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Elucidation of microbial characterization of aerobic granules in a sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal at varying carbon to phosphorus ratios

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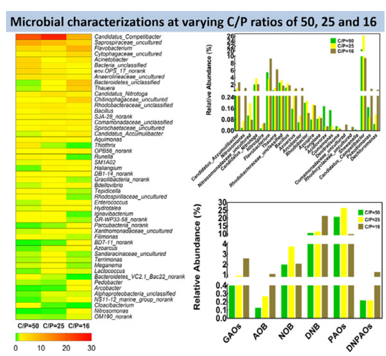
HIGHLIGHTS

- An aerobic granule SNDPR system was evaluated at varying C/P ratios.
- A C/P ratio of 25 was found as a watershed.
- Decreasing C/P ratios decreased TP removal while unaffected carbon and nitrogen.
- C/P ratios shaped the microbial populations on both diversity and structure.
- GAOs, AOB, DNB and DNPAOs enriched via decreasing C/P ratios.

GRAPHICAL ABSTRACT



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ABSTRACT

An aerobic granules simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system was evaluated in terms of the reactor performance and microbial population dynamics with decreasing C/P ratios from 50 to 16. The effects of C/P ratios on organic carbon and nutrients removal were investigated, as well as the alpha diversity of the bacterial community and bacterial compositions by using Illumina MiSeq pyrosequencing technology. Finally, the relative abundances and distribution patterns were identified and assessed given the key functional groups involved in biological nutrients removals to reveal the effects of C/P ratios to aerobic granules in the SNDPR from the molecular level.

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1. Introduction

Aerobic granular sludge (AGS) is an attractive alternative for activated sludge flocs due to their advantages in excellent settleability, high biomass retention, and the ability to withstand high organic loading rates and tolerance to toxic shocks (Adav et al., 2008). Therefore, aerobic granules have experienced extensive investigations in numerous researches for domestic and industrial wastewater treatment in recent years (Adav et al., 2008; Liu and Tay, 2004). Granules system has been widely used for domestic wastewater treatment due to their effective and reliable capacity

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for simultaneous organic carbon, nitrogen and phosphorus removal (Wei et al., 2014; Yilmaz et al., 2008). He et al. (2015, 2017, 2016a) and Yilmaz et al. (2008) employed the simultaneous nitrification, denitrification and phosphorus removal (SNDPR) system by using aerobic granules and achieved excellent simultaneous organic carbon and nutrients removal efficiencies. It was well confirmed that the use of the SNDPR by combining AGS technology would meet the strict effluent standards for domestic wastewater treatment (He et al., 2015, 2016a; Yilmaz et al., 2008).

The key microorganisms involved in biological nitrogen and phosphorus removal contains ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), denitrifying bacteria (DNB), phosphorus accumulating organisms (PAOs) and denitrifying PAOs (DNPAOs) (Oehmen et al., 2010). Biological nitrogen removal is achieved by nitrification and denitrification processes, where AOB and NOB are responsible for nitrification process, and DNB conducts denitrification. Both PAOs and DNPAOs can perform biological phosphorus removal, while DNPAOs simultaneously remove nitrogen and phosphorus via nitrite (NO_2^--N) or nitrate (NO_3^--N) (Zhang et al., 2016). Besides, the competition between heterotrophic glycogen accumulating organisms (GAOs) and PAOs/DNPAOs largely affect the biological nutrients removal when the carbon source is limited (Kishida et al., 2006; Wang et al., 2015b). Zhang et al. (2016) also reported the competition between DNPAOs and DNB for nitrate/nitrite during biological nutrients removal.

Previous studies have revealed that the ratio of carbon to phosphorus in the feeding water affects the phosphorus removal by shaping the structure of bacterial communities involved in enhanced biological phosphorus removal (EBPR) and the focus is the competition between PAOs and GAOs (Chuang et al., 2011; Jiang et al., 2015; Muszynski and Milobedzka, 2015). Numerous studies have found that a high C/P ratio (e.g. >50) in influent tends to favor the growth of GAOs instead of PAOs, therefore, a lower C/P ratio (e.g. 10–20) will favor the enrichment of PAOs (Chuang et al., 2011). Community structure within aerobic granules is one of the most interesting topics for researchers, especially in a system related to simultaneous carbon, nitrogen and phosphorus removal (He et al., 2016b; Lee et al., 2010). Exploring the relative abundances and distributions helps demonstrate the inner mechanism for biological nitrogen and phosphorus removal (Zhang et al., 2016). Kong et al. (2002) analyzed the community composition of a sequencing batch reactor by Fluorescence *in situ* hybridization (FISH) technology and found that the C/P ratios (50 to 10) largely influenced the microbial community. However, up to date little is known about the microbial community shifts in response to variations of C/P ratios. Besides, systematic investigations on the relative abundances and spatial distributions of key functional groups involved in nitrogen and phosphorus removal in terms of GAOs, PAOs/DNPAOs, AOB, NOB, and DNB are limited.

