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# Kinetic characteristics and microbial community of Anammox-EGSB reactor

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ABSTRACT

The present study reports kinetic characteristics of Anammox (anaerobic ammonium oxidation) EGSB (Expanded Granular Sludge Bed) reactor after feeding with strong ammonium-containing synthetic wastewater. The microbial communities were analysed based on their 16S rRNA gene sequences. The results showed that the volumetric nitrogen loading rate (NLR) and volumetric nitrogen removal rate (NRR) reached up to 22.87 kg N/($m^3$ d) and 18.65 kg N/($m^3$ d), respectively, when the influent nitrogen concentrations were 1429.1 mg N/L. Monod and Haldane models both proved to be suitable in characterizing the kinetic behavior of the reactor. Based on Haldane model, the relationships among the ammonium, nitrite, nitrogen conversion rates and substrate concentrations were established with corresponding correlation coefficients of 0.992, 0.993 and 0.993, respectively. The maximum ammonium, nitrite and nitrogen conversion rates ($q_{max}$) by the granular sludge were 381.2, 304.7 and 731.7 mg N/(gVSS d), half saturation constants ($K_s$) were 36.75, 0.657 and 29.26 mg N/L and inhibition constants ($K_i$) were 887.1, 13,942.1 and 1779.6 mg N/L, respectively. Anammox-EGSB reactor was found tolerant to substrate and capable of treating strong ammonium-containing wastewater. The dominant microbial population of the granular sludge in the reactor was *Candidatus Kuenenia stuttgartiensis*.



## 1. Introduction

Anaerobic ammonia oxidation (Anammox) is a biotechnological process in which ammonium is directly converted to dinitrogen gas with nitrite as the electron acceptor under anoxic conditions [1]. The Anammox process which was initially discovered in a denitrifying pilot plant reactor [2]. It is a novel and promising alternative to conventional nitrogen removal processes. The application of Anammox process to nitrogen removal would lead to a reduction of operational costs up to 90% [3]. The first full-scale Anammox reactor was constructed for the Dokhaven–Sluisjesdijk wastewater treatment plant in Rotterdam in 2002, treating sludge dewatering effluent from a Sharon (single reactor high activity ammonia removal over nitrite) process. The maximum NRR for the first Anammox reactor was 9.5 kg N/($m^3$ d) [4].

Ammonium is widely present in wastewaters (e.g., industrial wastewater, agricultural wastewater and domestic sewage). Most of the wastewaters contain ammonium concentrations below 1000 mg/L. However, the ammonium concentrations reach up to 10,000–17,000 mg/L in wastewaters like ion-exchange wastewater from the production units of monosodium L-glutamate. Ammonium and nitrite serve as substrates for Anammox bacteria; however, these act as inhibitors of Anammox bacteria when their concentrations exceed a certain level [5–7]. Although the Anammox process was initially designed to treat ammonium-rich wastewaters [8], the toxic substrate concentrations were mostly controlled below 1000 mg/L for the process [5,6,9,10]. The treatment of ammonium-rich wastewaters (the ammonium concentrations greater than 1000 mg/L) through Anammox will reduce the engineering investment as well as operational cost. In order to study the kinetic characteristics of Anammox reactor and to verify its adaptability to ammonium-rich wastewaters, the Anammox-EGSB reactor was run and its performance was monitored for more than 130 days, the microbial community under high substrate concentrations was also analysed. This paper reports the performances, kinetic characteristics and the microbial community of the Anammox-EGSB reactor.

## 2. Materials and methods

### 2.1. Synthetic wastewater

Ammonium and nitrite were supplemented to mineral medium as needed in the form of $NH_4Cl$ and $NaNO_2$, respectively. The composition of the mineral medium was (g/L except for trace element solution): $KH_2PO_4$, 0.01; $CaCl_2{\cdot}2H_2O$, 0.18; $MgSO_4{\cdot}7H_2O$, 0.30; $KHCO_3$, 1.250; EDTA, 0.005; $FeSO_4$, 0.00625, and 1 mL/L of trace element solution. The trace element solution contained

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(g/L): EDTA, 15; $H_3BO_4$, 0.014; $MnCl_2·4H_2O$, 0.99; $CuSO_4·5H_2O$, 0.25; $ZnSO_4·7H_2O$, 0.43; $NiCl_2·6H_2O$, 0.19; $CoCl_2·6H_2O$,0.24; $NaMoO_4·2H_2O$, 0.22; $NaSeO_4·10H_2O$, 0.21 and $NaWO_4·2H_2O$, 0.05 (adapted from [11]).

### 2.2. Anammox inoculum

Anammox granular sludge was collected from a lab-scale Anammox reactor and was used as the seed sludge for Anammox-EGSB reactor. The volatile suspended solids (VSS) content of seed sludge was 31.31 g/L. The lab-scale Anammox reactor was initially inoculated with anaerobic granular sludge taken from a paper mill wastewater treatment plant (100 $m^3$, located in Zhejiang Province, China). The average diameter of the anaerobic granular sludge was 2.2 mm, and the VSS/TSS (total suspended solids) was 85%. The reactor was successfully started up and subsequently operated stably for 2 years before conducting the experiments.

