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Culture of denitrifying phosphorus removal granules with different influent wastewater

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ABSTRACT

Fed with three different kinds of influent, i.e. sodium acetate-based synthetic wastewater, synthetic wastewater with 10-mg L^{-1} Ca^{2+} ions, and raw domestic wastewater, respectively, compact of different influent wastewater on aerobic granulation was investigated in three sequencing batch reactors (corresponding to R1–R3) operating in an alternating anaerobic/oxic/anoxic mode. As a result, denitrifying phosphorus removal granules with an average diameter above 600 μm were successfully cultivated within 42 d when inoculated with flocculent sludge. The granules showed some characteristics, e.g. low in moisture content, high in specific gravity, and specific oxygen uptake rate. Reactors' performance throughout operation presented that effluent concentration of COD was always lower than 40-mg L^{-1}; TN, NH_4^+-N, and TP was often lower than 1-mg L^{-1}. In addition, cycle test displayed that efficiencies of COD, NH_4^+-N, TN, and TP removal in R1–R3 reached up to 90.25, 92.98, 92.96%; 99.29, 99.57, 91.70; 90.83, 92.80, 89.56%; 94.06, 96.76, 90.71%, respectively, the maximal specific phosphorus release rate was 14.34, 8.32 and 2.32 mg P g $MLVSS^{-1}$ h^{-1} in R1–R3, respectively, and the maximal specific phosphorus uptake rate was 14.13 mg P g $MLVSS^{-1}$ h^{-1} in R1 and 2.34 mg P g $MLVSS^{-1}$ h^{-1} in R2, respectively.

Keywords: SBR; Granular sludge; Denitrifying phosphorus removal; Denitrifying phosphorus-accumulating organisms; A/O/A mode

1. Introduction

In conventional biological nutrient removal processes, nitrogen is removed by aerobic nitrification and anoxic denitrification [1], while phosphorus removal is achieved by anaerobic phosphorus release and aerobic phosphorus uptake under anaerobic/aerobic conditions [2]. Thus, simultaneous nitrogen and phosphorus removal can be only accomplished by cooperation of nitrifying and denitrifying bacteria, polyphosphate-accumulating organisms (PAOs) [3,4]. However, both denitrification and phosphorus release require organic carbon source, resulting in the competition for organic carbon in influent [5,6]. Therefore, the phosphorus removal rate often decreases when organic carbon available is not enough [7].

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Denitrifying polyphosphate-accumulating organisms (DNPAOs) are a group of micro-organisms which can perform denitrification and phosphorus removal simultaneously using the same carbon source [8]. DNPAOs can take up external carbon substrates and store them as polyhydroxyalkanoates in the cells under anaerobic conditions, and use nitrate/nitrite instead of oxygen as an electron acceptor to remove phosphorus under anoxic conditions [9,10]. The cell yield decreases by 20–30% for DNPAOs are 40% less efficient in generating energy [11]. Therefore, the utilization of DNPAOs offers many advantages in simultaneous nitrogen and phosphorus removal [12,13].

Aerobic sludge granules have been developed widely for the following excellent attributes, regular, smooth and nearly round in shape, excellent settleability, dense and strong microbial structure, high biomass retention, ability to withstand at high organic loading, and tolerance to toxicity [14–17]. It's been reported that simultaneous nitrogen and phosphorus removal can be obtained in the layered oxic/anoxic/anaerobic conditions within granules due to the limited oxygen penetration depth inside [11,14,16].

Denitrifying phosphorus removal sludge granules have been reported cultivated in sequencing batch reactor (SBR), and had a good performance in biophysical and chemical characteristics and nutrients' removing capacities. [11,18,19].

In this work, three different kinds of wastewater—normal synthetic wastewater, synthetic wastewater with 10-mg L^{-1} Ca^{2+} ions, and domestic wastewater—were fed into R1–R3 under anaerobic/oxic/anoxic (A/O/A) mode to investigate effects of different influent wastewater on the culture of denitrifying phosphorus removal granules. Some biophysical and chemical characteristics of obtained granules as well as COD and nutrients' removal performance during the granulation process in R1–R3 were studied. Mechanism of aerobic granulation was also discussed to contribute to further studies of granulation of denitrifying phosphorus removal sludge.

