to the reduction of coenzyme Q or chytochromes for the efficient utilization of the H_2 in natural ecosystems [27].

4. Conclusions

Following conclusions are drawn based on the results obtained in this study:

(1) Combination of GG and GFB is proposed as an electrode material for biocathode to increase efficiency and sustainability of MFCs. The MFC with the combined cathode, R1 was enriched in a short period (186 h) and produced higher maximum power of $99.83 \pm 3.72\,\mathrm{W\,m^{-3}}$ than those with biocathode of GG or GFB alone.
(2) Combined biocathode of GG and GFB could meet the needs of increasing the electrode surface area and the surface properties for growth of electrochemically active bacteria which catalyzed biological oxygen reduction. Thus R1 decreased ohmic resistance and charge transfer resistance, and increased power production.
(3) FISH analyses indicated that the biocathode of R1 was populated with higher density of bacteria than those of R2 and R3. Small ribosomal RNA gene sequence analyses showed that biocathode of R1 contained chemoautotrophic bacteria which were affiliated with *Nitrobacter* sp., *Achromobacter* sp., *Acinetobacter* sp., and *Bacteroidetes*.

Acknowledgements

The authors gratefully acknowledge funding from Project 50776024 and Project 50821002 (National Creative Research Groups) supported by National Nature Science Foundation of China, Key Projects in the National Science & Technology Pillar Program during the 11th Five-Year Plan Period (2006BAC19B04), and partial supports by State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology (2010DX17).

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