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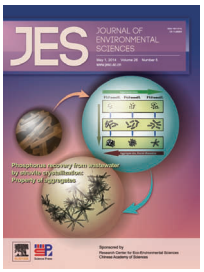

Effects of ferrous and manganese ions on anammox process in sequencing batch biofilm reactors

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ABSTRACT

Ferrous and manganese ions, as essential elements, significantly affect the synthesis of Haem-C, which participates in the energy metabolism and proliferation of anammox bacteria. In this study, two identical sequencing batch biofilm reactors were used to investigate the effects of ferrous and manganese ions on nitrogen removal efficiency and the potential of metal ions serving as electron donor/acceptors in the anammox process. Fluorescence *in situ* hybridization analysis was applied to investigate the microbial growth. Results showed that the nitrogen removal increased at high concentrations of Fe^{2+} and Mn^{2+} and the maximum removal efficiency was nearly 95% at Fe^{2+} 0.08 mmol/L and Mn^{2+} 0.05 mmol/L, which is nearly 15% and 8% higher than at the lowest Fe^{2+} and Mn^{2+} concentrations (0.04 and 0.0125 mmol/L). The stabilities of the anammox reactor and the anammox bacterial growth were also enhanced with the elevated Fe^{2+} and Mn^{2+} concentrations. The Fe^{2+} and Mn^{2+}were consumed by anammox bacteria along with the removal of ammonia and nitrite. Stoichiometry analysis showed Fe^{2+} could serve as an electron donor for NO_3^--N in the anammox process. Nitrate could be reduced with Fe^{2+} serving as the electron donor in the anammox system, which causes the value of NO_2^--N/NH_4^+-N to decrease with the increasing of N-removal efficiency.

Introduction

The anaerobic ammonium oxidation (anammox) process is a biochemical process for nitrogen removal found at the end of 20th century. Under anaerobic conditions, ammonium is oxidized into nitrogen gas by the anammox bacteria along with nitrite reduction (Vandegraaf et al., 1995). Anammox bacteria are widely distributed in the aquatic N cycle, and have been discovered in marine (Engstrom et al., 2009; Kuypers et al., 2003; Thamdrup and Dalsgaard, 2002), freshwater (Penton et al., 2006; Schubert et al., 2006), terrestrial (Humbert et al., 2010; Zhu et al., 2011) and wastewater ecosystems (Gao and Tao, 2012). Anammox bacteria have a profound impact on the global cycling of nitrogen. The anammox process offers many advantages for ammonium removal in the wastewater treatment process, such as lower energy requirements, no requirement for aeration and organic carbon resources and no CO_2 emission. Based on this knowledge, the anammox process, a new innovative technology in wastewater treatment, is often applied for the removal of inorganic N species from different wastewaters having high ammonium concentration (Dapena-Mora et al., 2006; Joss et al., 2011; Kumar and Lin, 2010; Yamamoto et al., 2006). However, anammox bacteria are sensitive to their surroundings (substrate concentration, temperature, dissolved oxygen , etc.), and may thrive only under the known optimal conditions (operation conditions, substrates) (Dapena-Mora et al., 2004; Ni, 2008; Strous et al., 1998; Trigo et al., 2006).

The genome analysis of *Kuenenia stuttgartiensis* showed that two hundred genes directly involved in

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catabolism and respiration exceeds the number possessed by most other bacteria (Strous et al., 2006). There are a large number of encoded c-type cytochromes in *Kuenenia stuttgartiensis*, and many c-type cytochromes have been found in the metal-respiring organisms *Geobacter sulfurreducens* and *Shewanella oneidensis* (Strous et al., 2006). Therefore, metal ions should have a significant effect on the anammox process.

The purpose of this article was to focus on how different metal ion (Fe^{2+}, Mn^{2+}) concentrations affect the anammox system and the potential of metal ions to serve as electron donors in the anammox process. Fluorescence *in situ* hybridization (FISH) was applied for the identification of anammox bacteria in the reactors with different metal ions. The potential of Fe^{2+} and Mn^{2+} to serve as electron donor/acceptor was assessed by calculating the conversion of ammonium, nitrite and nitrate. In addition, the utilizable ratio of metal ions was also analyzed. This research may provide a better understanding of the role of Fe^{2+} and Mn^{2+} in the anammox process, which might explore a good way for the anammox bacteria to thrive in limited conditions, and increase the opportunity for anammox process application in a variety of wastewaters.

