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Research article

Effects of gibberellin on the activity of anammox bacteria

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ABSTRACT

The enhancement of gibberellin (GA) on the activity of anaerobic ammonium oxidation(anammox) bacteria in short-term batch experiments(500 mL serum bottle) was studied in this paper. To make sure the accuracy of the data, each experiment group was conducted some statistical analysis. The results showed that GA played an important role in improving anammox activity when the GA dosage ranged from 0.1 to 1.5 mg L^{-1}, and the total nitrogen removal rate (NRR) was increased by 34% when the GA dosage was 1 mg L^{-1}. The monitoring results of extracellular polymeric substances (EPS) and biomass of anammox bacteria indicated that GA addition improved the secretion of EPS and the biomass increasing, whose amount achieved maximum under the GA dose of 1 mg L^{-1}. Compared to the control test, the maximum improvement ratio of the EPS and biomass was 28.6% and 34%, respectively. In addition, the cloning results also indicated that the anammox bacterial community structure shifted in species level of *Candidatus Brocadia* genus during the experiment, and the most dominant anammox bacteria were *Candidatus Brocadia fulgid*.

1. Introduction

Anammox is a novel biological process of converting NH_4^+ and NO_2^- into N_2 under anaerobic conditions (Strous et al., 1999). Compared to conventional nitrification and denitrification process, it produces less sludge, CO_2 and N_2O with no need of oxygen and external carbon source, thus saving operational costs (Zekker et al., 2012a; Tenno et al., 2016, 2017, 2018). Therefore, the research of anammox has received increasing attention (Kimura et al., 2011), and there were more than 100 full-scale anammox plants put into operation worldwide (Lackner et al., 2014). In complete WWTP partial nitrification can be used with respective oxygen consumption, nevertheless deammonification occurs with denitrification in real conditions and can be applied, such as partial nitritation-anammox process, which also reduced consumption and lower organic carbon demand (Rikmann et al., 2018; Zekker et al., 2012a). Although anammox bacteria are widely distributed in natural ecosystems (Zhu et al., 2013), the cultivation of anammox bacteria is still facing challenges in practical applications. The major obstacle is the slow growth of anammox bacteria whose doubling-time reaches nearly 11 days (Strous et al., 1998), which largely extends the start-up time of anammox process (Joss et al., 2009). Besides, anammox bacteria are sensitive to external environment and usually inactivated by many factors, such as substrate, DO, pH, temperature, and illumination (Raudkivi et al., 2017; Zekker et al., 2015; Zhang et al., 2016; Jin et al., 2012).

In order to shorten the start-up time of anammox reactors and enrich anammox bacteria, many researchers have employed various methods to retain anammox biomass in the system or to accelerate the activity of anammox bacteria (Ibrahim et al., 2016). These methods mainly include the design of reactors (Jin et al., 2008), the application of carriers (Tomar and Gupta, 2016), the addition of anaerobic granular sludge (Tomar et al., 2015), and other physical and chemical methods (Yin et al., 2015a). The up-flow anaerobic sludge blanket (UASB) was found to be a suitable anammox reactor, where the maximum NRR reached 74.3–76.7 kg N m^{-3} day^{-1} (Tang et al., 2011). Other types of anammox treatment systems could be noted as a promising wastewater treatment technology, For example, moving bed biofilm systems, which used buoyant carrier media as a biofilm growth support material and retained anammox bacteria effectively (Zekker et al., 2012b,c). Also, some municipal water treatment experiments were conducted at low temperature in biofilms as comparison at −5, 10 and 15 °C, which could be used also as a source of nutrient removal tank (Daija et al., 2016). In addition, some kinds of metal ions have been verified to be useful for enhancing anammox bacterial activity, such as Fe(II), Fe(III), Cu(II), and Ni(II) (Liu and Horn, 2012; Chen et al., 2014). Yin et al. (2015a) discovered that electric field could enhance the activity of

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anammox bacteria and increase the crude enzyme activities and cell quantities. Duan et al. (2011) indicated that the maximum NRR could be increased by 25.5% when the ultrasound intensity was 0.3w cm^{-2}. Wang et al. (2013) and Yin et al. (2015b, 2016) enhanced the activity of anammox bacteria by adding graphene oxide in system, with an increase of the maximum NRR of 27.4%.

GA, a kind of plant hormone, can regulate the growth of plant and impact various developmental stages, including stem elongation, germination, seed dormancy, flowering, sex expression, aging leaves and suppression of fruits. The most prominent role of GA is to accelerate cell elongation and promote cell division and expansion (Cui et al., 2000). However, few studies have addressed the effects of GA on the bacteria. Wu (2012) showed that GA could accelerate the growth and activity of photosynthetic bacteria by promoting cell metabolism, increasing nutrient uptake, and strengthening cell viability. Liu et al. (2013) have shown that GA has a significant role in promoting the growth of rhizobia. Furthermore, no studies have ever considered the effect of GA on the anammox bacteria so far.

Hence, the present study is aimed at investigating the short-term impacts of GA on anammox bacterial activity and evaluating the changes in the properties of anammox granular sludge under the stimulation of GA. Additionally, the effect of different concentrations of GA on the microbial community structure of anammox bacteria was examined. This research may provide a better understanding of the role of GA in the anammox process, which might explore a good way for improving the activity and growth of anammox bacteria, as well as increasing the potential for solving the problem of the lack of anammox biomass.

