Metabolic activity of an anammox population affected by major environmental factors

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Abstract: Anammox is known as a cost-effective approach to nitrogen removal from ammonium-rich wastewater. However, the eco-physiological characteristics of anammox population have not well been understood so far, which has obstructed the practical application of anammox process. In the present research work, an enrichment culture of anammox population was collected from an expanded granular sludge bed (EGSB) reactor, and the anammox activity was evaluated by various pH, temperature and reactant density, progressively. The results showed that the optimal ecological amplitude of pH and temperature for the anammox population was ranged from 7.0-7.5 and 30℃-35℃, respectively. To make anammox process more efficient, the density of NH_4^+-N and NO_2^--N should be around 10.0 and 4.0 mmol·L^{-1}, respectively. It was found that anammox population was more sensitive to NO_2^--N density than NH_4^+-N density, and then NO_2^--N density was proposed as a key indicator to practicing anammox process in engineering. With the findings, the present research will be significant in practicing annamox process.

1 Introduction

Water eutrophication has been widely considered as a major environmental issue all around the world. For the restoration of natural water environment, discharge standards of ammonia (NH_4^+-N) and total nitrogen (TN) have become stricter than ever before in China. Though combined anoxic-aerobic (A/O) processes have been extensively practiced in engineering for nitrogen removal from various wastewater, the managers are still suffering from the high engineering investment and operating costs. The A/O processes are constructed based on the traditional theory of biological denitrification. In other words, NH_4^+-N has to be oxidized to nitrite (NO_2^--N) or nitrate (NO_3^--N) in the aerobic reactor, which would be returned to the anaerobic reactor for heterotrophic denitrification [1,2]. Therefore, the traditional nitrification-denitrification processes not only requires higher energy consumption but also exhibits a limitation in TN removal when the organics in wastewater could not meet the requirement of heterotrophic denitrification [3]. Though the next developed shortcut nitrification-denitrification process has obviously reduced the energy consumption in wastewater treatment process, the nitrogen removal efficiency is still unsatisfactory [4].

The novel anammox is considered as the most cost-effective biological nitrogen removal process in which no organic carbon source and aeration are required [5]. In the anammox process, anammox bacteria use NH_4^+ and NO_2^- as the electron donor and electronic acceptor, respectively, to form NO_3^- and gaseous nitrogen (N_2) under anaerobic conditions, as expressed as the following equation [6].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.12H^+ \rightarrow$$
$$1.0N_2 + 0.26\,NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$

Though aeration is still required to oxidize partial NH_4^+ to NO_2^- in ammonium-rich wastewater treatment, practicing anammox has attracted an increasing interesting all around the world because of the advantages as follows [7-11]. 1) The nitrogen removal efficiency is no longer restricted by organic carbon source in wastewater; 2) energy consumption is remarkably reduced because of the less oxygen supplement; 3) less excess sludge will be produced in the treatment process.

However, anammox bacteria are very difficult to be enriched in organic wastewater treatment processes because of their slow growth [12], though widely distributed in natural environment. Therefore, it is significant for practical application to understand the environmental factors affecting the metabolic activity of anammox bacteria [13-18]. Unfortunately, any pure culture of anammox bacteria has not been obtained so far, which is a serious impediment to understanding the eco-physiological characteristics of anammox bacteria [19].

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In the present research work, an enrichment culture of anammox bacteria was obtained from an expanded granular sludge bed (EGSB) reactor. And then the effects of pH, temperature and density of reactants (NH_4^+-N and NO_2^--N) as the key environmental factors were investigated on the activity of annamox population by batch tests. With the findings of the optimal conditions for annamox population, the present research will be helpful to practice annamox in engineering.

2 Materials and methods

2.1 Basic medium for anammox bacteria

The basic medium for the enrichment of anammox bacteria was an inorganic salt culture medium composed of (per liter): $(NH_4)_2SO_4$ 0.33 g, $NaNO_2$ 0.35 g, $KHCO_3$ 1.50 g, KH_2PO_4 0.03 g, $CaCl_2 \cdot 2H_2O$ 0.14 g, $MgSO_4 \cdot 7H_2O$ 0.20 g, and microelement solution 1.0 mL. The pH of the medium was above 7.0 naturally. The microelement solution including per liter: EDTA 5.00 g, $FeSO_4$ 5.00 g, $ZnSO_4 \cdot 7H_2O$ 0.43 g, $CoCl_2 \cdot 6H_2O$ 0.24 g, $MnCl_2 \cdot 4H_2O$ 0.99 g, $CuSO_4 \cdot 5H_2O$ 0.25 g, $NiCl_2 \cdot 6H_2O$ 0.19 g, H_3BO_3 0.014 g, $Na_2MoO_4 \cdot 2H_2O$ 0.22 g, $NaSeO_4 \cdot 10H_2O$ 0.21 g.