2. Materials and methods

2.1. Reactor operation

Investigation was carried out in three SBRs with a relatively low height/diameter (D/H) of 6.3 and a working volume of 7 L. 3.5 L of influent wastewater was fed into each reactor at the start of every cycle. The hydraulic retention time was 16 h. Air was introduced by a fine-bubble pump fixed at the bottom of each reactor. Stirring speed was set at 300 rpm. The test was carried out at room temperature of 18–25℃ during the operation. Dissolved oxygen (DO) was controlled above 5-mg L^{-1} in the oxic phase. Influent pH of synthetic wastewater was adjusted to 7–8, and was 6–8 in raw domestic wastewater without control.

R1–R3 were operated on a 8-h cycle, operation time was changed by steps according to settleability and reactors performance, in general, consisting of 5 min of feeding, 180 min of anaerobic phase, 180–140 min of oxic phase, 90–148 min of anoxic phase, 20–2 min of settling time, and 5 min of effluent discharge periods.

2.2. Inoculated sludge and feeding composition

Activated sludge inoculated to start up SBRs was obtained from Shahu Wastewater Treatment Plant operated with an A^2O process configuration; therefore, inoculated sludge had certain capacity to SNPR at the beginning of start-up period. The collected seed sludge was evenly inoculated into R1–R3 after one-day acclimatization with normal synthetic wastewater. The initial inoculated sludge in R1–R3 had a mixed liquor suspended solids concentration of approximately 5,000 mg L^{-1}.

R1 was treated with sodium acetate-based synthetic wastewater with the following composition (per liter): 300–350 mg COD, 8–14 mg TP, 40–50 mg NH_4^+-N, and 1-mL trace solution per 15-L influent. R2 was the same in composition as R1 with 10-mg L^{-1} Ca^{2+} ions (with $CaCl_2$ as the ions' provider) additionally. R3 was treated with domestic wastewater from an urban residence in Chagang District, Wuhan University. The trace solution contained as follows (per liter): 0.9-mg $FeCl_3$, 0.15-mg H_3BO_3, 0.18-mg KI, 0.03-mg $CuSO_4{\cdot}5H_2O$, 0.06-mg $MnCl_2{\cdot}4H_2O$, 0.12-mg $ZnSO_4{\cdot}7H_2O$, 0.15-mg $CoCl{\cdot}7H_2O$, 0.06-mg $Na_2MoO{\cdot}2H_2O$, and 10-mg EDTA.

2.3. Analytical methods

COD, NH_4^+-N, NO_3^--N, NO_2^--N, TN, and TP were measured according to the standard methods for the examination of water and wastewater [20]. The pH and DO were measured by pH meter (pHS-25) and DO meter (YSI5000). The granular sludge morphology was analyzed by electronic microscope. Moisture content and specific gravity were measured by weighting method [21]. SOUR was measured by breathing method [22].

3. Results and discussion

3.1. Granules attributes

Disperse inoculated activated sludge with loose structure and no filamentous bacteria on the surface developed into fine particles gradually during the start-up period. As a result, compact, plump granules with smooth surface, and close structure, in spherical/ellipsoidal shape and yellow/brown-yellow in color were obtained at the end of process.

Table 1 shows some biophysical and chemical characteristics of granules in R1–R3. As shown in Table 1, granules obtained in R1–R3 performed excellent attributes in average diameter, moisture content, specific gravity, and SOUR than reported activated sludge, which had a typical diameter of 70 μm, moisture content higher than 99%, specific gravity of 1.002–1.006, and average SOUR of 8-mg O_2 g $MLVSS^{-1}$ h^{-1} [23–25], which suggested that granules in R1–R3 were much compact in structure, dense in microbial content, and strong in organisms' activity than inoculated activated sludge.

