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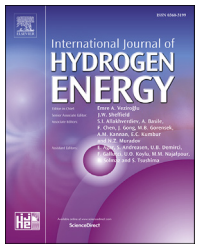

Cathodic reducing bacteria of dual-chambered microbial fuel cell

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ABSTRACT

Bacteria on biocathode can facilitate cathodic reduction reaction for a microbial fuel cells. This study profiled the cathodic bacterial community with high reduction activity using the Illumina pyrosequencing method, from which the highly diversified and novel population structure was found. The cathodic biofilm had a community structure dominated by Proteobacteria (42.7%), Firmicutes (25.0%), 9.7% Bacteroidetes (9.7%), Actinobacteria (7.7%) and Aquificae (7.1%). And the species of *Acidovorax*, *Soehngenia*, *Clostridium*, *Sulfurihydrogenibium*, *Flexibacter*, *Mycobacterium* and others were the predominant populations. Function composition analysis showed that the membrane transport is the most important metabolic activity for this community.

Introduction

A proton exchange membrane fuel cell (PEMFC) oxidizes hydrogen at anode to release electron and proton to cathode surface on which oxygen combines with the electron (via external electric circuit) and protons (via diffusion through liquid medium) to form water [1,2]. The microbial fuel cell (MFC) is a device with exoelectrogens attached on anode and/or cathodes to work as the electrode catalysts [3–5]. Owing to the capability of functional strains in the MFC cathodic biofilms is presented as a focus of research and development, including inoculum selection, component/reactor design, and operation strategies [6–8].

The anode respiring bacteria produces the oxidizing power to transform the fed fuel to its oxidized form (such as from acetate to CO_2) [9,10]. Meanwhile, the biocathode reducing microorganisms yields the reducing power to reduce the fed oxidant to its reduced form (such as from O_2 to H_2O) [11]. The resistances for the entire MFC are often largely contributed by the anodic biofilm transport/reaction [12,13]. Therefore, the anodic biofilm has been widely studied. Conversely, although the bacterial cathode has many advantages, such as improving MFC performance and sustainability [14,15],

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removing pollution (organic substance like dyes and chlorobenzene [16–18], heavy metals like Cr^{6+}, U^{6+} and V^{5+} [19,20], and inorganic substances like NO_3^-, NH_4^+, and CO_2 [21–23]), and producing useful products like H_2 and CH_4 [24–26], their role in MFC performance is not comprehensively studied [27]. The bacterial communities that develop in biocathode show great diversity, ranging from (α- and γ-) Proteobacteria [14], to (α-, β, γ-, and δ-) Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes, Planctomycetes, and et al. [28–30]. In addition, many microbial populations remain to not be discovered, limited by enrichment and detecting methods [31,32]. Community analysis reflected that there is no single important bacteria or 'winner' in the microbe communities that develop on the biocathode. Although some of the pure culture isolates obtained from the cathode biofilm were proved to increase the power output of MFC vs a non-inoculated control, they were unable to reach the levels attained by a mixed population [33]. It indicated that there is much cooperation among these bacteria in the cathode biofilm, and the community should be studied as a whole system.

The microorganisms in biocathode respond to the reduction function of the cathodic chamber of an MFC. However, the detailed community structure for cathode reducing bacteria has not been comprehensively studied. This work provides a detailed profile for the microbial community in mature biofilm on a cathodic surface for a dual-chambered MFC.

Experimental

Biocathode microbial fuel cells

The dual-chambered MFCs were composed of both anode and cathode compartments identical rectangular shape (120 mm × 120mm × 25 mm) with the same working volume (100 mL). Both the cathode and anode comprised graphite fiber brush (carbon fibers STS40 24 K, 650 ± 17 $m^2\ m^{-3}$, average fiber diameter of 7.0 μm, 1.7 Ω cm^{-1}, Toho Tenax) and graphite granules (diameter of 1–5 mm, 55 $m^2\ m^{-3}$, 0.5–0.6 Ω/granule, Jiuxin Carbon Goods Company, Jilin Province, China). A Nafion 117 PEM from Dupont with a sectional area of 12 cm × 12 cm separated the anode and the cathode chambers. The preparation procedures for PEM and electrodes are listed in Ref. [34]. The Ohmic resistance of the whole cell was estimated as 10.8 ± 1.6 Ω based on current interrupt method.

