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Response of a three dimensional bioelectrochemical denitrification system to the long-term presence of graphene oxide

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HIGHLIGHTS

- Increasing GO decreased denitrification efficiency and altered community abundance.
- Denitrifying genes abundances were decreased under high GO stress.
- Long-term presence of GO caused chronic impacts onto the microorganisms.

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ABSTRACT

In this study, a three dimensional bioelectrochemical denitrification system (3D-BEDS) was operated under long-term graphene oxide (GO) condition to treat high nitrate polluted water. When GO concentration increased from 0 to 100 mg L^{-1}, nitrate removal efficiency slightly decreased from 99.52% to 94.81%. However, when GO concentration was further increased to 150 mg L^{-1}, the denitrification efficiency dramatically decreased to 74.95%. Increasing GO concentration in this BEDS resulted in decreased community richness, and the abundances of the dominant bacterial communities presented obvious shift. The abundances of denitrifying genes *napA*, *nirS*, and *nirK* showed no obvious changes with GO concentration lower than 50 mg L^{-1}. However, the abundances of the three genes decreased when GO concentration was further increased to higher than 100 mg L^{-1}. The increased lactate dehydrogenase (LDH) release and reactive oxygen species (ROS) production demonstrated that long-term presence of GO caused chronic impacts onto microorganisms in this BEDS.

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1. Introduction

Nowadays, graphene oxide is widely used in many kinds of industries such as pipes, packaging, biosensors and etc. because of its particular electrochemical properties (Ahmed and Rodrigues, 2013; Brody, 2006). Besides, GO has been broadly utilized to remove environmental contaminants such as heavy metals, dyes, and some refractory organic pollutants in recent years due to its abundant oxygen containing functional groups and big specific surface areas (Huang and Pan, 2016; Jiang et al., 2016; Wang and Chen, 2015). However, because of its strong dispersity in waters, GO will potentially present in wastewaters without effective collection measures after utilization. Therefore, GO may bring potential threats to the conventional biological wastewater treatment process because nanomaterial might easily accumulate in the microorganism bodies and affect the pollutants metabolic process (Qu et al., 2015).

Recently, numerous studies have reported the bioelectrochemical denitrification system is effective and promising for nitrate removal (Ghafari et al., 2008; Zhou et al., 2007). For instance, Zhou et al. (Zhou et al., 2007) developed a three-dimensional bioelectrochemical reactor and demonstrated that this reactor performed excellent denitrification ability. Wan et al. (Wan et al., 2009) investigated the nitrate remove process in a combined bioelectrochemical and sulfur autotrophic denitrification system and achieved satisfied results. Kondaveeti et al. (Kondaveeti et al., 2014) investigated the effects of electron acceptors in a bioelectrochemical denitrification system and evaluated the bacterial communities. These researches focused on the nitrate removal efficiency and even bacterial community to evaluate the performance and analyze the mechanism of BEDS, and demonstrated that BEDS was effective and promising for nitrate removal. However, there has been very little research conducted on the response of bioelectrochemical system to the presence of GO during the denitrification process.

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In the present study, a three dimensional bioelectrochemical denitrification system was developed to treat high nitrate wastewater under long-term presence of GO condition. The objective of this study was to evaluate the effects of increasing GO concentration on the microbial communities, functional genes and denitrification efficiencies. In addition, lactate dehydrogenase release and reactive oxygen species production analyses would reveal the integrity of cells and the oxidation stress induced by GO.

2. Materials and methods

2.1. Reactor operation

The total volume of the 3D-BEDS was 100 L, and the working volume was 40 L (Fig. 1). The bioelectrochemical system was composed of a graphite rod (diameter 8 mm) as anode, a piece of carbon fiber felt as cathode, and sulfur granule (diameter 5–8 mm) as carrier. The cathode and anode were connected by a direct current power. The system was initially started for 50 days with enough anaerobic sludge (MLSS 3500 mg L^{-1}) collected from Erlangmiao Municipal Wastewater Treatment Plant, Wuhan, China. The medium was: NO_3^--N 500 mg L^{-1}, HCO_3^- 500 mg L^{-1}, $MgCl_2$ 10 mg L^{-1}, $ZnCl_2$ 0.50 mg L^{-1}, $CoCl_2$ 2.00 mg L^{-1}, $MnSO_4$ 1.00 mg L^{-1}, $NiCl_2$ 0.30 mg L^{-1}, $CuCl_2$ 0.30 mg L^{-1}, $FeSO_4$ 0.20 mg L^{-1}, $CaCl_2$ 0.50 mg L^{-1}, Na_2MoO_4 0.30 mg L^{-1}. During the whole operation, NO_3^--N concentration was maintained at 500 mg L^{-1}, while current, hydraulic retention time (HRT), pH, and temperature were remained at 100 mA, 16 h, 7.0, and 25 °C, respectively. Especially, the GO (which was prepared in our previous study (Chen et al., 2016)) concentration was adjusted to 0, 50, 100, 150 mg L^{-1} for each 50 days. The concentrations of NO_3^-, NO_2^-, SO_4^{2-}, and N_2 were determined to evaluate the nitrate removal efficiency in this 3D-BEDS.

