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Comparison of performance, microorganism populations, and bio-physiochemical properties of granular and flocculent sludge from denitrifying phosphorus removal reactors

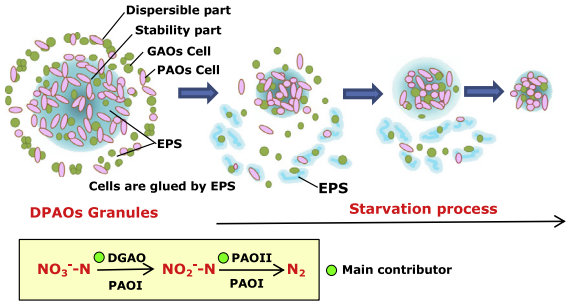
Yayi Wang [a,*], Xuxin Jiang [a], Hong Wang [a], Gang Guo [b], Jianhua Guo [c], Jian Qin [a], Shuai Zhou [a]

[a] State Key Laboratory of Pollution Control and Resources Reuse, College of Environmental Science and Engineering, Tongji University, Siping Road, Shanghai 200092, PR China
[b] Department of Civil & Environmental Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong
[c] Advanced Water Management Centre (AMWC), The University of Queensland, St. Lucia, Brisbane, Queensland 4072, Australia

HIGHLIGHTS

- Denitrifying P removal and nitrite tolerance was compared between flocs and granules.
- Granules had higher resistance to nitrite/FNA due to the mass transfer resistance.
- Granular sludge had a higher PAOs content than flocs sludge.
- PAOII dominated in both systems and performed anoxic P removal cooperated with DGAOs.
- PAOs and GAOs existed in different depths in granules.

GRAPHICAL ABSTRACT



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ABSTRACT

Granule formation frequently occurs in denitrifying phosphorus (P) removal systems. The differences between granules and flocculants, including P removal capacities, bio-physiochemical properties, and microorganism distribution and diversity, are however still poorly understood. Physical and biochemical characteristics of granules and sludge flocs were investigated through two laboratory-scale sequencing batch reactors (SBRs). One reactor was operated as a flocculent SBR and the other was operated as a granular SBR, using a granule diameter of 1.92 ± 0.78 mm. Granular sludge had a higher tolerance to inhibitors (nitrite/free nitrite acids (FNAs)), and a higher percentage of P accumulating organisms (PAOs) (72% vs. 64% in flocs) than flocculent sludge. The granule crush tests and higher PAOII (unable to use nitrate as an electron acceptor) to PAOs ratios (over 72%) by fluorescent *in situ* hybridization showed that in both reactors, glycogen accumulating organisms (GAOs) were mainly responsible for nitrate to nitrite reduction, and PAOII further reduced nitrite to nitrogen gas in association with anoxic P uptake; GAOs washout weakened the mutual relationship between GAOs and PAOII to some extent, which made denitrification of nitrate to nitrite inefficient and weakened subsequent anoxic P removal. GAOs existed mainly on the surface of the granules, whereas PAOs (PAOI + PAOII) were distributed both on the surface and in the interior of the granules. Thus, GAOs had easier access to carbon sources but were at risk of suffering from exposure to FNA.

* Corresponding author. Tel./fax: +86 21 65984275.
E-mail address: yayi.wang@tongji.edu.cn (Y. Wang).

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1. Introduction

Denitrifying phosphorus (P) accumulating organisms (DPAOs) have received much attention because of their many advantages, including effective use of organic carbon substrates and low sludge production [1,2]. DPAOs are enriched under alternating anaerobic and anoxic conditions and are capable of using nitrate (NO_3^-) or nitrite (NO_2^-) as electron acceptors instead of oxygen to achieve satisfactory phosphate uptake and nitrogen (N) removal at the same time [2]. This, however, frequently leads to the accumulation of nitrite/free nitrous acids (FNAs) in DPAOs systems when the denitrification process is interrupted [3]. Nitrite and FNAs are toxic to a wide range of microorganisms, and PAOs are sensitive to FNAs at concentrations as low as 0.0017 mg NO_2^--N/L [3,4]. The fact that P accumulating organism (PAO) activities tend to be inhibited by nitrite or FNAs makes the DPAOs process less stable than that of conventional biological P removal (BPR) systems [3], and is a challenge for the practical application of denitrifying P removal technology.

Granular sludge has been proposed as a promising technology for biological wastewater treatment. It has been successfully formed in sequencing batch reactors (SBRs) designed for denitrifying P removal [1,5]. There are many advantages associated with the use of biogranulation technologies in wastewater treatment, including high biomass retention, strong microbial structures, the ability to withstand high-strength wastewater, shock loadings, and also a high tolerance to toxicity [6,7]. If DPAOs are enriched in granular sludge, it is thought that the constraints of the accumulated nitrite or FNA might be eliminated, primarily owing to (i) the dense distribution of biomass at the outer layer of the granular sludge, and (ii) the thicker, extracellular polymeric substance (EPS) matrix that is built up by biofilm cells, which means that granules are more resistant to hazardous materials (e.g., nitrite/FNA) [6]. These unique structural and bio-chemical properties of granular sludge protect the functional organisms from the harsh external environment, and hence help maintain efficient nutrient removal and the stability of the processes. Further, if DPAOs granules build up a good resistance to nitrite/FNA, it may be possible to use nitrite as an acceptor instead of nitrate. Thus, the denitrifying P removal can be coupled with short-cut nitrification, which will result in more cost-effective and sustainable nutrient removal systems.

