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Short Communication

Enhancement of anammox activity by addition of compatible solutes at high salinity conditions

Mu Liu, Yongzhen Peng *, Shuying Wang, Tiantian Liu, Han Xiao

Key Laboratory of Beijing for Water Quality Science and Water Environment Recovery Engineering, Engineering Research Center of Beijing, Beijing University of Technology, Beijing 100124, PR China

HIGHLIGHTS

- Domestication time was shortened by 1.65 times by CS addition.
- Higher NRR, higher TTC-DHA and lower EPS was observed with CS addition.
- Microbial population structure was promoted by CS addition.
- CS addition was feasible to counteract saline inhibition of anammox.

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ABSTRACT

The enhancement effect of compatible solutes on anammox activity under salinity stress was investigated. Glycine betaine (GB) was the most effective in alleviating salt toxicity, although all the compatible solutes (GB, trehalose and ectoine) were found to be valid. Acclimation potential of anammox biomass under salinity of 30 g/L increased significantly with GB addition. The recovery time in the reactor with GB addition (R_B) (49 days) accompanied by a more stable stoichiometric ratio was 2.65 times shorter than in the control reactor (R_C) (130 days). After 49 days, the extracellular polymeric substances and the tetrazolium chloride-dehydrogenase activity were 217.9 mg/gVSS and 38.7 μg TF/gVSS/h in R_B, 1.86 times lower and 3.17 times higher than the levels in R_C, respectively. R_B possessed evident superiority in the aspects of microbial population proportion. And thus, compatible solutes addition was regarded as one of the feasible solution to counteract saline inhibition on anammox.

1. Introduction

Anaerobic ammonium oxidation (anammox) process is recognized as a novel, environmentally friendly and cost-effective technology (Jin et al., 2012). However, the widespread application of anammox process is limited by long start-up period, the low temperature, the presence of organic matters, high salinity and other inhibitors. The high salt in saline wastewater originated from many industries can induce cell plasmolysis or death due to the dramatic increase in osmotic pressure and thus may alter biochemical properties of activated sludge or the microbial community structure (Ma et al., 2012).

Currently, the acclimatization of anammox to saline condition is supposed to be one of the main methods to reduce salt inhibition. Kartal et al. (2006) found that anammox bacteria acclimated to salinity conditions can be used to treat up to 75 g/L initial NaCl concentration. However, Liu et al. (2009) reported that even with long-term domestication, freshwater anammox sludge cannot tolerate salinity higher than 30 g/L. Moreover, there are massive swings in salinity for many industrial wastewaters, while a gradual and long exposure to salinity is essential to achieve high anammox activity with saline wastewater (Jin et al., 2012).

In addition, the addition of compatible solutes (CS) has previously been shown to encourage sludge activity under saline condition. CS are low molecular-weight organic compounds, named so because they do not interact with macromolecules in detrimental ways. They can increase the cell's osmotic potential and result in the maintenance of an appropriate turgor pressure (Cyplik et al., 2012). Yerkes et al. (1997) demonstrated that 1 mM of glycine betaine (GB), one of the CS, could create a positive effect on pure cultures of *Methanosarcina* and *Methanosaeta* under highly saline conditions. Vyrides et al. (2010) found that methane produced during batch anaerobic digestion in salt toxicity level (35 gNaCl/L) was

* Corresponding author. Tel./fax: +86 10 67392627.
E-mail address: pyz@bjut.edu.cn (Y. Peng).

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three times higher when GB was added. Nevertheless, to the best of our knowledge, no studies have focused on the effect of CS addition on the anammox process for the treatment of saline wastewater.

Hence the main objective of this work was to examine the possibility of enhancing anammox activity under saline conditions by CS addition. Specific anammox activity (SAA), nitrogen removal rate (NRR), stoichiometric ratios, tetrazolium chloride-dehydrogenase activity (TTC-DHA) and extracellular polymeric substances (EPS) were examined as indicators to explore the feasibility and underlying mechanism of this strategy. Also, fluorescent in situ hybridisation (FISH) analysis was manipulated to investigate the relationship between performance and evolution of microbial community structure.

2. Methods

2.1. Parent reactor operation

Synthetic wastewater was prepared according to Miao et al. (2014). $(NH_4)_2SO_4$ and $NaNO_2$ were added to control the NH_4^+-N and NO_2^--N at 200 and 260 mg/L, respectively. Anammox biomass obtained from a pilot-plant UASB was introduced to a sealed SBR (50 L) as parent reactor (PSBR) with a volatile suspended solids (VSS) of 8.45 g/L. The PSBR was fed with synthetic wastewater and operated with three cycles each day for 12 months at a NRR of over 2.0 kg N/m^3/d. The initial pH was fixed at 7.5 and temperature at 33 ± 1 °C. Each cycle (480 min) consisted of 10 min feeding, 360 min mixing, 40 min settling, 10 min drainage, and 60 min idle time and the exchange volume was fixed at 25%.