We could also learn from Table 1 that, although sludge granules cultivated in R2 had the largest diameter size and was the lowest in moisture content and the highest in specific gravity, granules in R3 was much higher in SOUR than that in R1 and R2. Therefore, we could be convinced that the different influent wastewater in R1–R3 led to the different exhibition attributes of granules. Acetate-based synthetic wastewater was more favorable to bigger, denser, and more compact granules since soluble organic carbon was abundant and easy to absorb, while raw domestic wastewater acclimatized microbial activity due to its complicated composition.

Table 1
Characteristics of granules from R1 to R3 treated with different influent wastewater

	On the day 42		
Characteristic	R1	R2	R3
Average diameter[a]	800.00	1008.00	598.02
Moisture content[b]	97.00	96.70	97.10
Specific gravity	1.016	1.023	1.014
SOUR[c]	26.93	54.97	116.77

[a]Units: μm.
[b]Units: %.
[c]Units: mg O_2 g $MLVSS^{-1}$ h^{-1}. SOUR for inoculated sludge was 13.58-mg O_2 g $MLVSS^{-1}$ h^{-1}.

3.2. COD removal

COD removal performance during the start-up period in R1–R3 is shown in Fig. 1. The average removal efficiencies of COD in R1–R3 were 90.67, 90.95, and 80.19%, respectively, while the average effluent COD were 31.1, 28.6, and 27.4 mg L^{-1} over the whole operation process. COD removal rate changed slightly in R1 and R2, but fluctuated about 20% in R3.

The different influent wastewater would be responsible for the variation of removal behaviors. Soluble organic carbon available in R1 and R2 were

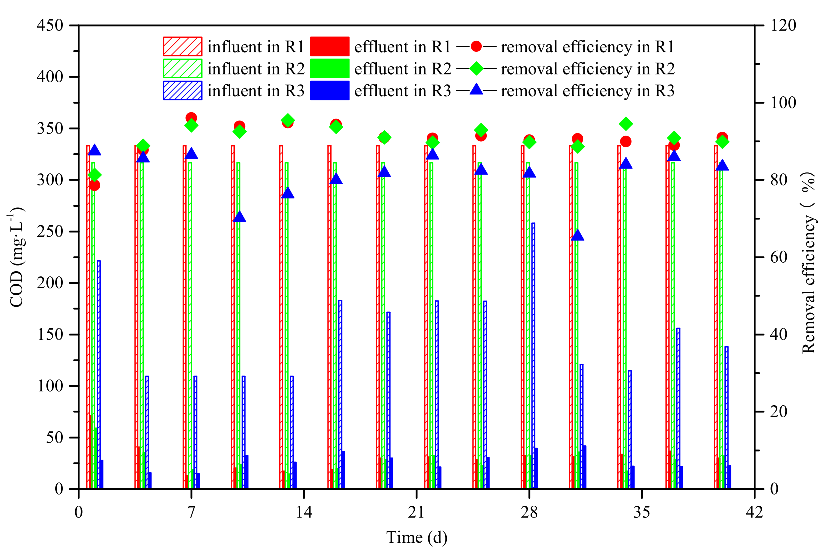

Fig. 1. COD removal.

