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Morphological characteristics and nutrient removal efficiency of granular PAO and DPAO SBRs operating at different temperatures

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Abstract. Biological nutrient removal is gaining increasing attention in wastewater treatment plants; however, it is adversely affected by low temperatures. This study examined temperature effects on nutrient removal and morphological stability of the granular and denitrifying phosphorus accumulating organisms (PAO and DPAO, respectively) using sequencing batch reactors (SBRs) at 5, 10, and 20 °C. Lab-scale SBRs were continuously operated using anaerobic-anoxic and anaerobic-oxic cycles to develop the PAO and DPAO granules for 230 d. Sludge granulation in the two SBRs was observed after approximately 200 d. The average removal efficiency of soluble chemical oxygen demand (SCOD) and PO_4^{3-}-P remained >90% throughout, even when the temperature dropped to 5 °C. The average removal efficiency of NO_3^--N remained >80% consistently in DPAO SBR. However, nitrification drastically decreased at 10 °C. Hence, the removal efficiency of NH_4^+-N was decreased from 99.1% to 54.5% in PAO SBR. Owing to the increased oxygen penetration depth at low temperatures, the influence on nitrification rates was limited. The granule in DPAO and PAO SBR was observed to be unstable and disintegrated at 10 °C. In conclusion, morphological characteristics showed that changed conversion rates at low temperatures in aerobic granular sludge altered both nutrient removal efficiencies and granule formation.
Keywords: biological nutrient removal; granular sludge DPAO; granular sludge PAO; morphological characteristics; temperature dependency

1. Introduction

As effluent water quality standards are being strengthened in Korea, nutrient removal through biological nutrient removal (BNR) in wastewater treatment plants (WWTPs) is garnering interest (You *et al.* 2018). Temperature is a critical operating parameter in BNR systems. Enhanced biological phosphorus removal (EBPR) processes involving phosphorus-accumulating organisms (PAO) and denitrifying phosphorus-accumulating organisms (DPAO) are effective for simultaneously removing N and P in WWTPs (Mackey 2019, Yun *et al.* 2019, Park *et al*. 2020, Jang *et al*. 2022). The granular sludge system is a novel technology for biological wastewater treatment (Iorhemen *et al.* 2017, Purba *et al.* 2020, Jung *et al*. 2019, Rashidi *et al*. 2020). Compared to conventional activated sludge, using granular systems allows for enhanced settleability and accommodates higher organic loads in sludge. Therefore, recently, several studies have been conducted in Korea for applying EBPR through a granulation system in WWTPs.

Low temperature adversely affects the BNR system. In Korea, sewage temperature usually varies from 5.8–12 °C and 15–30 °C in winter and summer, respectively (Korean Society on Water Environment 2010). Therefore, investigating the effect of. temperature on aerobic granular sludge is necessary, before this technology is applied practically and efficiently scaled-up. In nitrogen removal, autotrophic nitrification and heterotrophic denitrification activation are substantially decreased at low temperatures (Choi *et al*. 1998, McClintock *et al*. 1993). In phosphorus removal, low temperature might not reduce the removal efficiency (Barnard *et al*. 1985, Erdal *et al*. 2003). However, many researchers have reported contradictory results (Lee *et al*. 2005, Lee and Yun, 2014). EBPR temperature dependency has been explored through the conversion of relevant compounds for biological phosphorous removal at 5, 10, 20 and 30 °C in separate batch tests (Brdjanovic *et al*. 1997). A continuous increase was observed at 5–30 °C for the conversion rates under aerobic conditions, and the rate of anaerobic phosphorous release (or acetate-uptake) mechanism reached its maximum value at 20 °C (Brdjanovic *et al*. 1997).

DPAO simultaneously removes N and P under anaerobic-anoxic (An-Ax) conditions and uses 48% less carbon energy than N and P removal by PAO (Choi *et al*. 1998, Lee *et al*. 2005, Zeng *et al.* 2003b). The granular system has a high settling velocity and maintains a high biomass concentration (Beun *et al.* 1999). These are advantages of sequencing batch reactors (SBR) operation using granular sludge, resulting in granular BNR systems being widely researched (Coma *et al*. 2012, de Kreuk *et al*. 2005, Kishida *et al*. 2009, Yilmaz *et al*. 2008, Zeng *et al*.

