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Variation in the performance and sludge characteristics of anaerobic ammonium oxidation inhibited by copper

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ABSTRACT

This study investigated the short-term inhibitory effects of Cu(II) on anaerobic ammonium oxidation (anammox) performance and sludge characteristics. The short-term inhibition, kinetic characteristics, and cumulative inhibition of Cu(II) on anammox sludge and recovery from this inhibited activity by washing with buffered solution were investigated by batch testing. The results showed that the half inhibition concentration (IC_{50}) was 32.5 mg L^{-1} and that inhibition by Cu(II) on anammox was non-competitive. The cumulative toxicity of Cu(II) was severe. After the biomass was washed with buffered solution for 10 h, the suppression of anammox activity by 5 mg L^{-1} Cu(II) could be remitted, whereas no more than 73.0% of the pre-inhibited level of activity was regained at higher Cu(II) concentrations (over 15 mg L^{-1}). Additionally, 8 mg L^{-1} Cu(II) inhibited anammox performance considerably during continuous operation, with the nitrogen removal rate (NRR) decreasing from 12.4 to nearly 0 kg m^{-3} d^{-1}. In contrast, the anammox consortia could tolerate Cu(II) stress after domestication with a low level of Cu(II) (5 mg L^{-1}). The performance of recovery similar to the re-startup features were simulated by a modified Boltzmann model.

1. Introduction

As nitrogen pollution has become an increasingly important issue, several novel nitrogen removal processes have been developed, including completely autotrophic nitrogen removal over nitrite (CANON), oxygen limited autotrophic nitrification denitrification (OLAND), single-reactor system for high activity ammonia removal over nitrite (SHARON), and anaerobic ammonium oxidation (anammox) [1]. Anammox allows significant savings in aeration energy, reduces the need for organic carbon addition and lowers sludge production [2]. Although the anammox process has many advantages for wastewater treatment, due to the slow growth rate of anammox consortia (doubling time of approximately 11 days) [3] and their susceptibility to environmental conditions, especially heavy metals, antibiotics, phenols, sulfides and other inhibitors [4–8], the application of anammox processes in treating wastewater containing such inhibitors is limited.

At low concentrations, heavy metals, such as Cu(II), Zn(II), Co(II), Fe(II), Mn(II) and Ni(II) are essential micronutrients for vital cofactors of metalloproteinases and certain enzymes [9,10]. However, excessive heavy metal concentrations would be inhibitory or even toxic to biochemical reactions and microorganisms due to the chemical binding of heavy metals to enzymes, resulting in the disruption of enzymatic structure and activities [11]. Heavy metals can accumulate by fast and relatively nonspecific metal transport systems, which are constitutively expressed [9]. Additionally, heavy metals are non-biodegradable and can accumulate in living tissues, causing deterioration of microbial activity in synthetic wastewater treatment systems [9,10], which in turn results in reduced process efficiency or even process collapse.

Cu(II), a necessary and redox-active metal, can catalyze the production of hydroxyl radicals and promote stress through redox-cycling activity, resulting in impairment of membrane function [12]. Many researchers have reported the effects of Cu(II) in various wastewater treatments, such as activated sludge processes [13], fermentative hydrogen production processes [14], fermentative methane production [15,16], and nitrification and denitrifying processes [17,18]; However, to our knowledge, little has been reported on the inhibition of anammox processes by Cu(II) [7]. Additionally, the short-term recovery property of anammox biomass by washing with buffered solution has not been widely investigated in a Cu(II)-suppressed anammox system.

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The objectives of this study were (i) to investigate the short- and long-term effects of Cu(II) on anammox performance, (ii) to track the evolution of sludge characteristics under Cu(II) inhibition and its recovery process, and (iii) to evaluate the application of regulation tools in recovering performance.

2. Materials and methods

2.1. Synthetic wastewater and inoculums

The anammox bacteria were cultivated in an autotrophic environment, and ammonium and nitrite were added to the mineral medium as needed in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, respectively, along with bicarbonate; trace elements were introduced into the influent as nourishment. The composition of the synthetic medium was similar to that of Yang and Jin [19]. Cu(II) was added to the synthetic wastewater in the form of $CuCl_2{\cdot}2H_2O$ as required. The additional Cu(II) noted below did not include the Cu(II) added as a trace element.

The anammox granular sludge with a specific anammox activity (SAA) in the range of 5.18–16.6 mg-NO_2^--N g^{-1}-VSS h^{-1} was harvested from several laboratory-scale anammox up-flow anaerobic sludge blanket (UASB) reactors with nitrogen removal rates of 10.5–10.7 kg m^{-3} d^{-1} and was mixed as the inoculums of the anammox reactor under continuous operation in the present study. After inoculation, the initial biomass, characterized by the volatile suspended solids (VSS) concentration in the reactor, was 16.7 g L^{-1}.

