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Microbial metabolism and activity in terms of nitrate removal in bioelectrochemical systems

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ABSTRACT

Bioelectrochemical systems (BESs) are a promising technology for generating energy while treating wastewater. By utilizing the electron transfer between the anode and cathode, nitrate can be effectively removed from the BES. Our previous studies show that the carbon source and C/N ratio influences nitrate removal performance. The study presented here investigates how biofilm formation, nitrate removal and signaling molecule release are related in the BESs fed with glucose, starch and HCO_3^-. The results indicate that increasing the current can benefit signaling molecule (DSF) release and extracellular polymeric substances (EPS) excretion, which improves biofilm formation. However, when the current exceeds the optimum value, the influence becomes adverse. Nitrate removal was also improved with increased current, though different carbon sources showed different trends. The highest nitrate removal efficiency of 1.23 ± 0.27, 1.38 ± 0.09, 1.80 ± 0.02 mmol L^{-1} d^{-1} for the BESs fed with glucose, starch and HCO_3^- were achieved, respectively. This paper studied the bacterial habits in a BES to better acquire and regulate the reaction process, with the aim of achieving good pollutant removal performance.

1. Introduction

Nitrate pollution of water sources has gained worldwide attention as nitrate can induce gastric cancer, methemoglobinemia and Hodgkin's lymphoma [1–4]. There are several nitrate removal technologies, with biological denitrification being most widely used. In engineered systems, the heterotrophic denitrification process is usually used to remove nitrate [5]. To achieve high treatment efficiency, external organic matter is required and can result in excessive biomass production.

In recent years, bioelectrochemical systems (BESs) have been widely studied. They are capable of converting the chemical energy of organic wastes into electricity or valuable hydrogen/chemical products. The energy for production is sourced directly from the wastewater [6,7]. Based on electron transfer characteristics between the anode and cathode, nitrate can be effectively removed in a BES. Microbial fuel cells (MFCs) are the most widely investigated type of BES. In an MFC, electrons produced by bacterial metabolism are transferred to the anode and flow to the cathode by conductive materials [8]. Substances such as nitrate can be reduced at the cathode because of the low redox potential. Clauwaert et al. [9] and Puig et al. [10] have successfully achieved autotrophic denitrification in an MFC. However, the denitrification rate is low in the MFC. Biofilm-electrode reactors (BER) have also been developed and applied to treat nitrate-contaminated water [11,12]. In a BER, the hydrogen produced via the electrolysis of water is used as the electron donor, which allows hydrogenotrophic denitrification to proceed.

For all reactors, microorganisms are essential for reducing nitrate. It was reported that bacterial enzyme activity could be enhanced by appropriate electric field stimulation [13]. Electrochemically active bacteria achieve electron transfer between the electrode and the organic or oxide material present in the reactor [14,15]. Biofilm formation on the electrode surface significantly influences a system's performance. Our previous studies have shown that the source of carbon has a significant influence on nitrogen removal [16]. However, the mechanism of microbial activity was still unclear. Biofilm formation is the result of environmental selection. Environmental stress from toxic compounds, nutrient availability or high shear forces can affect and regulate the architecture of a biofilm [17]. In the BER, efficient nitrogen removal can be achieved by increasing the electric current [18]. Microbial activity may also differ under various electric currents.

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It has been shown that cell-to-cell signaling, known as quorum sensing (QS), is important for initial biofilm formation and can be used to determine the behavior of biofilm communities [19,20]. QS signaling takes place via chemical signal molecules such as acyl-homoserine lactones (AHLs), peptides, diffusible signal factors (DSF) and pseudomonas quinolone signals (PQS) [21]. QS also affects the electron transfer in BESs. Venkataraman et al. [22] found that QS can control the production of phenazines and mediated current generation by the anode-respiring bacterium *Pseudomonas aeruginosa* in the MFC. Similarly, Yong et al. [23] investigated the impact of genetic over-expression of QS cassettes on the electrochemical activity of *P. aeruginosa.* This study indicated that under different QS expression patterns, different electron shuttles were used by *P. aeruginosa.* However, the effect of signaling molecule excretion on nitrate removal in BES was unclear. To better understand and enable more precise manipulation of BESs, it is important to explore this microbial process.

This study aims to investigate how biofilm formation, nitrate removal and signaling molecule release are related in BESs. The production of extracellular polymeric substances (EPS) was also studied, as it is crucial to biofilm formation.

2. Materials and methods

2.1. Experimental configuration and operating conditions

The experimental configuration was the same as our previous study [16]. In brief, bottles with an effective volume of 500 mL were used as reactors. Rectangular graphite electrodes (15 cm long and 8 cm wide) were used as electrodes. Synthetic wastewater, consisting of (L^{-1}) 176 mg $NaNO_3$, 4 mg $MgSO_4{\cdot}7H_2O$, 2.08 mg KH_2PO_4, 1.76 mg K_2HPO_4, 0.96 mg NaCl, 1.12 mg $CaCl_2$, 1.92 mg $FeCl_3{\cdot}6H_2O$, was used to simulate nitrate-contaminated water. In this synthetic wastewater, trace elements with components of (L^{-1}) 30 μg $MnCl_2{\cdot}4H_2O$, 30 μg $Na_2MO_4{\cdot}2H_2O$, 10 μg $CuCl_2{\cdot}H_2O$, 70 μg $ZnSO_4{\cdot}7H_2O$, 1000 μg EDTA, 300 μg H_3BO_3, 600 μg $CoCl_2{\cdot}6H_2O$, 20 μg $NiCl_2{\cdot}6H_2O$ were supplied. The glucose, starch and $NaHCO_3$ used as carbon sources were added to the synthetic water. The ratio of chemical oxygen demand (COD) to nitrate was controlled at about 2.0.

