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# Nitrous oxide emission in an aerobic granulation sequencing batch airlift reactor at ambient temperatures



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## 1. Introduction

# Aerobic granulation sludge technology, a new and promising environmental biotechnological process, is increasingly drawing interest of researchers engaging in work in the area of biological wastewater treatment. Aerobic granules were considered to be a special case of self-immobilized microbial consortium [\(Seviour](#page--1-0) [et al., 2010\)](#page--1-0). Compared to conventional activated sludge systems, aerobic granules have the advantages of compact microbial structure, good settling ability, high biomass retention, and have the ability to withstand high organic loading rate [\(A dav et al., 2008;](#page--1-0) [Xavier et al., 2007\)](#page--1-0).

Aerobic granular sludge was first reported in an aerobic up flow sludge blanket reactor by [Mishima and Nakamura \(1991\).](#page--1-0) Formation of granules in aerobic conditions has been possible and appears as a promising technique for high strength or highly toxic wastewater treatment. During the last 20 years, aerobic granules have been successfully applied to the treatment of high strength organic wastewater, toxic organic wastewater, heavy metals and dyes, dairy and brewery wastewater, and even low-strength

#### **ABSTRACT**

This study aims to investigate the nitrous oxide  $(N_2O)$  emission in an aerobic granulation sequencing batch airlift reactor (SBAR) and the associated microbial community of aerobic granular sludge at ambient temperature (18  $\pm$  3)°C. After 48 days of operation, 1–2 mm granules were obtained and excellent chemical oxygen demand (COD) and ammonium  $(NH_4^+ - N)$  removal efficiencies were stably achieved.  $N_2O$  concentration in the off gas was maximal at the beginning of the aerobic period and stabilized at a lower concentration after an initial peak. (0.60  $\pm$  0.17, n = 3) % of the total nitrogen load to the SBAR was emitted as  $N_2O$ . A dramatic change in the microbial community structure was noted between the initial seed sludge and the final mature aerobic granular sludge. Nitrosospira was identified to be the dominant ammonium oxidizing bacteria (AOB) which was attributed as the dominant source of N2O production in aerobic granular sludge by analysis of 16S rDNA sequences.

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domestic wastewater in laboratory studies ([A dav et al., 2008\)](#page--1-0). However, it is not yet established as a large-scale application. Aerobic granulation can be affected by a number of operational parameters, such as seed sludge, substrate composition, organic loading rate, feeding strategy, reactor design, settling time, exchange ratio, and aeration intensity (hydrodynamic shear force) etc. Inside the granules, aerobic and anoxic zones are present that can simultaneously perform different biological processes in the same system, i.e. simultaneous nitrification-denitrification [\(Beun](#page--1-0) [et al., 1999](#page--1-0); [Qin and Liu, 2006\)](#page--1-0).

The research efforts have been focused on the cultivation conditions and factors influencing granulation. As aerobic granular sludge is a complex biological system, the emission of nitrous oxide (N2O) as an intermediate or end product in the metabolism of both nitrification and denitrification can not be neglected. The greenhouse impact of  $N_2O$  is about 300 times of carbon dioxide.  $N_2O$  can also contribute to ozone layer depletion. As the reason of  $N_2O$  has long estimated half-life (approximately 120 years), even a small amount of  $N_2O$  accumulation may cause destructive effects for centuries [\(Ravishankara et al., 2009](#page--1-0)). It was reported that about two thirds of the overall  $N_2O$  was emitted by microbial processes. Therefore, a better understanding and controlling of  $N_2O$  emission are urgently required for minimizing the adverse effect of  $N_2O$ . Controlling the emission of  $N_2O$  has become an interesting topic of

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biological wastewater treatment. However, there is little information available in literature about the emission of  $N_2O$  in aerobic granulation.

Microbial processes are the major emission source of the  $N_2O$ . Few researches have been focused on the relationship between  $N_2O$ emission and microbial community structure, especially in aerobic granulation. With the rapid development of molecular biological techniques, many microbial molecular ecological techniques have been applied to monitor the variance of the bacterial community in activated sludge ([Forney et al., 2004](#page--1-0)). Among these molecular methods, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) seems to be a more practical and useful approach for bio-monitoring the bacterial community [\(Zhang et al.,](#page--1-0) [2011\)](#page--1-0). In combination with a wide range of other micro-scale techniques such as scanning electron microscopy (SEM), researchers are now able to better investigate microbial evolution and granule morphology during sludge granulation ([Li et al., 2008\)](#page--1-0).

In this study, we focused on investigating the  $N<sub>2</sub>O$  emission in an aerobic granulation sequencing batch airlift reactor and the microbial community of the aerobic granular sludge at ambient temperatures. Aerobic granules were achieved in sequencing batch airlift reactor (SBAR). The physical and chemical properties of the aerobic granules were determined. PCR-DGGE was employed for revealing the microbial community structure. This work offers a detailed investigation on the nitrous oxide emission in aerobic granulation at ambient temperatures. It could be useful for the development of the pilot- and full-scale application.

#### 2. Materials and methods

#### 2.1. Experimental set-up and operation

A double-walled cylindrical column gastight sequencing batch airlift reactor was used with an internal diameter of 8 cm. The reactor contained an internal riser (80 cm high, 5 cm internal diameter, bottom clearance 1.5 cm) with a working volume of 3.6 L. Air was introduced via a fine bubble aerator at the bottom of the reactor with an airflow rate of 3 L/min during the aeration phase. During the experiment temperature was controlled at ambient temperature (18  $\pm$  3) $\degree$ C.

