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The joint inhibitory effects of phenol, copper (II), oxytetracycline (OTC) and sulfide on Anammox activity

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HIGHLIGHTS

- The combination of two or three factors had varying effects on the Anammox activity.
- The joint toxicity of OTC and copper (II) on the Anammox activity was antagonistic.
- The toxicity of the combinations of OTC and S^{2-} or of phenol and S^{2-} was synergistic.
- The joint toxicity of phenol and copper (II) was dependent on the level of phenol.
- The activity inhibition can be ranked in the order: NO_2^-–N > copper (II) > OTC.

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ABSTRACT

A batch test was employed to analyze the joint toxicity of copper (II) and oxytetracycline (OTC), OTC and sulfide, phenol and sulfide (S^{2-}), phenol and copper (II), and OTC, copper (II) and substrate on an Anammox mixed culture. The joint toxicity of OTC and copper (II) on the Anammox mixed culture was antagonistic, whereas the interaction between OTC and S^{2-} and between phenol and S^{2-} was generally synergistic. The joint toxicity of phenol and copper (II) was dependent on the level of phenol: the joint toxicity was antagonistic at a high phenol level of 300 mg L^{-1}, whereas the joint toxicity was synergistic at a low phenol level of 75 mg L^{-1}. The joint toxic effect of OTC, copper (II) and NO_2^-–N on the Anammox activity can be ranked in the following order: NO_2^-–N > copper (II) > OTC.

1. Introduction

As an innovative and promising process for biological nitrogen removal, the anaerobic ammonium oxidation (Anammox) process has enormous potential for the treatment of nitrogenous pollution in wastewater with a low level of organic carbon (Strous et al., 1999a; Jaroszynski et al., 2012; Jin et al., 2012a,b). Under anaerobic conditions, the Anammox bacteria, which are the dominant species in the Anammox system, oxidize ammonium to produce nitrogen gas using nitrite as the electron acceptor (Strous et al., 1999a). Although some full-scale Anammox processes have been established successively (van der Star et al., 2007; Gao and Tao, 2011), there are still many inhibitory factors for nitrogen-containing wastewater that hinder the widespread application of the Anammox process (Gao and Tao, 2011; Jin et al., 2012b), which need to be further studied.

The complexity of real wastewater provides challenges for the removal of nitrogen from the wastewater through the Anammox process. Phenol, copper (II), oxytetracycline (OTC) and sulfide (S^{2-}) are four pollutants that are commonly found in wastewater (Beristain-Cardoso et al., 2009, 2011; Álvarez et al., 2010). Nitrogen-rich wastewater often contains two or more of these substances, such as piggery wastewater, which contains OTC, copper (II) and S^{2-}; wastewater from the production of OTC, which is rich in OTC and S^{2-}; wastewater from coke ovens, which contains phenol and S^{2-}; and some types of petrochemical wastewaters that contain phenol and copper (II). In addition, under anaerobic conditions, SO_4^{2-} can be reduced to sulfide or H_2S (Dapena-Mora et al., 2007; Chen et al., 2008). Therefore, it is necessary to determine the combined effects of these inhibitors on the mixed culture to obtain information on the applicability of the Anammox process. Because of the toxic effects of substrates (ammonium and nitrite), many researchers have focused on the substrate inhibition on the Anammox systems and the improvement of the Anammox performance (Jin et al., 2012b).

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Few studies were conducted to explore the inhibitory effects of phenol or sulfide on the Anammox process. Toh and Ashbolt (2002) acclimated the Anammox bacteria to the phenol-containing wastewater at a low nitrogen-loading rate of no more than 62 g NH_4^+–N m^{-3} d^{-1} over a long period (more than 15 months). The activity of the Anammox sludge used was low and the inhibitory effects of antibiotic (chloramphenicol) which was used to inhibit nitrite reduction via denitrification on the Anammox process was neglected (Toh and Ashbolt, 2002). The effects of sulfide on Anammox were distinct in different studies. In batch tests, van de Graaf et al. (1996) reported that the Anammox activity was enhanced at a sulfide-S concentration of 1 or 5 mg L^{-1}, however, Dapena-Mora et al. (2007) reported that the Anammox activity was completely lost at the S^{2-} concentration of 5 mg L^{-1}. The joint inhibitory effects of sulfide and other inhibitors were not found in literatures.

Almost no report of the OTC or copper (II) inhibition on the Anammox process is found up to now. OTC is an antibiotic. At present, the studies on the inhibition of antibiotics on the Anammox process were limited to the chloramphenicol (van de Graaf et al., 1995; Fernandez et al., 2009), penicillins (van de Graaf et al., 1995), ampicillin (van de Graaf et al., 1995), tetracycline hydrochloride (Fernandez et al., 2009) and pharmaceutical wastewater containing the polypeptide antibiotics colistin sulfate and the macrolide kitasamycin (Tang et al., 2011). Although the Anammox process has been used to treat the nitrogen-rich wastewater containing heavy metal ions, such as landfill leachate (Egli et al., 2001; Jin et al., 2012b), there are no reported literatures on the copper(II) inhibition on the Anammox process (Jin et al., 2012b).