The objective of the present study was to obtain a comprehensive understanding of the aerobic granules SBR in a SNDPR system in terms of the reactor performance and microbial population dynamics. Therefore, simultaneous removals of carbon, nitrogen, and phosphorus were monitored over the operation with varying C/P ratios from 50, 25 to 16, along with the microbial diversity and structure by using Illumina MiSeq pyrosequencing technology. Key functional groups including GAOs, PAOs/DNPAOs, AOB, NOB, and DNB were identified and explored in terms of relative abundances and spatial distributions as well as their roles in the SNDPR system.

2. Materials and methods

2.1. Experimental setup

Experiments were conducted in a lab-scale SBR with a working volume of 3.6 L. Mature granules from our previous study (He et al., 2016b) was inoculated into the reactor with an initial concentration of about 4.4 ± 0.5 g/L. The granules were with an average diameter of 1.5 ± 0.5 mm and a sludge volume index at 5 min (SVI_5) of 22.58 ± 0.69 mL/g. To achieve simultaneous nitrification, denitrification and phosphorus removal, an anaerobic/oxic/anoxic mode was adopted and configured as previous research (He et al., 2016a). Each cycle consisted of 2 min of feeding, 120 min of anaerobic phase, 90 min of aeration phase, 144 min of anoxic phase, 2 min of settling time and 2 min of discharge periods, making the hydraulic retention time (HRT) of 12 h. Synthetic domestic wastewater was supplied in the present work with compositions as follows (per liter): 200 mg chemical oxygen demand (COD), 20 mg ammonia nitrogen (NH_4^+-N), 10 mg calcium ions and 10 mg magnesium ions, and 1 mL trace solution as described by He et al. (2015). The concentration of phosphorus was varied with the average C/P ratios of 50, 25 and 16, respectively, by increasing the influent phosphorus concentration. 1.8 L of synthetic wastewater was fed into the reactor at the beginning of each cycle thus making the exchange ratio of 50%. No manual sludge discharge was conducted during the operation period, with a sludge retention time (SRT) of about 25 days. The dissolved oxygen during the oxic phase was about 5.0 mg/L. The temperature was not controlled during the treatment period and the water temperature was 17–20 °C. A mechanical stirrer was operated constantly at 250 rpm through the anaerobic, oxic and anoxic phases to prevent sludge settling.

2.2. MiSeq pyrosequencing

Aerobic granules samples from the reactor were collected on day 25, 50 and 75 (i.e., the end of each phase before decreasing the C/P ratios). Genomic DNA extraction was conducted using the PowerSoil DNA kit (MoBio Laboratories, Carlsbad, USA). PCR amplification, and pyrosequencing were conducted sequentially using the primer sets 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) as the procedures by previous studies (Wang et al., 2015a). Sequencing of the targeted genes of the collected samples was conducted using the Illumina MiSeq platform (PE30, CA, USA) following the manufacturer's instructions. The bioinformatics analysis was run as previous work (He et al., 2016b; Wang et al., 2015a). The original gene sequences of the three samples under C/P ratios of 50, 25 and 16, respectively, to NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra/), and the accession number is SRR5518931.

2.3. Analytical methods

The COD, nitrogen (including NH_4^+-N, nitrate (NO_3^--N), nitrite (NO_2^--N)), TP, MLSS, MLVSS, sludge volume index at 5 min (SVI_5) were measured according to the standard methods (APHA, 2005). Total inorganic nitrogen (TIN) was regarded as the sum of NH_4^+-N, NO_3^--N, NO_2^--N (He et al., 2016a). The pH and DO were measured using a pHS-25 meter and YSI5000 meter.

3. Results and discussion

3.1. Reactor performance

The aerobic granules SBR performing simultaneous carbon, nitrogen, and phosphorus removal was run for 75 days, with different C/P ratios of 50, 25 and 16 for a period of 25 days each, respectively (Fig. 1). Continuous operation of the aerobic granular SBR exhibited reliable removal for COD (average effluent of 22.38 mg/L and removal rate of 89.15%), NH_4^+-N (average effluent of 0.50 mg/L and removal rate of 97.37%) and TIN (average effluent

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