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# The performance of high-loading composite anammox reactor and its long-term recovery from extreme substrates inhibition



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#### HIGHLIGHTS

- Composite anammox reactor combined granules and biofilm in one single SBR.
- Performance of high-loading anammox reactor under fluctuations.
- The composite reactor presented significant recovery potential.
- Anammox granulation from flocculent sludge directly during recovery operation.
- The composite reactor achieved nitrogen removal rate of 18.01 kg N m<sup>-3</sup> d<sup>-1</sup>.

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#### ABSTRACT

A composite reactor combined with granule and biofilm in one single SBR was developed for high-rate anammox operation. Low activity flocculent inoculum was used as seed sludge and the nitrogen loading rate reached 10.62 kg N m $^{-3}$  d $^{-1}$  after 93 days' operation. Subsequently, the performance of high-loading anammox reactor under fluctuation condition was investigated. Due to the extremely short hydraulic retention time and high-strength substrates, the reactor presented dramatic substrates accumulation which resulted in severe inhibition subsequently. Nevertheless, the composite reactor presented significant recovery potential even after biomass hydrolysis caused by inhibition, mainly due to the protection and promotion supplied by biofilm. Besides, granules were gradually formed and accumulated from the flocculent sludge directly during the recovery operation, further elevating the reactor performance. The nitrogen loading rate and corresponding nitrogen removal rate achieved 20.30 and 18.01 kg N m $^{-3}$  d $^{-1}$  with total nitrogen in the influent of 1500 mg/l finally.

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#### 1. Introduction

In recent years, serious eutrophication resulted from the discharge of excessive nitrogen with the accelerating industrialization and urbanization, has been especially concerned in water environmental protection. The conventional technology for nitrogen removal, nitrification and denitrification, required plenty of energy to create aerobic condition and organic compounds for nitrate reduction. This approach would be especially costly and difficult for treating wastewater with high concentration of ammonia while small amount of carbon compounds. For that, anaerobic ammonia oxidation (anammox) has been considered as a promising alternative to the traditional process for its energy-efficient and cost-saving (Kuenen, 2008; Furukawa et al., 2009).

In the anammox reaction, ammonia is partially oxidized to nitrite firstly, then the anammox would combine the formed nitrite and ammonia to dinitrogen gas with a small amount of nitrate production (van de Graaf et al., 1997). Due to the cost-saving in oxygen and organics consumption, the anammox-based technology has been applied into full-scale with more than 100 installations around the world (Lackner et al., 2014).

In previous researches, short hydraulic retention time (HRT) rather than high-strength influent was preferred for high-loading anammox operation. Additionally, strict operation conditions were also required to maintain the stability of reactor (Tang et al., 2011; Tsushima et al., 2007). However, in practical application, the raw wastewater treated by anammox process mostly contained high-strength nitrogen compounds with total nitrogen concentration above 1000 mg N/l. Besides, the reactor frequently met operation fluctuations (e.g. shock load, out of control in temperature) which would cause reactor inhibition consequently and this situation might be especially severe under high-loading condition (Lackner

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et al., 2014). For these reasons, the capacity to treat high-strength influent stably and the recovery potential after inhibition should be especially concerned for the high-loading anammox operation. Previous researches have focused on different reactor configurations and sludge forms, and further revealed the respective advantages of different reactors. Among them, granules were considered as the alternative for high-loading anammox operation due to their high specific anammox activity and the diameter of granules should be maintained within relatively small range in case of disintegration and floatation (Lu et al., 2012). This, however, reduced the resistance for extreme adverse condition accordingly (An et al., 2013). By contrast, the biofilm was recognized for its excellent capacity for shock resistance and recovery potential (Ren-Cun et al., 2012), while poor performance in mass transfer and detachment. For these reasons, a composite reactor combined these advantages would be a promising alternative for high-loading anammox operation and manage the potential fluctuations well in practical application.

In this study, we established a composite SBR combined the granules and biofilm in one single reactor for anammox operation and elevated the nitrogen loading rate (NLR) to relatively high level. Under this circumstance, the out of control in temperature was simulated to investigate the performance of high-loading anammox reactor under fluctuation circumstance. The fluctuation resulted in the dramatic accumulation of substrates and further led to severe inhibition. Nevertheless, the reactor showed excellent recovery potential under extreme substrates inhibition and further developed into the combined granule-biofilm reactor. It was proved that the composite anammox SBR was qualified to treat high-strength wastewater under high-loading condition.