2.2 Enrichment culture of anammox population for batch tests

The enrichment culture of anammox bacteria for batch tests was collected from an expanded granular sludge bed (EGSB) reactor. Fed with the basic medium containing NH_4^+-N and NO_2^--N of the same 20.0 $mmol \cdot L^{-1}$, the EGSB had been continuously operated for over 270 days with a constant hydraulic retention time of 8 h at 30℃. Removal of NH_4^+-N and NO_2^--N in the last steady phase was kept at about 91.5% and 98.3%, respectively. The enrichment culture was brick-red granules with a specific NH_4^+-N and NO_2^--N removal of about 2.90 and 3.48 $mmol \cdot g^{-1} \cdot d^{-1}$ in terms of mixed liquid volatile suspended solids (MLVSS).

The enrichment culture collected from the EGSB was washed for 3 times with $NaHCO_3$ buffer (pH 7.1) to remove the residual matrix and then used as inoculum for the batch tests. Biomass in the inoculum was about 5.85 $g \cdot L^{-1}$.

2.3 Experimental process

Effect of pH, temperature and the density of NH_4^+-N and NO_2^--N on the metabolic activity of anammox population in the enrichment culture were evaluated by batch tests, progressively. All of the batch tests were conducted in 50 mL anaerobic bottles. Each of the bottle was load with 28 mL basic medium and then inoculated with 2 mL of the pretreated enrichment culture.

With the same 5.0 $mmol \cdot L^{-1}$ of NH_4^+-N and NO_2^--N in the basic medium, nine pH gradients within 5.0-9.0 were tested firstly. At the obtained optimal pH, seven temperature gradients ranged from 4℃-50℃ were further evaluated. With the obtained optimal pH and temperature, effect of reactants densities, i.e. the concentration of NH_4^+-N and NO_2^--N, on the metabolic activity of anammox population were finally investigated. Seven NH_4^+-N gradients within 2.0-16.0 $mmol \cdot L^{-1}$ and eight NO_2^--N gradients within 0.5-6.0 $mmol \cdot L^{-1}$ were tested with a constant NO_2^--N or NH_4^+-N of 5.0 $mmol \cdot L^{-1}$, respectively.

Each of the tests was conducted in triplicate. All of the constructed anaerobic bottles were incubated for seven days in a constant temperature air bath shaker (HZQ-C, Harbin Donglian Electronic Technology Development Co. Ltd., China) at 120 rpm. Except the temperature tests, the other anaerobic bottles were incubated at 30°C.

2.4 Analytical methods

Supernatant in each of the anaerobic bottles was sampled once per six hours for the determination of pH, NH_4^+-N, NO_2^--N and NO_3^--N. In addition to MLVSS, all of the analysis items were also conducted following the standard methods [20]. Metabolic activity of the enrichment culture in each of the anaerobic bottles was evaluated by specific anaerobic ammonium removal rate in terms of MLVSS ($mmol \cdot g^{-1} \cdot d^{-1}$), which was calculated based on the data collected from the logarithmic phase, normally in the first day.

3 Results and discussion

3.1 Effect of pH on the activity of anammox population

It is well known that pH is one of the most important environmental factors on the growth and metabolism of microorganisms. The enrichment culture of anammox population was collected from the EGSB and the influence of pH on the anammox activity was firstly investigated by batch tests. The results (Fig. 1) showed that a weakly alkaline environment was more favourable to anammox activity in the enrichment culture. As shown as Fig. 1, the anaerobic ammonium removal rate was gradually enhanced with the pH increased from 5.0 to 7.5. When the pH was over 8.0, the anaerobic ammonium removal rate was decreased remarkably. Though anammox activity could be kept by the enrichment culture at the pH of as low as 5.0 or as high as 9.0, the optimal ecological amplitude was identified as 7.0-8.0 with the anaerobic ammonium removal rate ranged from 0.14-0.17 $mmol \cdot g^{-1} \cdot d^{-1}$. Similarly, Daverey et al. reported that the maximum specific anammox activity was observed at pH of 7.5 [21]. Moreover, a wider optimum pH range of 6.5-9.3 was also found for the growth and activity of anammox bacteria [22]. In summary, anammox bacteria are sensitive to pH changes, and pH control is important during process operation.

