Bioresource Technology 260 (2018) 186–195

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of temperature on anammox performance and community structure

Shilong He[a,*], Yi Chen[a], Meng Qin[a], Zhen Mao[a], Limei Yuan[a], Qigui Niu[b], Xicheng Tan[a]

[a] School of Environment and Spatial Informatics, China University of Mining & Technology, Xuzhou 221116, China
[b] School of Environmental Science and Engineering, Shandong University, No. 27 Shanda South Road, Jinan 250100, China

ARTICLE INFO

Keywords:
Anammox
Temperature
UASB
SAA
Ca. Kuenenia

ABSTRACT

A lab-scale anammox up-flow anaerobic sludge blanket (UASB) reactor was run to investigate the influence of temperature on anammox performance and community structure. The anammox system had a higher substrate tolerance at 13 °C than at 18 °C. The adverse effects caused by the use of a lower temperature (8 °C) could be restored. The nitrogen removal rate (NRR) decreased with decreasing in situ specific anammox activity (SAA). Interestingly, the ex situ SAA acclimated at 23 °C, when exposed to ex situ temperatures of 33 and 28 °C, was higher than for those acclimated at 33 and 28 °C. No shift was observed in the optimum temperature for ex situ SAA in the whole lowering process of anammox UASB. More extracellular polymeric substances were produced in response to cooler conditions (18 °C and 13 °C). *Ca.* Kuenenia became much more abundant (55.18% of the microbial community) and had a competitive advantage over other anammox bacteria (AnAOB) at 13 °C.

1. Introduction

Nitrogen pollution owing to human activity has increased over the last 40 years because of the increasing world population. Discharge of nitrogen into natural waters can lead to eutrophication and oxygen depletion, and make the waters toxic to aquatic life (Hulle et al., 2010). Anaerobic oxidation of ammonium (anammox) (Mulder et al., 1995) is recognized as an attractive alternative N-removal process, which when coupled with nitrite reduction, releases N_2, a harmless gas, as the end product in natural and manmade ecosystems (Ma et al., 2013) (Eq. (1).

$$NH_4^+ + 1.32NO_2^- + 0.0664HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 2.03H_2O + 0.066CH_2O_{1.5}N_{0.15} + 0.26NO_3^- \quad (1)$$

Compared to other nitrogen removal processes, the anammox process uses lesser oxygen, lesser sludge yield, and lesser or no organic carbon sources. Therefore, it has been widely used in wastewater treatments plants (WWTP) to treat concentrated ammonia (Tang et al., 2011). It has been reported that anammox bacteria (AnAOB) have the highest activity at the medium temperature range (around 35 °C). However, the temperature of most actual wastewaters is often lower (10–20 °C), and at higher latitudes in particular, the wastewater temperature could be lower than 10 °C. This condition is a challenge for a typical anammox process. Therefore, achieving acceptable nitrogen removal performance by using the anammox process at low temperatures has been the focus of many works in recent years. It has been reported (Ma et al., 2013) that the nitrogen removal rate (NRR) decreased with decrease in temperature, but could be sustained at 2.28 kgN m^{-3} d^{-1} at 16 °C. The anammox reaction occurred at a low temperature of 6 °C, and the nitrogen removal efficiency (NRE) was around 50% (Isaka et al., 2008). After cold acclimation of the AnAOB, the characteristics of the anammox process changed. Hu et al. (2013) showed that for the microbes acclimated at 12 °C, the optimum temperature for the optimum activity was around 25 °C, instead of 30–35 °C. It has also been showed that when marine anammox species were enriched in lab reactors operated at a higher temperature (15 °C or 23 °C), a higher temperature optimum of 25–30 °C was observed (Van et al., 2008). However, others (Lotti et al., 2014) reported that the optimum temperature of 30 °C had not shifted when AnAOB was adapted to 10 °C and 20 °C for a long period. To resolve these inconsistencies, further study was needed to determine whether the optimum activity temperature of anammox was shifted by low-temperature acclimation and whether change in the community structure was responsible for this phenomenon.