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2003a, Purba *et al*. 2020). Diffusion of substrates and dissolved oxygen (DO) interact with each other inside the granules. According to Bao *et al*. (2009), an organic substrate in the influent of the reactor can penetrate deeper into the anaerobic layer than DO, depending on the concentration gradient. DO transfer limitation and higher mixed liquor suspended solids (MLSS) content were conducive to an anaerobic selection condition at low temperatures (Liu and Liu 2006, Martins *et al*. 2004).

Bao *et al*. (2009) evaluated the effect of low temperatures (10 °C) on aerobic granule development and nutrient removal. A decrease in influent chemical oxygen demand (COD) concentration was found to reduce the denitrification ability (Bao *et al*. 2009). However, only a few studies have been conducted to determine the temperature effects on the granular DPAO system. Several authors reported that at low temperatures, granulation process can be inhibited, and the generated biomass can be washed out (de Kreuk *et al*. 2005b, Jiang *et al*. 2016). Additionally, operations at temperatures as low as 8 °C have shown process instability due to excessive growth of filamentous microorganisms (Adav *et al*. 2010, Jiang *et al*. 2016). An outgrowth of filamentous organisms and irregular structures in biofilms or aerobic granular sludge can result from the concurrent availability of oxygen and readily biodegradable substrate at low concentrations, leading to significant substrate concentration gradients inside the granules (Van Loosdrecht *et al*. 1997, de Kreuk and Van Loosdrecht 2004, McSwain *et al*. 2004). This indicates there is no feast-famine regime under these circumstances, which is crucial for stable aerobic granule formation (Beun *et al*. 1999, de Kreuk and Van Loosdrecht 2004, McSwain *et al*. 2004).

Circumstances are advantageous for filamentous growth due to the substrate availability during the aerobic period and the low temperature. In activated sludge systems, an increased chance of sludge bulking during winter and spring has often been observed (Eikelboom *et al*. 1998, Kruit *et al*. 2002). Similar conditions during this experiment led to the outgrowth of filaments, bad settling characteristics, and biomass washout.

There are reports on simultaneous N and P removal using granular sludge under tropical climate conditions (30 °C) (Ebrahimi *et al*. 2010, Winkler *et al*. 2011). Therefore, this study evaluated the morphological characterization of two aerobic granular sludge, the DPAO and PAO SBRs, performing simultaneous nitrogen and phosphate removal, operated at different temperatures (5, 10, and 20 °C).

2. Materials and methods

2.1 Experimental procedure

The experiment was performed using laboratory-scale SBR under the An-Ax and An-Ox conditions. Detailed design and specifics of the reactors were reported in our previous study (Yun *et al*. 2019). Table 1 shows the characteristics of influent synthetic wastewater and operating conditions for the granular DPAO and PAO SBRs. Synthetic wastewater is prepared using propionic acid (HPr) as carbon source, NH_4Cl and KNO_3 as nitrogen source, and KH_2PO_4 as phosphate source. The reason for using HPr was to exclude glycogen accumulating organism (GAO), which is known to inhibit EBPR and selectively cultivate PAO and DPAO (Oehmen *et al.* 2005, Lopez- Vazquez *et al.* 2009). The An-Ax DPAO SBR was sequentially operated as follows: 1) fill (0.5 h), 2) anaerobic (2.5 h), 3) anoxic (4 h), 4) settling (0.5 h), and 5) decant (0.5 h). The An-Ox PAO SBR was sequentially operated as follows: 1) fill (0.5 h), 2) anaerobic (2.5 h), 3) aerobic (4 h), 4) settling (0.5 h), and 5) decant (0.5 h).

