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Short Communication

# Long term effect of $MnO_2$ powder addition on nitrogen removal by anammox process

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## HIGHLIGHTS

- Nitrogen removal performance of anammox process increased with $MnO_2$ powder addition.
- The crude enzyme activity of anammox biomass with $MnO_2$ powder addition increased 78.2%.
- The T-Mn content of anammox biomass with $MnO_2$ powder increased 50-fold.
- The filament-like structure and larger particles in anammox cell of the reactor with $MnO_2$ powder were observed.

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## ABSTRACT

This study examined long-term effect of $MnO_2$ powder (average diameter of 4–7 μm) on nitrogen removal in anammox process. Two lab-scale up-flow anammox reactors were operated for 380 days, one with and one without $MnO_2$ powder addition. During the period when only substrate concentrations varied, the maximum nitrogen removal rate in the reactor with $MnO_2$ addition reached 920.9 g-N/m$^3$/d. This value was 2-folds higher than that (464.6 g-N/m$^3$/d) of the reactor without $MnO_2$ addition. The crude enzyme activities of the anammox biomass from the two reactors was measured as 0.531 ± 0.019 and 0.298 ± 0.007 μmol cytochrome *c* reduced/mg protein/min, respectively. Transmission electron microscopy observation demonstrated more undefined particles existing inside anammox bacterial cell in the reactor with $MnO_2$ powder addition. Furthermore, filament-like structures inside anammoxosome were observed, which formed a net-like structure with particles as the connecting nodes. The experiment results demonstrated that $MnO_2$ improved nitrogen removal performance of anammox process.



## 1. Introduction

Anaerobic ammonium oxidation (anammox) process is now recognized as a novel and important process in biological nitrogen removal, which can directly convert $NO_2^-$–N to $N_2$ gas with $NH_4^+$–N as the electron donor under anaerobic conditions (Strous et al., 1999). Compared with the conventional nitrification–denitrification processes, anammox process offers significant advantages such as no demand for oxygen and organic carbon, low sludge production and reduced $CO_2$ or $N_2O$ emissions (Schmid et al., 2003). The first full-scale anammox reactor (75 m$^3$) in the world was reported to treat anaerobic sludge digester liquor with average N removal rate of 750 kg-N/d (Van der Star et al., 2007). Recently, Tang et al. (2010) reported a very high nitrogen removal rate of 74.3–76.7 kg-N/m$^3$/d in a lab-scale anammox UASB reactor, in which the biomass concentration was as high as 42.0–57.7 g-VSS/L. These results suggested high potential of anammox process in biological nitrogen removal from wastewaters. However, extremely slowly growth rate of anammox bacteria with a doubling time of 11 days (Strous et al., 1999) causes the longer start-up period. Consequently, enhancing the bacterial activity of anammox bacteria or shortening the start-up period of anammox reactors is subject of great interest and challenge.

Recently, a number of exciting studies have also been published, which utilized external field energy such as magnetic field, electric field and low intensity ultrasound to increase the activity of anammox bacteria. For instance, Liu and Yang (2009) reported the maximum nitrogen removal rate of anammox biomassn increased by

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30% at magnetic value of 60.0 mT and 20% at an electrode voltage of $-0.05V_{SCE}$. Similarly, Duan et al. (2011) demonstrated that total nitrogen removal rate of anammox bacteria increased by 25.5% by applying ultrasound intensity of 0.3 w/cm$^2$ with the optimal irradiation time of 4 min. The ultrasound effect could last for 6 days after ultrasound applied once. Zhang et al. packed with a Fe-electrode to enhance the activity of anammox biomass, who found that the N removal performance with the Fe-electrode was about 24% higher than that of the reactor without Fe-electrode. Zhang et al. speculated that the released $Fe^{2+}$ from the Fe-electrode was in favor of the growth of retaining of anammox biomass (Zhang et al., 2012).

Luther et al. (1997) and Hulth et al. (1999) have suggested that the interaction between Mn-oxides and nitrogen compounds under anaerobic condition will catalyze the production of dinitrogen gas, as shown in Eqs. (1) and (2).

