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Effect of different preservation conditions on the reactivation performance of anammox sludge

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ABSTRACT

The preservation of anaerobic ammonium oxidation (anammox) bacteria is a key element in the study and application of the anammox process. Different cryoprotectants (dimethyl sulfoxide and trehalose) at storage temperatures of 4 °C and −40 °C were selected to construct a preservation system. After 2 months of storage, the performance of the reactors and the particle characteristics from the activity recovery test revealed the advantages and disadvantages of the different storage methods. Ultimately, the preservation method adopted was storage without cryoprotectant at 4 °C. With the aid of this optimized method, the nitrogen removal rate reached 4.41 kg m^{-3} d^{-1} during the recovery period. Among the cryoprotectants assessed, dimethyl sulfoxide was found to be an appropriate cryoprotectant, and trehalose had surprising and unexpected effects. The modified Stover–Kincannon model was suitable for describing the nitrogen removal performance of the reactors with sludge after storage, except for the two reactors in which the granules were exposed to trehalose during the storage period.

1. Introduction

Anaerobic ammonium oxidation (anammox) is a microbial reaction that uses NH_4^+ as the electron donor and NO_2^- as the electron acceptor [1]. The application of the anammox process has already improved energy savings and treatment efficiency and inspired substantial research. Anammox cultures are the sole source of material for the anammox process. Research into anammox bacteria translated from theoretical research to practical use after their application in a pilot-scale denitrification system in the 1990s [2,3]. Moreover, the contribution of anammox bacteria to marine denitrification accounted for approximately 50% of the total [4].

However, anammox bacteria grow slowly, and the doubling time is approximately 11 d [5]. Such a long doubling time will lead to cyclical swing in nitrogen removal efficacy, and consequently, it is difficult to treat a steady flow of wastewater effectively [6]. Furthermore, full-scale anammox plants are located in different places throughout the world, such as the Netherlands and Japan. The long travel distances make the transport of the anammox enrichment culture extremely difficult [7–10]. For the above reasons, appropriate storage of anammox bacteria is a critical topic in the study and application of the anammox process. Several techniques exist for the preservation of microbial cultures, including liquid paraffin, liquid nitrogen [11], refrigeration, freezing, lyophilization and gel encasement in an appropriate growth media with or without a cryoprotectant [12–14]. However, using these technologies to store anammox sludge does not fully preserve the bacteria, although the cultures retain some activity. The anammox bacteria are susceptible to environmental conditions such as temperature and dissolved oxygen. Appropriate preservation methods should be identified to protect most of the morphological and structural characteristics of the anammox bacteria.

Current research on preservation techniques for anammox granular sludge remains incomplete. Refrigeration is superior to other preservation techniques because it protects the bacteria from variations in temperature. Rothrock Jr et al. [14] stored lyophilized anammox bacteria in liquid nitrogen (−200 °C) for 4 months and achieved similar stoichiometric ratios to those of the parent bioreactor during the reactivation period. By contrast, Heylen et al. [13] used dimethyl sulfoxide (DMSO), trehalose and trypticase soy broth as cryoprotective agents at −80 °C for 29 weeks, and the activity of the culture after preservation was the highest compared with those of other tested combinations of cryoprotectants. Therefore, the addition of a suitable cryoprotectant (e.g., glycerol, trehalose or DMSO) further retains anammox bacterial activity. Vogelsang et al. [12] stated that nitrifying sludge stored with

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10 mM trehalose exhibited better initial activity compared with the original sludge.

In addition to identifying ideal preservation conditions, rational evaluation criteria are also important to establish an optimal preservation method. The objective of this study is to investigate the effects of the storage temperature and the type of cryoprotectant on the anammox reactor performance and on the physical properties and microbial activity of anammox granules during the reactivation process. Moreover, the optimal preservation conditions for maintaining the stability and rapid activation of anammox granules are explored.

2. Materials and methods

2.1. Anammox sludge origin

Anammox sludge was collected from a 1 L anammox up-flow anaerobic sludge bed (UASB) bioreactor. This bioreactor, hereafter called the parent bioreactor (R0), operated at ambient temperature. The parent bioreactor was used at a hydraulic retention time (HRT) of 2.64 h with influent NH_4^+-N and NO_2^--N concentrations of 210 mg L^{-1}, maintaining the stoichiometric ratio of the substrate at 1.0. Specific features of the original anammox sludge are provided in Table 1.