Table 1 Characteristics of wastewater and operating conditions for granular sequencing batch reactors

System parameters		Granular DPAO SBR	Granular PAO SBR
Average influent (mg/L)	SCOD	169.2 ± 0.9	169.2 ± 0.9
	NH_4^+-N	9.6 ± 0.5	9.6 ± 0.5
	PO_4^{3-}-P	4.8 ± 0.3	4.8 ± 0.3
Average external NO_3^--N (mg/L)		12.2 ± 1.0	-
C/N/P ratio		35.5: 4.6: 1.0	35.5: 5.0: 1.0
SRT (d)		25.7	25.4
MLSS (mg/L)		5,388	5,776
Operating day	20.2 ± 0.6 °C	20.2 ± 0.6 °C	20.3 ± 0.4 °C
	Period II: 10 °C (72 days)	10.4 ± 0.5 °C	10.5 ± 0.6 °C
	Period III: 5 °C (58 days)	5.5 ± 0.4 °C	5.6 ± 0.3 °C

In the An-Ax DPAO SBR, 9.6 ± 0.5 mg/L NH_4^+-N was injected into the influent for microbial synthesis, and an external electron acceptor was added with 12.2 ± 1.0 mg NO_3^--N/L. In the An-Ox PAO SBR, 24.0 ± 0.7 mg/L of NH_4^+-N was injected into the influent, such that the TN concentration of the An-Ax SBR would be equal to that of the An-Ox SBR. For the aerobic condition in the An-Ox PAO SBR operation, an oval air stone of 140 mm diameter and an air pump (LP-40A, Yong-nam Co.) were installed for aeration, such that the flow rate was 20 L/min and gas flow rate was 1.5 m/s. At this time, aeration was supplied to maintain an oxic DO concentration of approximately 2–3 mg/L, and SRT was maintained for 20 d during steady-state operation based on the measured total suspended solids (TSS) and effluent TSS concentrations in both SBRs. The average SRT of the An-Ax SBR was 27 d, whereas that of the An-Ox SBR was 25 d. The average mixed liquor suspended solids (MLSS) of An-Ax SBR was 2,226 mg/L, and that of An-Ox SBR was 2,812 mg/L.

To temperature control was installed using a constant-temperature water tank (1,000 (L) × 400 (W) × 300 (H) mm) (DAB-075, Daeil cooler Co. Ltd) and electronic thermostat (DH4-1000A, Donghwa electronics). In this experiment, temperature was decreased in two steps, from 20 to 10 to 5 °C. The average operating temperature (Table 1) of the granular PAO and DPAO SBR was 20.3 ± 0.4 °C, 10.5 ± 0.6 °C, and 5.6 ± 0.3 °C, and 20.2 ± 0.6 °C, 10.4 ± 0.5 °C and 5.6 ± 0.4 °C, respectively, for a total 220 operating days.