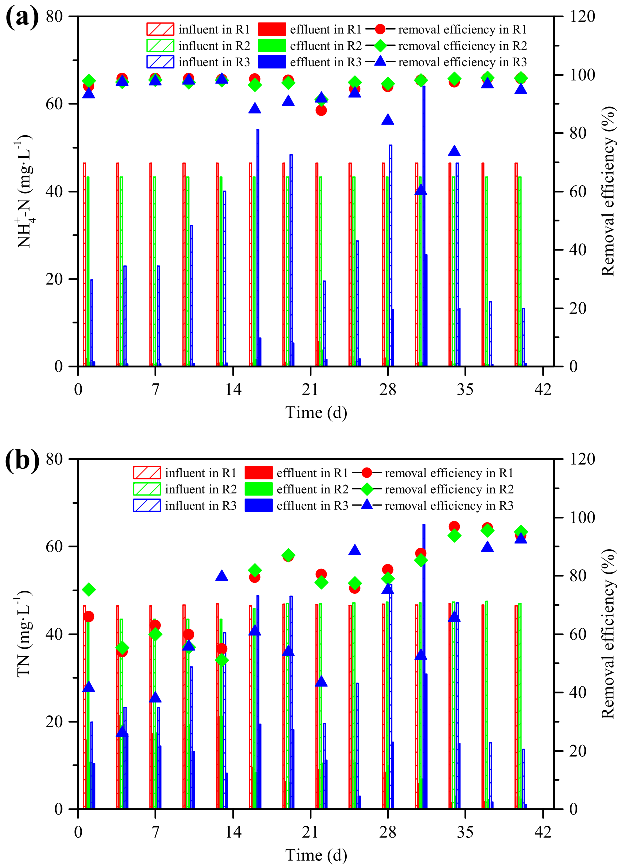

Fig. 2. Ammonia nitrogen and TN removal: (a) ammonia nitrogen and (b) TN.

sufficient enough and easy for uptake; however, domestic wastewater fed into R3 changed more frequently in influent COD concentration and the system in R3 was still weak in resistance to impact loading. The influent COD concentration difference between R1 and R2 might lead to their slight disparity of removal performance.

3.3. *N removal*

Results of ammonia nitrogen and TN removal during the start-up period are presented in Fig. 2(a). The removal efficiencies were often higher than 90% in R1–R3 and changed slightly over the whole operation process, except for a decreasing removal rate at the 16th and 21st days due to a sharp increase in the influent ammonia nitrogen loading. Just as the case in removal of COD in R3, the ammonia nitrogen removal rate fluctuated due to its lack of resistance for compact loading. However, the recovery capacity for ammonia removal exceeded that of COD as shown in Fig. 1. Overall, the ammonia nitrogen received an average removal efficiency of 97.03, 97.35, and 88.43%, and an average effluent concentration of 1.38, 1.15, and 7.27 mg L^{-1} in R1–R3, respectively.

As depicted in Fig. 2(b), the variation of TN in R1–R3 fluctuated frequently over the study period. The average removal efficiency of R1–R3 increased obviously from around 60 to 90% at the end of operation process. A similar trend between TN and ammonia nitrogen removal could be drawn out from Fig. 2(a) and 2(b). R1 and R2 got a higher removal rate of 76.86 and 76.39%, and lower effluent TN of 10.79 and 10.56 mg L^{-1}, respectively, compared with 60.34% and 14.93 mg in R3.

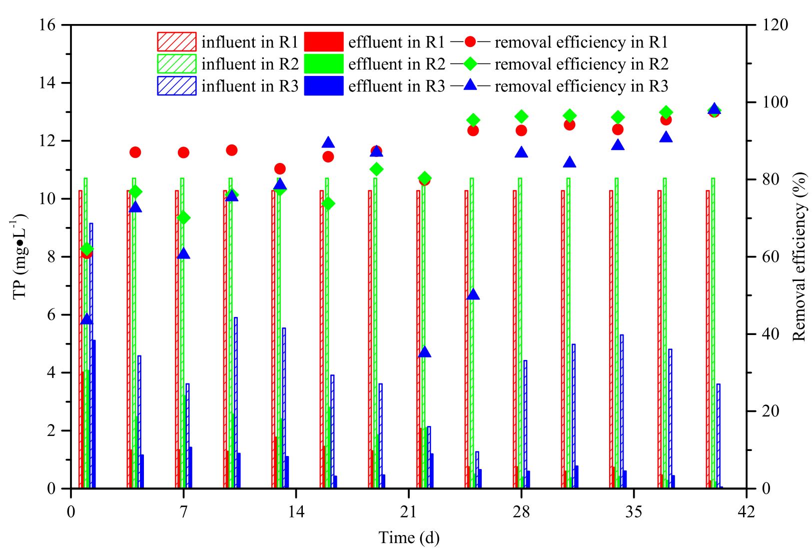

Fig. 3. TP removal process.

