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Performance and microbial community of anammox in presence of micro-molecule carbon source

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HIGHLIGHTS

- Effects of acetate on N and COD removal in an anammox reactor were studied.
- Nitrogen removal performance was promoted at low COD concentration ($\leq 251 \pm 7$ mg L^{-1}).
- Ca. Kuenenia first decreased then increased with increasing COD in the system.
- Anammox and heterotrophic denitrification could coexist at COD of 730 ± 9 mg L^{-1}.

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ABSTRACT

Because ammonium (NH_4^+-N) coexists with organic matter in some wastewaters, the possible adverse influences of organic matter become a major concern in the applications of anaerobic ammonium oxidation (anammox). In this study, the effects of acetate, as a representative of micro-molecule organic matter, on anammox were investigated. Efficient nitrogen removal was realized because denitrifying bacteria and anammox bacteria (AnAOB) had a better synergistic effect under the condition of chemical oxygen demand (COD) concentrations lower than 251 ± 7 mg L^{-1}. Furthermore, the nitrogen removal efficiency (NRE) decreased to $82.02 \pm 3.14\%$ when COD was increased to 730 ± 9 mg L^{-1}, and effluent free ammonia (FA) reached 21.93 ± 4.71 mg L^{-1} might be one of factors leading to inhibition. However, the nitrogen-removal contribution rate of anammox remained steady at $61.97 \pm 2.84\%$ at COD of 730 ± 9 mg L^{-1}, which indicated that anammox was still dominant in the system. AnAOB, such as Ca. Kuenenia and Ca. Jettenia, and denitrifying bacteria, such as Denitratisoma and Thauera, were found to coexist in the reactor. Interestingly, Ca. Kuenenia presented in the trend of first decreased then increased with the increasing of organic matter concentration, which might be one of reasons that anammox played an important role in nitrogen removal at high COD concentration.

1. Introduction

Anaerobic ammonium oxidation (anammox) is a novel biological nitrogen removal process, and the anammox bacteria (AnAOB) can use nitrite (NO_2^--N) as electron acceptor to directly convert ammonium (NH_4^+-N) to nitrogen gas under anoxic conditions according to Eq. (1) (Strous et al., 1998). Compared with conventional nitrification-denitrification processes, anammox process has some benefits, such as lower cost, lower sludge production, and lower/no requirement of organic carbon sources (Sliekers et al., 2003), which has been the focus of many studies in environmental engineering field.

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

However, AnAOB with slow growth rate was vulnerable to external environment (Wang et al., 2016). Organic matter, which often coexists with NH_4^+-N in some wastewaters, was considered to be one kind of harmful substances for AnAOB, and the possible effect of organic matter on anammox has caused a widespread

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concern. Some reports stated that a low organic matter concentration in anammox systems can improve the nitrogen removal efficiency (NRE) by promoting synergism between the anammox and denitrification processes (Ni et al., 2012). Li et al. (2016) reported that more than 95% of total nitrogen (TN) removal could be achieved by increasing the chemical oxygen demand (COD) in an anammox reactor to 150 mg L^{-1}. Too much organic matter in an anammox reactor would result in excessive multiplication of heterotrophic bacteria, which would compete with AnAOB for electron acceptors, thereby inhibiting the activities of AnAOB (Molinuevo et al., 2009). Tang et al. (2013) observed that the anammox activity in sequencing batch reactor decreased severely with COD (from glucose) of 800 mg L^{-1} and COD/N of 1. Chamchoi et al. (2008) found that anammox bacteria were overtaken by heterotrophic denitrification bacteria if the COD/N ratio rose above 2 at a COD (from fat milk) of 400 mg L^{-1}. Sánchez Guillén et al. (2014) reported that nitrogen removal efficiency (NRE) in an up-flow anaerobic sludge bed (UASB) reactor decreased to 73% at COD (from starch) of 360 mg L^{-1} and COD/N of 6. However, in the literatures, there is no consensus on the specific range of the organic matter concentration (expressed as COD) and COD/N ratio that inhibits/or affects the anammox process. The reasons for this might be caused by the difference in type of reactors, operating conditions, enrichment of AnAOB, etc. Moreover, the difference in the structure of organic matter might be also responsible for these discrepancies, because denitrifying bacteria and heterotrophic bacteria have different utilization capacities for different organic matters (Xie et al., 2012), and then would affect the competition between AnAOB and denitrifying bacteria, which need to be further identified.

Meanwhile, AnAOB was reported to have diversified metabolic pathways, which can simultaneously carry out the oxidation of the organic acids (formate, acetate, and propionate, etc) and anaerobic ammonium oxidation (Güven et al., 2005; Strous et al., 2006); it may also transform NH_4^+-N to nitrate (NO_3^--N) by an enzymatic anoxic oxidation mechanism (Sabumon, 2007). However, thermodynamically speaking, denitrifying bacteria seemingly have a higher competitive advantage than AnAOB when presenting micro-molecule organic matter in wastewater according to Eqs. (2) and (3) (Wang et al., 2016). Nevertheless, what would happen in the competition between AnAOB and denitrifying bacteria in presence of micro-molecule organic matter needs to investigate in depth.

