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Comparing two start up strategies and the effect of temperature fluctuations on the performance of mainstream anammox reactors

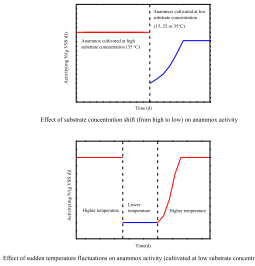
Guopeng Wang, Dong Zhang*, You Xu, Yu Hua, Xiaohu Dai**

State Key Laboratory of Pollution Control and Resources Reuse, School of Environmental Science and Engineering, Tongji University, 1239 Siping Road, Shanghai, 200092, China

HIGHLIGHTS

- Shift of substrate concentration prolonged anammox mainstream reactor startup.
- Anammox cultured with low substrate concentration favored mainstream reactor startup.
- Anammox activity changed quickly as temperature changed in the mainstream reactor.

GRAPHICAL ABSTRACT



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ABSTRACT

Anammox cultivated with high substrate concentration (NH_4^+-N, 150 mg/L; NO_2^--N, 200 mg/L) at 35 °C was first used as seed sludge to start up reactors at 35 (Ra), 20 (Rb) and 15 °C (Rc) with low substrate concentration (NH_4^+-N 30 mg/L, NO_2^--N 40 mg/L). The results showed that anammox activity initially decreased in the three reactors, but that activity levels and nitrogen loading rate (NLR) increased as the bacteria gradually adapted to the new conditions (12–30 days). Temperature and concentration shift affected anammox activity jointly. In the process, the abundance of mRNA of the key functional genes of *hdh* and *nirS*, changed with time but this change did not reflect the change of anammox activity. When the reactors reached a stable state after 40 d, the effect of temperature fluctuations was tested. The results showed that anammox adapted to low temperatures as soon as temperature decreased (i.e., decreased from 35 °C to 15 °C). When temperature increased, 2–3 days were needed for activity recovery. From this result, it may be concluded that reactors with low temperatures and low substrate (mainstream) concentrations can be started up using anammox cultivated at a higher temperature (35 °C) with low substrate. Then anammox in Ra was used to start up a mainstream reactor at 15 °C and it was operated for 60 days. The results showed that the activity in Ra decreased sharply to the level as that of Rc at the stable state. After the experiment, microbiological analysis showed that the anammox was stable and that *Candidatus Kuenenia* was the dominant species.

1. Introduction

There are currently more than 100 full-scale installations worldwide that use partial nitritation/anammox (PN/A) for biological nitrogen removal. In this process, the need for organic

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangdong_2011@aliyun.com (D. Zhang), daixiaohu@tongji.edu.cn (X. Dai).

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carbon, aeration, and sludge production are lower by 100%, approximately 60%, and approximately 90%, respectively, compared to traditional nitrification–denitrification processes, making PN/A an energy saving technology (Gilbert et al., 2015; Lackner et al., 2014). The wastewater streams currently treated with PN/A have relatively high nitrogen concentrations (NH_4^+-N>500 mg N/L) with relatively high temperatures (>30 °C)(Gilbert et al., 2015; Hendrickx et al., 2012). Successful application at lower temperatures and nitrogen concentrations (mainstream system) would expand its applications in municipal sewage treatment, creating new possibilities for the design of energy-producing wastewater treatment plants (WWTPs) (Gilbert et al., 2015; Lotti et al., 2015). Anammox is the one of the key processes for the successful application of PN/A in mainstream reactors and there had been much research in recent years on the performance of anammox in these reactors. Researchers enriched a cold-adapted anammox species and, fed with 61 mg (NH_4^+ + NO_2^-)-N/L, operated at 10 °C. *Candidatus Brocadia fulgida* was the dominant species in the enriched biomass, with a specific activity of 30–44 mg N/(g VS·d)(Hendrickx et al., 2014, 2012). Lotti et al. (2014b) operated an anammox reactor for more than 10 months at 10–20 °C. The volumetric nitrogen removal rates obtained by the system were comparable to or higher than those of conventional nitrogen removal systems, with values higher than 0.4 g N/(L·d) when operated at 10 °C; *Candidatus Brocadia fulgida* was the dominant species. They also reported a reactor operated at 10–20 °C using ammonium (60 and 160 mg N/L) as the only nitrogen compound, at a hydraulic retention time (HRT) of 0.23–0.3 d (Lotti et al., 2014a). Laureni et al. (2015) studied the activity and growth of anammox biomass on aerobically pretreated municipal wastewater and these investigations strongly supported the feasibility of municipal wastewater treatment via anammox. Gilbert et al. (2014) used a moving bed biofilm reactor (MBBR) with a carrier material to test the tolerance of the overall PN/A process to temperatures ranging from 10 to 20 °C. They found that an MBBR, with its 10 mm thick biofilm carriers, was sufficient to sustain enough biomass to allow anammox activity at even 10 °C.