2. Materials and methods

2.1. Seed sludge and synthetic wastewater properties

The anammox sludge was taken out from the stably laboratory-operated SBR reactor whose sludge concentration was 4300 mg L^{-1}, nitrogen loading rate (NLR) was 120 g $N{\cdot}m^{-3}{\cdot}day^{-1}$ and NRR was 39.6 mg $N{\cdot}g^{-1}$ VSS$\cdot day^{-1}$ after 200 days of operation. In addition, the temperature and the rotate speed of the SBR reactor were controlled at 30 ± 1 °C and 140 rpm, respectively. The anammox sludge obtained was washed in phosphate buffer (0.14 g L^{-1} KH_2PO_4 and 0.75 g L^{-1} K_2HPO_4) to eliminate the influence of nitrogen background value. Then the mixed liquor was transferred to 50 mL centrifuge tubes and centrifuged at 4000 rpm for 3min. The supernatant was abandoned, and granular sludge was recycled in the phosphate buffer. The above steps were repeated twice. In this study, we used a serum bottle as the anammox reactor, whose total volume and liquid-phase volume were 500 mL and 450 mL, respectively. Each serum bottle was inoculated with 2 g sludge (wet weight), and the initial biomass concentration was approximately 0.755 g volatile suspended solids (VSS) L^{-1} in each serum flask. The inoculum sludge was dark brown with a VSS/TSS ratio of 0.73 and SVI of 66 mL g^{-1}. The characteristics of inoculum sludge are depicted in Table 1. Synthetic wastewater was used in this study, and the initial concentrations of substrates were 50 mg L^{-1} of NH_4^+-N ($(NH_4)_2SO_4$) and 65 mg L^{-1} of NO_2^--N ($NaNO_2$). The composition of the mineral medium was (g L^{-1}) KH_2PO_4: 0.0065; $NaHCO_3$: 1.85; $FeSO_4{\cdot}7H_2O$: 0.018; $MgSO_4{\cdot}7H_2O$: 0.2; $CaCl_2{\cdot}2H_2O$: 0.3. Composition of trace element solution (mg L^{-1}) was as follows: EDTA: 1500; $ZnSO_4{\cdot}7H_2O$: 430; $CoCl_2{\cdot}6H_2O$: 240; $MnCl_2{\cdot}4H_2O$: 990; $CuSO_4{\cdot}5H_2O$: 250; $NaMoO_4{\cdot}2H_2O$: 220; $NiCl_2{\cdot}2H_2O$: 190; $H_2SeO_4{\cdot}10H_2O$: 210; H_3BO_4: 14; and 1 mL trace element solution was added into 1 L water every day.

Table 1
Physical characteristics of inoculum and granular sludge.

Parameters	Inoculum sludge
Sludge source	Laboratory-operated SBR anammox sludge
Influent wastewater	Synthetic Wastewater
Sludge volume index (SVI), mL g^{-1} SS	66
VSS g L^{-1}	0.755
VSS/TSS ratio	0.73
Temperature	Ambient(30 °C)

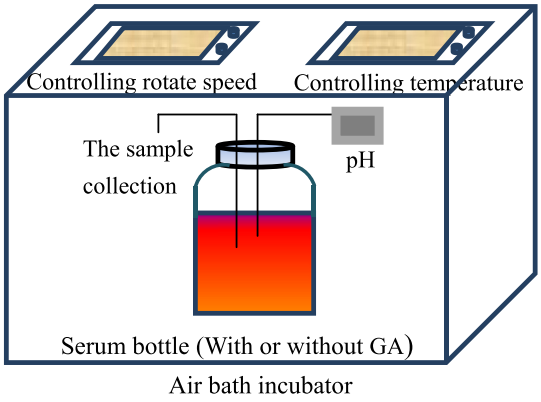

Fig. 1. Schematic diagram of the reactor configuration.

2.2. Experimental procedures

Batch tests were carried out to compare the activity of anammox bacteria with and without additional GA. The serum bottle used in the experiment was shown as Fig. 1. Glass serum flasks were specially designed to avoid the influence of external O_2, and these bottles were wrapped with aluminum foil to avert the adverse effect of the light on the anammox bacteria. All test bottles were incubated in an air bath incubator (Chang zhou, China) at 30 ± 1 °C, and the rotate speed was controlled at 140 rpm. The pH value was adjusted to 7.5 ± 0.01 with HCl (1 mol/L) and NaOH (1 mol/L) and the initial alkalinity was 250 mg L^{-1}. Afterwards, the reactor was purged with nitrogen to eliminate O_2 for 10 min. The bottle without GA was served as control, and the rest four groups of experimental samples were added with different doses of GA to investigate GA's effect on the activity of anammox bacteria. The NLR of the influent was relatively low due to the fact that the synthetic water was meant to simulate domestic sewage. Each serum bottle was conducted for duration of 9 days to avoid potential result of a low nitrogen removal performance. Each of experimental conditions was triplicated in this study, and the average value and the standard deviation were calculated for analysis.

2.3. The analysis of anammox bacteria activity

Batch experiments were performed with glass serum bottles to measure the NRR,Extracellular polymers (EPS), and the gene copies number of anammox bacteria. And then the short-term impact of GA stimulation on anammox activity was analyzed using these results.

2.4. Analytical methods

Water samples were collected and filtered through 0.45 μm filter membranes every day to determine the nitrogen concentrations, which were analyzed according to standard methods (APHA, 2012). The biomass concentration (VSS) of sludge was also measured through standard methods (APHA, 2012). The EPS were extracted through the methods described by Yu et al. (2013), and the main components, protein (PN) and polysaccharide (PS), were analyzed according to the methods described by Lowery et al. (1951) and Dubois et al. (1956) respectively. GA concentrations were determined using High