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Hydrazine addition enhances the nitrogen removal capacity in an anaerobic ammonium oxidation system through accelerating ammonium and nitrite degradation and reducing nitrate production

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ABSTRACT

Hydrazine is an important intermediate for anaerobic ammonium oxidation (anammox), in which ammonium and nitrite are converted to nitrogen gas. Hydrazine addition is known to improve the nitrogen removal capacity in anammox-based processes. However, the underlying mechanism remains unknown. This study investigated the effects of hydrazine addition on conversion performance for normal anammox substrates (ammonium, nitrite and nitrate) using batch experiments with various combinations of nitrogenous as substrates for the anammox bacteria in the presence or absence of hydrazine. Hydrazine addition improved specific anammox activity (SAA) by 42%, of which 5% was attributed to increased ammonium removal by strengthening anammox, 25% to acceleration of nitrite degradation by the bioreaction where exogenous hydrazine was reacted with nitrite to generate azide, and 12% to reducing nitrate production rate through selective inhibition of nitrite-nitrate oxidoreductase (NXR) activity by generating azide. A model in which hydrazine addition enhances the nitrogen removal capacity in an anammox system was established, which can provide theoretical guidance for engineering applications of anammox with trace hydrazine addition.

1. Introduction

Anaerobic ammonium oxidation (anammox) is a cost-effective nitrogen removal process that plays a major role in the Earth's nitrogen cycle and is used in energy-efficient wastewater treatment [1–3]. The process was first discovered in 1995 in a denitrifying pilot plant reactor in Delft, the Netherlands [4], and is being applied worldwide today [5–7]. Anammox is performed by chemoautotrophic bacteria of the phylum Planctomycetes that catalyze the oxidation of ammonium (NH_4^+), using nitrite (NO_2^-) as an electron acceptor in the absence of oxygen [8], generating nitrogen gas (N_2) as the major final product [9]. The anaerobic and autotrophic nature of these microorganisms permits significant savings in aeration energy, obliterates the need for organic carbon, and reduces sludge production [10].

One of the most striking aspects of anammox bacteria is the synthesis and metabolism of hydrazine, a highly unusual metabolic intermediate [11]. Hydrazine is an energy-rich compound used as a rocket fuel and toxic to most organisms [12,13]. Some bacteria (nitrite oxidizing bacteria (NOB) or nitrate reducing bacteria (NRB)) are inhibited through its toxic effects [14,15], whereas others (anammox bacteria) can use it as an energy source [11,16]. Anammox culture was able to convert and tolerate 1 mM hydrazine, and the half-saturation constant and inhibition constant of N_2H_4 were 10.42 mg N/L and 1393.88 mg N/L, respectively [11,17]. The addition of trace amounts of hydrazine determines anammox activity recovery after exposure, even long-term, to high concentrations of nitrite [18,19]. Long-term addition of hydrazine could recover and enhance the autotrophic nitrogen removal capacity in completely autotrophic nitrogen removal over nitrite (CANON) [20,21]. A similar phenomenon has been observed in moving bed biofilm reactors and batch experiments with anammox enrichment culture [18,22]. It was speculated that the electrons released from exogenous hydrazine oxidation substituted the electrons from nitrite oxidation to nitrate to reduce nitrate production or additional hydrazine exerted an inhibitory effect on NOB while an positive effect on anammox bacteria, even or hydrazine addition increases the nitrite conversion rate in anammox culture [11,20,21].

The mechanism underlying the improvement of anammox performance by trace hydrazine addition and the effects of exogenous

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hydrazine on the conversion performance of anammox substrates (ammonium, nitrite and nitrate) remain unclear, which hampers the prediction, modeling, and design of anammox-based technologies. Therefore, we conducted an in-depth study of the effect of hydrazine addition on the conversion performance of anammox substrates (especially, nitrite and nitrate) in an anammox system. We expect our findings to provide theoretical guidance for engineering applications of anammox with trace hydrazine addition.

2. Materials and methods

2.1. Origin of the biomass

Anammox granular sludge was cultivated and maintained in a laboratory-scale expanded sequencing batch reactor (8 L) fed with a synthetic medium at a volume load of 0.97 kg N/(m^3·d) (Fig. A.1). The temperature in the reactor was maintained at 32 °C using a water bath, and the sludge was maintained in a suspended state by means of a stirrer. This culture was used as an inoculum to start up the reactor, which was operated for 300 days before the experiments were performed. The volatile suspended solids (VSS) content of the biomass in the laboratory reactor was approximately 90% of the total suspended solids (TSS). The average size of the anammox granules was 1.1 ± 0.5 mm (calculated by image analysis of a photograph of the granular sludge sample, using ImageJ software). Bacteria from the *Candidatus* "Brocadia" were the dominant anammox microorganisms in the sludge granules [23].