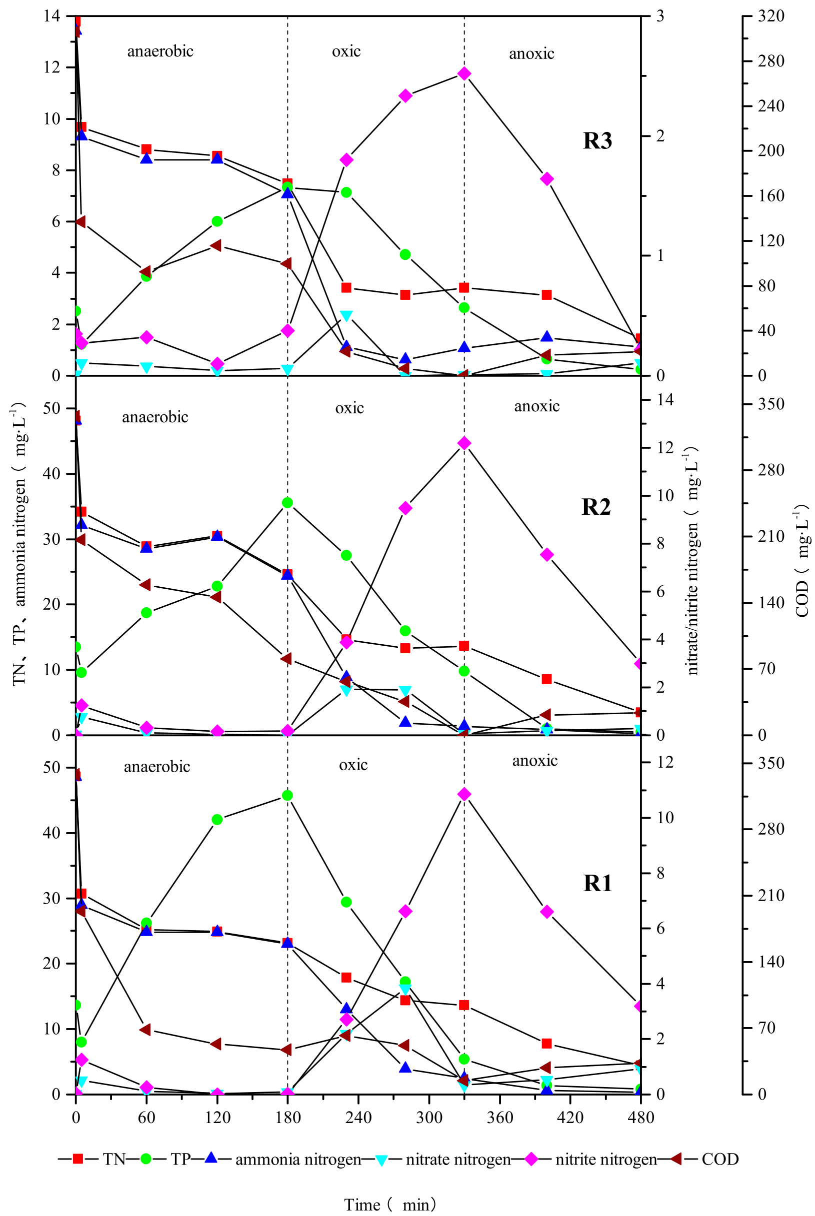

Fig. 4. Cycle removal of COD, N, and P.

Both ammonia nitrogen and TN removal were enhanced over the culture process, especially that of TN in R1–R3. However, TN removal process was not synchronous with ammonia removal during the first several days since both nitrification and denitrification were necessary in nitrogen removal process.

3.4. P removal

Although fed with much higher TP loading in R1 and R2 than R3, the removal efficiencies excelled over the study period, which were 87.39, 84.21, and 74.29% in average, respectively, corresponding to an effluent TP concentration of 1.30, 1.69, and 1.09 mg L^{-1}, respectively. Acetate-based synthetic wastewater enhanced phosphorus removal in R1 and R2 mainly for the adequate soluble organic carbon feeding and speeded enrichment of denitrifying phosphorus-accumulating organisms. However, removal performance showed no straight difference between R1 and R2, illustrating that removal of TP remained unaffected with the addition of 10-mg L^{-1} Ca^{2+} ions. Moreover, the much lower TP loading in R3 led to the lowest effluent TP. It could be concluded from Fig. 3 that with the enrichment of DNPAOs, the removal efficiencies promoted in R1–R3.