$$NH_4^+ + 4MnO_2 + 6H^+ \rightarrow 4Mn^{2+} + NO_3^- + 5H_2O \quad (1)$$

$$2NO_3^- + 5Mn^{2+} + 4H_2O \rightarrow 5MnO_2 + N_2 + 8H^+ \quad (2)$$

Luther et al. (1997) provided field and laboratory evidence that $N_2$ could be produced by the oxidation of $NH_3$ and organic-N with $MnO_2$. While the reduced $Mn^{2+}$ could be reoxidized to $MnO_2$ by $O_2$ in a catalytic cycle that affected nitrogen speciation and marine nitrogen cycle. However, Thamdrup and Dalsgaard (2000) demonstrated that the oxidation of $NH_4^+$ with $MnO_2$ was insignificant. Thamdrup and Dalsgaard (2002) measured the average rate of anaerobic ammonium oxidation of samples taken from three sediments, which were 30, 99, and 83 μM/d, respectively. Thamdrup and Dalsgaard (2002) deduced that anaerobic ammonium oxidation might be particularly favored over denitrification as a sink of nitrate in manganese oxide-rich sediment. Engström et al. (2004) found that anammox process contributed to almost 80% $N_2$ production in the sediment with Mn-oxides concentration over 300 μmol/g dry sediment. Engström et al. (2004) speculated that the presence of Fe- or Mn-oxides possibly enhanced buffering capacity, acted as an oxidant potentially significant for the limited organic carbon in marine sediments. Fe- or Mn-oxides may stimulate and contribute to conditions more favorable for anammox than traditional denitrification. Hulth et al. (1999) and Engström et al. (2004) also suggested that availability of Mn and Fe-oxides seemed to occasionally accelerate total rates of $N_2$ formation from anammox process compared to traditional denitrification in surface marine sediments. Anammox, in the presence of Mn-oxides to produce nitrate, was also suggested to occur in natural marine sediments on the basis of co-occurrence of peak of Mn (II) and $NO_3^-$ (Bartlett et al., 2008). Furthermore, *Candidatus Kuenenia stuttgartiensis* (one kind of anammox bacteria) was proved to have a versatile lifestyle (Kartal et al., 2007). It could also employ $Fe^{3+}$, Mn-oxides and nitrate as electron acceptor in their metabolism (Strous et al., 2006), which supported the role of Fe- and Mn-oxides in anammox process. The results of the above studies demonstrated that Mn-oxides favored $N_2$ production and anammox process in the natural ecosystem, especially in the marine sediments environments. The effects of Mn-oxides on nitrogen conversion and anammox process in man-made ecosystem, such as bioreactor system or municipal wastewater treatment plants, are still not well known or explored.

The objective of this study was to investigate long term effect of $MnO_2$ on nitrogen removal in anammox bioreactor system. Two lab-scale up-flow anammox reactors were operated in parallel, one with and one without $MnO_2$ powder addition. In addition to the nitrogen removal performances, we also examined the crude enzyme activity of anammox bacteria cells and cell morphotypes with transmission electron microscopy (TEM) technique in two parallel. The possible mechanism of effect of Mn-oxides on anammox biomass was also discussed.

## 2. Methods

### 2.1. Microorganisms and feed substrates

The anammox sludge used for inoculation originated from a laboratory-scale anammox upflow column reactor in our lab. Anammox bacteria, belonging to two groups: *Candidatus Kuenenia stuttgartiensis* and *Candidatus Jettenia asiatica,* accounted for 57% of the total biomass (Duan et al., 2011). The substrates used in the experiments mainly consisted of ammonium and nitrite in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The composition of the trace mineral medium was as same as described by Third et al. (2001), only removing the compound of $MnCl_2{\cdot}4H_2O$ to avoid its interference. The detailed information is shown Table 1.

### 2.2. Batch experiments

In order to ascertain the initial effects of addition of $MnO_2$ on $N_2$ gas production in anammox process, three sets of batch experiments were conducted with the same biomass. The tests were carried out in three 120 ml serum vials containing 100 ml medium. Three serum vials contained only anammox biomass, only $MnO_2$ powder (5 g dry weight, 90%, activated at 200 °C for 6 h, average diameter of 4–7 μm), and both anammox biomass and $MnO_2$ powder, respectively. The anammox mixed liquor volatile suspended solid (MLVSS) concentration of each batch experiments was set as 2240 mg/L (wet weight 5 g). Biomass samples were taken from the reactors and washed three times with mineral medium to remove residual nitrogen. The pH was adjusted to 7.5 and the temperature was maintained at 35 ± 1 °C. The serum bottle contents were purged with argon gas to remove dissolved oxygen. Initial $NH_4$–N and $NO_2$–N concentrations were set at 50 mg-N/L. Samples were taken at the same interval to analyze the $NH_4$–N, $NO_2$–N, $NO_3$–N, total Mn concentrations and $N_2$ contents in spare space of the serum vials.

### 2.3. Continuous experiments

Two identical upflow fixed bed column reactors A and B had the working volumes of 0.5 L with the inner diameter of 5 cm and the height of 25.5 cm. Both reactors contained 50 g (wet weight) anammox biomass having a MLVSS concentration of 1320 mg/L. 50 g $MnO_2$ powder were added to the bottom of reactor A only to study the long-term effect of $MnO_2$ on the performance of anammox reactors. The two reactors were continuously fed with the substrates, which was purged with 99.5% $N_2$ to maintain dissolved oxygen below 0.5 mg/L. The pH of the influent was adjusted to 7.5 ± 0.2 by dosing with 2 M HCl and the two reactors' temperature was maintained at 35 ± 1 °C using a water bath (please see Fig. S1).

### 2.4. Analytical methods

Concentrations of nitrite and nitrate were determined by using ion-exchange chromatography (ICS-1100, DIONEX, AR, USA) with an IonPac AS18 anion column after filtration with 0.22 μm pore size membranes. $NH_4$–N, mixed liquor suspended solid (MLSS) and MLVSS concentrations were measured according to the Standard Methods (APHA, 1995). pH measurement was done using a digital pH meter (PHS-25, Leici Company, China), while DO was measured using a digital DO meter (YSI, Model 55, USA). $N_2$ was determined by a gas chromatograph (GC-17A, Shimadzu, Japan). The anammox bacterial samples for TEM observations were taken from reactors A and B on day 240, which was carried out according to the methods described by Duan et al. (2011).

For determining the content of total Mn in the microbial cells, 0.3 g (wet weight) of biomass was taken from the both reactors