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Enhanced effects of maghemite nanoparticles on the flocculent sludge wasted from a high-rate anammox reactor: Performance, microbial community and sludge characteristics

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ABSTRACT

Magnetic nanoparticles (NPs) have been widely applied in environmental remediation, biomass immobilization and wastewater treatment, but their potential impact on anaerobic ammonium oxidation (anammox) biomass remains unknown. In this study, the short-term and long-term impacts of maghemite NPs (MHNPs) on the flocculent sludge wasted from a high-rate anammox reactor were investigated. Batch assays showed that the presence of MHNPs up to 200 $mg\,L^{-1}$ did not affect anammox activity, reactive oxygen species production, or cell membrane integrity. Moreover, long-term addition of 1–200 $mg\,L^{-1}$ MHNPs had no adverse effects on reactor performance. Notably, the specific anammox activity, the abundance of hydrazine synthase structural genes and the content of extracellular polymeric substance were increased with elevated MHNP concentrations. Meanwhile, the community structure was shifted to higher abundance of *Candidatus Kuenenia* indicated by high-throughput sequencing. Therefore, MHNPs could be applied to enhance anammox flocculent sludge due to their favorable biocompatibility.

1. Introduction

Engineered nanoparticles (NPs) are extensively used in many industrial and consumer products due to their specific chemical, physical and optical properties (Yang et al., 2013; Zhang et al., 2017a). In recent years, the synthesis and utilization of engineered magnetic NPs (MNPs) has drawn much attention (Xu et al., 2012). Maghemite (γ-Fe_2O_3), hematite (α-Fe_2O_3) and magnetite (Fe_3O_4) are the most common magnetic nanomaterials applied in remediation and water treatments (Tang and Lo, 2013). As a kind of nanosorbent, maghemite NPs (MHNPs) and magnetite NPs (MNNPs) are the most promising materials for heavy metal removal (e.g., Cr(VI) and As(V)) because their use is convenient for magnetic separation (Tang and Lo, 2013). MNPs have also been proposed to degrade organic pollutants or reduce their toxicity due to the enhanced photocatalysis effect (Tang and Lo, 2013). In addition, in view of their biocompatibility, chemical stability, and magnetic behavior, they are widely used in biomedical sciences and for immobilization of biomass such as fungi and microalgae (Xu et al., 2012). A previous study reported that the immobilization of sludge on MHNPs (25 $mg\,g^{-1}$) enhanced the hydrolysis step of dark-photo fermentation process and promoted the anaerobic conversion of starch wastewater into biohydrogen (Nasr et al., 2015). Similarly, the addition of 100 $mg\,L^{-1}$ MNNPs increased the populations of bacteria and archaea and improved the activity of key enzymes in the anaerobic digestion of activated sludge (Wang et al., 2016a). Moreover, the addition of MNNPs at 50 $mg\,L^{-1}$ could enhance the granulation of aerobic activated sludge, the compact structure of the granules, and the retention of biomass (Liang et al., 2017).

However, the extensive use of MNPs in consumer and industrial products has also raised public concerns about their potential environmental impacts. A previous study reported that chitosan-coated MNNPs showed significant antimicrobial activity against *Bacillus subtilis* and *Escherichia coli* (Arakha et al., 2015). The addition of MNNPs at 60 $mg\,L^{-1}$ could lead to oxidative stress and cell membrane damage to the bacteria in activated sludge (Ma et al., 2017a). Another study demonstrated that acute exposure to MNNPs at 50 $mg\,L^{-1}$ inhibited the activated sludge process, but long-term exposure to 50 $mg\,L^{-1}$ MNNPs eventually enhanced wastewater nitrogen removal performance and increased the abundance of nitrifying bacteria (Ni et al., 2013). This resistance to MNPs was also observed for periphyton aggregates (Tang et al., 2017).

Anaerobic ammonium oxidation (anammox) bacteria are ubiquitous

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in various natural environments and are the key microorganisms in the biological nitrogen removal process (Zhang et al., 2017b). The number of full-scale anammox installations around the world had exceeded 100 according to the previous survey (Lackner et al., 2014), and it is expected to be continuously applied to practical engineering because of their essential merits of high-efficiency and low-cost (Zhang et al., 2017a, 2015). However, to the best of our knowledge, it is unknown whether the introduction of MNPs into wastewater would disturb anammox process and whether MNPs could be used to enhance the characteristics of anammox sludge. Therefore, in this study, the impacts of MHNPs on the flocculent sludge wasted from a high-rate anammox reactor were investigated by i) testing acute toxicity and ii) monitoring long-term performance, microbial community and sludge characteristics.

2. Materials and methods

2.1. Origin of anammox biomass and nanoparticles

Anammox seeding sludge was harvested from two laboratory-scale up-flow anaerobic sludge blanket (UASB) reactors using an 80-mesh sieve in order to collect anammox flocs with a mean diameter less than 0.2 mm. The operation conditions of the parent reactor and the characteristics of those flocs are detailed later.

Commercially produced MHNPs (20 nm, 99.9% purity) were purchased from the Aladdin Reagent Co. Ltd., China. A stock suspension ($2\,g\,L^{-1}$, pH 7.5) was prepared before each use according to the methods described previously (Mu et al., 2011). Briefly, 0.1 mM sodium dodecylbenzene sulfonate (SDBS) was added to the stock suspension as a dispersing reagent, and then, the stock suspension was sonicated for 1 h in an ultrasonic bath (25 °C, 40 kHz, 250 W) for homogeneity. Finally, the stock suspension of MHNPs was immediately added to the synthetic wastewater as needed. The synthetic wastewater containing ammonium, nitrite, minerals, and trace elements is summarized in Table 1. The potential effects of SDBS (the maximum load was $3.4\,mg\,L^{-1}$ in this study) itself on anammox sludge were negligible according to the previous study (Zhang et al., 2017b).

