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Using graphene oxide to reactivate the anaerobic ammonium oxidizers after long-term storage

Gang Wang*, Xiaochen Xu, Fenglin Yang*, Hanmin Zhang, Dong Wang

Key Laboratory of Industrial Ecology and Environmental Engineering (China Ministry of Education), School of Environmental Science and Technology, Dalian University of Technology, Linggong Road 2, Dalian 116024, PR China

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ABSTRACT

In this study, the reactivation of three kinds of anammox sludge was investigated, including the sludge stored at room temperature (sludge A), at 4 °C without and with 0.1 g/L graphene oxide (GO) (sludge B and C). The reactors were operated for about 5 weeks. The reactivation of anammox bacteria was evaluated by measuring the total nitrogen removal rate, special anammox activity (SAA) and extracellular polymeric substances (EPS). The characterization of microorganisms was observed by using scanning electron microscope (SEM) and fluorescence in situ hybridization (FISH). The activity and properties of sludge C were optimal. The final total nitrogen removal rate of reactor B and C achieved about 1200 mg/L/day, while reactor A achieved only 773.6 mg/L/day. According to Mastersizer, the particle size of reactor A, B and C increased to 153, 189 and 230 μm, respectively. The time reaching the original NRR (700 mgN/L/day) was 35 days, 21 days and 12 days, and the specific anammox activity (SAA) eventually fixed at 0.30, 0.42, 0.44 g N (g VSS day)$^{-1}$, respectively. The total EPS, the polymers carbohydrate and proteins in the extracted EPS decreased individually. Scanning electron microscope (SEM) showed the granulation process and the change of particle size in the cultivated sludge. FISH analysis confirmed the existence of other bacteria and anammox bacteria became the dominant population. All results indicated that the sludge stored at 4 °C was easier to reactivate, and the addition of 0.1 g/L GO would further promote the reactivation of anammox.

Introduction

Historically, biological nitrogen removal from wastewater is achieved by the combination of two processes, autotrophic nitrification and heterotrophic denitrification. However, the conventional nitrogen removal process treated wastewater with low C/N ratio, the nitrification/denitrification process is uneconomical and complicated stemming from the oxygen supply requirements of nitrification and the requirement of an organic carbon source for denitrification. Anaerobic ammonium oxidation (anammox) is a recent advance in microbial biotechnology which has led to significant progress in wastewater treatment. Anammox process was carried out by microbial oxidation of ammonia with nitrite to dinitrogen gas occurring under anoxic conditions [1,2]. This process is a novel, environmental friendly, cost-effective technology that has a high nitrogen removal potential [3]. The anammox reaction oxidizes ammonia to nitrogen gas using ammonium as an electron donor and nitrite as an electron acceptor under anaerobic conditions [4].

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 0.26NO_3^- + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad (1)$$

Anammox bacteria is widely discovered, no matter in wastewater treatment systems or in nature [5,6]. However, anammox bacteria are strictly anaerobes and autotrophs and difficult to enrich with a doubling time of about 11 days. The bacteria are susceptible to environmental changes such as temperature, pH, dissolved oxygen and organic matter. Inappropriate operation often reduces anammox activity, even led to anammox process collapse [7,8]. There have been numerous researchers attempting to the fast start-up of anammox process and enrichment of anammox bacteria, but paying less attention to the storage and reactivation of anammox bacteria. Recently, the application of graphene oxide in microorganisms has aroused great interest, owing to its incredibly large specific surface area and layered sheets with the abundant oxygen-containing functional groups (e.g., epoxide, carboxyl, carbonyl and hydroxyl groups) on their basal planes and edges [9]. These oxygen functional groups enabled GO to be readily dispersed in water, which is important for GO in microbiology applications. According to previous studies, GO is so highly biocompatible that can effectively facilitate the proliferation of some bacteria. For example, GOs coated on filters induce the faster growth of *Escherichia coli* bacteria [10]. Moreover, Ruiz et al. have proved that GO could be used as a scaffold for anammox bacteria attachment. In Wang's research [11], she mentioned that the appropriate GO dose could efficiently stimulate the increase of EPS. Thus,

* Corresponding authors.
E-mail addresses: kingwg01@163.com (G. Wang) yangfl@dlut.edu.cn (F. Yang).