Seed sludge was collected from the Qige Wastewater Treatment Plant (Hangzhou, China).The result of 16S rDNA sequencing indicated that the inoculated sludge was composed byα-proteobacteria (35.41%), β-proteobacteria (21.48%), γ-proteobacteria (13.45%), Sphingobacteria (1.35%), Gram$^+$ (1.97%) and other bacteria (26.34%). Sludge was cultured without supplying oxygen and nitrate for about one week to form anoxic microorganisms. 200 mL sludge was inoculated to the reactor and a constant current (5 mA) was applied by a DC power supply. COD was decreased to 60 mg L^{-1} while nitrate was gradually increased to 30 mg L^{-1}. Control reactors were set up identically but with an open electrical circuit. The synthetic wastewater was replaced every 24 h and the temperature maintained at 30 ± 2 °C in a greenhouse. The wastewater exchange procedure was carried out as follow: 250 mL solution was extracted out by siphon and another 100 mL solution was extracted through syringe. The total volume of 350 mL solution was collected in a beaker (1 L). Then 350 mL synthetic wastewater was injected into the reactor. After the sludge was settled for about 0.5 h, the same procedure were repeated twice. Sludge in the beaker was also added into the reactor. The influence of different currents of 5, 15 and 25 mA on biofilm formation, EPS excretion, signaling molecule production and nitrate removal performance was investigated.

2.2. Bioassay for signaling molecules

In this study, the signaling molecule of AHLs, PQS and DSF was detected. Signaling molecules were extracted from the effluent as described in previous reports [24] with some modification. 50 mL of effluent was centrifuged at 22,087 × *g* for 5 min, the supernatant was extracted three times with an equal volume of ethyl acetate. A moderate amount of anhydrous sodium sulfate was added to dry the organic phase. The dehydrated organic phase was further dried using a rotary evaporator. The residual signaling molecule extract was dissolved in 2 mL of 50% HPLC-grade acetonitrile and stored at −70 °C before analyzing. The extracted signaling molecule was analyzed by high performance liquid chromatography (Waters Co., Massachusetts, USA), with an X Bridge C-18 column (5 μm *d*, 4.6250 × mm).

2.3. Bioassay for biofilm formation

Bioassay for sludge biofilm formation was performed with some modifications to the process described in Maeda et al. [25]. Under different currents, fresh sludge was centrifuged at 17,000 × *g* at 4 °C for 5 min and the supernatant discarded after washing twice with a potassium phosphate buffer (pH 7.5). 5% (w/v) sludge was prepared with different medium before 96-well biofilm assay. An inorganic salt medium (ISM) supplied with glucose, starch, $NaHCO_3$ and nitrate acted as the carbon and nitrogen source. The ISM was composed of 0.2 g K_2HPO_4, 0.8 g KH_2PO_4, 0.2 g $MgSO_4$, 0.1 g $CaSO_4{\cdot}H_2O$, 0.0033 g $NaMoO_4$ and 0.005 g $FeSO_4{\cdot}H_2O$ in 1 L of distilled water. 200 μL of 5% sludge with the medium was then inoculated at 37 °C for 24 h. The sole medium was used as a control. Biofilm formation was measured according to the procedure in Herzberg et al. [26].

2.4. Bioassay for EPS extraction and measurement

5 mL of sludge was extracted for the EPS assay using the heat EPS extraction procedure described by Yang and Li [27]. The content of extracellular protein and extracellular polysaccharides were respectively determined by Coomassie brilliant blue assay [28] and the phenol/sulfuric acid method [29]. The content of extracellular DNA was measured using the diphenylamine chromogenic method [30].

2.5. Scanning electron microscopy (SEM) image

The cathodic biofilm was first scraped from the electrode and then centrifuged in 10,000 rpm for 5 min. By discarding supernatant, a suitable amount of 2.5% glutaraldehyde was added and the biofilm was kept in it for about 24 h. Then the biofilm was rinsed triple with phosphoric buffer (0.1 M, pH 7.0) for 15 min. The sludge was treated with 1% (w/v) osmic acid for 1.5 h and then rinsed triple with phosphoric buffer for 15 min. Then the biofilm was ordinally treated with 50%, 70%, 80%, 90%, 95% and 100% ethanol, each for 15 min. After dehydrating with ethanol, mixture of ethanol and isoamyl acetate (v/v = 1/1) was added into the sample to treat for 30 min. At last, the biofilm was treated with isoamyl acetate for 1.5 h and then dried. After coating a layer of gold, cathodic biofilm morphology was observed with SEM (Quanta 200, FEI Co., Ltd. Czech Republic)

2.6. Other analysis

The phenate method and ultraviolet spectrophotometric screening method were used to respectively measure nitrogen as NH_4^+ and NO_3^-. Nitrogen in the form of NO_2^- was measured using the colorimetric method. Standard methods [31] were used. Total