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Increased salinity improves the thermotolerance of mesophilic anammox consortia

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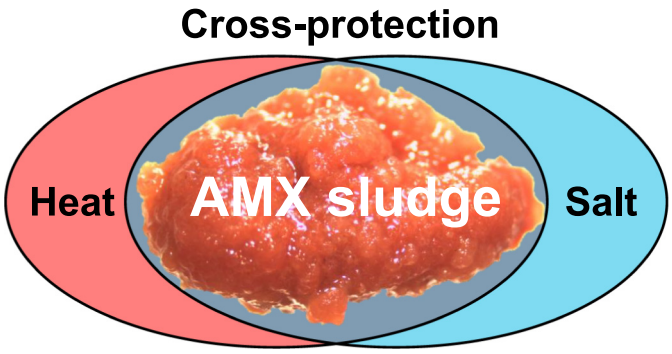
HIGHLIGHTS

- The thermotolerance of mesophilic anammox consortia was first investigated.
- Salt addition alleviated the shock of 40–50 °C to mesophilic anammox consortia.
- Thermophilic reactor performance could be enhanced by increased salinity.
- A nitrogen removal rate of 0.53 kgN m^{-3} d^{-1} was finally obtained at 50 °C.

GRAPHICAL ABSTRACT



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ABSTRACT

While the application of anammox-based process for mesophilic sidestream treatment is at present the state of the art and mainstream treatment at ambient temperature is also in development, the feasibility of thermophilic anammox process is still unclear. This study investigated the effects of salinity on the thermotolerance of mesophilic anammox consortia. In batch activity tests, 45 °C seems to be the critical temperature for the tolerance of mesophilic anammox consortia without acclimatization or amendments. The optimal anammox activity at 40, 42.5, and 45 °C can be achieved with the amendment of salt at 5–8, 8–10, and ~12 g NaCl L^{-1}, respectively. However, this improvement effect was limited at 50 °C or when the shock duration was longer than 24 h even at 45 °C. In continuous-flow bioreactors, mesophilic anammox consortia could gradually adapt to 40–50 °C under a transition of 2.5 °C, and the performance was enhanced by an increase in salinity, which may be associated with the increase in extracellular polymeric substances. A nitrogen removal rate of 0.53 kgN m^{-3} d^{-1} was finally obtained at 50 °C. Overall, these interesting results facilitate further opportunities for thermophilic anammox process.

1. Introduction

It is generally known that organic pollutants and nitrogen compounds (mainly in the form of ammonium) are the two main targets of wastewater treatment plants (WWTPs) (Morales et al., 2015). In the classical disposal processes, biodegradable organic pollutants are mainly removed by denitrification, while ammonia is removed by a two-step process: ammonium is first oxidized to nitrate via nitrification, and the nitrate is then reduced to N_2 via denitrification. Nevertheless, autotrophic anaerobic ammonium oxidizing (anammox) bacteria can directly convert ammonium to N_2 using nitrite as the electron acceptor under anaerobic conditions (Kartal et al., 2010). Obviously, combined

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with partial nitritation, the anammox process can greatly reduce the cost of aeration energy and additional carbon sources in the nitrification-denitrification process (Siegrist et al., 2008). Meanwhile, organic pollutants in wastewaters can be efficiently converted to energy resources by the anaerobic digestion process and recovered in the form of biogas (Kuenen et al., 2011). The sustainability of WWTPs is dependent on their technical reliability, economic feasibility and environmental impact, giving importance to the aspects of energy consumption, sludge production, and greenhouse gas emissions (Morales et al., 2015). Accordingly, the development of anammox-based process provokes revolutionary changes in the biological wastewater treatment (Kartal et al., 2010; Morales et al., 2015).

The anammox-based process has been commercially applied to mesophilic sidestream treatment and is being developed for mainstream treatment at ambient or low temperature (Gilbert et al., 2014; Lackner et al., 2014; Laureni et al., 2016; Reino et al., 2018). Nevertheless, thermophilic anammox-based process, if available, is particularly advantageous for the removal of nitrogen from thermophilic digestion effluent (De la Rubia et al., 2013; Liu et al., 2010), municipal wastewater in tropical climates or at high seasonal temperatures (Lapara and Alleman, 1999), and warm industrial wastewater (e.g., fertilizer, paper and pulping industries) (Shore et al., 2012). Benefits can be achieved from the lower sludge yields and cheaper cooling costs of warm wastewaters (Courtens et al., 2014a; Courtens et al., 2014b; Lapara and Alleman, 1999). To date, however, no continuous thermophilic anammox enrichments with a potential of practical application have been documented to our knowledge. Indeed, temperature is a crucial operational parameter for the anammox process. The optimum temperature for anammox bacteria is in the range of 30–40 °C (Jin et al., 2012). Low temperatures below 15 °C can seriously inhibit anammox activity, while temperatures above 45 °C can also induce an irreversible loss of anammox activity (Dosta et al., 2008; Lotti et al., 2014). Maintaining the high performance of biological nitrogen removal above 40 °C remains a major challenge. Although the presence and activity of anammox bacteria have been detected in hot habitats in natural environments, such as hot springs (>52 °C) (Jaeschke et al., 2009) and deep-sea hydrothermal vents (>60 °C) (Byrne et al., 2008), no anammox bioreactors have been successfully applied to remove nitrogen from hot wastewaters. Unlike mesophilic anammox consortia, thermophilic anammox consortia cannot be enriched from mesophilic municipal sludge by a direct temperature shift to 55 °C (Toh et al., 2002).