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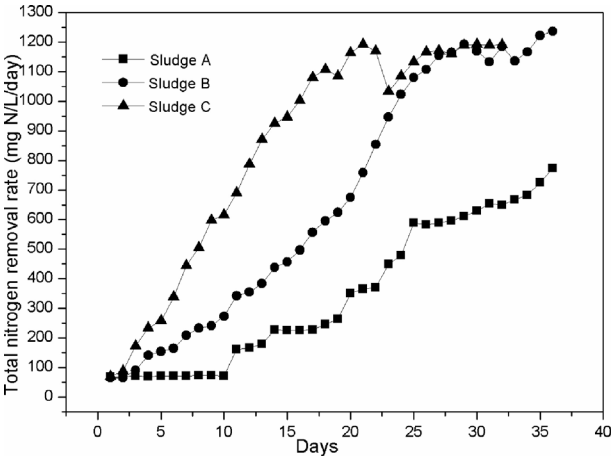

Fig. 1. Total nitrogen removal rate of sludge A, B and C.

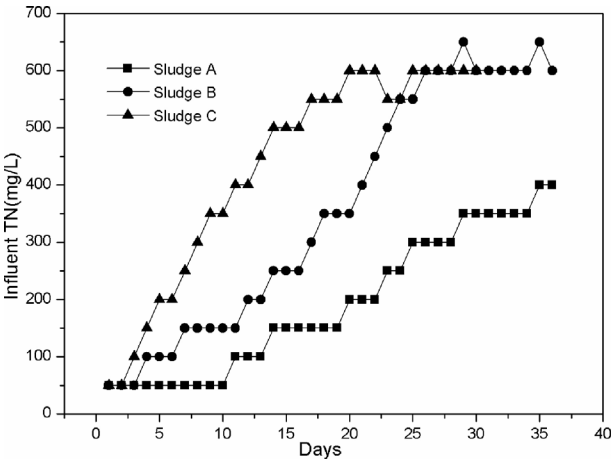

Fig. 2. Influent total nitrogen of sludge A, B and C.

the application of GO is an effective method to enhance the activity of anammox bacteria.

The present study aims for investigating the reactivation of anammox bacteria and probing into a method to reactivate anammox. The seed sludge was collected from different storage conditions and inoculated into three reactors in parallel. The sludges stored at room temperature and 4 °C were sludge A and B, respectively. Sludge B with 0.1 g/L GO (sludge C) which may be conductive to enhance the biological activity [11] was inoculated in reactor C. During the operation, we compared the sludge reactivation in the three reactors by determining sludge production quantity, sludge particle size and EPS. Meanwhile, we also estimated the influent nitrogen load, the ratio of NO_2^--N and NH_4^+-N removal and SAA. When anammox process operated stably and efficiently, the microbial morphology and community structure of anammox were analyzed by scanning electron micrographs (SEM) and fluorescence in situ hybridization (FISH).

