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Microbial population dynamics during sludge granulation in an A/O/A sequencing batch reactor

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HIGHLIGHTS

- Granulation was realized at an organic loading rate as low as 0.3 kg COD/(m^3·d).
- Simultaneous COD, nitrogen and phosphorus removal were achieved over operation.
- Bacterial population dynamics were explored by MiSeq pyrosequencing.
- Dominant functional groups for granulation and pollutants removal were identified.

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ABSTRACT

The evolution of the bacterial population during formation of denitrifying phosphorus removal granular sludge was investigated using high-throughput pyrosequencing. As a result, mature granules with a compact structure were obtained in an anaerobic/aerobic/anoxic (A/O/A) sequencing batch reactor under an organic loading rate as low as 0.3 kg COD/(m^3·d). Rod-shaped microbes were observed to cover with the outer surface of granules. Besides, reliable COD and simultaneous nitrogen and phosphorus removal efficiencies were achieved over the whole operation period. MiSeq pyrosequencing analysis illustrated that both the microbial diversity and richness increased sharply during the granulation process, whereas they stayed stable after the presence of granules. Some microorganisms seemed to contribute to the formation of granules, and some were identified as functional bacterial groups responsible for constructing the biological reactor.

1. Introduction

With the rapid industrialization and urbanization, water eutrophication has become a global challenge in the field of wastewater treatment (Bassin et al., 2012). Multiple studies have found that the excess discharge of wastewater rich in nitrogen and phosphorus, especially phosphorus, is the main cause resulting in eutrophication (de Kreuk et al., 2005a). Stringent wastewater discharge regulations about nitrogen and phosphorus concentration limits have thus been put forward worldwide. Biological wastewater treatment technology has been extensively applied for both nitrogen and phosphorus removal owing to its cost-effective and environmentally friendly advantages (Chen et al., 2009). The denitrifying phosphorus removal technology is a newly-developed approach for simultaneous nitrogen and phosphorus elimination. In this process, denitrifying polyphosphate-accumulating organisms (DNPAOs) take up volatile fatty acids (VFAs) and store them as poly-β-hydroxyalkanoates (PHAs) under anaerobic condition. Interestingly, phosphorus is taken up under anoxic condition by utilizing nitrate (NO_3^--N) or nitrite (NO_2^--N) as the electron acceptor instead of oxygen. Therefore, both denitrification and phosphorus removal processes are conducted using the same carbon sources (Kishida et al., 2006). The denitrifying phosphorus technology is 40% less efficient in energy and 20–30% lower in sludge yield compared with the conventional processes (Wang et al., 2012).

Aerobic granular sludge has been intensively investigated due to its advantages in fast settling velocity, dense microbial quantity, compact and strong structure etc. Meanwhile, reliable nitrogen and phosphorus removal can be obtained by granular sludge via nitrification, denitrification and phosphorus uptake (de Kreuk et al., 2005a; Wang et al., 2014). Thus, aerobic granular sludge has some potential role in the construction of efficient, novel and compact wastewater treatment systems. Some researchers seek to link the

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denitrifying phosphorus removal technology with aerobic granular sludge, aiming to enrich DNPAOs within granular sludge (Kishida et al., 2006; Zhang et al., 2011b). In the denitrifying phosphorus removal granular sludge system, the utilization of DNPAOs can relieve the competition for carbon sources; in turn, the layered structure within the granular sludge can provide excellent environments for the cultivation and enrichment of DNPAOs. Therefore, this efficient and innovative technology combines the advantages of denitrifying phosphorus removal and granular sludge (Kishida et al., 2006).

Though numerous studies have been conducted to explore the denitrifying phosphorus removal, aerobic granular sludge, and denitrifying phosphorus removal granular sludge processes for simultaneous nitrogen and phosphorus removal, little work has been performed on revealing the microbial community dynamics during the aerobic granulation process conducting denitrifying phosphorus removal, nor on identifying the functional bacterial groups within the biological granular reactor. Therefore, the major purpose of the present study is to realize granular sludge formation with synthetic wastewater for simultaneous nitrogen and phosphorus removal, besides, the bacterial population dynamics during the aerobic granulation are explored through high-throughput pyrosequencing.

2. Materials and methods

2.1. Set-up and operation

The Plexiglas reactor was 120 mm in inner diameter and 800 mm in height (Fig. 1), giving an effective volume of 7 L and a ratio of height to diameter (H/D) of 5.2. 3.5 L of synthetic wastewater was fed into the reactor at the beginning of every cycle at an exchange ratio of 50%. Air was introduced by a fine-bubble aerator from the bottom of the reactor with a constant airflow rate of 2.5 L/min, and the dissolved oxygen (DO) concentration was controlled at about 5.0 mg/L during the aerobic phase. A stirring speed of 250 rpm was set throughout the anaerobic, aerobic and anoxic phases by a mechanical stirrer. The water temperature of the reactor was kept at 25 ± 3 °C.