2.2. Experimental design

Short-term effect of CS on anammox biomass from the PSBR at different salinity was conducted by SAA test with VSS of 1 g/L. Initial concentrations of substrates were 70 mg/L of NH_4^+-N and 70 mg/L of NO_2^--N. According to CS concentration (0.1, 1 and 5 mM), SAA test was divided into three groups. Each group was carried out by adding the following CS separately to the medium: GB ($C_5H_{11}NO_2$), trehalose ($C_{12}H_{22}O_{11}$) and ectoine ($C_6H_{10}N_2O_2$) under five salinity conditions adjusted by adding NaCl. The CS were provided by Sangon Biotech (Shanghai) Co., Ltd. In addition, a control test was also conducted without addition of CS at different salinity. All experiments were repeated three times.

To investigate long-term effect of CS, two identical sealed SBR (6 L) were inoculated from the PSBR with VSS of 8.26 g/L. The two SBR, one with the addition of 1 mM GB (R_B) and a control reactor without any CS (R_C), were fed with the same synthetic wastewater and operated with the same mode as PSBR at salinity of 30 g/L for 181 days except that the biomass was washed three times with synthetic wastewater to maintain a stable medium before the start of each cycle. The initial pH was fixed at 7.5 and temperature at 33 ± 1 °C both in short-term and long-term test.

2.3. Analytical methods

TN, NH_4^+-N, NO_2^--N, NO_3^--N and VSS were analyzed according to standard methods (APHA, 1998). SAA was conducted according to Tang et al. (2009). The TTC-DHA was determined according to Filipic et al. (2012). The EPS was extracted using the heating method according to Adav and Lee (2008). Carbohydrates in the EPS were taken by the anthrone method with a glucose standard. Proteins were determined with the Lowry method using bovine serum albumin as a standard. FISH was conducted using EUB338 probe special to all the eubacterium, plus AMX368 specific to the anammox bacteria and Azo664, Thau646, Curvi997 specific to denitrifying bacteria, respectively. Sample fixation and hybridization steps of FISH were carried out as described by Kartal et al. (2007).

3. Results and discussion

3.1. Addition of different CS to anammox biomass at different salinity

As depicted in Fig. 1, CS addition barely resulted in any enhancement under low salinity (5–10 g/L) and significantly enhanced SAA at salinity of 15–30 g/L. In addition, GB was the most effective in coping with a sudden increase in the salinity on anammox biomass, followed by trehalose and then ectoine. For instance, at salinity of 25 g/L, the SAA with the addition of 1 mM GB, trehalose and ectoine was 12.4, 11.8 and 10.9 mg N/gVSS/h and increased by nearly 59.0%, 51.3% and 39.7% over the control (7.8 mg N/gVSS/h), respectively. According to the theory put forward by Vyrides et al. (2010), it can be inferred that GB may be the most easy to be transported through the membranes and absorbed by the anammox bacteria, followed by trehalose and then ectoine. Furthermore, 1 mM GB addition (Fig. 1(b)) had a more positive effect on reducing salt inhibition compared with 0.1 mM GB (Fig. 1(a)), however, no further obviously promotion was observed with 5 mM GB (Fig. 1(c)). Thus 1 mM GB addition

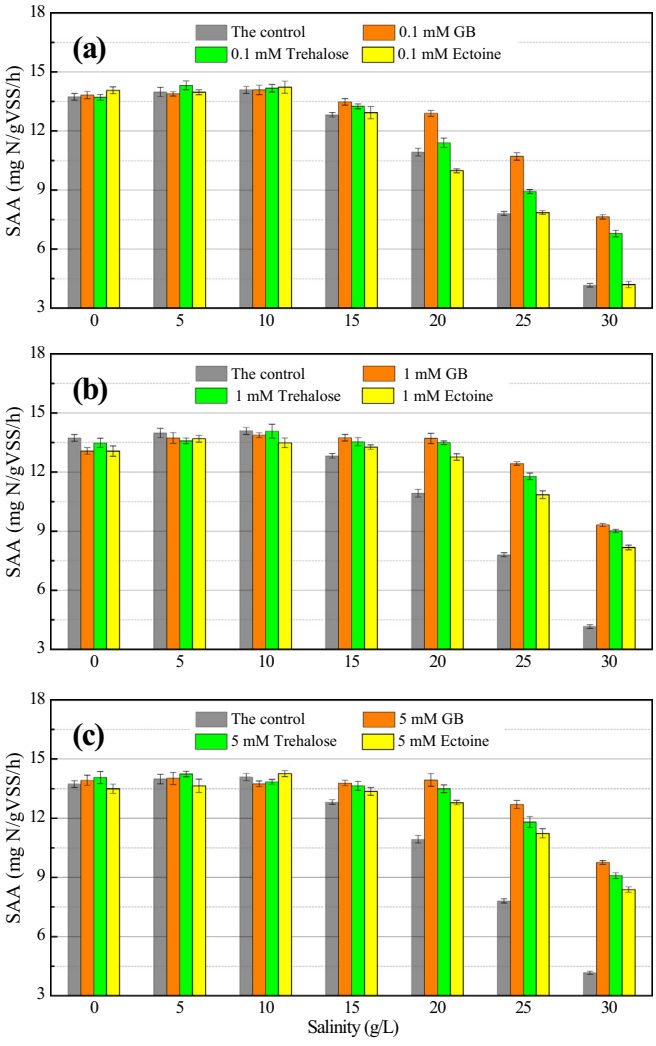

Fig. 1. SAA of anammox biomass at different salinity with (a) 0.1 mM, (b) 1 mM and (c) 5 mM of GB, trehalose and ectoine added.