1 Materials and methods

1.1 Sequencing batch biofilm reactor

Experiments were carried out in sequencing batch biofilm reactors with a working volume of 1.5 L. The reactors were filled with polyethylene beads which were 1 cm in diameter, 0.965–0.968 aspect ratio, and 3 m^2/g in specific surface area. The polyethylene beads were fixed by a polyethylene net. The pumps and timing of the operational cycles were controlled by a time cycle controller system. Four cycles of 6 hr were performed each day in both reactors, including settling (30 min), decanting (15 min), feeding (15 min), and mixing (300 min).

1.2 Biomass and growth conditions

The biomass used for inoculation originated from an anammox-sequencing batch biofilm-reactor that had worked steadily more than 1 year. Temperature was controlled at 35 ± 1°C using a thermostatic jacket. Dissolved oxygen (DO) in the influent was maintained as 0.6 ± 0.2 mg/L and pH of influent ranged between 7.8 and 8.2. Complete mixing inside both reactors was achieved using magnetic stirrers at rotating speeds of 80 r/min. Synthetic wastewater contained mainly nitrite and ammonia to support anammox activity. Potassium bicarbonate was used as the carbon source. Trace elements and other nutrient substances were added to the inflow according to Van de Graaf et al. (1996). The two reactors were run in three stages using elevated Fe^{2+} concentrations at 0.04, 0.06, and 0.08 mmol/L (Reactor 1) and Mn^{2+} at 0.0125, 0.025, and 0.05 mmol/L (Reactor 2), respectively.

1.3 Analytical methods

Ammonium, nitrite and nitrate content were measured according to standard methods (Van de Graaf et al., 1996). The changes of metal ion concentrations in both reactors were analyzed by flame atomic absorption spectrometry (Varian SpectrAA 220, USA).

1.4 Fluorescence *in situ* hybridization analysis (FISH)

Samples (1.5 mL) from both reactors (Reactor 1: day 127, day 171, day 216; Reactor 2: day 85, day 125, day 173) were mixed with paraformaldehyde for fixation. FISH analysis was performed as described by Schmid et al. (2005). After washing, fixation and dehydration, the cells were hybridized with the following fluorescently-labeled oligonucleotide probe: Amx-368 with the sequence CCTTTCGGGCATTGCGAA labeled with Cy3 (Sangon, Shanghai, China). Samples were counterstained using mounting medium containing 4,6-diamidino-2-phenylindole (Sangon, Shanghai, China). An Olympus fluorescence microscope (BX2U-MWU2, Japan) was used for observation.

2 Results

2.1 N-removal performance in different metal ion concentrations

The ammonium and nitrite removal efficiencies all increased with the addition of metal ions in both reactors (**Fig. 1**). After start-up, the simultaneous removal of ammonium and nitrite was detected in the two reactors, which indicated the occurrence of the anammox reaction. In Reactor 1, the concentrations of NH_4^+-N and NO_2^--N in the effluent declined. The average ammonium removal efficiency at the three stages was 85.23% ± 4.85%, 92.60% ± 1.93% and 95.22% ± 1.90%, respectively, and the average nitrite removal efficiency was 87.81% ± 2.60%, 88.23% ± 1.32%, and 93.70% ± 2.11% respectively. The maximum removal efficiencies of ammonium and nitrite (98.57% and 97.33%) were all obtained at Fe^{2+} 0.08 mmol/L. The nitrate concentration in effluent was not changed obviously. In Reactor 2, NH_4^+-N and NO_2^--N concentrations in the effluent declined with the increase of Mn^{2+} concentration. The average ammonium and nitrite removal efficiencies were 86.62% ± 3.23% and 94.56% ± 2.00% at Mn^{2+} 0.025 mmol/L and 87.93% ± 1.97%, 94.20% ± 2.81% at Mn^{2+} 0.05 mmol/L. The maximum removal efficiencies of ammonium and nitrite (99.39% and 98.70%) were obtained at Mn^{2+} 0.05 mmol/L. The nitrate concentration in the effluent increased slightly.