A 6-h working cycle was applied over the entire experiment. The reactor was operated in a sequencing fed batch mode and the different periods are displayed in Fig. 1. One cycle consisted of five successive phases including: (1) 10 min feeding, (2) 317 min aerobic reaction, (3) 3 min settling, (4) 5 min discharging and (5) 30 idling. The exchange volume was 50% resulting in a hydraulic retention time (HRT) of 12 h. The sludge settling time was reduced gradually from 20 to 3min after 108 cycles in 27 days and the aeration time was increased accordingly from 300 to 317 min.

Typical cycle was performed regularly by measuring the control parameters (pH, DO and ORP), nitrogen compounds (ammonium, nitrite and nitrate) and the emission of  $N_2O$  throughout the 6-h cycle. During the aeration reaction phase, the emission gas was directly collected in gas sampling bags at the interval of 30 min. During settling, discharging, idling and feeding phases,  $N_2$  was blown in from one side of the reactor's top cover and the injected  $N<sub>2</sub>$  transported the emitted gas into sampling bags ([Kong et al.,](#page--1-0) [2013](#page--1-0)).

#### 2.2. Seed sludge and synthetic wastewater

Activated sludge was used as the seed sludge for the reactors at an initial sludge concentration of 6490 mg/L in mixed liquor volatile suspended solids (MLVSS) collected from a full-scale municipal wastewater treatment plant in Jinan, China.



Fig. 1. Sequencing operation of the SBAR.

The comparison of synthetic wastewater were as follows (per liter): 0.52 g glucose; 0.83 g NaAC; 0.24 g NH<sub>4</sub>Cl; 0.058 g K<sub>2</sub>HPO<sub>4</sub>; 0.024 g KH<sub>2</sub>PO<sub>4</sub>; 0.067 g CaCl<sub>2</sub>; 0.042 g MgSO<sub>4</sub>·7H2O; 0.042 g EDTA;  $0.25$  g NaHCO<sub>3</sub>; 1 ml trace element solution. One liter of trace element solution contained: 1.5 g FeCl<sub>3</sub>  $\cdot$  6H<sub>2</sub>O; 0.15 g H<sub>3</sub>BO<sub>3</sub>; 0.03 g CuSO<sub>4</sub> $\cdot$ 5H<sub>2</sub>O; 0.03 g KI; 0.12 g MnCl $\cdot$ 4H<sub>2</sub>O; 0.06 g NaMoO<sub>4</sub> $\cdot$ 2H<sub>2</sub>O; 0.12 g ZnSO<sub>4</sub> $\cdot$ 7H<sub>2</sub>O; 0.15 g CoCl<sub>2</sub> $\cdot$ 6H<sub>2</sub>O. NaHCO<sub>3</sub> was dosed into the feeding solution to maintain the reactor pH in the neutral range between 7.0 and 7.8.

#### 2.3. Analytical methods

Ammonium (NH<sup>+</sup><sub>4</sub> – N), nitrite (NO<sub>2</sub> – N), nitrate (NO<sub>3</sub> – N), sludge MLVSS concentration, zone settling velocity (ZSV), sludge volume index at 10 min (SVI10) and effluent volatile suspended solids (EVSS) were measured regularly according to standard methods ([APHA, 2005](#page--1-0)). On-line data were collected by probes for pH, dissolved oxygen (DO), oxidation-reduction potential (ORP) and temperature (HACH HQ40d, USA).

The morphology of the seed sludge and granules in the reactors was observed under an optical microscope (BX53, Olympus, Japan) equipped with a digital camera (DP72, Olympus, Japan). In addition, the microstructure of mature granules was examined with a scanning electron microscope (SEM) (S-520, Hitachi, Japan) following the sample treatment procedure detailed by [Diao et al.](#page--1-0) [\(2004\)](#page--1-0). The emission of  $N_2O$  was measured by a gas chromatography (SP-3410, China) with an electron capture detector (ECD) and a Poropak Q column, using 30 mL/min high-purity nitrogen as the carrier gas. The temperature of the detector and oven were set at 360 $\degree$ C and 50 $\degree$ C, respectively ([Wu et al., 2009\)](#page--1-0).

#### 2.4. Calculation of  $N<sub>2</sub>O$  emission rate and emission quantity

The quantity of  $N_2O$  emission with time was calculated from the following equation Eq. (1):

$$
m_{N_2O} = \sum_2^n \left[ Q \cdot (c_{N_2O,n} + c_{N_2O,n-1}) \cdot \Delta t \cdot M_{N_2O} \cdot P/(R \cdot T \cdot 2) \right]
$$
 (1)

where  $m_{N_2O-N}$  is the N<sub>2</sub>O emission quantity (g) varying with time; Q is the volumetric flow rate of air during aerobic reaction phase or of  $N_2$  during mixing, settling, discharging and feeding phase (L min-<sup>1</sup>);  $c_{N_2}$  ois the N<sub>2</sub>O concentration in the emission-gas (mol/mol); *n* is the number of sampling points;  $\Delta t$  is the time interval between each sampling point (30 min);  $M_{N_2O}$  is the molecular weight of N<sub>2</sub>O (44.02 g mol<sup>-1</sup>); *P* is the atmospheric pressure (1 atm); R is the gas constant (0.082 L atm/(K mol); T is room temperature (K).

The  $N_2O-N$  emission in the aerobic granulation sequencing batch airlift system of the incoming nitrogen load was calculated from the following equation Eq. [\(2\):](#page--1-0)

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