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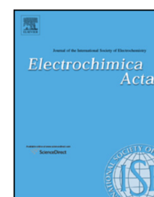

Anodic ammonia oxidation to nitrogen gas catalyzed by mixed biofilms in bioelectrochemical systems

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ABSTRACT

In this paper we report ammonia oxidation to nitrogen gas using microbes as biocatalyst on the anode, with polarized electrode (+600 mV vs. Ag/AgCl) as electron acceptor. In batch experiments, the maximal rate of ammonia-N oxidation by the mixed culture was ~ 60 mg L^{-1} d^{-1}, and nitrogen gas was the main products in anode compartment. Cyclic voltammetry for testing the electroactivity of the anodic biofilms revealed that an oxidation peak appeared at +600 mV (vs. Ag/AgCl), whereas the electrode without biofilms didn't appear oxidation peak, indicating that the bioanode had good electroactivities for ammonia oxidation. Microbial community analysis of 16S rRNA genes based on high throughput sequencing indicated that the combination of the dominant genera of *Nitrosomonas*, *Comamonas* and *Paracocus* could be important for the electron transfer from ammonia oxidation to anode.

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1. Introduction

Ammonia-N pollution, which can cause eutrophication and be toxic to aquatic species, is usually converted into nitrogen gas via anaerobic ammonium oxidation (ANAMMOX) or nitrification/denitrification [1]. However, additional nitrite-N is necessary for anammox reaction and the cultivation of the anammox bacteria is difficult [2]. Besides, the nitrification/denitrification process need two stages starting with nitrification, which is the aerobic oxidation of ammonia-N to nitrite-N or nitrate-N, followed by heterotrophic denitrification under anaerobic conditions [3].

Although the electrochemical method has been shown effective for ammonia removal from wastewater [4], the catalytic oxide materials are confined to alloy electrode. Different anodic materials such as Pt [5], boron-doped diamond (BDD) [6] and dimensionally stable anode (DSA) [7,8], have been extensively assessed. What's more, the rigorous reaction conditions and the expensive electrode materials limit its application in wastewater treatment. Therefore, it is necessary to develop a low-cost electrochemical system that could removal nutrients under mild conditions.

Recently, bioelectrochemical systems (BESs) have been proposed as a promising alternative for contamination removal, energy generation and biosynthesis because of sustainable bacterial metabolic reactions [9]. In the device, the bioanode is the terminal electron acceptor for substrates oxidation including glucose [10], acetate [11] and sulfur [12]. However, to date, there is no report on ammonia oxidation to nitrogen gas with microbial anode as electron acceptor. In fact, the Gibbs free energy of ammonia is high than that of the nitrogen molecule, so ammonia has the potential to be oxidized to nitrogen gas [13]. Furthermore, recent researches have pointed out that potential control is an effective method to domesticate electroactive biofilms [14] and control substrates oxidation [15,16]. Therefore, the study on microbially catalyzed anode ammonia oxidation controlled by potential is feasible and indispensable.

In this study, the characteristic of ammonia oxidation was investigated in a BES with polarized microbial anode (+600 mV vs. Ag/AgCl) as electron acceptor. The electroactivity of the biofilms was determined by cyclic voltammentry test. In addition, the microbial community of the bioanode was analyzed through 16S rRNA genes analysis based on high throughput sequencing, which was responsible for ammonia oxidation to nitrogen gas with anode as electron acceptor.

2. Experimental

2.1. BESs setup and operation

The BES used in this study consisted of two compartment (made of polymethyl methacrylate), physically separated by an anion exchange membrane (AEM, Zhejiang Qianqiu Co.,Ltd, anion

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exchange capacity 1.9 mmol g^{-1}). Gas vent was connected on the top of each chamber (5 cm × 5 cm × 6 cm) for collecting gas. Both anode (working electrode) and cathode (counter electrode) electrodes were carbon felt of 4.0 cm × 4.0 cm. Both chambers were filled with 130 ml medium containing the following components (per liter of distilled water): 0.14 g KH_2PO_4, 3.2 g $Na_2HPO_4 \cdot 12H_2O$, 2.0 g $NaHCO_3$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$ and 1.0 ml of trace mineral mix [17]. In addition, the anode compartment was added 10 ~ 30 mM NH_4Cl (140 ~ 420 mg L^{-1} ammonia-N). Both champers were flushed with argon gas for 20 min to strip out the dissolved air, and then connected to a potentiostat. Ag/AgCl (sat. KCl, 0.197 V vs. SHE) electrode was used as reference electrode. The pH of the two champers was maintained at 7.7 ± 0.2 by adding $NaHCO_3$ and 1 mM HCl. The abiotic control experiments were also performed under closed circuit conditions without microbial culture while the biotic control experiments were carried out under open circuit conditions.

The mixed culture inoculated in the anode chamber was originated from a wastewater treatment plant of Chengdu, China. In order to produce hydrogen at cathode, the mixed biofilms were acclimated with anode potential set to +600 mV (vs. Ag/AgCl). The domestication of biofilms was monitored by measuring the change of ammonia-N in the anode compartment. The performance of nitrogen removal was repeated several times at 28 ± 2 °C until obvious and consistent ammonia removal was determined.