Table 2
Comparison of the denitrifying phosphorus removal from this work with the activated sludge study by Zeng and AOAGS study by Kishida

Literatures	Ratio of phosphate uptake to NO_3^--N consumption by DNPAOs[a]	Denitrification phosphorus removal rate[b]	SPRR[c]	SPUR[c]
Zeng [27]	–[d]	–[d]	48.05	17.36
Kishida [11]	2.1 [3]	50	22.32	3.72
R1	2.0*	42.01	14.34	14.13
R2	2.0*	60.95	8.32	9.06
R3	2.0*	–[d]	2.32	2.34

[a]Units: g P/g NO_3^--N.
[b]Units: %.
[c]Units: mg P g $MLVSS^{-1}$ h^{-1}.
[d]No data.
*An average reported ratio by Kim [8] and Kuba [3].

Due to a circuit problem at the day 22, TP in R1–R3 had an acute drop for lack of sludge discharge as shown in Fig. 3. With the remedy of the operation mistake, removal capacity for TP resumed soon.

3.5. Cycle performance

Fig. 4 shows cycle nutrient removal performance in R1–R3 at the end of operation. As shown in Fig. 4, efficiencies of COD, NH_4^+-N, TN, and TP reached up to 90%, higher than that in the start-up period, and effluent concentrations of nutrients reduced further.

Fig. 4 represents that the amount of increase in NO_3^--N in R1–R3 were 75.85, 84.04, and 15.01 mg, respectively, while the amount of decrease in NH_4^+-N were 143.66, 160.91, and 41.87 mg, respectively. According to the stoichiometric formula of nitrification by U.E.P. Agency [26], nitrate generation by nitrification could be calculated. Therefore, it is suggested that 64.85, 73.56, and 26.00 mg of NO_3^--N was removed by denitrification in R1–R3. Therefore, DNPAOs as well as other denitrifiers inside the granules were responsible for denitrification jointly.

Comparison between this study and works of activated sludge and granular sludge is shown in Table 2. Both SPRR and SPUR were relatively low compared with that in activated sludge because diffusion resistance of substrate and nutrients exists in the granular sludge. In addition, PAOs would not act due to the lack of oxygen inside the granular sludge in the oxic phase. Although DNPAOs might act in anoxic zones inside the granular sludge, the anoxic phosphorus uptake rate is generally lower than the oxic uptake rate.

It's highlighted that reported ratios were not applicable for domestic wastewater-based system, probably because of the complicated source of ammonia and organic carbon in R3; therefore, further study on the compact of raw domestic wastewater on denitrifying phosphorus removal and related calculations are required.

After a 42-day operation, the removal efficiencies of COD, nitrogen, and phosphorus reached up to 90%, and effluent concentrations reduced further. The removal capacity of the denitrifying phosphorus removal granules were greatly enhanced and maintained.

3.6. Mechanism of granulation

Selective pressure driven hypothesis is favored by researchers in mechanism of granulation [25]. Only particles that settle within a given time can be retained in the reactor, while those with poor settleability are washed out [25]. Hydraulic shear force is believed to be another selective pressure contributing to granulation [28].

This work provides selection pressure by steps as follows:

(1) Reducing settling time from 20 to 2 min. The complete SBR cycle time is 8 h. The settling time was set 20 min at the beginning of the start-up, gradually decreased to 15, 10, 5, and 2 min, thus, selecting particles with good settling properties.
(2) Constant aeration and electrical stirring. Aeration and stirring process created high shear environments, contributing to micro-organisms secreting extracellular polymeric substances to resist damage of suspended cells.

(3) A/O/A alternating operation mode. This was favor to enrichment of DNPAOs and acted as a source of mass transfer power.

