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Effect of organic matter strength on anammox for modified greenhouse turtle breeding wastewater treatment

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HIGHLIGHTS

- Nitrogen could be removed efficiently by anammox reaction from modified greenhouse turtle breeding wastewater.
- Anammox contribution to nitrogen removal was always dominant with the different COD strengths.
- Anammox bacteria, denitrifiers and ammonia-oxidizing bacteria coexist for synergetic removal of nitrogen.

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ABSTRACT

Anaerobic ammonium-N removal from modified greenhouse turtle breeding wastewater with different chemical oxygen demand (COD) strengths (194.0–577.8 mg L^{-1}) at relatively fixed C/N ratios (~2) was investigated using a lab-scale up-flow anaerobic sludge blanket (UASB) anammox reactor. During the entire experiment, the total nitrogen (TN) removal efficiency was about 85% or higher, while the average COD removal efficiency was around 56.5 ± 7.9%. Based on the nitrogen and carbon balance, the nitrogen removal contribution was 79.6 ± 4.2% for anammox, 12.7 ± 3.0% for denitrification + denitratation and 7.7 ± 4.9% for other mechanisms. Denaturing gradient gel electrophoresis (DGGE) analyses revealed that *Planctomycete*, *Proteobacteria* and *Chloroflexi* bacteria were coexisted in the reactor. Anammox was always dominant when the reactor was fed with different COD concentrations, which indicated the stability of the anammox process with the coexistence of the denitrification process in treating greenhouse turtle breeding wastewater.

1. Introduction

Turtle aquaculture is one of the major Chinese agricultural sectors, and the production value has exceeded $1.63 billion per year. Nowadays, almost all of the turtle aquaculture units are operated in the isothermal (the temperature maintained at 30 ± 1 °C) and airtight greenhouse. As 70–85% nitrogen in feed waste and turtle feces are directly discharged into the cultivation water, the greenhouse turtle breeding wastewater contains relatively high ammonium-N (80–250 mg L^{-1}) and COD (10–700 mg L^{-1}). With fluctuated water quality and irregular discharge, the greenhouse turtle breeding wastewater is difficult to treat with nitrification–denitrification processes for nitrogen removal.

Anaerobic ammonia oxidation (anammox), a lithoautotrophic biological conversion process mediated by a group of *Planctomycete* bacteria, is considered to be a novel and cost-effective alternative to traditional processes for treating wastewater with low C/N ratios (Ni et al., 2012). Recently, the anammox process has been successfully implemented for treatment of anaerobic digested fish canning effluents (Dapena-Mora et al., 2006), pig manure effluents (Molinuevo et al., 2009), urban landfill leachate (Ruscalleda et al., 2008), and pharmaceutical wastewater (Tang et al., 2011). However, there have been no reports investigated the possibility of using the anammox process for greenhouse turtle breeding wastewater treatment.

As shown in Eq. (1), anammox is a biological process in which ammonium-N is directly converted to nitrogen gas (N_2) with nitrite-N as the electron acceptor under anoxic conditions (Tsushima et al., 2007). Compared to the traditional nitrification–denitrification process, the anammox process combines with a partial nitrification process, consumes 100% less biodegradable organic carbon

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and at least 50% less oxygen (Tal et al., 2006), and need 90% less operation cost (Jetten et al., 2001).

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O + 0.066CH_2O_{0.5}N_{0.15} \quad (1)$$

The greenhouse turtle breeding wastewater contains a certain amount of organic matter, which, combined with high concentration of nitrite-N, would negatively affect anammox bacteria. This is because of the competition for nitrite-N between anammox bacteria and heterotrophic denitrifiers (Jianlong and Jing, 2005; Molinuevo et al., 2009) as the anammox process requires nitrite-N as an electron acceptor Eq. (1), while the denitritation process reduces nitrite-N to N_2 gas Eq. (2) below by consuming organic matter (Ahn et al., 2004). Theoretically, denitrification reactions are thermodynamically more favorable than anammox reactions because anammox bacteria have very slow growth rates (the biomass yield, $Y = 0.066 \pm 0.01$) compared to heterotrophic denitrifiers ($Y = 0.3$) (Jetten et al., 1998; Molinuevo et al., 2009; Strous et al., 1999b). For example, COD concentrations over 300 mg L^{-1} in wastewater was found to inactivate or eradicate the anammox communities (Chamchoi et al., 2008). COD concentrations above 237 mg L^{-1} for post-digested effluent and above 290 mg L^{-1} for partially oxidized effluent led to complete inhibition of the anammox reaction (Molinuevo et al., 2009). Concentrations of 50 mM of acetate resulted in 70% inhibition of the anammox process (Dapena-Mora et al., 2007). The anammox bacteria are no longer able to compete with heterotrophic denitrifiers at C:N ratios above 1:1 (Güven et al., 2005). The nitrite-N concentration in the reactor reached a level of more than 70 mg L^{-1} would induce a complete loss of anammox activity (Strous et al., 1999a).