In this study, the performance of an anammox reactor was evaluated during the incremental change in temperature, and the effects of low temperature on ex situ and in situ specific anammox activity (SAA) were investigated. The changes in the physical and chemical properties of sludge at various temperatures were analyzed. In addition, the bacterial communities in the anammox sludge at different temperatures were phylogenetically characterized based on 16S rRNA gene sequences.

* Corresponding author.
E-mail address: hslongrcees@163.com (S. He).

https://doi.org/10.1016/j.biortech.2018.03.090
Received 16 January 2018; Received in revised form 13 March 2018; Accepted 17 March 2018
Available online 30 March 2018

2. Methods

2.1. Synthetic wastewater

Synthetic wastewater was used with substrates (ammonium nitrogen: NH_4^+-N and nitrite nitrogen: NO_2^--N), nutrient elements, and trace elements. NH_4^+-N and NO_2^--N were introduced as NH_4Cl and $NaNO_2$, respectively, and the ratio of the addition of NO_2^-/NH_4^+ was 1.32. The nutrient components included 0.25 g L^{-1} $KHCO_3$, 0.01 g L^{-1} KH_2PO_4, 0.3 g L^{-1} $MgSO_4$ $7H_2O$, and 0.0056 g L^{-1} $CaCl_2$ $2H_2O$. Trace elements were supplied in 1 mL L^{-1} of wastewater, which contained 15 g L^{-1} EDTA, 5 g L^{-1} $FeSO_4$, 0.43 g L^{-1} $ZnSO_4{\cdot}7H_2O$, 0.24 g L^{-1} $CoCl_2{\cdot}6H_2O$, 0.99 g L^{-1} $MnCl_2$ $4H_2O$, 0.25 g L^{-1} $CuSO_4{\cdot}5H_2O$, 0.22 g L^{-1} $NaMoO_4{\cdot}2H_2O$, and 0.19 g L^{-1} $NiCl_2{\cdot}2H_2O$.

2.2. Biomass

The sludge used for long-term operation was drawn from an anammox up-flow anaerobic sludge blanket (UASB) reactor that had been operated at 35 °C for more than 300 days in this lab, and its NRR and NRE reached at 4.30 kgN m^{-3} d^{-1} and 87.42%, respectively. The average granule diameter of inoculated sludge was 2.69 mm and biomass concentration was 17.6 gVSS L^{-1} and the ratio of VSS/SS was 0.28.

2.3. Experimental set up

2.3.1. Batch test

In order to investigate the short-term and long-term effects of temperature on SAA, the ex situ and in situ SAA at different temperatures were determined by the batch tests. The batch tests carried out according to the method reported elsewhere (Dapena-Mora et al., 2007). For this, 70 mL vials were used at 13–33 °C. Five gram (wet weight) of anammox granular sludge collected from the reactor was washed three times. The sludge sample was inoculated in the form of a 50 mL solution containing 75 mg L^{-1} NH_4^+-N and 75 mg L^{-1} NO_2^--N in a 70 mL vial. The vial was sealed with a butyl rubber stopper and an aluminum cap, and purged with nitrogen gas for 15 min to remove oxygen. The pH was set at approximately 7.5. The vials were anaerobically incubated on a temperature-controlled shaker (150 rpm) in a water bath and set to 13, 18, 23, 28 and 33 °C, respectively. Water samples were taken out periodically from the vials for chemical analysis. The SAA was calculated based on the sum of the NH_4^+-N and NO_2^--N removal rates per g-VSS of the sludge (expressed as gN g^{-1} VSS d^{-1}).

2.3.2. Long-term operation

A UASB reactor was used as the anammox reactor to treat synthetic wastewater (shown in Fig. 1). It had a volume of 2.5 L with an inner diameter of 7 cm and outer diameter of 12 cm. The reactor was covered with black sponge to avoid light and prevent the growth of phototrophic organisms. The reactor was temperature controlled (8–33 °C) using a thermostatic bath connected to the double wall of the reactor. The influent pH value was adjusted to 7.5–7.9. Table 1 summarizes the operating conditions applied to the UASB at different temperature ranges (I–VI) over a period of 210 days.

