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Effect of electricity on microbial community of microbial fuel cell simultaneously treating sulfide and nitrate

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HIGHLIGHTS

- Effect of electricity on microbial community on sulfide and nitrate removal was studied in MFC.
- All of four MFCs showed similar good capacity to remove substrate simultaneously.
- The MFCs displayed different characteristics in electricity generation.
- Significant correlation was existed between Richness of community and generated electricity.
- PCA showed that the two MFCs suffered current shock showed similar suspension communities.

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ABSTRACT

The effect of electric current on microbial community is explored in Microbial Fuel Cells (MFCs) simultaneously treating sulfide and nitrate. The MFCs are operated under four different conditions which exhibited different characteristics of electricity generation. In batch mode, MFCs generate intermittently high current pulses in the beginning, and the current density is instable subsequently, while the current density of MFCs in continuous mode is relatively stable. All operational parameters show good capacity for substrate removal, and nitrogen and sulfate were the main reaction products. Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analysis is employed to obtain profiles of the bacterial communities present in inoculum and suspension of four MFCs. Based on the community diversity indices and Spearman correlation analyses, significant correlation exists between Richness of the community of anode chamber and the electricity generated, while no strong correlation is evident between other indexes (Shannon index, Simpson index and Equitability index) and the electricity. Additionally, the results of Principal Component Analysis (PCA) suggest that MFCs suffering from current shock have similar suspension communities, while the others have diverse microbial communities.

1. Introduction

Microbial fuel cells (MFC) offer a novel approach in the field of wastewater treatment, which can generate electricity through degradation of the substrates [1]. The process has been applied for the treatment of domestic wastewater in 1991 [2]. MFC technology is gaining popularity and most of the studies involved the use of wastewater containing organic substrates like glucose, acetate, sucrose, etc [3].

Generally, wastewaters contain both organic and inorganic substances such as sulfide. Sulfide-containing waste streams are generated by many industries such as tanneries, petrochemical plants, viscose rayon factories etc. [4]. Sulfide is a toxic substance, which exerts various toxicological influences on human health and environmental ecology [5]. The sulfide is treated by various physical, chemical and biological technologies. The biological processes are cost effective as they operate under natural ambient conditions without any requirement for expensive chemicals and catalysts [6]. It has been demonstrated that nitrate may be employed in the control of sulfide species under anoxic or anaerobic conditions mediated by some bacterial species [7]. For such reasons, the

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simultaneous anaerobic sulfide and nitrate removal process has been recently developed.

$$5HS^- + 2NO_3^- \rightarrow 5S^0 + N_2 + 6H_2O + 7e^-$$
$$\Delta G_m^\theta = -1264\ \text{KJ mol}^{-1} \quad E^0 = 1.87\ \text{V}$$

Based on stoichiometric considerations, the simultaneous anaerobic sulfide and nitrate removal may be accomplished in MFCs. However; a few studies have reported this process in MFCs so far. Lee et al. studied the interactions between denitrifying sulfide removal in a MFC process and confirmed that the MFC was capable of the simultaneous sulfide and nitrate removal using monoculture of *Pseudomonas* sp. C27 [8]. We operated a two-chambered MFC simultaneously treating sulfide and nitrate using anaerobic activated sludge process [9,10]. It was concluded that electricity output enhanced through the microbial enrichment with the operation; suggesting that an active bacterial consortium capable of substrate removal and electricity generation had established in the MFCs.

Microorganisms of the anodic chamber play an important role in substrate removal and electricity generation in MFCs [11]. Many researchers have investigated the microbial communities on the electrode surface and those in the anode chamber of MFCs [12–15]. The microbial communities involved in MFC have been characterized in the previous work, generally studies focused on variations of substrate, reactor design or monocultures of novel organisms [16]. It is well established fact that the microorganism may affect the electricity production in a MFC. It is speculated that the electricity would also have adverse influence on the community of suspended microorganisms in MFCs. Very little information on the effect of electricity on the microbial community of the MFCs is available to date. However, bacterial communities associated with MFC treating sulfide or nitrate in the wastewater are well established [17–19], still information on the microorganism responsible for simultaneous sulfide and nitrate removal in the MFC is limited.

