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Short Communication

Ammonia as carbon-free substrate for hydrogen production in bioelectrochemical systems

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ABSTRACT

In this study, ammonia was used as an anode substrate for cathode hydrogen generation by controlling the anode potential in two compartment bioelectrochemical systems. During batch experiments, the total quantity of electric charge increased from 129 C at 10 mM ammonia to 185 C at 20 mM ammonia, and it reached 281 C at 30 mM ammonia. Besides, the nitrogen removal efficiency reached 41%, 56% and 48% at 10, 20 and 30 mM ammonia respectively. Correspondingly, the productions of nitrogen were respectively 5.1 ml, 13.3 ml and 14.3 ml, and the productions of hydrogen were respectively 7.2 ml, 13.4 ml and 14.6 ml. However, there were no nitrogen and hydrogen production, as well as only a little electric charge of 10 C when there was no ammonia addition. The coulombic efficiency was higher than 50% during 5 days operation, which demonstrated that the bacteria growing on the anode were able to utilize most of the available ammonia for current generation. In addition, ammonia-oxidizing bacteria and ammonia-assimilating bacteria were detected by microbial community analysis of 16S rRNA genes based on high throughput sequencing, but anammox bacteria were not found at the anode biofilms, which suggested that there were two different pathways for anodic ammonia transformation.

Introduction

Renewable energy productions have attracted increasing attention because of the shortage of fossil fuels and the negative impacts of its usage on environment [1]. Hydrogen is a clean energy without carbon footprint, which has been claimed to be a feasible alternative to fossil fuels [2,3]. Recently, bioelectrochemical systems (BESs) which use microorganisms to catalyze the reaction at the anode and/or cathode [4] are suggested to be a promising new approach for contamination removal, energy generation, biological hydrogen production and biosynthesis for value products

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using a wide variety of substrates [5–7]. In this device, the hydrogen carriers are mainly focused on the organic matters including fermentable substrates (glucose, lactose and sucrose) and non-fermentable substrates (acetate, ethanol and methanol) [8–11].

Unlike these substrates, ammonia is carbon-free at the end user, which makes it an ideal hydrogen carrier (17.6 wt %) [12]. Although ammonia has been reported as an anodic substrate for electricity generation, the coulombic efficiency (CE) is very low, only 0.34% and 1.1% reported by two papers respectively [13,14], which indicates that the ability of ammonia oxidation with electrode as electron acceptor need to be improved, and that there are may be others metabolic pathways for anodic ammonia transformation. In addition, the products of ammonia oxidation are nitrite and nitrate [15,16], which are not suitable for nitrogen removal. Ideally, nitrogen gas and hydrogen gas should be generated at anode and cathode respectively in the process of anodic ammonia oxidation. However, to our knowledge, there is no report on hydrogen generation from ammonia in the promising BESs.

Hence, in this study, ammonia was used as an anode substrate for hydrogen generation in two compartment BESs by controlling the anode potential. The products of anode and cathode compartments were analyzed, and the performance of ammonia oxidation in BESs for hydrogen generation was also evaluated. Furthermore, the pathway of ammonia metabolism by mixed cultures was investigated in view of nitrogen balance and microbial community.

Materials and methods

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 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 by glass syringe. 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. 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 7.6–7.8 by adding $NaHCO_3$ and 1 mM HCl. The abiotic control experiments were also performed under identical conditions without microbial culture.

The mixed cultures inoculated in the anode chamber were originated from a wastewater treatment plant of Chengdu, China. In order to produce hydrogen at abio-cathode, the anodic mixed biofilms were acclimated at a potential of +0.6 V (vs. Ag/AgCl) controlled by a potentiostat. The development of biofilms was monitored over a period of 3 months by measuring the change of ammonia-N in the anode compartment. The solution was decanted when all ammonia-N removed, and then fresh medium was added. The performance of the BESs was repeated several times at 28 ± 2 °C after obvious and consistent nitrogen removal.

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 H_2 were detected by gas chromatography with thermal conductivity detector (GC-1690), with a chromatographic column of 2 m length × 3 mm filled with 13× molecular sieves as a separating column (GC system: high-purity helium carrier gas at 50 ml min^{-1}; Column temperature at 30 °C; Inlet temperature at 60 °C; Detector temperature at 80 °C). The current of working electrode was collected every 50 s by CHI 1000C with a Power Laboratory 8SP unit connected to a computer.

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 [19,20]. The detail PCR conditions were carried out as described previously [21]. 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.

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/). The coulombic efficiency was calculated to address the recovery of electron as electrical current from the oxidation of ammonia to nitrogen as described in our previous report [16]. The cumulative electric charge (eq_i) that was transferred at the electrodes was calculated by integrating the current (A) over the period of electrode polarization. Cumulative reducing equivalents (eq_p) that were used for the formation of reduced products were calculated from the measured amounts of H_2. Cathode efficiency was accordingly calculated as η (%) = (eq_p/eq_i) × 100.