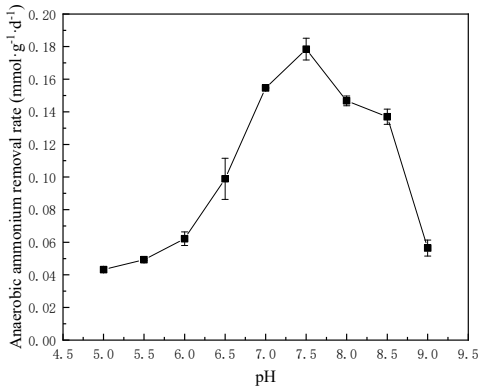

Fig. 1. Anaerobic ammonium removal rate of the enrichment culture with the increase of pH

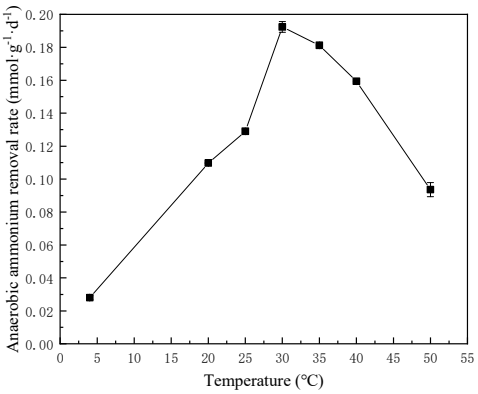

Fig. 2. Anaerobic ammonium removal rate of the enrichment culture with the increase of temperature

3.2 Effect of temperature on the activity of anammox population

As another important environmental factor on the growth and metabolism of microorganisms, the effect of temperature on anammox activity in the enrichment culture was further evaluated at pH 7.0. The results (Fig. 2) showed that pH also had an obvious effect on the anammox population. As illustrated in Fig. 2, the anaerobic ammonium removal rate of the enrichment culture was improved rapidly along with the temperature increased from 4℃ to 30℃. When the temperature was increased from 30℃ to 35℃, the anaerobic ammonium removal rate was slightly decreased from 0.19 to 0.18 $mmol \cdot g^{-1} \cdot d^{-1}$. However, a sharp decrease in anaerobic ammonium removal rate was observed when the temperature was further increased to over 40℃. The inhibition of anammox activity at the higher temperature was likely due to biomass lysis. Also operated at 30℃, the anaerobic ammonium removal rate in the EGSB was as high as 2.90 $mmol \cdot g^{-1} \cdot d^{-1}$ (Section 2.2). The results suggested that the enzymes involved in anammox were most active at the temperature around 30℃ [23]. This is consistent with the previous studies reporting that the optimal temperature for most anammox species used in wastewater treatment are between 30°C and 40°C [24]. As known, the optimum temperatures for the two typical anammox species i.e. *Candidatus Brocadia anammoxidans* and *Candidatus Kuenenia stuttgartiensis* were reported to be 40°C and 37°C, respectively [24]. It can be concluded that the dominant anammox bacteria in the enriched anammox population could be *Candidatus Kuenenia stuttgartiensis*.

In addition, temperature along with pH also affect the concentration of free ammonium (NH_3) and free nitrous acid (HNO_2) which can strongly inhibit the anammox process. However, the mechanisms of the anammox activity affected by NH_3 and HNO_2 are still unclear. Furthermore, the threshold values of NH_3 and HNO_2 for suppressing anammox bacteria are various reported in previous studies [22]. Therefore, in next step study, the inhibition mechanisms and threshold values of the NH_3 and HNO_2 need to be further investigated.

3.3 Effect of reactant density on the activity of anammox population

NH_4^+ and NO_2^- are the dominant reactants served as electron donor and electronic acceptor, respectively, in anammox reaction [6]. And it is known that the specific substrates have an outstanding effect on the growth and metabolism of microorganisms. Therefore, the effects of NH_4^+-N or NO_2^--N density on the metabolic activity of anammox population were further evaluated at the optimal pH 7.0 and temperature 30℃.

