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Magnetite nanoparticles accelerate the autotrophic sulfate reduction in biocathode microbial electrolysis cells

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ABSTRACT

The aim of this study was to investigate the effect of magnetite nanoparticles on the performance of autotrophic sulfate-reducing biocathode in microbial electrolysis cell (MEC). The biocathodes in MECs were start-up using cathode medium with and without magnetite nanoparticles addition (with initial iron content of 0.64 mM), respectively. With magnetite, the sulfate reductive rate reached 152 ± 7.0 g m^{-3} d^{-1}, which was improved by 122% than the MEC without magnetite. The electron recovery efficiencies in MECs with and without magnetite were 56.1% and 29.6%, respectively. With the effect of magnetite, the peak currents in cyclic voltammograms of the biocathode were about two times higher than that without magnetite, indicating its higher electrochemical activity. Analyses of the scanning electron microscope and the energy dispersive spectrometer showed that pilus-like substance and iron-sulfide were formed on the magnetite-biocathode. Confocal laser scanning microscopy showed the increase of the biomass, thickness, and viability of biofilm in the biocathode with magnetite compared to those without magnetite. The relative abundance of *Desulfovibrio* sp. was 72.2% in the biocathode with magnetite compared to 27.5% without magnetite. The conductive magnetite could affect the development of electrochemical active biofilm directly and accelerate sulfate reduction in the MEC.

1. Introduction

The wastewater discharged from industrial processes (e.g., pharmaceutical, chemical plant, and paper mill) contains large quantities of sulfate, which can be harmful to the environment and human health [1]. Physicochemical methods to treat sulfate in wastewater, including chemical precipitation, ion exchange, and liquid membrane separation, are facing various challenges, such as high technological costs and secondary pollution [2]. For traditional biological method, sulfate-rich wastewater is usually deficient in electron donors and requires external supplement, causing chemical waste and pollution [3]. Hence, other biological methods using autotrophic sulfate-reducing bacteria (SRB) have rapidly caught the attention of scientific experts.

The microbial electrolysis cell (MEC) with biocathode has been recently proposed as a new and sustainable technology for bioremediation of contaminated environment [4]. In the MEC, microorganisms can accept electrons from the cathode for the reduction of contaminants, such as nitrate, sulfate, Cr^{6+}, nitrobenzene [5]. It has been reported that autotrophic SRB can catalyze sulfate reduction with a polarized electrode (−400 mV vs. Ag/AgCl) as the sole electron donor without via electron shuttles or hydrogen production [4]. Blázquez and Gabriel [6] showed that effective treatment of high strength sulfate wastewater and simultaneous recovery of elemental sulfur using an autotrophic biocathode under oxygen-limiting conditions. More and more attention has been paid to autotrophic biocathode for sulfate reduction in MECs, because the method is inexpensive, sustainable, and resistant to sulfide poisoning [7,8]. To optimize the performance of autotrophic biocathode, many factors have been considered, such as external applied voltage [9], operation mode of electrolytes and potential value of cathode [10], pH value of catholyte [11], structure of reactor [6]. Although these factors improve MEC performance by indirectly affecting biofilm, effective methods are still needed to optimize the biofilm development directly.

Conductive and semi-conductive minerals (e.g., pyrite, magnetite, and hematite) can serve as electric conduits to promote electron transport between microorganisms [12]. The unique electronic properties and redox propertiesof Fe(III) and Fe(II) in magnetite can enhance electron transfer to various electron acceptors [13–16]. A pilus-deficient mutant of *Geobacter sulfurreducens* can attach to Fe(III) oxides, forming highly conductive pili that may

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serve as biological nanowires [17]. Magnetite particles can enhance the kinetics of TCE dechlorination and boost the electrocatalytic activity of *Desulfovibrio paquesii* biocathodes for hydrogen production [18,19]. In addition, conductive minerals are less costly for interspecies electron transfer and abound in nature soils and sediments [20]. Therefore, development of the electrochemical active biofilm using magnetite particles should be an important alternative for optimization of biocathode. Nevertheless, it is with little information how the magnetite affects the cathode biofilm with mixed culture.

The objective of this study was to explore the effect and mechanism of magnetite particles on autotrophic biocathode to treat sulfate-rich wastewater. Two-chamber MECs with SRB biocathode were start-up using medium with and without magnetite addition, respectively. During stable operation period, the MECs were then operated using medium without magnetite addition. The MEC performance was evaluated using the current output, sulfate reduction, and electron recovery efficiency. The mechanism was investegated based on development of biocathode biofilm.

2. Materials and methods

2.1. Magnetite nanoparticles preparation

Magnetite nanoparticles were prepared using the co-precipitation method [21]. Briefly, 5.2 g of $FeCl_3$ and 2.0 g of $FeCl_2$ were successively dissolved in an acid solution with stirring. The resulting solution was added dropwise into 250 mL of 1.5 M NaOH solution under vigorous stirring. The last step generated an instant black precipitate, which was separated by placing a magnet, then purified by deoxygenated water. The synthesized magnetite stored in an anaerobic bottle with N_2 as the shielding gas.

