



Enhanced nitrogen removal in an aerobic granular sequencing batch reactor performing simultaneous nitrification, endogenous denitrification and phosphorus removal with low superficial gas velocity



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HIGHLIGHTS

- An aerobic granule SNDPR system were established for reliable C, N and P removal.
- Enhanced nitrogen removal achieved via strengthening SND and NUR with dropping SGVs.
- GAOs and PAOs were enriched with decreasing SGVs.
- Lower SGV favored the growth of AOB while suppressed the NOB.

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ABSTRACT

An aerobic granular simultaneous nitrification, endogenous denitrification and phosphorus removal (SNDPR) system was configured for simultaneous carbon and nutrients removal fed with low-strength synthetic wastewater (0.3 kg COD/(m³·d)). The SNDPR system was operated on an anaerobic/oxic/anoxic (AOA) mode with a gradually dropping superficial gas velocity (SGV) from 0.17, 0.11 to 0.04 cm/s. Long term operation over 120 days revealed decreasing SGV resulted in poorer settleability and microbial activity as well as larger particle sizes, which were in great linear correlations with the sharp decrease of protein/polysaccharides (PN/PS). Excellent removal of chemical oxygen demand (COD) and phosphorus were achieved during the whole process, while enhanced nitrogen removal was observed by dropping the SGV. Both simultaneous nitrification and denitrification rate (SND) and nitrite accumulation rate (NUR) increased significantly, as with the removal efficiency for nitrogen. Illumina MiSeq pyrosequencing displayed glycogen-accumulating organisms (GAOs) *Candidatus_Cometibacter* predominated the SNDPR system, while the PAOs *Candidatus_Accumulibacter* grew notably with decreased SGV. Lower SGVs favored the enrichment of ammonia-oxidizing bacteria (AOB) while suppressed the accumulation of nitrite-oxidizing bacteria (NOB). This study might contribute to the application of SNDPR system for simultaneous carbon, nitrogen and phosphorus removal.

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Abbreviations: AOA, anaerobic-oxic-anoxic; AOB, ammonia oxidizing bacteria; AOR, ammonia oxidizing rate; COD, chemical oxygen demand; COD_{consum}, anaerobic organic carbon consumption; COD_{ex}, COD taken by exogenous denitrification; COD_{intra}, COD taken as the intracellular carbon source; DGAOs, denitrifying GAOs; DNPAOs, denitrifying PAOs; DO, dissolved oxygen; GAOs, glycogen-accumulating organisms; H/D, ratio of height to diameter; HRT, hydraulic retention time; MLSS, mixed liquor suspended solids; MLVSS, mixed liquor volatile suspended solids; MLVSS/MLSS, ratio of MLVSS to MLSS; NAR, nitrite accumulation rate; NH₄⁺, N-ammonia nitrogen; NO₃⁻, N-nitrate nitrogen; NO₂⁻, N-nitrite nitrogen; NOB, nitrite oxidizing bacteria; NUR, nitrite accumulation rate; OTUs, operational taxonomic units; PAOs, phosphorus accumulating organisms; PN, protein; PS, polysaccharides; PN/PS, ratio of PN to PS; PRA, phosphorus released amount; PRR, phosphorus released rate; PUA, phosphorus uptake amount; PUR, phosphorus uptake rate; RDP, ribosomal database project; SBR, sequencing batch reactor; SBAR, sequencing batch airlift reactor; SGV, superficial gas velocity; SND, simultaneous nitrification and denitrification rate; SNDPR, simultaneous nitrification, (endogenous) denitrification and phosphorus removal; SOUR, specific oxygen uptake rate; SRT, sludge retention time; SVI, sludge volume index; TIN, total inorganic nitrogen; TP, total phosphorus.

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1. Introduction

Aerobic granules are self-aggregates under aerobic conditions with no carrier materials [1,2]. In recent years, aerobic granular sludge technology has raised much attention due to its superiorities over conventional activated sludge, such as compact and strong microbial structure, excellent settleability, high biomass retention and the capacity to withstand high loading rates [3]. Besides, simultaneous carbon, nitrogen and phosphorus is achieved by aerobic granules. There are aerobic, anoxic and anaerobic zones within granules under aeration conditions due to the layered structures of aerobic granules [4]. Wang et al. [5] revealed that endogenous denitrification and phosphorus removal exhibited effective removal capacity by inserting an anaerobic phase before the oxic period. He et al. [6,7] reported the feasibility of applying the aerobic granular sequencing batch reactor (SBR) under an anaerobic/oxic/anoxic mode for simultaneous nitrification, endogenous denitrification and phosphorus removal (SNDPR), which could efficiently utilize the carbon source in the influent by strengthening the anaerobic intracellular carbon storage.

Previous investigations have reported that the superficial gas velocity (SGV) not only shapes the structures of aerobic granules, but also affects the biological removal performances [8]. Compared with the high SGV, limited SGV is always accompanied by larger volume, less compact structure and poorer settling. Besides, microbes resided within granules can get sufficient energy supply due to the lower mass transfer resistance in loosely structured flocs [9]. In addition, from the viewpoint of energy saving, low SGV is more favorable for operation [10]. Present studies have been focused on the effects of SGV on aerobic granulation process, however, few have been conducted on the operation of the aerobic granular reactor, nor combining with the SNDPR [4,8,9]. Besides, the long-term stable operation of aerobic granules is a major concern for wider application of this promising technology, which might disintegrate with inappropriate conditions. There is a lack of information concerning the operation of SNDPR system by aerobic granules in a SBR under low SGV conditions.