2.2. Experimental setup and operational strategy in continuous operation

A UASB reactor (1 L effective volume) fabricated from Plexiglas was employed to investigate the long-term effects of Cu(II) on the anammox process. Influent synthetic wastewater was continuously introduced into the reactor using a peristaltic pump. The reactor was placed in a thermostatic room at 35 ± 1 °C and was covered with black cloth to prevent inhibition by light. The pH of the influent in the experiments was set to 7.5–8.0. The long-term testing of Cu(II) inhibition was divided into six phases (P_0–P_5) according to the influent Cu(II) concentration and experimental objectives, as detailed in Table 1.

2.3. Batch testing

Batch assays were performed in serum flasks with a total volume of 160 mL and liquid phase volumes of 120 mL. Basal mineral medium (100 mL), with a composition similar to that of the synthetic wastewater and anammox biomass (2.04 g-VSS L^{-1}) employed in the serum flasks, was applied to evaluate the short-term effects of Cu(II) stress on anammox activity and to determine the SAA of the anammox sludge. The serum flasks were placed in a 35 ± 1 °C thermostatic shaker at 180 rpm. The initial pH was adjusted to 7.5 by adding 1 mol L^{-1} hydrochloric acid or sodium hydroxide. The SAA determination and calculation methods were consistent with those described by Yang et al. [7].

2.3.1. Batch Cu(II) toxicity experiments at a fixed initial substrate level

As listed in Table 1, with different Cu(II) concentrations at a fixed initial substrate level, batch tests were conducted to determine the half inhibitory concentration (IC_{50}) of Cu(II).

2.3.2. Batch Cu(II) toxicity experiments at a varied initial substrate level

The kinetic characteristics were analyzed at a consistent Cu(II) concentration (15 mg L^{-1}) with different substrate concentrations, as presented in Table 2.

2.3.3. Batch cumulative toxicity tests

Trials on cumulative toxicity at three Cu(II) levels were conducted in 650-mL serum bottles. Fresh substrate (400 mL; 200 mg-TN L^{-1} with NH_4^+–N to NO_2^-–N, 1:1), without or with Cu(II), was added to the control or test bottles, respectively. The anammox community was exposed to Cu(II) for 12 h, followed by a 12-h interval without Cu(II) exposure; this cycle was repeated for 4.5 days. Water samples were obtained at regular intervals using a syringe with a needle.

The cumulative impact could be analyzed using the empirical coefficient K, as shown in Eq. (1).

$$K = \frac{ED_{50(n)}}{ED_{50(1)}} \quad (1)$$

where $ED_{50\ (n)}$ is the cumulative dosage of half inhibition under multiple exposures to Cu(II) and $ED_{50(1)}$ is the dosage of half inhibition under single exposure to Cu(II). If the K was in the range of 0–1, the cumulation was severe; if the K ranged at 1–3, the cumulation was obvious; if the K was of 3–5, the cumulation was medium. Otherwise, the cumulation was slight [20].

2.3.4. Short-term activity recovery tactics

When the control test (without Cu(II)) was stopped, the anammox sludge from all tested bottles was washed with buffer solution (synthetic medium without the substrate); the determined SAA was termed SAA_i. Subsequently, the sludge was incubated in synthetic medium for 10 h without Cu(II). Then, the sludge was washed by buffer solution and fresh substrate was supplied, in which the monitored SAA was the remitted SAA, denoted as SAA_r. The SAA_r represented the recovered activity after Cu(II) interaction.

2.4. Analytical procedures

The effluent of the reactor was collected for spectrophotometric measurements of ammonium, nitrite and nitrate concentrations [21]. Additionally, the pH was detected using a pH meter (PHS-9V), and the suspended solids (SS), VSS, settling velocity (V_S), and upward floating velocity (F_S) of the granules was determined using standard methods [21]. The SAA of the sludge was determined according to the method described by Yang and Jin [19]. The heme c content was quantified using the method described by Berry and Trumpower [22]. The extracellular polymeric substance (EPS) extraction method was employed following Sheng et al. [23]. The carbohydrate and protein in loosely and tightly bound EPS were measured with the modified Lowry method using bovine serum

Table 1
Initial substrate and Cu(II) concentrations in short-term tests.

Experiment objective	Cu (II) concentration (mg L^{-1})	Substrate concentration (NH_4^+–N, NO_2^-–N) (mg L^{-1})
Short-term effects at a fixed initial substrate level	0, 5, 15, 25, 35, 45	100, 100
Short-term effects at a various initial substrate level	15	(70, 70), (140, 140), (210, 210), (280, 280), (350, 350), (420, 420)
Cumulative toxicity	0, 1, 5, 10	100, 100
Short-term activity recovery	0, 5, 15, 25, 35, 45	100, 100