Fig. 3 illustrated the effect of NH_4^+-N density on the anaerobic ammonium removal rate of the enrichment culture with a constant NO_2^--N density of 5.0 $mmol \cdot L^{-1}$. The results indicated that the anaerobic ammonium removal rate of the enrichment culture was remarkably enhanced from 0.29 to 0.64 $mmol \cdot g^{-1} \cdot d^{-1}$ with the NH_4^+-N density increased from 0.5 to 10.0 $mmol \cdot L^{-1}$. When the NH_4^+-N density was further increased to 12.0 and 16.0 $mmol \cdot L^{-1}$ step by step, the anaerobic ammonium removal rate was severely dropped to 0.59 and 0.46 $mmol \cdot g^{-1} \cdot d^{-1}$, respectively. The results suggested that the NH_4^+-N density of 10.0 $mmol \cdot L^{-1}$ was more favorable for conducting anammox process.

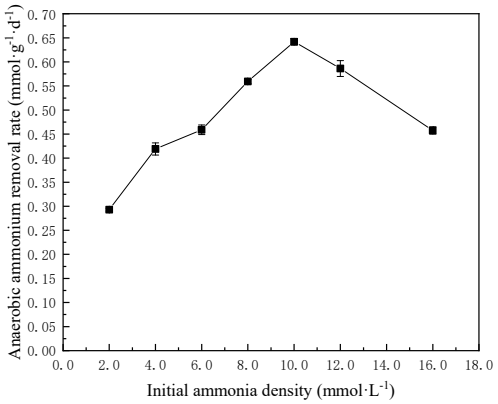

Fig. 3. Anaerobic ammonium removal rate of the enrichment culture with the increase of NH_4^+-N density

Contrary to the NH_4^+-N density, the NO_2^--N density less than 2.0 mmol·L^{-1} had no obvious effect on the anammox activity of the enrichment culture (Fig. 4). However, the anaerobic ammonium removal rate was sharply improved from 0.09 to 0.24 mmol·g^{-1}·d^{-1} when the NO_2^--N density was enhanced from 2.0 to 2.5 mmol·L^{-1}. When the NO_2^--N density was further increased, no obvious improvement of anaerobic ammonium removal rate was observed anymore. Even an obvious decrease of anaerobic ammonium removal rate was found when the NO_2^--N density was further enhanced to 6.0 mmol·L^{-1}. The highest anaerobic ammonium removal rate of 0.27 mmol·L^{-1} came up to the NO_2^--N density of 4.0 mmol·L^{-1}.

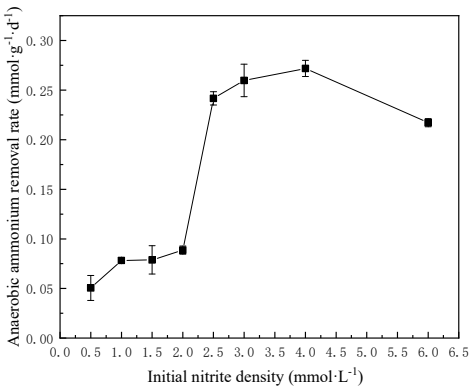

Fig. 4. Anaerobic ammonium removal rate of the enrichment culture with the increase of NO_2^--N density

The above results showed that anammox population were much more sensitive to NO_2^--N density (Fig. 4) than that to NH_4^+-N density (Fig. 3). It has been reported that NO_2^--N could function as a strong biological inhibitor of anammox population [25]. Thus, NO_2^--N density was proposed as a key indicator to practicing anammox process in engineering.

4 Conclusion

In this study, the effects of pH, temperature and reactant density on the activity of annamox population were investigated by batch tests. Based on the experiment results, following conclusions can be drawn:

(1) The optimal ecological amplitude of pH and temperature for the anammox population was ranged from 7.0-7.5 and 30℃-35℃, respectively.

(2) Anammox process would be more efficient with the NH_4^+-N and NO_2^--N density of 10.0 and 4.0 mmol·L^{-1}, respectively.

(3) NO_2^--N density was proposed as a key indicator to practicing anammox process in engineering because anammox population was more sensitive to NO_2^--N density than NH_4^+-N density.

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