Operational conditions

The anode camber of MFC was inoculated with activated sludge samples from a wastewater treatment plant in Qingdao, China. The anodic medium contained (in g/L): 17.1 $Na_2HPO_4{\cdot}12H_2O$, 3.0 KH_2PO_4, 0.3 NaCl, 0.494 $MgSO_4{\cdot}7H_2O$, 0.01 $CaCl_2$, 2 glucose and trace elements as described [35]. The topsoil obtained from the turf at Qingdao Agriculture University. The cathodic medium was (in g/L) 1.0 NH_4Cl, 1.2 K_2HPO_4, 0.5 $MgSO_4$, 0.5 KCl, 0.14 KH_2PO4, 0.01 $Fe_2(SO_4)_3{\cdot}H_2O$ and trace elements (the contents to be same as the anode medium). The cathode chambers were continuously aerated at 100 mL min^{-1} to provide dissolved oxygen at the catholyte, and the experiments were conducted in fed-batch mode at 25 °C.

Analytical methods

A multicenter voltage collection instrument (PISO-813, ICP DAS, Co., Ltd., Beijing, China) was adopted to record the voltage drop over an adjustable external resistor. The potentials of anodic electrodes were recorded by Ag/AgCl reference electrode (+0.197 V vs. standard hydrogen electrode (SHE), model RE-5B, BASi, Ningbo, China). The volumetric power density from the tested MFC was normalized by the anolyte volume. The steady-state voltage drop at varying external resistances determined the polarization curve of MFC, from which the maximum power density (P_{max}) can be estimated [36]. The slope of polarization curve estimated the internal resistance (R_{int}) of the cell.

Microbial community analysis

The cathodic biofilm samples were collected after the reactor was started up for 67 d. The DNA in the sample was extracted by applying PowerSoil™ DNA isolation Kit (MoBio, Carlsbad, CA, USA), whose amount was quantified by a nanodrop spectrophotometer with 260/280 nm absorbance ratio. The 16S rRNA genes fragments with variable V4 regions were amplified with 520F broad range forward primer 5'-barcode + GCACCTAAYTGGGYDTAAAGNG-3′ and the 802R reverse primer 5′- TACNVGGGTATCTAATCC-3'. The amplicons were cleaned, quantified, and sequenced with custom barcoded primers on the Illumina HiSeq platform [37,38], whose sequences were analyzed using the QIIME software package (Quantitative Insights Into Microbial Ecology) [39]. Taxonomy was subsequently assigned to each representative OTUs using the Greengenes database classifier with a minimum support threshold of 80%. The Krona software calculated interactive metagenomic visualization of the community structures [40]. Functional compositions were calculated using the PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) software based on KEGG (Kyoto Encyclopedia of Genes and Genomes) [41].

Results and discussion

Cell performance

The MFC voltage was increased to 756 mV at a fixed resistant (R_{ex} = 300 Ω) after 428 h of reactor startup, and the potentials of anode and cathode reached −371 mV and +385 mV, respectively (vs. Ag/AgCl reference electrode). Since this stage the biofilms on cathode and anode were regarded mature for further testing [35].

The open circuit voltage (OCV) MFC was 882 ± 6 mV, and the corresponding anode and cathode potentials were −430 mV and 453 mV (vs Ag/AgCl) (Fig. 1A). At R_{ex} was decreased to 10 Ω, the corresponding cell voltage, anode potential, and cathode potential were also reduced to 202 mV, −105 mV, +167 mV, respectively. From the slopes of the curves, the total cell R_{int} = 22.4 Ω, anode $R_{int,}$ = 11.8 Ω, and cathode R_{int} = 10.6 Ω. The R_{int} is a sum of ohmic losses ($R_Ω$), activation losses (R_{act}) and mass-transfer losses (R_{mt}), while

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