The objective of the current study was to investigate the effect of electricity on microbial community in MFCs treating sulfide and nitrate removal simultaneously. Four MFCs were operated under different conditions, in order to assess the impact of electricity. Denaturing Gradient Gel Electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S ribosomal RNA (rRNA) gene fragments to obtain profiles of the bacterial communities in inoculum and suspended sludge of four MFCs. Based on the community diversity indices (such as Richness, Dice index, Shannon Index, Simpson index and Equitability index) to present a detailed analysis of the community structure. Spearman correlation analyses were aimed at understanding the relationship between community diversity and electricity generation. Moreover, Principal Component Analysis (PCA) was used to investigate visual effects of electricity on the microbial community diversity in the anodic chamber.

2. Materials and methods

2.1. MFC construction

The MFCs consisted of anodic and cathodic chambers, each could accommodate total volume of 350 mL (300 mL net volume) as reported in our previous study [9]. Inoculum was collected from a UASB reactor simultaneously treating sulfide and nitrate operated under anaerobic lithoautotrophic conditions. The enriched sludge (100 mL) was inoculated in the anodic chambers of the MFCs. The anode chambers was also filled with synthetic influent containing $NaHCO_3$, $MgCl_2$, KH_2PO_4, (1 g L^{-1} each), $(NH_4)_2SO_4$ (0.24 g L^{-1}) and trace element solution (1 mL L^{-1}). The trace element solution was prepared according to Mahmood et al. [20]. The nitrate and sulfide concentrations were administered as KNO_3 and $Na_2S\cdot 9H_2O$, respectively; their concentrations were controlled according to the requirements of the experiment conducted. The influent pH was maintained at 7.0 ± 0.1 throughout the experiment. The medium in cathode chamber was a mixture of 50 mM PBS buffer (pH 7.0) and 100 mg L^{-1} $KMnO_4$ solution, which was recycled over the cathode compartment through a 2.5 L external buffer vessel by peristaltic pumps.

2.2. MFC operation

Four MFCs of same configuration were used in the experiment, which were denoted as MFC1, MFC2, MFC3 and MFC4, respectively. The MFCs were operated under different modes with diverse electrode materials. MFC1 was operated in continuous mode with graphite rod as electrodes, MFC2 was operated in batch mode containing graphite rod electrodes, and MFC3 was operated in continuous mode having graphite felt as electrodes, while MFC4 was also operated in batch mode equipped with graphite felt as electrodes. All MFCs were operated at room temperature (25 ± 2 °C).

While operated in batch mode, 200 mL synthetic wastewater was fed to the anode chamber on daily basis. Sulfide (540 mg L^{-1}) was added after the anodic chamber was flushed with N_2 for 5 min to remove O_2 from the solution. The nitrate concentration was added at S/N molar ratio of 5:2 based on stoichiometry of the chemical reaction.

During the MFCs operation in continuous mode, a peristaltic pump fed the synthetic wastewater to the system. Influent substrate concentrations were kept constant (300 mg L^{-1} and 52.5 mg L^{-1}, respectively), and the Hydraulic Retention Times (HRTs) were maintained around 10.6 h.

2.3. Analytical procedures

The influent and effluent nitrate-nitrogen, pH and sulfide were analyzed during the operation of MFC. Nitrate-nitrogen (NO_3^--N) was analyzed through ultraviolet spectrophotometric screening method on daily basis using spectrophotometer (Unico UV-2102 PC and 722S, China) [21]. The sulfide was determined by iodometric method and sulfate was measured through turbimetric method [21]. The pH was determined following standard method [21]. A three-point calibration of pH meter was performed on daily basis before pH determination. Total solids (TS) concentration was determined according to gravimetric method at 103 °C and volatile solids were analyzed through gravimetric method at 550 °C [21].

Voltage across the 1000 Ω resistor was recorded at an interval of every 10 min using a digital acquisition system (Agilent 34970A Data Acquisition/Switch Unit). Current density was normalized by the net surface areas of anode electrode.

2.4. DNA extraction and PCR–DGGE analyses

The activated sludge was collected from the anodic chamber of four MFCs during their optimal operation to analyze the dominant bacterial species. The activated sludge and inoculum were washed several times with the phosphate buffer solution before DNA extraction. Genomic DNA was extracted using 3S DNA isolation Kit for environmental samples (Shanghai Biocolors Company, China) according to the manufacturer's instructions. Quality of DNA extracts was detected by 1% (wt/vol) agarose gel electrophoresis. The extracted DNA was preserved at −20 °C.

The DGGE bacterial primers were GC-BAC338-F (ACT CCT ACG GGA GGC AG), added to the 5′ end of a 40-bp GC clamp (5′-CGC CCG CCG CGC CCC GCG CCC GTC CCG CCC CCG CCC-3′), and BAC805-R