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Enhancement of the adaptability of anammox granules to zinc shock by appropriate organic carbon treatment

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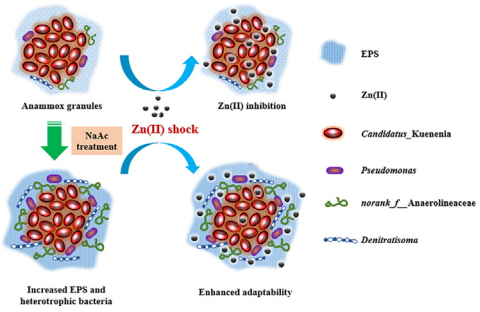
GRAPHICAL ABSTRACT



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ABSTRACT

Heavy metals, which are commonly present in high ammonia-containing wastewater, can cause inhibitory effects to anammox reaction. This study proposes a novel approach to enhance the adaptability of anammox granules to heavy metal [Zn(II)] shock by organic carbon (sodium acetate, NaAc) treatment, paying special attention to optimization of the treatment dosage and duration. For granules treated with 200 mg chemical oxygen demand (COD)/L NaAc for 2 d, the activity recovery (six cycles) efficiency after Zn(II) (40 mg/L) shock reached 127.4%. The extracellular polymeric substance (EPS) production increased by 168% and heterotrophic bacteria mildly proliferated (increased by 14%) in such granules compared with the control. The dramatic recovery capacity was likely due to the entrapment and barrier function of EPS and the outer-layer proliferated heterotrophic bacteria. This finding offers a useful process to enable maximum adaptability of anammox granules from heavy metals shocks, allowing anammox technology to be more widely applied.

1. Introduction

Anaerobic ammonium oxidation (anammox) technology has attracted widespread attention in wastewater biological nitrogen removal in recent years, owing to its advantages of substantial energy savings, no requirement for organic carbon and lower sludge production compared with the traditional nitrogen removal technology (Jetten et al., 2005; Kuenen, 2008). Concurrent with the emergence of novel anammox nitrogen removal processes such as CANON, DEMON, and OLAND (Ali et al., 2013; Lackner et al., 2014), anammox technology has been successfully applied to treatment of high ammonia-containing wastewaters such as sludge digester liquid, landfill leachate and semiconductor effluents, in which heavy metals such as Zn, Cu and Mn are generally present at concentrations of approximately 0.05–1000 mg/L

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(Radniecki and Ely, 2008), 5–100 mg/L (Ochoa-Herrera et al., 2011) and 0.05–1400 mg/L (Cecen and Gursoy, 2000), respectively. Heavy metals are not easily removed by biological means, and can accumulate in anammox sludge, causing severe inhibition to anammox activity (Jin et al., 2012; Zhang et al., 2015a).

Zn(II) is one of the most common heavy metals found in many ammonium-rich wastewaters (Scullion et al., 2007). Although it is an essential element for hydrogenase and dehydrogenase reactions in anammox bacteria (Daverey et al., 2014), excessive Zn(II) can cause toxicity to these bacteria. Lotti et al. (2012) studied the short-term effect (exposure time 24 h) of Zn(II) on the activity of anammox bacteria, and reported that the Zn(II) half inhibitory concentration (IC_{50}) of the studied anammox bacteria was 3.9 mg/L. On increasing Zn(II) concentration from 0 to 40 mg/L (Zhang et al., 2015a), the anammox activity decreased from 50 to 8 mg NO_2^--N/g VSS·h in an up-flow anaerobic granule blanket system. The influence of Zn(II) on the nitrogen removal, microbial community and biofilm properties was also investigated in an anammox biofilter (Zhang et al., 2017), and it was determined that although anammox bacteria could self-adapt to 1–10 mg/L Zn(II), the adaption was slow (over approximately 30 d) (Zhang et al., 2017), indicating a low self-recovery capacity of anammox bacteria after excessive Zn(II) shock without extra manipulation. In recent years, much attention has been paid to the toxic effects of heavy metals on anammox consortia and their mechanisms (Bi et al., 2014; Lotti et al., 2012), but less to practical methods to enhance the adaptability of anammox sludge to heavy metals shocks.

Extracellular polymeric substances (EPS) are an important barrier for anammox cells to avoid direct contact and interaction with heavy metal ions (Zhang et al., 2017). Ion exchange, complexation and surface precipitation are three main pathways responsible for the metal–EPS interactions (Li and Yu, 2014). Wei et al. (2017) compared the effects of EPS on the adsorption of Cu and Zn ions in activated sludge, anaerobic granular sludge and anaerobic flocculent sludge, and determined that EPS in granular sludge played a major role in heavy metals removal. Yan et al. (2017) demonstrated that EPS binding with heavy metals was mainly driven by enthalpy change, and proteins in the EPS were the major participants in the binding process. Accordingly, heavy metals removal in biomass reactors increased with increasing EPS production when cultivation parameters such as pH and temperature (Chug et al., 2016) or concentration of added glycerol (Nouha et al., 2016) were adjusted.

