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Short Communication

# Physical characteristics and formation mechanism of denitrifying granular sludge in high-load reactor

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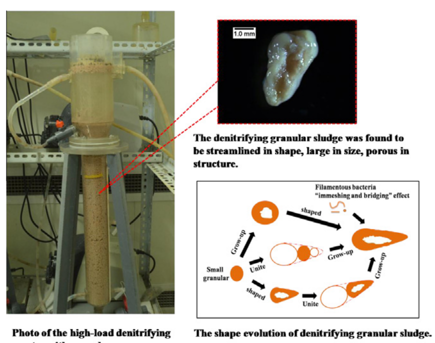
## HIGHLIGHTS

- The denitrifying reactor achieved a nitrate removal rate up to 35 kg/($m^3$ d).
- The denitrifying granule was found to be streamlined in shape at high load.
- The streamlined denitrifying granule showed a good settleability.
- The formation mechanism of streamlined denitrifying granule was revealed.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The physical characteristics of denitrifying granular sludge and their granulation mechanism were investigated in two denitrifying reactors (R1 and R2). The results showed that the denitrifying granular sludge was streamlined in shape with the aspect ratio (AR) of 0.62 ± 0.05, large in size with the diameter of 2.39 ± 0.05 mm, and fast in settlement with the average settling velocity (VS) of 126.36 ± 13.32 m/h at the nitrate loading rate (NLR) of 35.14 ± 0.38 kg/($m^3$ d). The dominance of denitrifying granular sludge with good settleability was attributed to the enhanced growth of granular sludge by extracellular polymers (EPS) and the shaping of granular sludge by shear force.



## 1. Introduction

Denitrifying reactor is a promising technology for denitrification which is a key link in the biological nitrogen removal (Franco et al., 2006; Jin et al., 2012). In denitrifying granular sludge bed reactors, the microorganisms often exist in the form of granular sludge (Rabah and Dahab, 2004; Franco et al., 2006), so the reactor performance is closely related to the amount and activity of granular sludge (Tang et al., 2011). The denitrifying granular sludge reactor was reported for the first time in 1975 (Miyaji and Kato, 1975), and after that it has been a research focus in the field of nitrogen removal from wastewaters.

The microbial residence time is largely dependent on the settling properties of granular sludge in the reactor. At different loading rates, the required settling velocity of granular sludge changes with the upflow velocity of fluid and gas (Rabah and Dahab, 2004; Jin et al., 2012). Jin et al. (2012) reported denitrifying granular

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sludge had notable settleability of 110 m/h. But most of the denitrifying reactors reported so far are low-loaded (Rabah and Dahab, 2004; Franco et al., 2006) and their maximum nitrate loading rate (NLR) is 25 kg/($m^3$ d) (Bode et al., 1987), no literature is available on the characteristics of denitrifying granular sludge and the granulation mechanism at higher NLR.

Recently, a high-rate denitrifying automatic circulate (DA**C**) reactor has been successfully developed in laboratory, and its NLR reached up to 35.14 ± 0.38 kg/($m^3$ d). So the novel DAC reactor was run to characterize the size, shape and settleability of denitrifying granular sludge and to make clear their granulation mechanism.

## 2. Methods

### 2.1. Experimental set-up

Two identical upflow DAC reactors (Fig. S1) with a working volume of 1.25 L were used. Two reactors (designated as R1 and R2) were initially inoculated with 1 L anaerobic granular sludge with the VSS/SS content of 70 ± 1%. The reactors were started up in parallel at the NLR of 2 kg/($m^3$ d) with a fixed recycling ratio about 2.0. R1 was kept to run at the NLR of 2 kg/($m^3$ d) since the high NLR for engineering applications was 1.9 kg/($m^3$ d) (Semon et al., 1997). R2 was operated with increasing NLRs up to 35 kg/($m^3$ d) by shortening hydraulic retention time (HRT) (Tang et al., 2011). The temperature was set at 30 ± 1 °C.

### 2.2. Synthetic wastewater

Sodium nitrate and methanol were pumped into the reactors at concentrations of 1 g $NO_3^-$–N/L and 5 g COD/L, respectively. The ratio of nitrogen to methanol was 1:3.33 to keep the nitrogen as the limiting substrate (Rabah and Dahab, 2004). The contents of the mineral medium were (g/L except for trace element solution): $KH_2PO_3$ 0.05, $CaCl_2$ 0.4, $MgSO_4·7H_2O$ 0.1 and 1 ml/L of trace elements solution. The trace elements solution contained (g/L): 5 EDTA, 5 $MnCl_2·4H_2O$, 3 $FeSO_4·7H_2O$, 0.05 $CoCl_2·6H_2O$, 0.04 $NiCl_2·6H_2O$, 0.02 $H_3BO_3$, 0.02 $(NH_4)_6Mo_7O_2·4H_2O$, 0.01 $CuSO_4·5H_2O$ and 0.003 $ZnSO_4$. The pH of synthetic wastewater medium was in range of 6.6–6.9.