2.2. Analytical techniques

NH_4^+-N, NO_2^--N and NO_3^--N were measured regularly according to the standard methods for the examination of water and wastewater [18]. N_2 and O_2 were analyzed using gas chromatography with thermal conductivity detector (GC-1690), with a chromatographic column of 2 m length × 3 mm filled with 13X molecular sieves as a separating column. Loading gas: high-purity helium; Flow rate of loading gas: 50 ml/min; Column temperature: 30 °C; Inlet temperature: 60 °C; Detector temperature: 80 °C.

The current of working electrode was collected every 50 s by CHI 1000 C with a Power Laboratory 8SP unit connected to a computer. Cyclic voltammetry measurements were performed with an electrochemical working station (EC550, China) at a scan rate of 10 mV s^{-1} in the potential range from 0.8 V to -0.6 V. Micrographs were taken by the scanning electron microscope (S-4800, Japan) as our previous described [14]. The Coulombic efficiency (CE) was calculated to address the recovery of electron as electrical current from the oxidation of ammonia to nitrogen as described in our previous study [19].

2.3. DNA extraction and MiSeq sequencing of 16S rRNA gene amplicons

DNA density and quality were checked using a NanoDrop Spectrophotometer. Extracted DNA was diluted to the concentration of 10 ng μL^{-1} and stored at -40 °C for downstream use. Universal primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with Illumina barcodes were used to amplify the V4 hypervariable regions of 16S rRNA genes for pyrosequencing using Miseq sequencer [20,21]. The PCR mixture (50 μl) contained 1× PCR buffer, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate at 0.4 μM, each primer at 1.0 μM and 1 U of TransStart Fast Pfu DNA Polymerase (TransGen, China) and 10 ng genomic DNA. The PCR amplification program included initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were subjected to electrophoresis using 1.0% agarose gel. The band with a correct size was excised and purified using Gel Extraction Kit (Omega Bio-tek, USA) and quantified with Nanodrop. The sequencing samples were prepared using TruSeq DNA kit according to manufacture's instruction. The purified library was diluted, denatured, re-diluted, mixed with PhiX (equal to 30% of final DNA amount) as described in the Illumina library preparation protocols, and then applied to an Illumina Miseq system for sequencing with the Reagent Kit v2 2 × 250 bp as described in the manufacture manual.

2.4. Data analysis

The sequence data were processed using QIIME Pipeline–Version 1.7.0 (http://qiime.org/tutorials/tutorial.html). All sequence reads were trimmed and assigned to each sample based on their barcodes. Multiple steps were required to trim the sequences, such as removal of sequences < 150 bp and average base quality score Q < 30. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier at a confidence level of 80% (http://pyro.cme.msu.edu/).

3. Results and Discussion

3.1. Ammonia oxidation catalyzed by the anode biofilms

Fig. 1 showed the characteristic of ammonia oxidation at anode with or without microbes as catalyst. Specifically, ammonia was oxidized into nitrogen gas without nitrite and nitrate accumulation in anode compartment with the presence of bacteria (Table 1 and Fig. 1a), and the nitrogen gas content increased from 34.1 ± 0.2% to 95.2 ± 0.5% when the concentration of ammonia-N increased from 140 mg L^{-1} to 420 mg L^{-1} (Fig. 1b). The maximal rate of ammonia-N oxidation by the mixed culture was ~ 60 mg L^{-1} d^{-1}, and the ammonia-N removal efficiency reached 41.3 ± 3.2%, 55.9 ± 5.5% and 47.7 ± 3.0% with different initial ammonia-N concentrations at 140 mg L^{-1}, 280 mg L^{-1} and 420 mg L^{-1}, respectively (Table 1). In contrast, there was almost no ammonia removal and no nitrogen gas generation (Fig. 1a and b) in the absence of microbes (abiotic experiments). During the experiments, ammonia was not transferred into cathode because of the presence of anion exchange membrane. In addition, there were also no ammonia, nitrite and nitrate detection in the cathode (data not show). What's more, oxygen was not detected in gas composition in all the experiments including the abiotic contrast experiments (Table 1). Although the theory oxygen evolution potential shifts to 0.57 V (vs. Ag/AgCl) at pH 7.7, which indicates that oxygen could have been generated at the potential examined here (0.6 V vs. Ag/AgCl) and have immediately been scavenged by the biofilms, the generated oxygen (in the form of dissolved oxygen) is insufficient to consume the metabolic ammonia. Here, we assume the saturated dissolved oxygen is 7.9 mg L^{-1} at 28 °C. In the abiotic experiments, there is no oxygen detection in the gas phase, so the maximum oxygen production may be 7.9 mg L^{-1} (0.25 mM) in the solution, which only oxidizes 4.62 mg L^{-1} ammonia-N (0.33 mM) to nitrogen gas, 2.38 mg L^{-1} ammonia-N (0.17 mM) to nitrite and 1.75 mg L^{-1} ammonia-N (0.125 mM) to nitrate. However, according to Table 1, in this study the total amount of removal ammonia-N reached 57.8 ± 4.5 mg L^{-1}, 156.5 ± 15.4 mg L^{-1} and 200.3 ± 12.6 mg L^{-1}, and the efficiency of ammonia-N transformation into N_2 reached 85%, 82% and 69% with different initial ammonia-N concentrations at 140 mg L^{-1}, 280 mg L^{-1} and 420 mg L^{-1}, respectively. Therefore, ammonia oxidation mainly happened with the anode as the electron acceptor catalyzed by microorganisms for nitrogen gas production.

In order to further determine ammonia oxidation with anode as electron acceptor, the generating current was evaluated at different

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