On the contrary, the anammox and partial denitritation process was used to treat successfully piggery waste in an UASB reactor; the high organic matter did not significantly influence the cooperation of anammox and denitrification cultures (Ahn et al., 2004). In addition, some kind of anammox bacteria has the capacity of oxidizing ammonium-N to N_2 gas using propionate (Ruscalleda et al., 2008). Therefore, there is a knowledge gap regarding the effects of different wastewater characteristics, the reactor systems, and operational conditions on (a) the performance of the anammox process (Molinuevo et al., 2009), (b) the competition between anammox bacteria and denitrifiers, and (c) contributions to nitrogen and/or COD removal by different trophs in a combined anammox and heterotrophic denitrification system.

Based on these arguments, the objectives of this study are to: (1) verify the feasibility of using an anammox-based UASB to treat the modified greenhouse turtle breeding wastewater with different concentrations; (2) assess the stability of the anammox process with the coexistence of the denitrification process in treating greenhouse turtle breeding wastewater; and (3) analyze the contribution of different trophs to the nitrogen and COD removal by means of denaturing gradient gel electrophoresis (DGGE) and analyses of clone libraries.

2. Methods

2.1. Experimental setup

2.1.1. Reactor and culture of biofilm (anammox) community

A lab-scale UASB reactor (modified by adding 267 pellets Spherical Plastic (SP) carriers) was used for greenhouse turtle breeding wastewater treatment (Fig. 1). The lower part of the reactor has an internal diameter of 10 cm, a height of 1 m and a liquid working volume of 10.8 L. The upper part of the reactor has an internal diameter of 20 cm, and a three-phase separator is installed there to separate water, sludge and gas. SP carriers (diameter = 25 mm and specific surface area = 460 m^2/m^3) were used to improve the anammox bacteria enrichment and minimize anammox biomass wash-out. These SP carriers were floating freely in the upper part of the reactor. The exhaust gas passes through a water-sealed bottle, and effluent is discharged from the top of the reactor and collected in an effluent bottle.

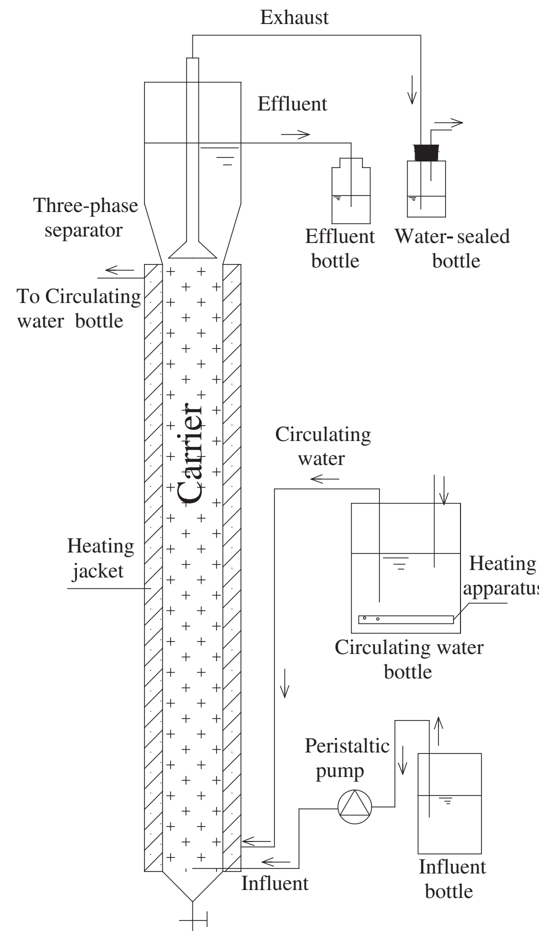

Fig. 1. Schematic diagram of the UASB reactor system.

The start-up period of the USAB reactor was 97 d for culturing anammox bacteria. The copy numbers of Anammox of 16S rRNA gene in the SP reactor reached up to 4.7×10^8 copies g^{-1} Volatile Suspended Solids in the steady phase. The feed of the USAB reactor was not greenhouse turtle breeding wastewater; instead it was synthetic wastewater with 40 mg/L of nitrite and ammonium nitrogen. Compared to the UASB reactor without carriers, SP addition could reduce the Anammox start-up time and increase the Anammox bacteria number of UASB reactors to some extent (Chen et al., 2012).

2.1.2. Substrate characteristics

Greenhouse turtle breeding wastewater was collected from one greenhouse turtle breeding plant (Zhejiang Lantian Ecological Agriculture Development Co., LTD, Hangzhou, China), which bred more than 2 million soft-shelled turtle *Trionyx sinensis*. Characteristics of the wastewater were pH = 7.5–8.1, total COD (COD_{cr}) = 529–624 mg L^{-1}, ammonium-N = 132–140 mg L^{-1} and TN = 138–145 mg L^{-1} (96% being ammonium-N with negligible nitrite-N and nitrate-N). Different pollutant concentrations were prepared for the experiment because of the fluctuated water quality of turtle breeding greenhouse wastewater. Once in the laboratory, the turtle breeding greenhouse wastewater was diluted for 1–3 times