2.2. Analytical methods

The effluent samples in the experiments were filtered by 0.45 µm membrane by a suction filter machine. NO_2^--N was measured using N-(1-naphthyl) ethylenediamine dihydrochloride spectrophotometric method at 540 nm. NO_3^--N was detected by ultraviolet spectrophotometric method at (220–275 × 2) nm.

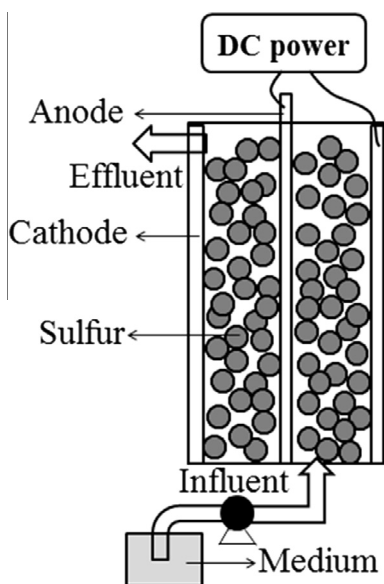

Fig. 1. Schematic diagram of the BEDS.

Nitrogen gas (N_2) was determined by an Agilent HP4890D gas chromatography. SO_4^{2-}-S concentration was measured using ion chromatograph (881 Compact IC pro, Metrohm, Switzerland). The pH was measured by a pH meter (PHS-3C, Kexiao Instrument, China). The water temperature was determined by a thermometer (TM827, Zhugongda Instrument, China). Lactate dehydrogenase (LDH) was measured using lactate dehydrogenase assay kit (Nanjing Jiancheng Bioengineering Institute, China) in accordance with the manufacturer's instructions. Reactive oxygen species (ROS) was measured using reactive oxygen species Assay Kit (Nanjing Jiancheng Bioengineering Institute, China) in accordance with the manufacturer's instructions. The specific procedures of LDH and ROS detections were also according to several previously published methods (Mu and Chen, 2011; Quan et al., 2015; Zheng et al., 2011).

2.3. DNA extraction and Miseq pyrosequencing

Four biofilm samples S1, S2, S3, S4 at GO concentrations of 0, 50, 100, 150 mg L^{-1} were collected and the bacterial genomic DNA was extracted by PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA). The PCR amplification, pyrosequencing procedure, and sequence analyses followed the method described in our previous study (Chen et al., 2015). Especially, the V3–V5 region of the bacterial 16S rRNA gene sequences were amplified, and the primers sequences were 338F (5′-ACTCCTACGGGAGGCAGCA-3′), 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Zheng et al., 2016).

2.4. Quantitative real-time PCR (q-PCR)

The qPCR was conducted using qPCR kit (SuperReal PreMix Plus (SYBR Green), TIANGEN BIOTECH, China) according to the manufacturer's instructions on a QuantStudioTM 6 Flex qPCR system (Applied Biosystems, CA, USA). Three functionally denitrifying genes *napA* (nitrate reductase gene), *nirS* and *nirK* (nitrite reductase genes) were evaluated. The specific primer for amplification of gene *napA* were napV67m (AAYATGGCVGARATGCACCC) and napV17m (GRTTRAARCCCATSGTCCA) (Miao et al., 2015). Gene *nirS* was detected by specific primer cd3aF (GTSAACGTSAAGGAR-ACSGG) and R3cd (GASTTCGGRTGSGTCTTGA) (Miao et al., 2015). The specific primer sets for amplification of *nirK* gene were F1aCu (ATCATGGTSCTGCCGCG) and R3Cu (GCCTCGAT-CAGRTTGTGGTT) (Throback et al., 2004). The PCR procedures for the three genes were according to our previous study (Chen et al., 2015). The calibration curves and the calculation method of gene copy number of the amplicons were according to the previously published study (Huang et al., 2015). The reaction efficiencies were greater than 90% with R^2 > 99%.

3. Results and discussion

3.1. BEDS performance

The concentrations of NO_3^-, NO_2^-, SO_4^{2-}, and N_2 in the effluent during the experimental phase were displayed in Fig. 2. When GO concentration increased from 0 to 100 mg L^{-1}, nitrate removal efficiency slightly decreased from 99.52% to 94.81%, which indicated that the denitrification efficiency was not obviously influenced by GO varied from 0 to 100 mg L^{-1}. However, when GO concentration was further increased to 150 mg L^{-1}, the denitrification efficiency dramatically decreased to 74.95%, suggesting that excessive GO could inhibit the denitrification process in this BEDS. The concentrations of intermediate product NO_2^--N always remained at low level closed to zero, while the maximum SO_4^{2-}-S concentration was within the recommended limits (250 mg L^{-1},

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