2.2. Autotrophic SRB enrichment

Sediment from the Pearl River Guangzhou section in south China was used as inoculums to enrich autotrophic SRB. Each of the sediment samples was acclimatized in a 100 mL anaerobic bottle, which contained 25 mL sediment and 25 mL sulfate medium. The medium contained (per liter of demineralized water): 0.72 g Na_2SO_4 (about 500 mg ofSO_4^{2-}), 2.0 g $NaHCO_3$, 6.84 g $NaH_2PO_4{\cdot}2H_2O$, 2.20 g $Na_2HPO_4{\cdot}12H_2O$, 0.3 g NH_4Cl, 0.1 g KCl, 0.04 g $MgCl_2$, 1 mL trace element solution, and 10 mL vitamin solution [10]. A gas mixture (H_2-CO_2 [80:20]) was fed as electron donor and carbon source. The acclimation process was operated in a batch mode with 4 d as a cycle. During the first 20 d, a half of the supernatant was replaced with fresh medium for each cycle. After that, all the supernatant was replaced at the end of the cycle. The concentration of sulfate ion (SO_4^{2-}) was analyzed every 96 h. When the removal rate of $SO_4{}^{2-}$ became stable (about 40 d), the acclimated sediment was used as inoculums of biocathode in the MEC.

2.3. Reactor construction

MEC reactors were constructed with two chambers separated by the cation exchange membrane (Ultrex CMI-7000, MI, USA). Each chamber was constructed by drilling a hole (30 mm diameter) in a solid block of polymethyl methacrylate. The graphite brush anode was composed of 10 bunches of graphite fiber (12 K, Xinka Carbon Industries CO., LTD, Shanghai, China), each of which was with 40 mm length. The anode was heated in a muffle furnace at 450 °C for 30 min and then placed in the center of anode chamber. Cathode was made of three pieces of polished graphite plate (BF3-4, Shandong, China), each of which was 25 mm width × 35 mm length × 2 mm height. All graphite plates were pretreated before use, washing in acetone and drying, followed by immersion in 1 M NaOH, and 1 M HCl for 24 h, then rinsed, and stored in deionized water [22]. After insertion of electrodes, each of the anode and cathode chambers had a volume of 28 mL. A voltage of 0.8 V was applied to the MEC using a programmable power supply (IT6720, Itech, China). An external resistor (10 Ω) was connected in series with the power supply negative lead and the cathode. A saturated $Hg/HgCl_2$ reference electrode (+0.242 V vs. SHE) was inserted into the cathode chamber to measure changes of the electrode potential.

2.4. Reactor start-up and operation

Anodes were initially inoculated with a mixture of anaerobic and aerobic sludge (1:1, 10.0 mL) collected from Liede Municipal Wastewater Treatment Plant of Guangzhou, China. Cathodes were inoculated with 10 mL acclimated sediment (i.e., the mixed culture of SRB). The anodic solution contained (per liter of demineralized water) 1 g CH_3COONa, 10.35 g $Na_2HPO_4{\cdot}12H_2O$, 3.31 g $NaH_2PO_4{\cdot}2H_2O$, 0.31 g NH_4Cl, 0.13 g KCl, 12.5 mL trace element solution, and 12.5 mL vitamin solution, and the pH of the solution was adjusted to 7.0 [10]. The cathode chamber was filled with the sulfate medium with the pH value adjusted to 6.0 using 1 M H_3PO_4. All the MEC reactors were protected from light and operated at 30 ± 3 °C in a constant temperature incubator (LRH-150B, Shaoguan, China). MECs (duplicate reactors) were start-up and operated at a fixed applied voltage of 0.8 V.

During the star-up period (0–720 h), magnetite particles were added into cathode medium for the magnetite-MECs with initial iron content of 0.64 mM. At the beginning (0–120 h), a half of the medium was replaced with fresh medium for each day. A gas mixture (H_2-CO_2 [80:20]) was used as an additional electron donor to accelerate biofilm growth on the cathode surface. After 120 h, the entire medium in anode and cathode chamber was replaced with fresh medium at the end of each 48 h cycle. To remove headspace air and dissolved oxygen after replacement, the cathode chambers were bubbled with ultrapure CO_2 (3 min per time) at the beginning of each cycle. The reactors were operated for about 30 d using medium with magnetite addition, which was considered as the start-up period.

To explore the enhancing effect of composite network structure that formed by magnetite particles and cathode biofilm on the performance of the MEC. After 30 d, magnetite particles were absent from the cathode medium of magnetite-MEC, which was considered as stable operation period (720–1008 h). The reactors were operated in a fed-batch mode with 48 h for each cycle. At the end of a cycle, the medium in each chamber was replaced with the fresh medium. For comparison, MECs were constructed using abiotic cathodes with addition of magnetite. To determine the electrochemical role of magnetite in the cathode chamber, an open circuit control (i.e., disconnected cathode) was also conducted with addition of magnetite. For each cycle, the anolyte and catholyte samples were periodically taken for analysis. The stability of the MEC was assessed based on the repeatable of current output and sulfate removal at least 3 cycles. The presented results except for current output are values of the mean value and standard deviation of data collected during 3 cycles.

2.5. DNA extraction and high-throughput pyrosequencing

After 45 d operation, cathode surface of duplicate MECs were crushed using sterile scissor for biofilm collection, which were used for the bacterial community analysis. Extraction, purification, and quantification of the genomic DNA were carried out using DNA kit (12888-50, MOBIO, USA) according to the manufacturer's instructions. The final DNA concentration and purification were determined by NanoDrop 2000 UV–vis spectrophotometer