2.2. Experimental setup

Batch exposure experiments were performed to investigate the cytotoxicity of MHNPs by monitoring three indicators: specific anammox activity (SAA), intracellular reactive oxygen species (ROS) production, and extracellular lactate dehydrogenase (LDH) activity. The detailed procedures were as previously described (Zhang et al., 2017b). The continuous-flow experiment was conducted in a UASB reactor with an internal diameter of 60 mm and an effective volume of 1.0 L. This reactor was inoculated with $12.0\,g^{-1}$volatile suspended solids (VSS) L^{-1} of anammox sludge to achieve full capacity and then placed in a dark and thermostatic room at 35 ± 1 °C. The influent ammonium and nitrite concentrations were both set at $280\,mgN\,L^{-1}$. The influent pH was maintained at approximately 7.8, and the hydraulic retention time was set at 1.2 h throughout the experiment. MHNPs were added to the influent of the reactor from Day 31, and the concentration was gradually increased from 1.0 to $200\,mg\,L^{-1}$ (as shown in Table 2).

Table 1
Composition of the synthetic wastewater.

Composition	Concentration
$MgSO_4{\cdot}7H_2O$	$58.6\,mg\,L^{-1}$
NaH_2PO_4	$10\,mg\,L^{-1}$
$NaHCO_3$	$840\,mg\,L^{-1}$
$CaCl_2{\cdot}2H_2O$	$73.5\,mg\,L^{-1}$
Trace element I[a]	$1.25\,mL\,L^{-1}$[c]
Trace element II[b]	$1.25\,mL\,L^{-1}$[c]
$(NH_4)_2SO_4$	Add as required[d]
$NaNO_2$	Add as required[d]

[a] The composition of trace element solution I was $5\,g\,L^{-1}$ EDTA and $9.14\,g\,L^{-1}$ $FeSO_4{\cdot}7H_2O$.

[b] The trace element solution II was composed of $15\,g\,L^{-1}$ EDTA, $0.014\,g\,L^{-1}$ H_3BO_4, $0.99\,g\,L^{-1}$ $MnCl_2{\cdot}4H_2O$, $0.25\,g\,L^{-1}$ $CuSO_4{\cdot}5H_2O$, $0.43\,g\,L^{-1}$ $ZnSO_4{\cdot}7H_2O$, $0.21\,g\,L^{-1}$ $NiCl_2{\cdot}6H_2O$, $0.22\,g\,L^{-1}$ $NaMoO_4{\cdot}2H_2O$ and $0.24\,g\,L^{-1}$ $CoCl_2{\cdot}6H_2O$.

[c] 1.25 mL of trace element solutions I and II were added per liter of wastewater.

[d] Equimolar ammonium and nitrite were supplied.

2.3. Microbial community analysis

Sludge samples were collected from the reactor on Days 30, 60, 90 and 130 for molecular analysis. DNA was extracted to measure the abundance of hydrazine synthase structural genes (*Hzs*A) and for high-throughput sequencing. Details are described in the Supplementary Materials.

2.4. Other analytical procedures

The levels of NH_4^+, NO_2^-, and NO_3^- were measured spectrophotometrically using phenol–hypochlorite, N-(1-naphthalene)-diaminoethane, and phenol disulphonic acid, respectively (APHA et al., 2005). Suspended solids (SS), VSS, and pH were determined according to standard methods (APHA et al., 2005). The mean diameter of sludge was measured using an image analysis system (QCOLite) with a Leica DM2LB microscope and a digital camera (Canon S30). Extracellular polymeric substance (EPS) was extracted and measured according to a previous study (Zhang et al., 2017c). Three-dimensional (3D) excitation–emission matrix (EEM) fluorescence spectra of the extracted EPS were obtained with a fluorescence spectrophotometer (F-4600, Hitachi Co., Japan). Fourier transform infrared (FTIR) spectra were measured using a Nicolet iS10 FTIR spectrometer (Thermo Fisher Nicolet, USA). The Fe content of the sample was determined after mixed-acid digestion using atomic absorption spectrometry (AA6300C, SHIMADZU, Japan) with an air/acetylene flame.

The tests performed in triplicate were expressed as the mean ± standard deviation. An analysis of variance (ANOVA) and Pearson correlation analysis were performed using SPSS 13.0 software (Statistical Package for the Social Sciences, USA), and $p < .05$ was considered statistically significant.

3. Results and discussion

3.1. The tale of two aggregates in aged UASB reactors

Two parent reactors (UASB, 2.0 L) have been operating under thermostatic (35 ± 1 °C) conditions for more than one year. The influent total nitrogen (TN) concentration was maintained at $560\,mgN\,L^{-1}$, while the HRT was gradually shortened to 1.2 h. Finally, a stable NRR of approximately $10\,kg\,N\,m^{-3}\,d^{-1}$ was achieved. Due to the plug-flow mode of UASB reactors, the substrate concentration decreased rapidly as the reactor height increased (as shown in the Supplementary Materials). 75% of the TN removal was achieved in the lower half of the sludge bed. The nitrite concentration in the upper half was below $70\,mg\,L^{-1}$. As a result, an oligotrophic environment was formed in the upper half of the UASB reactor without effluent recirculation. Similar observations were reported in previous studies in which almost 50–75% of the influent substrate was consumed in the first third of the height in the sludge bed (Ma et al., 2017b; Zhang et al., 2016). When fresh and sufficient substrate was pumped into the reactor, anammox bacteria could grow perfectly in the bottom of the reactor. However, in the top of the reactor where the substrate was inadequate and the pH was not optimal, the growth of anammox bacteria was retarded. As a result, the UASB reactor has a variation in