2.4. Analytical methods

2.4.1. Chemical analysis

NH_4^+-N, NO_2^--N, nitrate nitrogen (NO_3^--N), suspended solids (SS), and volatile suspended solids (VSS) were measured according to standard methods (Walter, 1998). The heating method was used to extract extracellular polymeric substances (EPS) according to the method reported elsewhere (Sheng et al., 2008). Carbohydrate measurements were taken using the anthrone method with a sucrose standard (Hendrickx et al., 2014). The protein concentration was measured using the modified Lowry method with bovine serum albumin as a standard (Bo et al., 1996). All samples were passed through a 0.45 μm filter before analysis.

The nitrogen loading rate (NLR, kgN m^{-3} d^{-1}), NRR (kgN m^{-3} d^{-1}), and NRE (%) were determined by Eqs. (2)–(4). In addition, the value of free ammonia (FA, mg-NH_3 L^{-1}) and free nitrous acid (FNA, μg-HNO_2 L^{-1}) could be calculated using Eqs. (5) and (6) (Anthonisen et al., 1976).

$$NLR = \frac{TN_{inf}}{1000 \times HRT} \quad (2)$$

$$NRR = \frac{TN_{inf} - TN_{eff}}{1000 \times HRT} \quad (3)$$

$$NRE = \frac{100 \times (TN_{inf} - TN_{eff})}{TN_{inf}} \quad (4)$$

$$FA = \frac{17}{14} \frac{TAN \times 10^{pH}}{\left[\exp\left(\frac{6334}{273 + °C}\right) + 10^{pH}\right]} \quad (5)$$

$$FNA = \frac{47}{14} \frac{TNN}{\left[\exp\left(-\frac{2300}{273 + °C}\right) \times 10^{pH}\right] + 1} \quad (6)$$

2.4.2. DNA isolation and quantitative PCR assay

DNA was extracted from granular sludge using the Fast DNA SPIN Kit for Soil (MP Biomedicals, LLC, Solon, OH). The primer set for the AnAOB was A438f (5′-GTCRGGAGTTADGAAATG-3′) and A684r (5′-ACCAGAAGTTCCACTCTC-3′). The V4 region of the 16S rRNA sequence was amplified using the bacterial primer set 515F (5′-GTGCC AGCMGCCGCGG-3′) and 907R (5′-CCGTCAATTCMTTTRAGTTT-3′). The PCR ABI7500 parameters for the AnAOB consisted of the following steps: 5 min at 95 °C and 30 cycles of 45 s at 95 °C, 45 s at 55 °C and 1 min at 72 °C, followed by a final extension step at 72 °C for 10 min (Ren et al., 2014).

Then, the 16S rRNA V4 region PCR products were quantified using TBS-380 (Turner Biosystems, USA). The DNA library was constructed and run according to the standard protocols on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd., to determine the microbial community. Statistical analysis of the biological information was performed using operational taxonomic units (OTUs) with 97% similarity. The database of Silva and Greengene was used.

3. Results and discussion

3.1. Operation of the anammox system

3.1.1. Nitrogen removal performance

The nitrogen removal performance of the anammox UASB reactor at 33–8 °C was investigated for 210 days, and the results are shown in Fig. 2. The start-up temperature of the anammox UASB reactor was 33 °C. In Phase I, HRT was shortened gradually from 3 to 1.2 h, resulting in increased NLR (up to 12.66 kgN m^{-3} d^{-1}). However, the NRE of the reactor decreased from around 87.42% (HRT = 3 or 2 h) to 77.33% (HRT = 1.2 h) in Fig. 2a. Meanwhile, the ratio of nitrite consumption and ammonium conversion (NO_2^--N/NH_4^+-N) and the ratio of nitrate production and ammonium conversion (NO_3^--N/NH_4^+-N) were lower than the theoretical values of 1.32 and 0.26 (Fig. 2c) (Strous et al., 1998), respectively. Some studies reported that growth of the AnAOB are always associated with nitrate production because these microorganisms oxidize part of nitrite to nitrate as the ultimate source for the electrons that are used for cell carbon fixation (Hu et al., 2013). Therefore, the lower ratio of NO_3^--N/NH_4^+-N (< 0.26) might mean the low yield production of AnAOB at the higher NLR caused by lower HRT. Moreover, overload is also disadvantageous