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Bio-electrochemical enhancement of anaerobic reduction of nitrobenzene and its effects on microbial community

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ABSTRACT

Nitrobenzene (NB) is listed as a priority pollutant in many countries due to its high toxicity and environmental risk. In this study, a microbial electrolysis cell (MEC) combined anaerobic reactor with a pair of Fe – carbon electrodes (R1) was designed to reduce NB to less-toxic and biodegradable aniline (AN). Applying a voltage of 0.5 V in R1 enhanced the NB reduction, AN production, and COD removal comparing to the reference reactors. Raising voltage from 0.5 to 1.2 V improved the conversion of NB to AN. The assistance of MEC intensified Fe^{2+} leaching and accelerated the process of sludge granulation. As compared with abiotic electrode, the cathode of R1 presented higher value of NB reduction rate constant (k). Real-time PCR and cloning sequencing analysis showed that the abundances of bacteria and methanogens were significantly higher than that of reference reactor, in which some species capable of reducing NB were dominant in R1.

1. Introduction

Nitrobenzene (NB) is widely used as an important chemical raw material for the production of aniline (AN), dyes, pharmaceuticals, and pesticides [1,2]. As a result of the improper disposal and accidental leakage from related factories [3], NB is frequently released to the water environment. Due to its recalcitrance and mutagenicity, NB has been listed as a priority pollutant by United States Environmental Protection Agency and other countries [2].

Advanced oxidation technologies such as ozonation process [4], Fenton oxidation [5], and photocatalysis [6] have been applied to oxidize NB directly by producing a powerful oxidizing agent •OH [7]. However, NB is still resistant to these chemical oxidation due to the electro-withdrawing effect of the nitro group [8]. Generally, most of these chemical processes need to consume more energy and/or require significant quantities of chemicals and precious metal catalysts, which are cost-intensive and may cause secondary pollution [4–7]. Comparatively, the biological process presents an environment-friendly and economically viable approach to remove NB. In general, NB could be reduced to AN first during the anaerobic process, and then AN is further mineralized into CO_2 and H_2O via aerobic treatment. But this method usually suffers from low treatment efficiency.

Electrochemical reduction is a promising approach to reduce bio-refractory and toxic compounds such as NB. In recent years, bio-electrochemical systems (BES) were developed to enhance denitrification, methanogenesis, and the reduction of perchlorate and chlorinated organic compounds [11–13]. Some researchers have also investigated the use of a BES to remove NB at the cathode, and they found that NB removal and AN formation rates were significantly enhanced, comparing to the traditional electrochemical cell [14,15]. However, the reducing ability of these single BES is limited because of the surface area of electrodes.

Zero valent iron (Fe^0), as a strong reductant, has been proved to be efficient for the reduction of NB due to its low redox potential ($E^0 = -0.44$ V) [9]. Under anaerobic conditions, NB can be reduced to AN by Fe^0 with nitrosobenzene as an intermediate product, meanwhile Fe^0 will be oxidized to Fe^{2+}. The overall reaction is as follows: $ArNO_2 + 3Fe^0 + 6H^+ = ArNH_2 + 3Fe^{2+} + 2H_2O$, in which Fe^0 is acted as the ultimate electron donor with two-electron reduction [9]. To further enhance the performance of NB reduction by Fe^0, a biotic Fe^0 system was developed which combined microbes and Fe^0 [10]. In this bio-Fe^0 system, the cathodic hydrogen produced via anaerobic iron corrosion can be used by microorganisms as

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electron donors for the reduction of NB. However, due to the limitation of iron dosage, the rate of hydrogen production becomes the rate-limiting step. It will further hinder NB to serve as electron accepter. We suppose that an electric field applied using Fe^0 anode possibly impel the oxidation reaction of Fe^0 ($Fe - 2e = Fe^{2+}$) and enhance its reducibility.

In this study, a microbial electrolysis cell (MEC) combined anaerobic reactor with a pair of Fe anode and carbon cathode was developed for enhancing anaerobic reduction of NB. This compact bio-electrochemical reactor integrated anaerobic reduction of NB and bio-electrochemical reduction of NB. It is believed that an electric field can intensify Fe^0 oxidation reaction and accelerate Fe^{2+} leaching, and carbon cathode served as electron donor can enhance NB reduction. However, there are few reports focus on electric driving Fe^0 anode to enhance anaerobic reduction of NB. Therefore, it is necessary to investigate the feasibility of bio-electrochemical enhanced NB reduction with Fe anode forced by electric field.

2. Materials and methods

2.1. Experimental set-up and operation

A pair of Fe plate anode (length 70 mm × width 40 mm × thickness 2 mm) and carbon felt cathode (length 70 mm × width 40 mm × thickness 3 mm) was inserted into a cylindrical acrylic plastic up-flow anaerobic blanket reactor (UASB) (Φ 120 mm × 250 mm) to form a MEC combined anaerobic reactor (hereafter referred to as R1). The top of the electrodes was located at 2/3 depth of the reactor. The electrodes were connected to a DC power source through an electric wire. The working volume of the reactor was 2 L. The control experiments were conducted in the following two reactors, a common UASB reactor that was the same as R1, but without electrodes (hereafter referred to as R2), and an abiotic electrochemical reactor that was the same as R1, except without anaerobic sludge (hereafter referred to as R3).