### 2.3. Anammox EGSB reactor

The Anammox-EGSB reactor system was illustrated in Fig. 1. The total volume of reactor was 1.2 L with a working volume of 1.0 L, reactor was covered with black cloth to avoid inhibition caused by light [12]. The sampling ports were distributed vertically on the reactor with the mutual distance of 250 mm. The influent was purged with 95% Ar–5% $CO_2$ in order to create anaerobic conditions. The synthetic wastewater was pumped at the bottom of the reactor by using peristaltic pump. The effluent was collected in the effluent tank after passing through the gas–liquid–solid separator. The granules settled at the bottom of the reactor and the gas escaped from the top of the reactor.

### 2.4. Operational conditions

To investigate the performance of EGSB reactor, the NLR was gradually increased through increasing ammonium and nitrite concentrations in the influent or by shortening the hydraulic retention time (HRT). The EGSB reactor was operated at $35 \pm 1$ °C. Influent pH was always kept in the range of 6.8–7.0 by dosing hydrochloric acid [10]. A recycling pump was used to mix the influent (substrate) and sludge (biocatalyst) well. The ratio of recycling flow to influent flow was set at 6. Six completely-mixed reactors of 100 mL capacity were used to study the kinetic characteristics of Anammox granular sludge in Anammox-EGSB reactor for 131 days. The kinetic experiments for Anammox-EGSB reactor were conducted at controlled conditions including controlled temperature, influent pH, reflux ratio, etc. The reactors used for kinetic experiments possessed same shape, but variable volumes compared with the Anammox-EGSB reactor. The kinetic experiments were conducted at the fixed HRT using variable substrate concentrations.

#### 2.4.1. Volumetric substrate conversion rate

Due to a greater recycling ratio of 6:1 and the large amount of gas production in Anammox-EGSB reactor, the operational pattern of the reactor tended to be completely mixed. Thus, the concentration of granular sludge could be characterized by mean value and the substrate concentrations inside the reactor were considered same as the substrate concentrations in the effluent. Under steady state, the mass balance for the reactor can be expressed as (Eq. (1)):

$$QS_0 - QS_e - V\left(\frac{dS}{dt}\right)_u = 0$$
$$\text{that is } \left(\frac{dS}{dt}\right)_u = \frac{Q}{V}(S_0 - S_e) \qquad (1)$$

where: $Q$ – influent flow (L/d); $V$ – reactor working volume (L); $S_0$, $S_e$ – substrate concentrations in influent and effluent (mg/L); $(dS/dt)_u$ – volumetric substrate conversion rate [mg/(L d)].

The volumetric substrate conversion rate could be calculated using Eq. (1).

#### 2.4.2. Monod model

Monod model (Eq. (2)) is the most popular model to describe the kinetics of pollutant biodegradation [13,14]. The maximal substrate conversion rate ($q_{max}$) and half saturation constant ($K_s$) can be obtained according to Monod model. The model is represented as follows:

$$q = \frac{(ds/dt)_u}{X} = \frac{Q(S_0 - S_e)}{VX} = \frac{q_{max}S}{K_s + S} \qquad (2)$$

where: $q$, $q_{max}$ – specific substrate conversion rate and the maximal substrate conversion rate, respectively (1/d); $X$ – the concentration of biomass in reactor (mg/L); $K_s$ – half saturation constant (mg/L); and $S$ – substrate concentration (mg/L).

#### 2.4.3. Haldane model

A number of substrates serve as nutrients at low concentrations, but they behave as inhibitors at high concentrations [15]. High substrate concentrations inhibit microbial growth and disturb their metabolism. Haldane model (Eq. (3)) is often used to describe the kinetics of pollutant biodegradation involving inhibition [16]:

$$q = \frac{(dS/dt)_u}{X} = \frac{Q(S_0 - S_e)}{VX} = \frac{q_{max}}{1 + (K_s/S) + (S/K_i)} \qquad (3)$$

where: $q$, $q_{max}$ – specific substrate conversion rate and the maximal substrate conversion rate, respectively (1/d); $X$ – the concentration of biomass in reactor (mg/L); $K_s$ – half saturation constant (mg/L); $K_i$ – substrate inhibition constant(mg/L); and $S$ – substrate concentration (mg/L).

### 2.5. Analytical methods

The influent and effluent samples were collected on daily basis and were analysed immediately or temporarily stored at 4 °C. The biomass concentration was determined as VSS. Water samples and VSS were analysed according to the standard methods [17]. The analysis of water samples was performed for ammonium concentrations, nitrite concentrations, nitrate concentrations and pH values. The dinitrogen gas was measured with a wet-gas flow meter and temperature was determined using a mercurial thermometer.

### 2.6. DNA extraction

For the DNA extraction, sludge was collected from the reactor during the initial (the inoculum-group 1) and final (on day 131-group 2). The granular sludge was washed several times using phosphate buffer solution prior to DNA extraction. DNA extraction was performed using DNA isolation kit v2.2 for environmental samples (Shenergy Biocolor Company, China). The extracted DNA was preserved at −20 °C.

### 2.7. PCR amplification

PCR reactions were performed on the extracted DNA. The primer pair pla46F (5′-GGATTAGGCATGCAAGTC-3′) [18] and 630R (5′-CAKAAAGGAGGTGATCC-3′) [19] was used in PCR amplification. PCR reactions were performed using 25 μL reaction volumes containing 2.5 μL dNTPs mixtures (2.5 mM) (Takara), 2.5 μL 10× PCR buffer (containing 15 mM magnesium ions) (Takara), 1 μL of each of the primers (10 mM) (Takara), 1 μL of the extracted DNA, 0.2 μL rTaq DNA polymerase (Takara) and 17.3 μL DEPC Rnase free $dH_2O$.