Community structure within aerobic granules is one of the most interesting topics for researchers, as well as the understanding of the key functional groups responsible for carbon and nutrients removal in the biological system. Therefore, the major purpose of the present study was to characterize simultaneous carbon, nitrogen and phosphorus removal via the aerobic granule SNDPR system for treating low-strength wastewater with low SGV. Mechanisms for carbon, nitrogen and phosphorus removal were investigated by establishing an aerobic granule SNDPR system. A further study employing MiSeq pyrosequencing explored the inner contributors for aerobic granular SNDPR system.

2. Materials and methods

2.1. SBR configuration and operation

The SBR used for present study contained: 1) a plexiglass reactor with a diameter of 100 mm and a height of 500 mm (giving an effective working volume of 3.6 L and a ratio of height to diameter (H/D) of 5); 2) two water pumps for feeding (from the top of the reactor) and discharging (from the middle of the reactor, making an exchange ratio of 50%), respectively; 3) an aerator at the bottom of the reactor (controlling the aeration rate to 800, 500 and 200 mL/min, corresponding to the superficial gas velocity of 0.17, 0.11, and 0.04 cm/s, respectively); 4) a mechanical stirrer with a speed of 150 rpm; and 5) a panel for controlling the whole system running automatically (operating on a 6-h-cycle (corresponding to the hydraulic retention time (HRT) of about 12 h) AOA mode con-

sisting of 2 min of feeding, 120 min of anaerobic phase, 90 min of oxic phase, 144 min of anoxic phase, 2 min of settling and 2 min of discharge periods according to our previous research [6]) (seen as Fig. S1). No manual discharge of sludge was conducted over operation with a sludge retention time (SRT) of about 25 days by natural sludge washout. Water temperature during the treatment period was controlled at 20 ± 2 °C.

2.2. Seed sludge and wastewater

Mature and stable aerobic granules from our previous study [11] were inoculated into the present reactor 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. The concentration of the mixed liquor suspended solids (MLSS) in the reactor was 4.4 ± 0.5 g/L. The system was fed with synthetic wastewater as follows (per liter): NaAc 256.27 mg, NH_4Cl 76.4 mg, KH_2PO_4 14.6 mg, $CaCl_2$ 10.6 mg and $MgSO_4 \cdot 7H_2O$ 90 mg and 1 mL of trace solution as described by He et al. [12], corresponding to the concentrations for COD, NH_4^+-N and TP of 200, 20 and 3 mg/L, approximately. The pH of influent wastewater was adjusted to about 7.5 using hydrochloric acid (HCl) or sodium hydroxide (NaOH) solutions without control during the operation. The configured system has been operated for enough long time to adapting to the low SGV conditions and followed by the present study with varying SGV from 0.17 to 0.04 cm/s.

2.3. Analytical methods

2.3.1. Water quality and sludge characterization

The COD, nitrogen (including NH_4^+-N , nitrate ($NO_3^- -N$), nitrite ($NO_2^- -N$)), TP, MLSS, sludge volume index at 5 min (SVI_5) were measured according to the standard methods [13]. Total inorganic nitrogen (TIN) was regarded as the sum of NH_4^+-N , $NO_3^- -N$, $NO_2^- -N$. The pH and DO were measured using a pHs-25 meter and YSI5000 meter.

2.3.2. EPS extraction and analysis

Aerobic granules were pretreated with 0.9% sodium chloride solutions and then subjected to EPS extraction with a modified heat extraction method. Protein (PN) content was determined by the Bradford method and polysaccharides (PS) content was analyzed using a sulfuric acid-anthrone colorimetric method [7]. EPS was regarded as the sum of PN and PS.

2.3.3. Quantification of SNDPR system

The quantification of SNDPR system was proceeded according to our previous study [6]. Namely, the process rates were measured based on the cycle performance of the AOA mode. The SND efficiency and rate were determined by the nitrogen loss at the oxic phase. The anaerobic organic carbon consumption (COD_{consum}) was defined as the consumption of influent COD at the anaerobic phase, which could be divided into two parts: the COD taken by the exogenous denitrification of $NO_3^- -N$ and $NO_2^- -N$ (COD_{ex}), and the COD stored by PAOs and GAOs as the intracellular carbon source (COD_{intra}) (Eqs. (1) and (2)).

$$COD_{consum} = COD_{ex} + COD_{intra} \quad (1)$$

$$COD_{ex} = 2.86NO_3^- -N + 1.71NO_2^- -N \quad (2)$$

where, 2.86 and 1.71 are the theoretical values of COD consumption for denitrification of unit $NO_3^- -N$ and $NO_2^- -N$, mg N/mg COD; $NO_3^- -N$ and $NO_2^- -N$ are referred to the concentrations reduced at the anaerobic phase, mg/L.

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