Science of the Total Environment 649 (2019) 440–447

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The revolution of performance, sludge characteristics and microbial community of anammox biogranules under long-term NiO NPs exposure

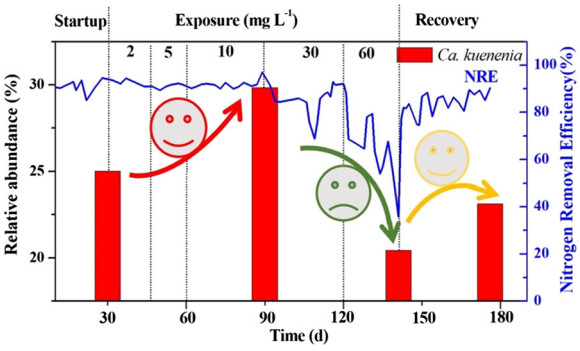
Jia-Jia Xu, Ya-Fei Cheng, Lian-Zeng-Ji Xu, Ying-Yi Liu, Bing-Qian Zhu, Nian-Si Fan, Bao-Cheng Huang *, Ren-Cun Jin *

College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China
Key Laboratory of Hangzhou City for Ecosystem Protection and Restoration, Hangzhou Normal University, Hangzhou 310036, China

HIGHLIGHTS

- Long-term effects of NiO NPs on the anammox system were firstly investigated.
- The anammox performance was enhanced below 10 mg L^{-1} NiO NPs and suppressed by 10–60 mg L^{-1} NiO NPs.
- The stability of the reactor operation could be controlled by the (F/M)/SAA ratio.
- "Ca. *kuenenia*" dominated the community in the presence of 1–60 mg L^{-1} NiO NPs.
- The performance of the anammox reactor could recover after withdrawing the NiO NPs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 July 2018
Received in revised form 21 August 2018
Accepted 27 August 2018
Available online 28 August 2018

Editor: Paola Verlicchi

Keywords:
Anammox
NiO NPs
Nitrogen removal
Granular sludge
High-throughput sequencing

ABSTRACT

Given the increasing applications of NiO nanoparticles (NPs) in battery products, the potential effects of NiO NPs on anaerobic ammonium oxidation (anammox) systems were studied for the first time. The results showed that the anammox system performance obviously differed under the stresses of different NiO NPs concentrations. After the withdrawal of NiO NPs, the nitrogen removal performance of the anammox reactor returned to nearly that of the initial phase within 35 days. Compared with 0 mg L^{-1} NiO NPs, the specific anammox activity first increased and then decreased to the minimum value of 116.8 ± 13.8 mg TN g^{-1} VSS d^{-1} at 60 mg L^{-1} NiO NPs. The variations in the heme c contents and extracellular polymeric substance amounts were similar to the variations in the specific anammox activity throughout the whole experiment. Additionally, the relative abundance of the dominant bacteria (*Candidatus kuenenia*) increased from 20.44% at 60 mg L^{-1} NiO NPs to 23.14% at the end of the last phase. Thus, the potential effects of NiO NPs on anammox systems should be a cause for great concern.

1. Introduction

With the broad and commercial utilization of nanotechnology, many different nanoparticles (NPs) have been extensively utilized in various industrial products, such as electrochemical devices, cosmetics, additives to food, catalysts, and biomedicine systems on account of their

* Corresponding authors at: College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China.
E-mail addresses: hbaocheng@163.com (B.-C. Huang), jrczju@aliyun.com (R.-C. Jin).

https://doi.org/10.1016/j.scitotenv.2018.08.386
0048-9697/

unique physicochemical characteristics (Maynard et al., 2006; Mu and Chen, 2011). However, NPs are inevitably released into the aquatic or terrestrial environments during the processes of manufacturing, transportation, consumption and disposal, and their potential risks present severe challenges to the environment. The released NPs could eventually be discharged into wastewater treatment plants (WWTP) (Zhang et al., 2017b; Brar et al., 2010) and have been detected in sewage and wastewater sludge (Ganesh et al., 2010). Previous reports have found NPs levels in municipal wastewater in the range of μg L^{-1} to mg L^{-1}, and NPs could be accumulated in sludge and then be partially released into the sludge digestate (Musee et al., 2011). Thus, an increasing number of researchers have been concerned about the adverse effects of NPs on the functional microbes used in WWTP biological treatments (Wang et al., 2017a). The impacts of NPs on aerobic nitrification (Dong et al., 2017; Wu et al., 2018), anoxic denitrification (Zheng et al., 2018; Zheng et al., 2014), anaerobic digestion (Tian et al., 2017; Mu and Chen, 2011) and the anaerobic ammonium oxidation (anammox) processes (Zhang et al., 2018a; Zhang et al., 2018c) have been investigated.

Due to their high capacitance characteristics, nickel oxide (NiO) NPs have been widely applied in light-emitting diodes, electro-chromic films, lithium-ion batteries, and other technologies (Wang et al., 2017b; Rao and Sunandana, 2008). Besides, the exponentially increasing use of NiO NPs in manufacturing, the potential impacts of NiO NPs on human tissues, aquatic and terrestrial animals, and microorganisms have attracted wide concern in recent years. For example, some researchers have found that NiO NPs can induce cytotoxicity and activate cells involved in inflammation in humans (Capasso et al., 2014; Duan et al., 2015). Lee et al. (2016) showed that NPs could recruit eosinophils by directly releasing intracellular eotaxin in the lungs of rats. Baek and An (2011) reported that NiO NPs have toxicity against *Escherichia coli* and *Bacillus subtilis*. Wang et al. (2017b) evaluated the reactor performance, microbial enzymatic activities, and microbial community structure and diversity of activated sludge and found some distinct changes in the presence of 0–60 mg L^{-1} NiO NPs in a sequencing batch reactor. However, no previous studies have investigated the potential impacts of NiO NPs on the nitrogen removal performance, physiological and physico-chemical properties or the microbial community of anammox systems.