$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O, \qquad \Delta G = -357.8 kJ/mol \tag{2}$$

$$\begin{aligned} NO_2^- + 3/8CH_3COO^- + 11/8H^+ &\rightarrow 1/2N_2 + 3/4CO_2 \\ &+ 5/4H_2O, \Delta G = -415.4 kJ/mol \end{aligned} \tag{3}$$

In this study, acetate as a representative of micro-molecule organic matters was added into the anammox reactor, and the long-term effects of organic matter on anammox process were investigated. In addition, the variation of the microbial community in operating process was analyzed to clarify the biological processes involved.

2. Materials and methods

2.1. Experimental setup

This experimental work was carried out in a 2.5 L (working volume) laboratory-scale UASB reactor with an internal diameter of 70 mm and a height of 750 mm (Fig. 1). This reactor was made of polyvinyl chloride (PVC). The reactor was equipped with an external water bath to maintain the temperature at 34 ± 1 °C. Black rubber sponge insulation board was wrapped around the reactor to avoid light interaction and conserve heat. The influent was continuously supplied to the reactor at hydraulic retention time (HRT) of 0.25 d, and an agitator was used to mix the medium uniformly. There was a three-phase separator in the top of the reactor. After granule sludge, gas and effluent were separated by the separator, granule sludge slid automatically to sludge bed in the bottom of the reactor, the gas was extracted from the gas chamber, and the effluent flowed from the water outlet.

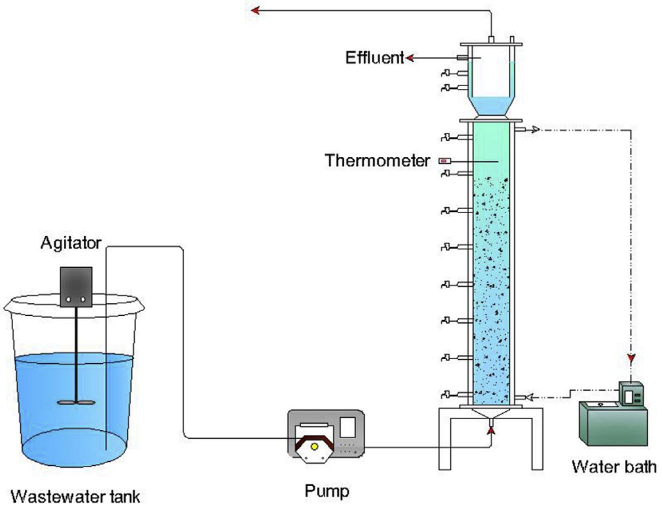

Fig. 1. Schematic diagram of the UASB reactor.

2.2. Influent and seed sludge

The influent was synthetic wastewater and comprised four parts: substrates, organic matter, mineral medium, and trace elements. The growth substrates, NH_4^+-N and NO_2^--N, were supplied by adding NH_4Cl and $NaNO_2$. The ratio of influent NO_2^--N to NH_4^+-N was kept at around 1.32 during operation. Organic matter was provided in the form of sodium acetate, and other components of the synthetic wastewater included $CaCl_2 \cdot 2H_2O$ (0.0056 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.30 g L^{-1}), $KHCO_3$ (1.25 g L^{-1}), KH_2PO_4 (0.01 g L^{-1}), trace element solution I (TES-1: 1 mL L^{-1}), and trace element solution II (TES-2: 1 mL L^{-1}). TES-1 was composed of EDTA (15 g L^{-1}) and $FeSO_4$ (5 g L^{-1}). TES-2 contained EDTA (15 g L^{-1}), $ZnSO_4 \cdot 7H_2O$ (0.43 g L^{-1}), $CoCl_2 \cdot 6H_2O$ (0.24 g L^{-1}), $MnCl_2 \cdot 4H_2O$ (0.99 g L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.25 g L^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.22 g L^{-1}), and $NiCl_2 \cdot 6H_2O$ (0.19 g L^{-1}) (deGraaf, 1996).

The seed sludge was taken from a stable anammox-UASB reactor located at the China University of Mining and Technology (Jiangsu, China) (Yang et al., 2018). It was operated at 34 ± 1 °C and treated low-strength synthetic wastewater. The seed sludge was repeatedly washed with nutrient solution to remove impurities and residual pollutants before inoculation. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) of the seed sludge were 75.46 g L^{-1} and 17.60 g L^{-1}, respectively.

2.3. Operating conditions

The COD/N ratio of this system was changed by varying the concentration of sodium acetate (0, 157.64, 315.28, 630.56, 945.85 mg L^{-1}). Table 1 shows the specific operation parameters of the reactor, which was operated over a period of 138 d.