The research described above demonstrated that anammox can remain active at low temperatures and that mainstream reactors can be operated successfully. However, starting up those reactors often took a long time as temperature was decreased gradually to prevent the inhibition of anammox activity. Additionally, temperature varies with time in municipal sewage treatment and the effect of drastic temperature fluctuations (TFs) in a short time has not been evaluated.

The objectives in this study were to explore the quick startup of mainstream reactors strategy and to study the effect of TFs on mainstream reactors. It was supposed that the activity of anammox cultivated under high substrate concentrations (NH_4^+-N and NO_2^--N concentration) would gradually decrease when anammox is placed in a mainstream systems and then finally reached a stable state. It would adapt to the mainstream condition more easily than anammox cultivated under the low substrate condition, so quick startup of mainstream reactors should be achievable. Firstly, high-activity anammox cultivated at 35 °C to treat wastewater with high substrate concentration (NH_4^+-N, 150 mg/L; NO_2^--N, 200 mg/L) was used to start up mainstream reactors (operated at 15 °C or 20 °C; NH_4^+-N, 30 mg/L; NO_2^--N, 40 mg/L) directly. One reactor at 35 °C with low substrate concentration (NH_4^+-N, 30 mg/L; NO_2^--N, 40 mg/L) was also set up. Cytochrome cd1 nitrite/nitric oxide oxidoreductase (nirS) and hydrazine dehydrogenase (hdh) are the key enzymes of anammox. In this study, mRNA of *nirS* and *hdh* was detected to assess the effect of temperature and concentration shift on their expression. Secondly, the effect of drastic TFs (temperature change by more than 5 °C in this research) on mainstream reactors performance was tested. Thirdly, a second method of quick start up mainstream reactor was proposed as contrast strategy. Anammox cultivated with low substrate at 35 °C was used for start up at 15 °C and the reactor was operated for 60 days.

2. Material and methods

2.1. Reactor start up using anammox cultivated with high substrate concentration and operation performance

Continuous stirred tank reactors (CSTR) were used in all of the experiments. The reactors operating at low temperatures and influent concentrations were started up with high-activity seed sludge (439 mg NO_2^--N/(g VSS·d)) that was cultivated in the laboratory for more than 1.5 years. The temperature of the seed sludge reactor was maintained at approximately 35 °C and the concentrations of NH_4^+-N and NO_2^--N in influent were approximately 150 and 200 mg/L, respectively. The diameter of the sludge particles was approximately 1–2 mm. The total suspended solid (TSS) content in the seed sludge was approximately 56.5 g/L, and volatile suspended solids (VSS) accounted for 84.1% of the sludge.

To start up the mainstream reactors, approximately 100 mL of seed sludge without further treatment was placed in two 1.5 L reactors (TSS 3.77 g/L, VSS 3.17 g/L), denoted as Rc and Rb, respectively. The temperatures of these reactors were maintained at 15 °C and 20 °C, respectively. NLR was adjusted through the control of the influent flow. Mixing in the reactor was achieved by a mechanical stirrer operated at a fixed rotating speed of 60 rpm. A third reactor, Ra, was started up simultaneously at 35 °C. The reactors were placed in three water baths with different temperature. Heating rods were used to heat the water baths and temperature was controlled at 35 °C. Two refrigerators were used to keep the temperature of the respective water baths at 15 or 20 °C. A schematic diagram of the CSTR system used in this study is shown in Fig. S1 (a). During 40 d of operation, NH_4^+-N and NO_2^--N in the effluent was maintained at approximately 4–7 mg/L to prevent substrate limitation.

2.2. Effect of TFs and reactors startup using anammox cultivated with low substrate concentration

After about 40 d of operation, all three reactors reached a stable state. Then, the effects of TFs on Ra, Rb and Rc performance were tested for two cycles. Each cycle included a 7 d TF phase and a 7 d recovery phase. The reactors were moved to a water bath of different temperature to change the temperature of the reactor and moved back to the original water bath for recovery.

In each cycle, when temperature decreased (at TFs phase or recovery phase), anammox activity was tested as soon as possible (0 h) to examine the temperature shock and then tested again after 24, 48, 72 and 168 h; when temperature increased (at TFs phase or recovery phase), anammox activity test was tested after 24, 48, 72, 96 and 168 h.

When this process was finished a second temperature change was performed; once again, it lasted 7 d before returning to the initial state. After this test, Rc and Rb remained stable for 60 days. The temperature of Ra decreased sharply to 15 °C and it became a low temperature reactor for 60 days. The experiment scheme is shown in Fig. S1 (b). Details on the temperature and NLR change are given in Table S1.

2.3. Synthetic wastewater

The following compounds were added to synthetic water: $KHCO_3$, 0.4 g/L; KH_2PO_4, 0.03 g/L; $CaCl_2·2H_2O$, 0.1 g/L; and $MgSO_4·7H_2O$, 0.30 g/L. The trace elements were adapted from Van