Anammox is recognized as a novel and sustainable process, and anammox bacteria are capable of converting ammonium to dinitrogen gas when coupled with nitrite reduction under anaerobic conditions (Strous et al., 1999; Tang et al., 2017). It is worth mentioning that some studies have shown that the impact of NPs on anammox systems might be related to their release of ions (Zhang et al., 2017a; Zhang et al., 2018d). Owing to the release of toxic Ni(II), the potential long-term risk to anammox systems posed by NiO NPs should be a cause for great concern. Moreover, given the widely large-scale production and usage of NPs, there might be high NPs loads in wastewater (Chen et al., 2012; Mu et al., 2012), and NPs might produce toxicity against bacteria (Song et al., 2018). Thus, not only the effects of NiO NPs at environmentally relevant concentrations on anammox granules but also the potential impacts of higher concentrations of NiO NPs are needed to be investigated.

Therefore, the purpose of the present study was to investigate the long-term impacts of NiO NPs on anammox systems. First, the nitrogen removal performance of anammox system at various NiO NPs concentrations was evaluated. Then, the sludge physiological characteristics, including specific anammox activity (SAA), heme c content, extracellular polymeric substances (EPS) amounts, Ni content and settling velocity (V_S), were assessed. Finally, the dynamics of the microbial community were investigated via high-throughput sequencing.

2. Materials and methods

2.1. Nanoparticles and seeding sludge

Commercially produced NiO NPs (30 nm, 99.5% purity) were obtained from Aladdin Reagent Co. Ltd., China. The stock suspensions of NiO NPs (2 g L^{-1}, pH 7.5, 0.1 mM sodium dodecylbenzene sulfonate (SDBS)) were prepared according to the methods reported in a previous study (Mu et al., 2012; Zhang et al., 2017a). Then, the stock suspension was homogenized in an ultrasonic bath (25 °C, 250 W, 40 kHz) to break up the aggregates before the suspension was introduced into the synthetic wastewater. The composition of the synthetic wastewater including substrates (ammonium and nitrite), minerals, and trace elements is summarized in Table 1.

The anammox mixed-culture inoculum was obtained from a laboratory-scale up-flow anaerobic sludge blanket (UASB) reactor that had been operating stably under thermostatic control (35 ± 1 °C) and in a dark room for nearly 2 years. The mature anammox granular sludge possessed SAA of 236.3 ± 26.7 mg TN g^{-1} VSS d^{-1}. The heme c content, EPS amount, and V_S were 1.59 ± 0.04 μmol g^{-1} VSS, 235.6 ± 7.9 mg g^{-1} VSS, and 74.1 ± 15.5 m h^{-1}, respectively.

2.2. Experimental setup

The continuous-flow experiment was performed in a UASB reactor with an effective volume of 0.8 L and an inner diameter of 6.0 cm. The reactor was wrapped in black cloth to prevent inhibition by light and then placed in a thermostatically controlled (35 ± 1 °C) dark room. To achieve a high volumetric nitrogen removal rate (NRR), the influent substrate concentration and hydraulic retention time (HRT) of the reactor were controlled at lower levels of 280 mg L^{-1} and 0.96 h, respectively. The influent pH was fixed at 7.7 during entire experiment.

Different levels of NiO NPs in the influent were achieved by mixing the NiO NPs stock suspension with the synthetic wastewater, which was then pumped into the reactor. Throughout the experiment, the addition of NiO NPs was terminated when the effluent NO_2^--N level of the reactor exceeded 100 mg L^{-1} and the influent substrate concentration decreased to 70 mg L^{-1}. The influent substrate concentration was increasingly enhanced with a gradient of 70 mg L^{-1} once the effluent NO_2^--N concentration dropped to below 10 mg L^{-1}. In detail, considering the different NiO NPs concentrations and the response of the anammox performance, the whole experiment included six phases: P0 (0 mg L^{-1}; 30 d), P1 (2, 5 mg L^{-1}; each 15 d), P2 (10 mg L^{-1}; 30 d), P3 (30 mg L^{-1}; 30 d), P4 (60 mg L^{-1}; 21 d) and P5 (0 mg L^{-1}; 35 d). P0 was set as the control phase, and within P5, NiO NPs were no longer added to the reactor in order to track the evolution of the recovery performance after it suffered long-term NiO NPs exposure.

2.3. Microbial community analysis

Sludge samples were obtained from the reactor at the end of P0, P2, P4 and P5 and were marked D-30, D-90, D-141, and D-176, respectively. DNA was extracted with a 5 mL anammox mixture based on the

Table 1
Composition of the synthetic wastewater.

Composition	Concentration
$(NH_4)_2SO_4$	Add as required[a]
$NaNO_2$	Add as required[a]
$MgSO_4 \cdot 7H_2O$	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[b]	1.25 mL L^{-1}[d]
Trace element II[c]	1.25 mL L^{-1}[d]

[a] Equimolar ammonium and nitrite were supplied.
[b] The composition of trace element solution I was 5 g L^{-1} EDTA and 9.14 g L^{-1} $FeSO_4 \cdot 7H_2O$.
[c] 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} $CoCl_2 \cdot 6H_2O$.
[d] 1.25 mL of trace element solutions I and II were added per liter of wastewater.