These three reactors were operated under a continuous mode at 35 ± 1 °C with a hydraulic retention time of 24 h, and the influent COD including sucrose and NB was maintained at 2000 mg/L (2 Kg COD/m^3/d) with the increasing of NB concentration as the following levels step by step: 0, 200, and 400 mg/L. Sucrose, NH_4Cl, and KH_2PO_4 were added in the feeding as the sole carbon, nitrogen, and phosphorus sources, respectively, to give a COD:N:P ratio of 200:5:1. The trace elements were added according to the following composition: 1 mL/L of a trace element solution containing $ZnSO_4{\cdot}7H_2O$ at 0.37 mM, $MnSO_4{\cdot}2H_2O$ at 2.5 mM, $CuSO_4{\cdot}5H_2O$ at 0.14 mM, Co $SO_4{\cdot}7H_2O$ at 8.4 mM, $NiCl_2{\cdot}6H_2O$ at 0.25 mM, H_3BO_3 at 0.8 mM, and EDTA at 3.4 mM. The pH of the influent wastewater was adjusted to 7.5 using $NaHCO_3$ solution.

The voltage for the electrodes was fixed at 0.5 V or 0 V (i.e., without voltage). During the initial 15 days, the reactor R1 was operated without voltage (0 V). From day 16 to day 44, the reactor R1 was supplied with a voltage of 0.5 V. To clarify the role of voltages on NB reduction, different voltages (0.5 V, 0.8 V, 1.2 V, and 0 V) were applied between the electrodes of R1 from day 46 to day 57. To further compare the effects of electrodes between reactor R1 and R3 on NB removal, the electrodes were taken out for a batch experiment after 57 days' operation. In this experiment, they were used to treat 100 mg/L NB in a 1 L airtight reactor (Φ 120 mm × 130 mm) with a voltage of 0.5 V. Before the experiment, the NB solutions were deoxidized using pure N_2 gas for 15 min.

Seed sludge was taken from a 10 L laboratory-scale UASB reactor treating sucrose-containing wastewater in our laboratory. 0.7 L anaerobic sludge was added into each reactor. The ratio of volatile suspended solids to total suspended solids (VSS/TSS) was 0.77 with initial TSS of 19.3 g/L.

2.2. Analytical methods

COD, VSS, and TSS were determined according to the standard methods [16]. The concentration of Fe(II) ions was measured using an UV spectrophotometer (Techcomp, UV-2301, Shanghai, China) at an absorbance of 510 nm. The pH was recorded using a pH analyzer (Sartorius PB-20, Germany). The concentration of NB and AN were determined by a high performance liquid chromatograph (HPLC, Waters 2695) equipped with a C18 column (15 cm × 4.6 mm × 5 μm) and a UV detector (254 nm). The CH_4 production was determined using a gas chromatograph (GC-14C, Shimadzu, Japan) equipped with a thermal conductivity detector. The average granular sludge size was measured using a Malvern Mastersizer 2000 (UK). These granular sludge were destructed with sulfuric acid for the roughly determination of Fe element. The morphology of the sludge was observed by a scanning electron microscope (SEM; Quanta 200 FEG, The Netherlands).

2.3. DNA extraction, PCR amplification, and sequencing

The genomic DNA of the samples were extracted using an extraction kit (Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. The general primers for bacteria were GM341F (forward primer: 5′-CCT ACG GGA GGC AGC AG-3′) and DS907R (reverse primer: 5′-CCG TCA ATT CCT TTG AGT TT-3′) [17]. The concrete steps of polymerase chain reaction (PCR) amplification was done according to previous illustration [17]. The PCR products were then subsequently cloned and sequenced by Sangon Biotech Co., Ltd., (Shanghai, China) and the sequences were screened against the GenBank database using the BLASTn program to identify the most similar sequences. In this study, approximately 50 bacterial clones of each sample were randomly selected. Sequences with 97% or higher similarity were grouped into operational taxonomic units. The nucleotide sequences were deposited in the GenBank with the accession numbers KP159421–KP159436.

2.4. Real-time PCR quantification

TaqMan based real-time PCR was used to quantify total bacteria, archaea, two hydrogenotrophic methanogens (MBT-set/Methanobacteriales and MMB-set/Methanomicrobiales), and two acetoclastic methanogens (Mst-set/Methanosaetaceae and Msc-set/Methanosarcinaceae) [18]. The sequences of all primers were listed in Supplementary Table S1. Quantitative real-time PCR was carried out using the StepOne Real-Time PCR System (ABI StepOne plus, USA) with the corresponding primer. Each real-time PCR reaction was conducted in a 25 μL PCR mixture contained 12.5 μL of SybrGeen qPCR Master Mix 0.5 μL of each primer, 2 μL of template DNA, and 9.5 μL of dH_2O. Cycling conditions of two step real-time PCR reaction were 2 min at 95 °C of initial denaturation, followed by 40 cycles of 10 s at 95 °C and 40 s at 60 °C. Standard plasmid was constructed by the method of synthetic genes. Then, the linear treatment of these plasmids was performed by an appropriate restriction enzyme, forming standard DNA. Each plasmid was diluted step by step with EASY dilution of 10 times gradient and served as template for real-time PCR reaction. Each reaction was conducted in triplicate to ensure the reproducibility of the results.

3. Results and discussion

3.1. NB reduction and AN production

Fig. 1 shows the treatment performances of reactor R1 and R2 in terms of NB reduction and AN formation. In the initial 6 days, the effluent COD in these two reactors ranged from 208.6 ± 13.3 to 225.4 ± 16.5 mg/L at a fixed influent COD of 2000 mg/L without