Meanwhile, it's believed that the addition of certain concentrations of Ca^{2+} ions could accelerate aerobic granulation since Ca^{2+} ions worked as the nucleus during cells coagulation process, promoting formation of granules [15,29]. The fact that granules in R3 were fast in granulation speed and bigger in size than in R1 and R2 could also verify the idea.

4. Conclusions

Compact of different influent wastewater on the culture of denitrifying phosphorus removal granules was investigated in this work. Following are the main outcomes:

(1) Denitrifying phosphorus removal granules with an average diameter above 600 μm were successfully obtained within 30 d in three SBRs fed with different influent wastewater, i.e. normal synthetic wastewater, normal synthetic wastewater with 10-mg L^{-1} Ca^{2+} ions, and raw domestic wastewater, respectively.

(2) Granules cultivated in synthetic wastewater were bigger in size and behaved better in moisture content and specific gravity; however, granules formed in raw domestic wastewater were more abundant in microbial species and well in activities. This work suggested that 10-mg L^{-1} Ca^{2+} ions contributed to the compact structure, while raw domestic wastewater promoted the microbial density.

(3) Nutrient removal performance was enhanced with the enrichment of DNPAOs and granulation of aerobic granules since limited oxygen penetration depth helped to create oxic/anoxic/anaerobic conditions. Nutrient removal performance at start-up period and cycle test revealed efficiencies of COD, NH_4^+-N, TN, and TP were 90% or so, the maximal SPRR was 14.34, 8.32, and 2.32-mg P g $MLVSS^{-1}$ h^{-1}, the maximal SPUR was 14.13, 9.06, and 2.34-mg P g $MLVSS^{-1}$ h^{-1} in R1–R3, respectively. Removal of COD, nitrogen, and phosphorus remained unaffected by 10 mg L^{-1} Ca^{2+} ions.

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References

[1] S. Morling, Performance of an SBR-plant for advanced nutrient removal, using septic sludge as a carbon source, Water Sci. Technol. 43 (2001) 131–138.
[2] J. Dou, G. Luo, X. Liu, Kinetic analysis of anaerobic phosphorus release during biological phosphorus removal process, FESE 1 (2007) 233–239.
[3] T. Kuba, G. Smolders, M.C.M. Van Loosdrecht, J.J. Heijnen, Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor, Water Sci. Technol. 27 (1993) 241–252.
[4] S.H. Kim, W.J. Kim, T.H. Chung, Release characteristics of nitrogen and phosphorus in aerobic and intermittent aerobic sludge digestion, Korean J. Chem. Eng. 19 (2002) 439–444.
[5] Y. Peng, H. Hou, S. Wang, Y. Cui, Y. Zhiguo, Nitrogen and phosphorus removal in pilot-scale anaerobic-anoxic oxidation ditch system, J. Environ. Sci. (China) 20 (2008) 398–403.
[6] H.N. Chang, R.K. Moon, B.G. Park, S.J. Lim, D.W. Choi, W.G. Lee, S.L. Song, Y.H. Ahn, Simulation of sequential batch reactor (SBR) operation for simultaneous removal of nitrogen and phosphorus, Bioprocess Eng. 23 (2000) 513–521.
[7] H. Shin, H. Jun, H. Park, Simultaneous removal of phosphorus and nitrogen in sequencing batch reactor, Biodegradation 3 (1992) 105–111.
[8] H.-T. Kim, S.-H. Oh, Y.-D. Lee, G.-S. Kim, The behavior of the DNPAOs at the anaerobic-anoxic process according to the change of the influent NO_3^--N loading, KSCE J Civ. Eng. 10 (2006) 399–403.
[9] N.Kishida, S.Tsuneda, J.H.Kim, R.Sudo, simultaneous nitrogen and phosphorus removal from high-strength industrial wastewater using aerobic granular sludge, J. Environ. Eng. 3 (2009) 153–158.
[10] Y. Wang, J. Geng, Y. Peng, C. Wang, G. Guo, S. Liu, A comparison of endogenous processes during anaerobic starvation in anaerobic end sludge and aerobic end sludge from an anaerobic/anoxic/oxic sequencing batch reactor performing denitrifying phosphorus removal, Bioresour. Technol. 104 (2012) 19–27.
[11] N. Kishida, J. Kim, S. Tsuneda, R. Sudo, Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms, Water Res. 40 (2006) 2303–2310.
[12] J.Y. Hu, S.L. Ong, W.J. Ng, F. Lu, X.J. Fan, A new method for characterizing denitrifying phosphorus removal bacteria by using three different types of electron acceptors, Water Res. 37 (2003) 3463–3471.
[13] S. Tsuneda, T. Ohno, J. Ahn, T. Daidou, A. Hirata, Quinone profiles reflecting population dynamics of denitrifying phosphate-accumulating organisms, Microbes Environ. 18 (2003) 69.
[14] S.S. Adav, D.J. Lee, J.Y. Lai, Aerobic granulation in sequencing batch reactors at different settling times, Bioresour. Technol. 100 (2009) 5359–5361.