2.2. Preservation treatments

A customized organic glass bottle with a gross volume of 1.5 L was selected as the sludge reservoir. The different storage methods included two temperatures and three kinds of cryoprotectant. Table 2 presents the details of the preservation treatments. Each storage system included 0.5 L original sludge and 0.5 L distilled water. The added proportions of DMSO and trehalose were 5% and 2%, respectively. During the preservation period, all the preservation systems were sealed and maintained for 2 months.

2.3. Reactivation procedure

After 2 months of storage, the anammox granules stored at −40 °C were removed and thawed in a 4 °C water bath for 8 h. Subsequently, the granules were washed 5 times with distilled water and then revived in 6 UASB reactors at a working volume of 0.5 L. Reactors R1, R2, R3, R4, R5 and R6 were inoculated with the same granule which was from parent bioreactor. Furthermore, A_1, A_2, A_3, B_1, B_2 and B_3 were different treatments which were acted on R1, R2, R3, R4, R5 and R6, respectively. The temperature of the reactor was maintained at 25 °C. During the initial phase of the start-up, all six reactors were controlled with the same operation parameters of 3 h HRT and influent NH_4^+-N and NO_2^--N concentrations of 70 mg L^{-1}. After the cells had partially recovered, the influent substrate concentrations were increased in steps of 28 mg L^{-1}.

2.4. Analytical methods

The influent and effluent samples were collected daily and either analyzed immediately or stored in a refrigerator at 4 °C until the analyses were performed. The measurements of NH_4^+-N, NO_2^--N, NO_3^--N, SS, VSS and the settling velocity were performed according to standard methods [15]. The specific anammox activity (SAA) assays were performed as previously described by Yang and Jin [16]. The pH of the samples was determined using a pH meter with a selective electrode. The extraction of extracellular polymeric substances (EPS) was performed as reported by Sheng et al. [17] and Wu et al. [18]. Carbohydrate measurements were performed using the anthrone method with glucose as a standard, and protein levels were measured with the modified Lowry method using bovine serum albumin as a standard [18]. The heme c content was determined according to Berry and Trumpower [19] and Sinclair et al. [20].

Table 2
Specific storage conditions of anammox biomass.

Treatment	Storage temperature (°C)	Protectant	Storage media
A_1	4	–	Distilled water
A_2	4	Trehalose	Distilled water
A_3	4	DMSO	Distilled water
B_1	−40	–	Distilled water
B_2	−40	Trehalose	Distilled water
B_3	−40	DMSO	Distilled water

2.5. Stover–Kincannon model

The modified Stover–Kincannon model is appropriate for predicting the nitrogen removal of anammox sludge [21,22]. The equation for the Stover–Kincannon model is as follows [21]:

$$\left(\frac{dS}{dt}\right)^{-1} = \frac{V}{Q(S_0 - S)} = \frac{K_B}{U_{max}}\frac{V}{QS_0} + \frac{1}{U_{max}} \quad (1)$$

where dS/dt is the substrate removal rate (kg m^{-3} d^{-1}); U_{max} is the maximum utilization rate constant (kg m^{-3} d^{-1}); and K_B is the saturation value constant (kg m^{-3} d^{-1}).

3. Results and discussion

3.1. Reactivation performance of stored anammox granules

3.1.1. Granule microbial activity

As a bioactivity readout, the SAA represents the catabolic activity of the microorganism, which can be used to compare the activity and biodegradation efficiency of the anammox granules [23]. The SAA of the original granules before storage was 0.14 g N g^{-1} VSS d^{-1}. Anammox granules maintained for 2 months under different storage conditions lost a portion of their initial metabolic activity. As shown in Fig. 1, the anammox granules in R1–R6 gradually resumed microbial activity upon reactivation. The granules at different storage conditions, except for those of R5, could successfully regain most of their bioactivity after 52 d of operation, but different storage conditions produced different performances.

Fourteen days after reactivation, the SAA retention percentages of R1, R2, R3, R4, R5 and R6 were 92.9%, 28.6%, 92.9%, 78.6%, 42.9% and 35.7%, respectively. The SAA of R1, R4 and R6 increased significantly as the culture recovered. Compared with R1, the SAA of R3 decreased with reactivation, which may be related to the toxicity of residual DMSO at ambient temperature. It seems likely that the toxicity of DMSO was reduced with lower storage temperature because the SAA for R6 was less affected by DMSO toxicity. How-

Table 1
The characteristics of anammox sludge before preservation.

Features	SS (g L^{-1})	VSS (g L^{-1})	SAA (g N g^{-1} VSS d^{-1})	Specific gravity	EPS (mg g^{-1} VSS)	Settling velocity (m h^{-1})	Heme c (μmol g^{-1} VSS)
Original sludge	19.1	17.3	0.14	0.86	21.2	74.93 ± 25.18	0.30