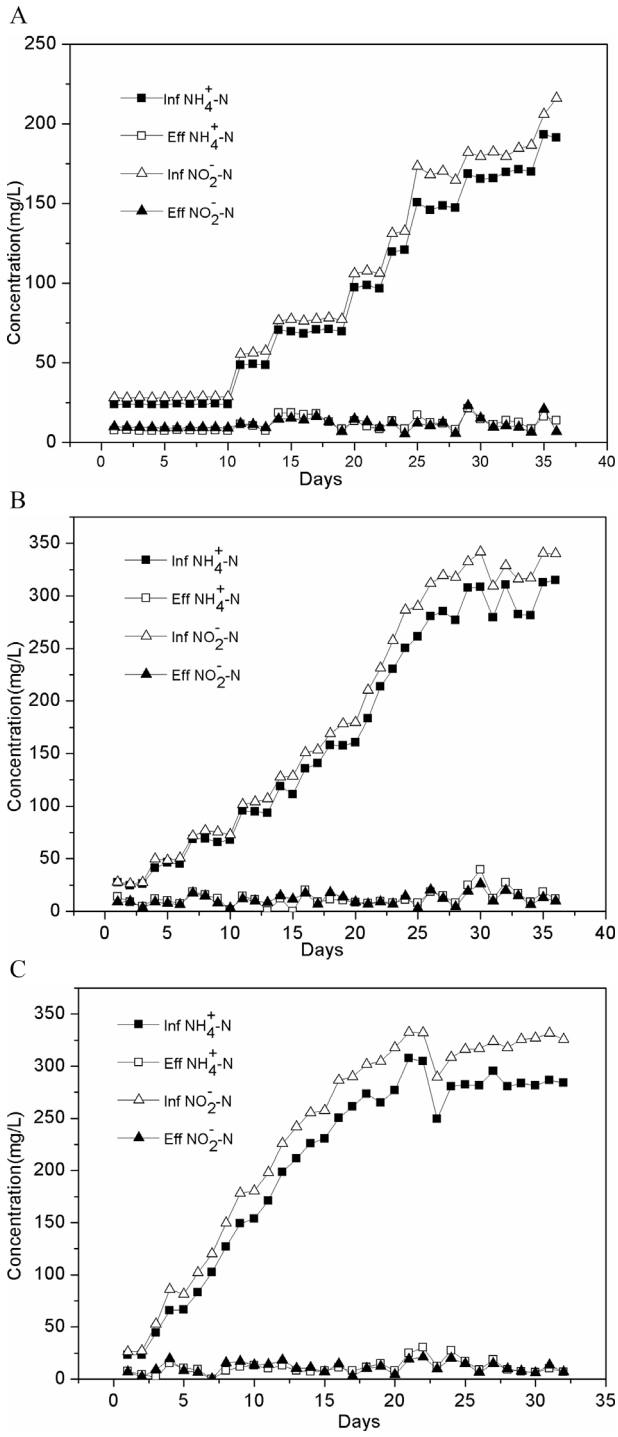

Fig. 3. Reactor performances during operation of the anammox process. (A–C) profile the concentrations of NH_4^+-N and NO_2^--N in influent and effluent of reactor A, B and C.

Materials and methods

Seed anammox sludge and synthetic medium

The seed sludge was harvested from a cylindrical acrylic plastic upflow anammox reactor that was fed with synthetic medium consisting of a NO_2^--N to NH_4^+-N with a molar ratio of 1.3:1.0 (courtesy of professor Yang Fenglin, Dalian University of Technology). The sludges have been stored for 2 months. Storage was made with proper concentration of nitrate and inorganic nutrient at room temperature and 4 °C, respectively. At the moment of anammox harvesting, the total nitrogen removal of the reactor had achieved about 700 mg N/L/day (NH_4^+-N: 359.4 mg/L/day; NO_2^--N: 378.8 mg/L/day). The MLVSS inoculated was about 2430 mg/L. In this study, the reactor was fed with synthetic wastewater with an NO_2^--N to NH_4^+-N molar ratio of 1.3 (actually, it was slightly lower than the ratio). During the operational period, synthetic wastewater with composition of NH_4^+-N 21.6–300 mg/L, NO_2^--N 28.4–400 mg/L, $KHCO_3$ 1250 mg/L, KH_2PO_4 25 mg/L, $MgSO_4{\cdot}7H_2O$ 200 mg/L, $CaCl_2{\cdot}2H_2O$ 300 mg/L, $FeSO_4{\cdot}7H_2O$