Recently, the enrichment of autotrophic thermophilic nitrifying bacteria from a nutrient-rich environment (50 °C) and the successful operation of a thermophilic nitrifying bioreactor have opened up a path for thermophilic nitrogen removal (Courtens et al., 2016; Courtens et al., 2014a). The adaptive capacities of mesophilic nitrifying consortia to high temperatures can be enhanced by acclimatization with the aid of the induction of salt stress (7.5 g NaCl L^{-1}).

Inspired by previous studies, we therefore investigated whether salt stress can improve the thermotolerance of mesophilic anammox consortia. First, the effects of salt stress (8–15 g L^{-1}) on the response of anammox activity at 40–50 °C were explored by batch assays. Subsequently, a continuous-flow reactor was employed to evaluate the effect of salt amendment on anammox performance at 40–50 °C. Moreover, the response of extracellular polymeric substances (EPS) to elevated temperatures was also tracked. Overall, the interesting results of this study promote opportunities for thermophilic anammox process.

2. Materials and methods

2.1. Origin of the mesophilic anammox inocula

Anammox granules were withdrawn from a mesophilic parent reactor for batch experiments. This parent reactor has been in operation for more than two years under thermostatic (35 ± 1 °C) conditions. These anammox granules dominated by "*Candidatus Kuenenia stuttgartiensis*" possess a mean diameter of 0.55 ± 0.64 mm, an EPS content of 174.5 ± 14.3 mg g^{-1} volatile suspended solids (VSS), and a specific anammox activity (SAA) of 522.3 ± 41.5 mgTN g^{-1}VSS d^{-1}.

2.2. Batch assays

Batch assays were performed in serum flasks with a liquid-phase volume of 120 mL. Fresh anammox granules withdrawn from the patent reactor were washed three times and re-suspended in a mineral medium. The mineral medium contained 10 mg L^{-1} KH_2PO_4, 5.6 mg L^{-1} $CaCl_2 \cdot 2H_2O$, 300 mg L^{-1} $MgSO_4 \cdot 7H_2O$, and 1250 mg L^{-1} $KHCO_3$. Then 100 mL of mineral medium and 0.15 mL of trace element solutions I and II were introduced to the serum flasks. Trace element solution I contained 5 g L^{-1} EDTA and 9.14 g L^{-1} $FeSO_4 \cdot 7H_2O$, and trace element solution II was composed of 15 g L^{-1} EDTA, 0.25 g L^{-1} $CuSO_4 \cdot 5H_2O$, 0.014 g L^{-1} H_3BO_4, 0.99 g L^{-1} $MnCl_2 \cdot 4H_2O$, 0.43 g L^{-1} $ZnSO_4 \cdot 7H_2O$, 0.22 g L^{-1} $NaMoO_4 \cdot 2H_2O$, 0.21 g L^{-1} $NiCl_2 \cdot 6H_2O$, and 0.24 g L^{-1} $CoCl_2 \cdot 6H_2O$. Each serum flask was inoculated with approximately 3 g VSS L^{-1} of anammox sludge. $(NH_4)_2SO_4$ and $NaNO_2$ were added as substrates for activity testing, and the salinity was adjusted as needed with NaCl. The pH was initially fixed at approximately 7.5. After removing the oxygen with argon (99.9% purity), the serum flasks were sealed with rubber stoppers and incubated at the designated temperature on an orbital shaker (180 rpm) in the dark. The SAA was calculated from the consumption rate of NH_4^+-N and NO_2^--N in bulk and expressed as mgTN g VSS^{-1} d^{-1}. Protocols 1 and 2 were designed to determine the response of SAA to elevated temperatures (35–50 °C) with and without the addition of NaCl, respectively (Table 1). Protocols 3 and 4 were designed to determine the response of SAA at 45 °C over time in the presence and absence of 8 g L^{-1} NaCl, respectively (Table 1). In Protocol 5, a total of 13 experiments (Table 2) were conducted, as a central composite design was used to study the interactive effects of exposure time and salinity on the response of anammox activity. The initial concentrations of NH_4^+ and NO_2^- were both set at 100 mg N L^{-1} for the determination of SAA.

2.3. Continuous-flow reactor setup and operation

Two identical up-flow anaerobic sludge blanket (UASB) reactors (R0 and R1) with a working volume of 1.0 L and an internal diameter of 6.0 cm were used for the continuous-flow experiments. The synthetic wastewater, as described above, was continuously pumped into the reactors with a hydraulic retention time of 6 h. The concentration of dissolved oxygen in the two reactors was lower than 0.1 mg L^{-1}. The initial nitrogen loading rate (NLR) was set at 2.11 kgN m^{-3} d^{-1} with a substrate level of 280 mgN L^{-1} for NH_4^+-N or NO_2^--N. The initial sludge concentration and the ratio of VSS/suspended solids (SS) were 13.6 g SS L^{-1} and 57.7%, respectively. The operational temperature was gradually increased from 40 to 50 °C at increments of 2.5 °C. R0 was set as the control without the addition of NaCl, and the imposed salinity of R1 is shown in Table 3.

2.4. Analytical methods

The levels of NH_4^+, NO_2^-, and NO_3^- were measured spectrophotometrically by the phenol-hypochlorite method, the N-(1-naphthalene)-diaminoethane method, and the phenol disulphonic acid method, respectively (APHA et al., 2005). The determinations of SS, VSS, and pH were according to standard methods (APHA et al., 2005). EPS were extracted using a heat-extraction method and quantified as the sum of the polysaccharides (PS) and proteins (PN) (Zhang et al., 2016a). PS was measured by the anthrone method with glucose as the standard, and PN was determined using the modified Lowry method with bovine serum albumin as the standard (Zhang et al., 2016a). Each test was performed in triplicate, and the results are expressed as the means ±