[15] S.S. Adav, D.J. Lee, K.Y. Show, J.H. Tay, Aerobic granular sludge: Recent advances, Biotechnol. Adv. 26 (2008) 411–423.
[16] Y. Kong, Y.-Q. Liu, J.-H. Tay, F.-S. Wong, J. Zhu, Aerobic granulation in sequencing batch reactors with different reactor height/diameter ratios, Enzyme Microb. Technol. 45 (2009) 379–383.
[17] B.Y.P. Moy, J.H. Tay, S.K. Toh, Y. Liu, S.T.L. Tay, High organic loading influences the physical characteristics of aerobic sludge granules, Lett. Appl. Microbiol. 34 (2002) 407–412.
[18] G. Yilmaz, R. Lemaire, J. Keller, Z. Yuan, Simultaneous nitrification, denitrification, and phosphorus removal from nutrient-rich industrial wastewater using granular sludge, Biotechnol. Bioeng. 100 (2008) 529–541.
[19] H. Zhang, F. Dong, T. Jiang, Y. Wei, T. Wang, F. Yang, Aerobic granulation with low strength wastewater at low aeration rate in A/O/A SBR reactor, Enzyme Microb. Technol. 49 (2011) 215–222.
[20] A.P.H. Association, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 2012.
[21] A. Jang, Characterization and evaluation of aerobic granules in sequencing batch reactor, J. Biotechnol. 105 (2003) 71–82.
[22] G.A. Hill, C.W. Robinson, Measurement of aerobic batch culture maximum specific growth rate and respiration coefficient using a dissolved oxygen probe, Biotechnol. Bioeng. 16 (1974) 531 –538.
[23] N. Abdullah, A. Yuzir, T.P. Curtis, A. Yahya, Z. Ujang, Characterization of aerobic granular sludge treating high strength agro-based wastewater at different volumetric loadings, Bioresour. Technol. 127 (2013) 181–187.
[24] J.J. Beun, M.C.M. van Loosdrecht, J.J. Heijnen, Aerobic granulation, Water Sci. Technol. 41 (2000) 41–48.
[25] D. Gao, L. Liu, H. Liang, W.M. Wu, Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment, Crit. Rev. Biotechnol. 31 (2011) 137–152.
[26] U.E.P. Agency, Process Design Manual for Nitrogen Control, EPA Technology Transfer, Washington, DC, 1975.
[27] R.J. Zeng, Z. Yuan, J. Keller, Model-based analysis of anaerobic acetate uptake by a mixed culture of polyphosphate-accumulating and glycogen-accumulating organisms, Biotechnol. Bioeng. 83 (2003) 293–302.
[28] C. Di Iaconi, R. Ramadori, A. Lopez, R. Passino, Hydraulic shear stress calculation in a sequencing batch biofilm reactor with granular biomass, Environ. Sci. Technol. 39 (2005) 889–894.
[29] H. Feng, Y. Ding, M. Wang, G. Zhou, X. Zheng, H. He, X. Zhang, D. Shen, J. Shentu, Where are signal molecules likely to be located in anaerobic granular sludge? Water Res. 50 (2014) 1–9.