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Effects of quinoid redox mediators on the activity of anammox biomass

Sen Qiao *, Tian Tian, Jiti Zhou

Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education, China), School of Environmental Science and Technology, Dalian University of Technology, Dalian 116024, PR China

HIGHLIGHTS

- RMs addition depressed TN removal performance by anammox biomass.
- RMs could markedly enhance the key enzymes activities of anammox bacteria.
- RMs was inferred to play the role as Q/QH_2 during anammox process.
- Ladderane as the main reason might block the contact between RMs and key enzymes.

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ABSTRACT

This study first explored the relationship between the activity of anammox biomass/key enzymes and quinoid redox mediators, which were anthraquinone-2,6-disulfonate (AQDS), 2-hydroxy-1,4-napthoquinone (LAW) and anthraquinone-2-carboxylic acid (AQC). Experimental results demonstrated that the total nitrogen removal performance showed a downward trend with all three redox mediators (RMs) dosage increasing. For instance, when the AQC addition increased to 0.8 mM, the TN removal rate sharply reduced to 17.2 mg-N/gVSS/h, only about 20% of the control. This phenomenon might be caused by microbial poisoning with the extracellular RMs additions. Nevertheless, the crude hydrazine dehydrogenase, nitrite reductase, and nitrate reductase activities were enhanced with RMs addition, about 0.6–3 folds compared to the control experiments without RMs addition. The RMs was inferred to play the role as ubiquinol/ubiquinone (Q/QH_2) during the anammox process. Furthermore, the specific ladderane membrane structure could block the contacting between RMs and the key enzymes inside anammoxosome. This might be the main reason for the contrary effects of RMs on anammox biomass and the key enzymes.

1. Introduction

Anaerobic ammonium oxidation (anammox) process is now recognized as a novel and important process in biological nitrogen removal, which can directly convert NO_2^- to N_2 gas with NH_4^+ under anaerobic conditions (Strous et al., 1999). Compared with the conventional biological processes (nitrification–denitrification), anammox process offers significant advantages such as no demand for oxygen and organic carbon, low sludge production and reduced CO_2 or N_2O emissions (Op den Camp et al., 2006). Recently, Tang et al. (2010) reported a very high nitrogen removal rate of 74.3–76.7 kg-N/m^3/d in a lab-scale anammox UASB reactor, which demonstrated high potential of anammox process in biological nitrogen removal from wastewaters. However, such a high nitrogen removal rate (NRR) was achieved through the continuous addition of anammox seed sludge into the targeted reactor, in which the biomass concentration increased as high as 42.0–57.7 g-VSS/L (Tang et al., 2010). Furthermore, the relative long doubling time of anammox bacteria will also cause a longer startup period and make the anammox system more vulnerable with low anammox bacteria abundance. Consequently, enhancing the bacterial activity of anammox biomass and further shortening the start-up period of anammox reactors are subjects of great interest and challenge.

Researchers have made numerous efforts to increase the activity of anammox biomass by utilizing external field energy (magnetic field, low intensity ultrasound) or adding some kinds of micronutrient. For instance, Liu et al. (2008) applied magnetic field successfully to enhance the activity of anammox bacteria whereby the maximum nitrogen removal rate increased by 30% at magnetic value of 60.0 mT in long term. Similarly, Duan et al. (2011) demonstrated that total nitrogen (TN) removal rate of anammox bacteria increased by 25.5% by applying ultrasound intensity of 0.3 W/cm^2 with the optimal irradiation time of 4 min, and this effect could last for about 6 days. Besides the application of external field, Qiao et al. (2012) demonstrated that the addition of MnO_2 powder could

* Corresponding author. Tel./fax: +86 411 84706252.
E-mail address: qscyj@mail.dlut.edu.cn (S. Qiao).

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also increase the nitrogen removal rate of anammox biomass about 2 times as high as that without MnO_2 powder addition.

Recently, redox mediators (RMs) were found to play an important role in the anaerobic transformation of organic and inorganic contaminants (Van der Zee and Cervantes, 2009). There were a few studies focused on the role of redox mediators on nitrogen removal by denitrification process. Aranda-Tamaura et al. (2007) investigated the impacts of different quinoid redox mediators on the simultaneous conversion of sulphide and nitrate by denitrifying biomass, including anthraquinone-2,6-disulfonate (AQDS), 2-hydroxy-1,4-naphthoquinone and 1,2-naphthoquinone-4-sulphonate (NQS). They demonstrated that NQS had the highest nitrate reduction rate using sulphide as electron donor (Aranda-Tamaura et al., 2007). Guo et al. (2010) explored the possibility of redox mediator catalyzing denitrification process with anthraquinone (AQ) immobilized by calcium alginate. They also found that addition of 500 anthraquinone immobilization beads would accelerate the denitrifying rate about 2 times. Liu et al. (2012) demonstrated that anthraquinone-2-sulfonate (0.04 mmol/L) immobilized into the functional electropolymerization biocarriers could increase the denitrification rate about 1.5 folds. Until now there was no report on the effects of RMs on anammox biomass.