Glycogen-accumulating organisms (GAOs) are recognized as the main competitors to PAOs in BPR systems. They take up carbon sources under anaerobic conditions but do not contribute to P removal. If GAO numbers increase, they can cause BPR failure [2]. A new method for separating PAOs from GAOs has been proposed from tests of granular sludge technology in lab-scale SBRs [8]. As PAOs accumulate high amounts of poly-P after aerobic/anoxic P uptake reactions, the settling velocity for PAO-dominated granules is higher than that of GAO-dominated granules. This means that biomass can be segregated by selectively removing sludge at different heights in a granular sludge bed. Using this method, competition between PAOs and GAOs can be controlled, and so it is a good method for manipulating P removal-related microbial populations.

Our current understanding of denitrifying P accumulating microbial granules has been gathered from studies of P removal in aerobic granules [1,5]. To date, there is limited information available about the micro-scale characteristics of denitrifying P accumulating granules in an anaerobic/anoxic/aerobic SBR. Similarly, the differences in (1) denitrifying P removal efficiency, (2) resistance to nitrite/FNA, and (3) the spatial distribution of organisms between flocs and granules are still unclear. Having this information would help us to better understand the advantages of granules for denitrifying P removal.

This study was therefore conducted to assess how sludge morphology (granules or flocs) affected the performance and microbial community structures of denitrifying P removal systems. We compared the resistance capacity of flocculants and granules with nitrite/FNA through dosing with various concentrations of nitrite. We analyzed the spatial distribution of PAOs and GAOs in granules by fluorescent *in situ* hybridization (FISH). We also examined the properties and PAO–GAO abundances in granules that were crushed through starvation tests to give an improved understanding of the mechanism for anoxic P removal in granules.

2. Materials and methods

2.1. The formation of flocs and granular sludge in flocculent SBR (F-SBR) and granular SBR (G-SBR)

DPAOs biomass was placed in two identical SBRs with a working volume of 7.5 L (an internal diameter of 16 cm and a height of 50 cm) [9] for acclimatization. Both SBRs were fed with synthetic wastewater (composition below) and were operated under alternating anaerobic–anoxic–aerobic conditions. They were operated at room temperature (20 ± 1 °C) with a cycle time of 8 h, which was split into a 15-min filling period, a 120-min anaerobic period, a 210-min anoxic period, a 30-min aerobic period, a 20-min sludge settling period, a 15-min effluent decanting period, and a 70-min idle phase. During the first 15-min feeding period, 5.5 L of synthetic wastewater was pumped into the reactors, and KNO_3 solution was pulse added into the reactor at the end of the anaerobic period, giving an initial NO_3^--N concentration of 30 ± 6 mg/L.

The rotation (mechanical mixers) speed was controlled at 150 ± 10 rpm during the reaction phases, and the airflow rate was controlled at 40 L/h via a gas-flow controller to keep the dissolved oxygen concentration at about 2–4 mg/L in the post-aerobic phases. Effluent was drawn from the port at 30 cm above the bottom, leaving 2.0 L of mixed liquor in the reactor. The solid retention time (SRT) of the two reactors was approximately 20 days. One hundred and twenty five microliters of mixed liquor was removed at the end of each aerobic period, and mixed liquid suspended solids (MLSS) were maintained at 4000 ± 200 mg/L. Liquid samples were collected at the end of the different phases of each cycle every 3 days.

The two SBRs achieved stable removals of phosphate and nitrate after 90 days operation (called Period I). In the subsequent period (called Period II), the rotation speed and aerobic airflow rate in one of the reactors (G-SBR) were increased to 300 rpm and 100 L/h, respectively, to facilitate the formation of granular sludge, while the other one (F-SBR) remained unchanged. After 240 days of operation (called Period IIb), the average diameter of the granules had reached equilibrium at 1.92 ± 0.78 mm, and stable and efficient denitrifying P removal was achieved in the two SBRs. To distinguish with Period IIb, the SBRs operation during days 90–240 was defined as Period IIa.

Cycle tests were conducted weekly by measuring the NH_4^+-N, PO_4^{3-}-P, NO_3^--N, NO_2^--N, volatile fatty acids (VFAs), dissolved N_2O and internal polymers (poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV), poly-3-hydroxy-2-methylvalerate (PH2MV), and glycogen concentrations every 30 min through the 8-h cycle. At the start of the cycle tests, the sludge in the two SBRs was washed three times with synthetic wastewater without propionate. Nitrogen gas was introduced into the headspace to ensure anaerobic conditions being maintained for P release. pH was continuously monitored online using a pH probe (pH 3310, WTW Inc., Munich, Germany) and was automatically controlled at 7.5 ± 0.1 by manual addition of 0.3 M HCl or 0.3 M NaOH. The MLSS