The reactor was operated on an 8 h cycle, while the time duration for each step was changeable mainly based on the settling performance of sludge within the reactor, generally consisting of 5 min of feeding, 180 min of anaerobic phase, 180–150 min of oxic phase, 90–138 min of anoxic phase, 20–2 min of settling time and 5 min of effluent discharge periods as given in Table 1. Activated sludge from the Shahu Wastewater Treatment Plant (WWTP) was inoculated to start up the SBR with an initial mixed liquor suspended solids (MLSS) concentration of 2752 mg/L. The sodium acetate-based synthetic wastewater was based on the water quality of urban municipal wastewater which is characterized by low chemical oxygen demand (COD). The composition of the influent media was as follows (per liter): 150 mg COD, 15 mg ammonia nitrogen (NH_4^+-N), 6 mg total phosphorus (TP), 10 mg Ca^{2+}, 10 mg Mg^{2+}, and 1 mL trace solution (He et al., 2015). Influent pH was kept around 7.5 without control during the operation.

2.2. Analytical methods

The COD, nitrogen (including NH_4^+-N, NO_3^--N, NO_2^--N), TP, MLSS, sludge volume index at 30 min (SVI_{30}) were measured according to the standard methods (APHA, 2005). The pH and DO were measured by a pHS-25 meter and YSI5000 meter. The morphology of the sludge flocs and granules was observed using an optical microscope (Olympus, Japan) and scanning electron microscope (SEM) (VEGA3, TESCAN). Samples for SEM were pretreated and analyzed as previous researches (Liu and Tay, 2015).

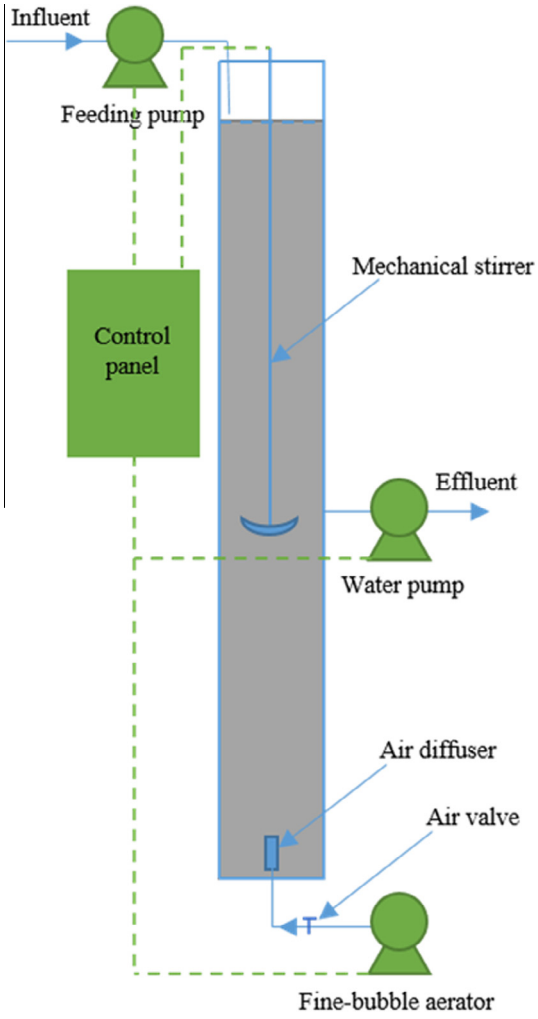

Fig. 1. Schematic diagram of the A/O/A sequencing batch reactor.

Table 1
The operational parameters for each phase during operation.

Steps	Day	Time duration (min)					
		Feeding	Anaerobic	Oxic	Anoxic	Settling	Discharge
I	1–14	5	180	180	90	20	5
II	15–19	5	180	170	105	15	5
III	20–25	5	180	160	120	10	5
IV	26–30	5	180	150	135	5	5
V	31–70	5	180	150	138	2	5

2.3. DNA extraction, polymerase chain reaction (PCR) amplification and pyrosequencing

The three samples G1, G2 and G3 were collected on day 1, 31 and 65, respectively, during the consecutive operation of the reactor (70 days in total). After appropriate treatment, the supernatants were used for DNA extraction, PCR amplification, and pyrosequencing using the primer sets 338F (5′-ACTCCTACGGGAGG CAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) (Liu et al., 2015) as the procedures by our previous research (Wang et al., 2015). The Illumina MiSeq platform (PE300, CA, USA) was applied for sequencing of the complete genome of collected samples