### 2.3. Sampling and analytical methods

The samples were collected when R1 and R2 were in steady state at the NLR of 2.00 ± 0.09 kg N/($m^3$ d) and 35.14 ± 0.38 kg N/($m^3$ d), respectively. The determination of pH, COD, nitrate, nitrite, ammonium, suspended solids (SS) and volatile suspended solids (VSS) were performed according to the standard methods (APHA, 2005). The granule size, aspect ratio and sphericity were determined using QICPIC system (Sympatec, Germany). The granule shape was observed with a stereoscope (Carl Zeiss, Germany). Settling velocity was measured by recording the time taken for individual granules to fall a certain height in a measuring cylinder. Extracellular polymers (EPS) were extracted from granular sludge by EDTA method (Sheng et al., 2005). The content of extracellular

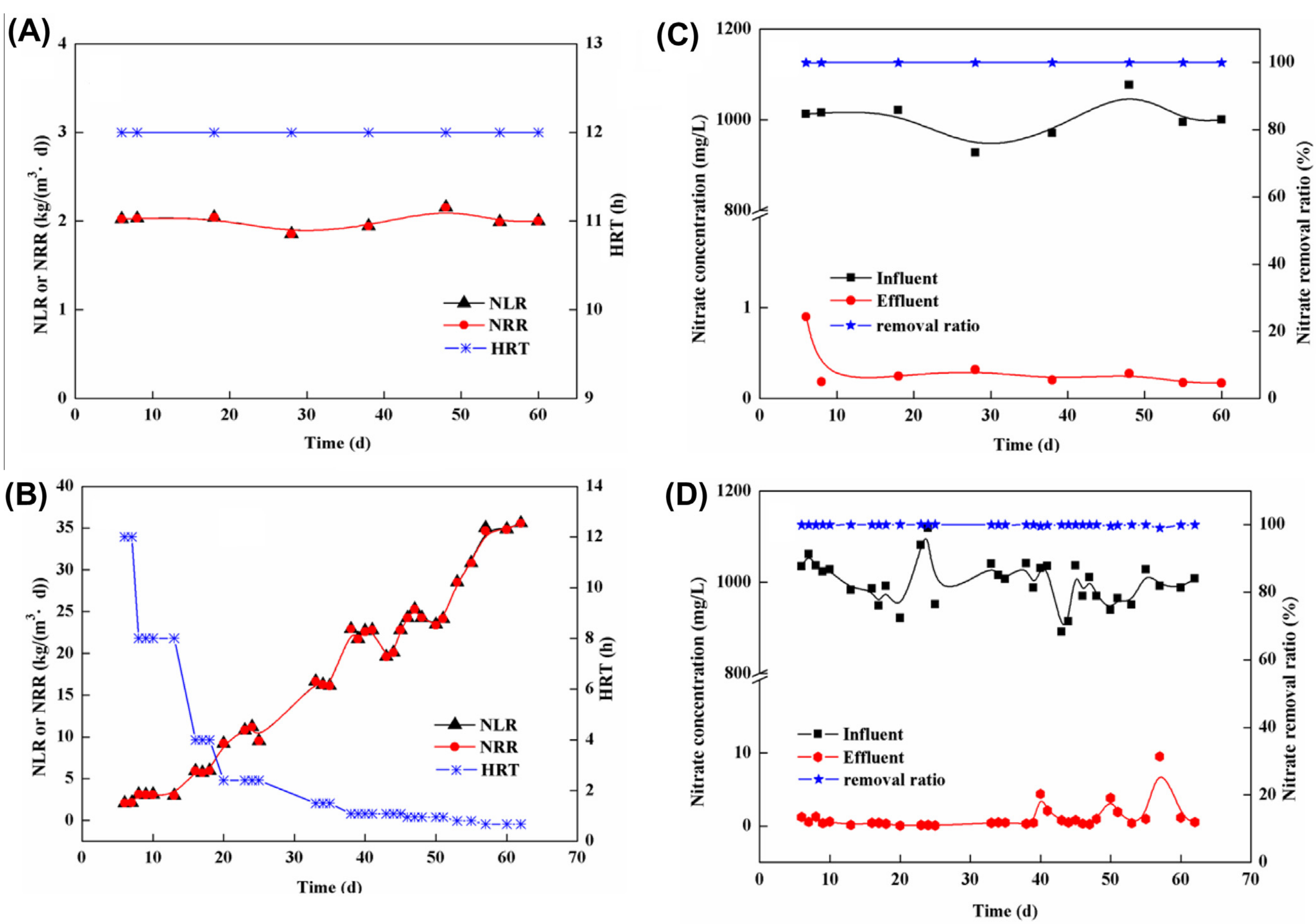


**Fig. 1.** Performance of nitrate removal of the two denitrifying R1 (A and C) and R2 (B and D).