#### 2. Methods

#### 2.1. Reactor configuration and operational condition

An SBR with working volume of 5 L was used to initiate the experiment, as shown in Fig. 1. The reactor was made by polymethyl methacrylate with inner diameter of 10 cm, and the temperature was kept at  $32\pm1$  °C within stable operation by means of a thermostat water jacket. For purpose of better mass transfer, impeller was installed at the bottom of reactor for mechanical agitation (120 rpm). With relatively weak shear force in the upper part of reactor, microbes could aggregate and cluster on the inner wall to form the biofilm and the biofilm could make contact with the granules adequately. The influent was introduced from the bottom and the effluent was drawn out from a tube inserted into the reactor by the peristaltic pumps.

The SBR was operated in the cycles consisted of 4 phases: feeding, reaction, settling and decanting, respectively. The duration of each phase varied along the long-term operation according to the performance of reactor, lasted for 5–10, 15–330, 3–10 and 3–10 min. During the start-up stage, the influent mainly consisted of ammonia and nitrite with concentration of 50 and 65 mg N/l, respectively. Pre-stirring phase lasting for 30 sec was introduced prior to each cycle when the concentration of total nitrogen in the influent increased to 460 mg/l on day 24, aiming to make the settled sludge dispersed before concentrated influent was introduced. The pH value was adjusted at  $7.8 \pm 0.2$  by adding 1 M HCl or NaHCO<sub>3</sub>.

To investigate the performance of high-loading anammox reactor under fluctuation conditions, the water bath was shut down while the reactor kept operated as usual (NH $_{\star}^{+}$ -N $_{in}$  = 500 mg/l, NO $_{z}^{-}$ -N $_{in}$  = 650 mg/l, cycle time = 39 min) on day 106 to simulate the perturbation process. The decrease of temperature resulted in dramatic suppression of reactor, and the recovery process would

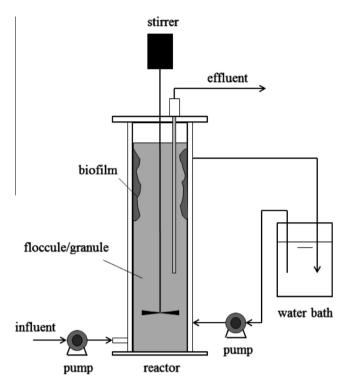


Fig. 1. Schematic description of the composite anammox reactor operated in SBR pattern.

be conducted after the inhibition experiment. The procedures for recovery were performed essentially as previously described (Scaglione et al., 2012): The supernatant was replaced by the fresh medium which mainly contained 50 mg N/l ammonia and 65 mg N/l nitrite to washout the high-strength substrates. Meanwhile, the water bath would be operated again to increase the temperature of the reactor to 32 °C.

#### 2.2. Inoculum and synthetic wastewater

The reactor was seeded with 2 L anammox sludge (volatile suspended solids = 2500 mg/l) from an anammox reactor in the same laboratory which has been shut down for 3 months in ambient temperature. The anammox sludge turned taupe and presented weak specific anammox activity (SAA) of  $0.14 \text{ kg N kg VSS}^{-1} \text{ day}^{-1}$ .

The SBR was fed with synthetic wastewater:  $NH_4CI = 50-650 \text{ mg N/l}$ ,  $NaNO_2 = 65-850 \text{ mg N/l}$ ,  $KH_2PO_4 = 15 \text{ mg P/l}$ ,  $CaCl_2 = 300 \text{ mg/l}$ ,  $MgSO_4 \cdot 7H_2O = 200 \text{ mg/l}$ ,  $NaHCO_3 = 400-800 \text{ mg/l}$ ,  $NaHCO_3 = 10-800 \text{ mg/l}$ ,

#### 2.3. Analytical methods

The influent and effluent were collected on a daily basis and were analyzed immediately or stored at 4 °C in a refrigerator until the analyses were carried out. The ammonia, nitrite, nitrate, suspended solids (SS), volatile suspended solids (VSS) were measured according to the standard methods (APHA, 2005). Each sample was analyzed in triplicate and the relative standard deviation was less than 5%. The total nitrogen (TN) was determined as the summation of ammonium, nitrite and nitrate. The temperature and pH were monitored using Hach HQ30d (Hach Inc., USA).

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