The inhibition of the Anammox bacteria, which is one of the obstacles in the application of the Anammox process, has become an increasingly important issue. Therefore, the development of control strategies for the prevention of the inhibition of the Anammox biomass has received extensive attention (Jin et al., 2012b). However, to the best of our knowledge, the study of joint inhibition by two or more inhibitors on the Anammox mixed culture has not been performed. Therefore, the objectives of this study were to investigate (1) the combined effects of two inhibitory factors on the Anammox mixed culture, including the combinations of (i) copper (II) and OTC, (ii) OTC and sulfide, (iii) phenol and sulfide, and (iv) phenol and copper (II) and (2) the combined effects of three factors, i.e., OTC, copper (II) and substrate (NO_2^-–N), on the Anammox mixed culture.

2. Methods

2.1. Synthetic wastewater

Inorganic synthetic wastewater was used as the basic feed. Ammonium and nitrite, in the forms of $(NH_4)_2SO_4$ and $NaNO_2$, respectively, were used to supplement the mineral medium as required. The composition of the synthetic wastewater was KH_2PO_4 (10 mg L^{-1}), $CaCl_2{\cdot}2H_2O$ (5.6 mg L^{-1}), $MgSO_4{\cdot}7H_2O$ (300 mg L^{-1}), $KHCO_3$ (1250 mg L^{-1}) and 1.25 mL of each of the trace element solutions I and II per liter of sterilized water. The composition of the trace element solution I (in g L^{-1}) was EDTA (5) and $FeSO_4{\cdot}7H_2O$ (9.14), whereas the trace element solution II was composed of (in g L^{-1}) EDTA (15), H_3BO_4 (0.014), $MnCl_2{\cdot}4H_2O$ (0.99), $CuSO_4{\cdot}5H_2O$ (0.25), $ZnSO_4{\cdot}7H_2O$ (0.43), $NiCl_2{\cdot}6H_2O$ (0.21), $NaMoO_4{\cdot}2H_2O$ (0.22) and $CoCl_2{\cdot}6H_2O$ (0.24). The mineral medium, which was used in the pretreatment of the sludge, was the inorganic synthetic wastewater without $(NH_4)_2SO_4$ or $NaNO_2$.

2.2. Inoculation

Anammox granular sludge, which was harvested from a high-rate Anammox upflow anaerobic sludge bed (UASB) reactor with a nitrogen removal rate (NRR) of 10.8 ± 0.6 kg m^{-3} d^{-1}, was used for the inoculation. The biomass concentrations that were measured as suspended solids (SS) and volatile suspended solids (VSS) were 45.5 ± 3.1 and 25.1 ± 2.8 g L^{-1}, respectively. The specific Anammox activity (SAA) of the inoculation was 20.6 ± 3.9 mg N g^{-1} VSS h^{-1}. The initial SAA and the biomass activity in the experimental containers were identical. Because the granular sludge used in our study had the ability to weaken the damage effects of inhibitors in a short test period, the characteristics of the granule were presumed to be not changed significantly under the inhibition of phenol, copper (II), OTC and sulfide.

2.3. Experimental container and batch tests procedure

Batch experiments were employed to analyze the combined effects of phenol, copper (II), oxytetracycline (OTC) and sulfide on the Anammox mixed culture. The assays were performed in serum bottles with a total volume of 160 mL and a liquid-phase volume of 120 mL. Each serum bottle was closed using a gas-tight butyl rubber plug. The Anammox sludge was first washed and resuspended in mineral medium. Approximately 15 mL of the sludge was then inoculated into each bottle. The initial pH was adjusted to 7.5 ± 0.2 through the dropwise addition of diluted hydrochloric acid or sodium hydroxide. The bottles were subsequently flushed with argon to remove any oxygen (Dapena-Mora et al., 2007) and thus prevent oxygen suppression of the Anammox biomass (Strous et al., 1997; Egli et al., 2001; Jung et al., 2007). The hermetically sealed bottles were then placed in a thermostatic shaker at 35 ± 1 °C and 180 rpm. Samples were obtained periodically using a syringe and needle and subsequently analyzed to determine the SAA (SAA = substrate consumption rate/ biomass concentration) over time. At the end of each experiment, the biomass concentration, which was measured as VSS, was determined.

2.4. Performed experiments

Because of the inhibitory effects of the substrates, especially nitrite at a high level (Jin et al., 2012b), an initial substrate level of 100 mg L^{-1} was used for both NH_4^+–N and NO_2^-–N in the study of the combined inhibitory effect of two factors. The initial levels of all the inhibitors used in the experiment to analyze the joint effect of two factors are shown in Table 1. The joint effects can generally be divided into four categories: antagonistic, synergistic, additive and irrelevant effects. An antagonist effect means that the joint effect is less than the sum of the individual effects of the two inhibitors. A synergistic effect indicates that the sum of the individual effects of the two inhibitors is less than the combined effect. An additive effect shows that the combined effect is equal to the sum of the individual effects. An irrelevant effect illustrates that the joint effect is simply the same as the effect of the strongest individual factor.

When determining the joint effect of oxytetracycline (OTC), copper (II) and substrate, the initial NH_4^+–N concentrations were 100, 200 and 300 mg L^{-1} with an initial ammonium-to-nitrite molar ratio of 1. It was previously reported that a concentration of ammonium less than 1 g N L^{-1} cannot inhibit the Anammox bacteria (Strous et al., 1999b); therefore, only the nitrite inhibition was considered. Three concentration levels were used for each factor in this test. The orthogonal experiment ($L_9(3^3)$) of the joint effect of oxytetracycline (OTC), copper (II) and substrate (NO_2^-–N) is presented in Table 2.

2.5. Analytical procedures

The concentrations of ammonium, nitrite, and nitrate were determined spectrophotometrically (APHA, 2005). The SS, the