2.2. Analytical methods

Prior to analyses, samples were filtered through 0.45-μm filters. Ammonium was analyzed by ion chromatography (DX-600 IC, Dionex). Nitrite (NO_2^--N; APHA-4110B), nitrate (NO_3^--N; APHA-4110B), total suspended solids (TSS; APHA-2540D), and volatile suspended solids (VSS; APHA-2540E) were determined according to standard methods [24]. The hydrazine concentration (measuring range 0–0.26 mg N/L) was determined by the Lange method based on a method described by Watt and Chrisp [25] at 458 nm using Hydraver 2 reagent, and nitrite interference was eliminated by adding 0.5% sulfamic acid [26]. The hydroxylamine (NH_2OH) concentration was measured by spectrophotometry at 705 nm according to Frear and Burrell [27]. The azide (N_3^-) concentration was measured by spectrophotometry at 555 nm according to Mehra [28]. Online monitors were set up in the reactors to measure the pH, temperature, and dissolved oxygen concentration (WTW Company, Germany). All data presented in this paper are mean values from triplicate experiments.

2.3. Batch bioassays

Batch assays were performed in duplicate and incubated in a constant-temperature incubator (135 rpm) in the dark at 32 ± 1 °C. Serum flasks (600 mL) were filled with basal mineral medium (200 mL) and anammox biomass (2.6 g VSS/L). The mineral medium was prepared using ultrapure water (Milli-Q; Millipore) and contained the following compounds (mg/L): KH_2PO_4 (10), $CaCl_2{\cdot}2H_2O$ (5.6), $MgSO_4{\cdot}7H_2O$ (300), and $KHCO_3$ (1250). The medium also contained two trace element solutions containing the microelements needed to avoid nutrient limitation, at a concentration of 1.25 mL/L [29]. The pH in these experiments was maintained at 7.7 using phosphate buffer solution. The serum flasks were sealed with rubber stoppers after the headspace and liquid phase (200 mL) were sparged with nitrogen gas to obtain anoxic conditions. In all treatments (Table 1), the liquid was sampled after addition of the nitrogenous substrates according to the set time of the experiments, for analyzing of NH_4^+, NO_2^-, NO_3^-, N_2H_4, NH_2OH, and N_3^-.

Table 1 summarizes the test conditions utilized in the various experiments.

Treatment 1: 1 mM hydrazine was added to anammox biomass supplied with classical substrates (ammonium, 2.7 mM; nitrite, 3.7 mM; and nitrate 1.5 mM) to study the effect of hydrazine addition on anammox substrate conversion performance, using anammox biomass without hydrazine addition as a control, and the concentrations of nitrogenous substrates and intermediates (ammonium, nitrite, nitrate, hydrazine, and hydroxylamine) were determined every 45 min over 225 min in total.

Treatment 2: Kinetic experiments were first performed to determine the conversion parameters for hydrazine addition in the anammox system. Batch experiments with nitrogenous combinations as substrates (Table 1) were conducted to further explore the conversion characteristics of hydrazine addition in anammox bacteria.

Treatment 3: The conversion performance of nitrite with/without

Table 1
Test conditions utilized in the various experiments.

Experiment	Treatment	Nitrogenous substances (mM)				Biomass
		NH_4^+	NO_2^-	NO_3^-	N_2H_4	
Effect of N_2H_4 addition on anammox characteristics	1	2.4	3.7	1.5	–	With
		2.4	3.7	1.5	1.0	With
N_2H_4 conversation performance in anammox system	2	4.0	–	–	2.0	With
		–	4.0	–	2.0	With
		–	–	4.0	2.0	With
		4.0	4.0	–	2.0	With
		–	4.0	–	0.12, 0.28, 0.51, 1.09, 1.79, 2.20	With
					1.09, 1.79, 2.20	
		–	–	–	0.11, 0.20, 0.47, 1.05, 2.50, 2.10	With
Effect of N_2H_4 addition on NO_2^- degradation performance	3	2.4	3.7	–	–	With
		2.4	3.7	–	1.0	With
		–	3.7	–	1.0	With/Without
		–	3.7	–	–	With
		–	0.18, 0.62, 1.30, 2.14, 3.03, 3.98	–	1.0	With
		–	0.17, 0.60, 1.30, 2.16, 3.10, 4.03	–	–	With
		–	4.7	–	1.43, 2.98, 4.82	With
Effect of N_2H_4 addition on NO_3^- accumulation	4	2.4	–	2.3	2.8	With
		–	4.7	2.3	4.7	With
		–	–	2.3	2.8	With/Without
		–	–	2.3	–	With