Moreover, different heterotrophic bacteria possess various coping strategies and resistance capacities for heavy metal ions (Blindauer et al., 2001; Labrenz et al., 2000; Robinson et al., 2001). Heterotrophic bacteria were found have a high tolerance to heavy metals such as Cu, Cd and Cr, exhibiting potential eco-physiological roles and survivability in aqueous environments containing these metals (Jeremic et al., 2016). The presence of heterotrophic bacteria can directly influence the aqueous form of heavy metals, then impact the toxicology of the heavy metal ions to bacteria (Abdulaziz et al., 2016; Jeremic et al., 2016), e.g., sulfate-reducing bacteria could decrease the zinc concentration in their microenvironment by formation of sphalerite (ZnS) deposits (Labrenz et al., 2000). Although the excessive proliferation of heterotrophic bacteria in anammox systems might disturb the anammox habitat by competing for substrate (nitrite) (Li et al., 2015), the presence of an appropriate amount of heterotrophic bacteria in the outer layer of flocs or granules, as well the secreted EPS, may protect anammox bacteria (which generally inhabit the internal layer of granules) from heavy metals shocks. Alleviation of heavy metals toxicity to anammox consortia is important, as once anammox bacteria are dead or washed out of the system by heavy metals shock, their reaccumulation takes a long time because of their low cellular yield (0.066 ± 0.01 mol carbon/mol ammonium) (Strous et al., 1998) and long doubling time (approximately 10–12 d) (van der Star et al., 2007). Anammox bacteria have been reported to be highly sensitive to various inhibitory substances, including substrates (ammonia and nitrite), organic matter, and heavy metals, which could hamper the robust nitrogen removal performance of an anammox bioreactor (Jin et al., 2012; van der Star et al., 2007).

In this study, the feasibility of improving the adaptability of anammox granules to heavy metal ion (Zn(II)) shocks was examined by adding organic carbon (sodium acetate, NaAc), paying special attention to the variation in specific anammox activity (SAA), microscopic morphology, EPS, and bacterial community structure of the NaAc-treated anammox granules. The aims of this study were to: 1) characterize the NaAc-treated anammox granules at various treatment dosages and durations; 2) examine the impacts of Zn(II) shock on the NaAc-treated anammox granules and optimize the NaAc treatment dosage and duration to enhance the adaptability of the anammox granules to Zn(II) shock; and 3) identify the possible mechanisms of NaAc-treatment-induced resistance to Zn(II) shock based on EPS and microbial community structure analysis. The results provide a new approach to enhance the capacity of anammox granules to resist and recover from heavy metals shocks when the anammox systems irregularly encounter heavy metals shocks during wastewater nitrogen removal.

2. Materials and methods

2.1. Parent anammox reactor setup and operation

The experimental anammox sludge was cultivated in a sealed laboratory-scale anammox sequencing batch reactor (SBR) with a working volume of 15 L. The temperature of the SBR was maintained at 35 ± 1 °C with a thermostatic water bath, and the stirring speed was 100 rpm. The SBR was operated with three cycles per day (8 h for each cycle), each consisting of a 20-min filling period, a 360-min anoxic mixing period, a 60-min sludge-settling period, a 20-min effluent decanting period, and a 20-min idle phase. Decanting volume was 50% of the working volume (7.5 L). The anammox system achieved a stable volumetric substrate (ammonium and nitrite) removal rate of about 0.99 kg N/m^3·d. The SAA of the reactor was stabilized at approximately 0.22 ± 0.01*g* N/g MLVSS·d after cultivation for 500 d. Fluorescence in situ hybridization (FISH) showed that anammox bacteria accounted for 80% of the total bacteria, and the dominant species were *Candidatus* Kuenenia stuttgartiensis (50%) and *Candidatus* Brocadia anammoxidans (22%). The detailed procedures for FISH assays are presented in the Supplementary Material.

2.2. Synthetic wastewater

The synthetic wastewater contained (g/L): NH_4Cl, 1.07 (280 mg NH_4^+-N/L, stable phase); $NaNO_2$, 1.77 (360 mg NO_2^--N/L, stable phase); $KHCO_3$, 1.25; KH_2PO_4, 0.02; $CaCl_2$, 0.02; $MgSO_4{\cdot}7H_2O$, 0.08; $FeSO_4{\cdot}7H_2O$, 0.015; Na_2EDTA, 0.02; and 1 ml/L of trace element solution. The trace element solution contained (g/L): EDTA, 15; H_3BO_3, 0.035; $ZnSO_4{\cdot}7H_2O$, 1.075; $CuSO_4{\cdot}5H_2O$, 0.625; $MnCl_2{\cdot}4H_2O$, 0.495; $NaMoO_4{\cdot}2H_2O$, 0.55; $CoCl_2{\cdot}6H_2O$, 0.6; and $NiCl_2{\cdot}6H_2O$, 0.475. The pH of the synthetic wastewater was adjusted to 7.0 by adding 2 M HCl.

2.3. Organic carbon treatment and Zn(II) shock/recovery batch tests

2.3.1. Organic carbon treatment approaches

The experimental anammox granules (approximately 15 g MLVSS (mixed liquor volatile suspended solids), diameter 0.5–2 mm) withdrawn from the parent reactor were sieved and washed three times with buffer solution (the synthetic medium without substrate). These anammox granules were evenly separated into fifteen 500-mL serum bottles. These serum bottles were divided evenly into three groups for organic carbon treatment for 1, 2, and 4 d, respectively. The experimental bottles were placed in a water bath (35 ± 1 °C) with magnetic stirring at 120 rpm. NH_4Cl, $NaNO_2$, and buffer solution were added to each bottle at initial concentrations of 75 mg/L NH_4^+-N and 80 mg/L NO_2^--N; NaAc was then added to achieve initial chemical oxygen