Most key enzymes of denitrifying biomass are located on the cell membrane or the cell membrane periplasma. Thus, RMs could contact these enzymes and accelerate the biodegradation rate of nitrate or nitrite. However, all the key enzymes of anammox bacteria are located inside anammoxosome, and on its membrane giving rise to a proton-motive-force and subsequent ATP synthesis by membrane-bound ATPases (shown in Fig. 1). From the outside of anammox bacteria into anammoxosome, RMs must cross cell wall, cytoplasmic membrane, intracytoplasmic membrane and anammoxosome membrane in order to contact with the key enzymes. The ladderane of the anammoxosome membrane consist of C18 and C20 fatty acids including either 3 or 5 linearly concatenated cyclobutane rings (Sinninghe et al., 2002). They are ester bound to a glycerol backbone or ether bound as alkyl chains (Sinninghe et al., 2005). Therefore, the ladderane might block the contacting between RMs and the key enzymes inside anammoxosome.

The objective of this study was to investigate the effects of three kinds of RMs on the activity of anammox biomass. The effects of RMs on the key enzymes (hydrazine dehydrogenase nitrate reductase and nitrite reductase) of anammox bacteria were also studied. The possible mechanisms of effects on both anammox biomass and the key enzymes were also discussed. The tested RMs included anthraquinone-2,6-disulfonate (AQDS), 2-hydroxy-1,4-naphthoquinone (LAW) and anthraquinone-2-carboxylic acid (AQC).

2. Methods

2.1. Microorganisms and feed media

The anammox sludge used for inoculation originated from a laboratory-scale anammox upflow column reactor in our lab. The inner diameter and height of the column-type reactor were 8 and 45 cm, respectively. The working volume of this reactor was 2 L and continuously operated under 35 ± 1 °C. The total nitrogen (TN) removal rate of this reactor reached 8.0 kg-N/m^3/d during 670 days' operation. Anammox bacteria of KSU-1 strain (AB057453.1) accounted for about 70–75% of the total biomass in seed biomass. The media used in the experiments mainly consisted of ammonium and nitrite in the form of $(NH_4)_2SO_4$ and $NaNO_2$. The composition of the trace mineral medium was as described by van der Graaf et al. (1996).

2.2. Batch experiments

In order to ascertain the effects of different RMs concentrations on specific anammox activity, seven sets of batch experiments were conducted with the RM concentration from 0 to 0.8 mM. The tests were carried out in seven 120 ml serum vials containing 100 ml medium, each containing anammox biomass (MLVSS concentration of 2000 mg/L) with varied RMs additions. Biomass samples were taken from the reactors and washed three times with mineral medium to remove residual nitrogen. The pH was adjusted to 7.5 and the temperature was maintained at 35 ± 1 °C in a water bath shaker. The shaking speed was set at 150 rpm to keep the full contact between biomass and media. The serum bottle contents were purged with dinitrogen gas to remove dissolved oxygen. Initial NH_4^+-N and NO_2^--N concentrations were set at 50 mg-N/L. Specific anammox activity was estimated from the peak of the curve indicated by the decrease of ammonium and nitrite concentrations per unit biomass concentration in the vials as time lapsed. The samples were collected every hour using a sterile syringe and purged through 0.45 μm pore size membranes to analyze the NH_4^+-N, NO_2^--N, NO_3^--N and RMs concentrations.

2.3. Analytical methods

Concentrations of nitrite and nitrate were determined by using ion-exchange chromatography (ICS-1100, DIONEX, AR, USA) with an IonPac AS18 anion column after filtration with 0.22 μm pore size membranes. NH_4-N, MLSS and MLVSS concentrations were measured according to the Standard Methods (APHA, 1995). pH

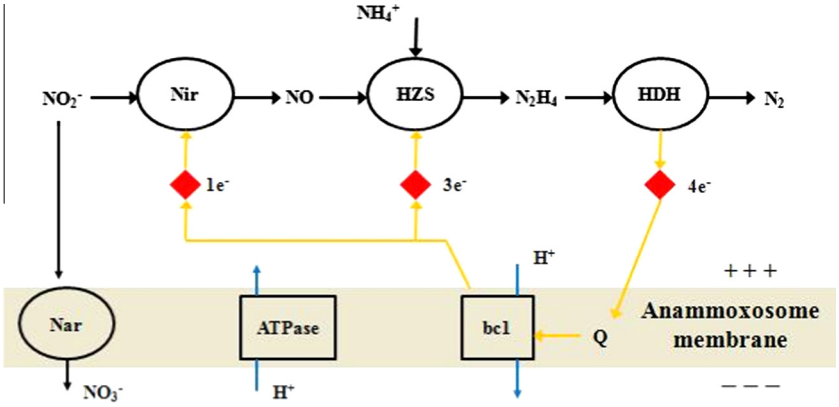

Fig. 1. Metabolic pathways of *K. stuttgartiensis* (revised according to Kartal et al., 2011). Nir, nitrite reductase; HZS, hydrazine synthase; HDH, hydrazine dehydrogenase; Nar, nitrate reductase; Grey line, anammoxosome membrane; Red diamonds, cytochromes; Yellow arrows, electron flow; Q, coenzyme Q. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)