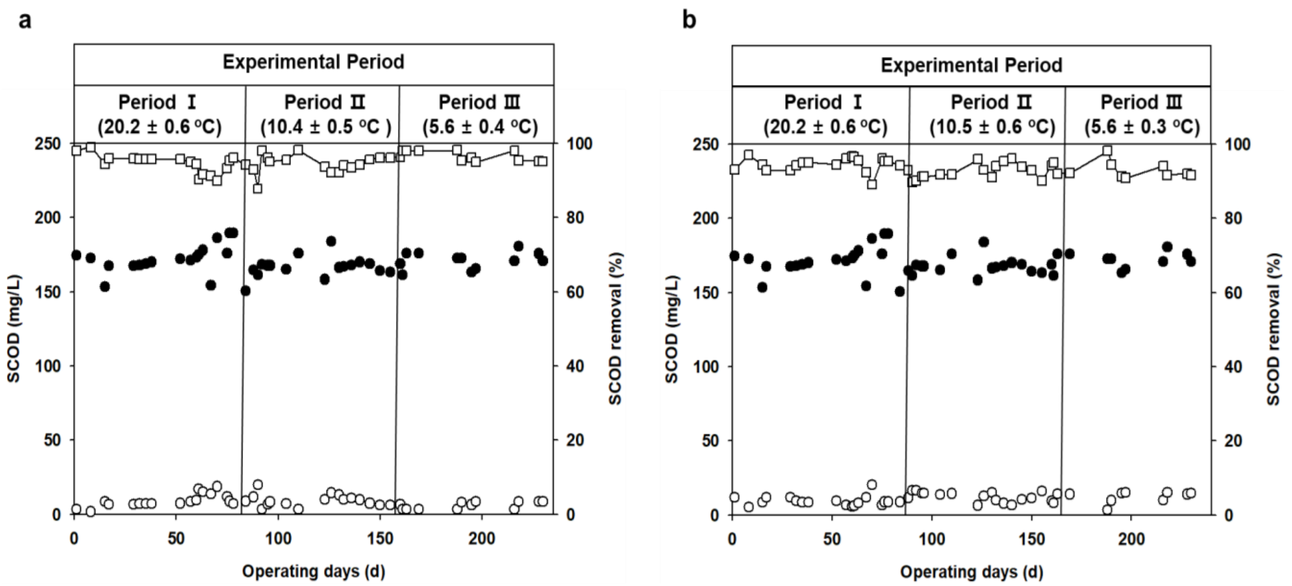

Fig. 1 SCOD concentrations in the influent (●) and effluent (○) and SCOD removal (□) in the long-term operation of the DPAO (a) and PAO (b) SBRs. SCOD, soluble chemical oxygen demand, DPAO, diritrifying phosphorus accumulating organisms, and PAO, phosphorus accumulading organisms.

2.2 Chemical analysis

All chemical measurements, including COD, nitrogen, phosphorus, and solids, were performed according to standard methods (APHA 2005). For soluble fraction analysis, the samples were filtered beforehand using a filter with a pore size of either 0.47 μm (SCOD, NH_4^+-N) or 0.2 μm (NO_2^--N, NO_3^--N, PO_4^{3-}-P) to remove suspended solids. The SVI30 was determined by reading the height of the settled bed in the reactor after 30 min of settling (after the settling and effluent withdrawal phases) and calculated from the settled bed volume and dry weight in the reactor (APHA, 2005). TSS and MLSS were determined using the sum of inorganic and organic fractions of a sample and analyzed using the 2540D method (APHA 2005). VSS and MLVSS, the organic fractions of the sample, were analyzed according to the 2540E method (APHA 2005).

2.3 Morphological analysis

Morphological characteristics of sludge were examined using optical (JSB-133, Green sci., South Korea) and environmental scanning electron microscopes (ESEM, FEI XL-30 FEG, Philips). To prepare for this, after sludge was obtained from the PAO and DPAO reactors at the anoxic and oxic stage, respectively, sample was harvested via centrifugation (10,000 *g*) for 10 min. For ESEM, samples were fixed to the sample holder with silver paste for precise measurements.

2.4 Data analysis

The rate of conversion process in a biological system depends on the temperature (Henze *et al*. 2006). This is described by a simplified derivation of the Arrhenius equation:

$$k(\mathrm{T})=k(20)\theta^{(\mathrm{T}-20)} \quad (1)$$

where, k(T)(h^{-1}) is the conversion rate at temperature T (°C) and θ is a constant that can be determined experimentally. The conversion rates were averaged from nine cyclic measurements. The removal efficiencies were determined as an average from a stable period in each experimental stage.

3. Results and discussion

3.1 Effects of temperature change on granular SBRs performance

The start-up of aerobic granular sludge SBR at room temperature was comprehensively studied. The measured profiles of nutrient components (SCOD, phosphate, and nitrogen compounds) obtained in the long-term operation of DPAO and PAO SBRs from the time of parallel operation are shown in Figs. 1-3, respectively. The average SCOD removal efficiency in both SBRs was >90% during the entire operation at the different temperatures (Fig. 1).

There were no changes in the observed phosphate and COD removal (SCOD consumption during anaerobic period and P removal efficiency was 95%). The COD consumption, phosphate release, and PHA formation were observed in the anaerobic phase. Then, PHA was decomposed in the presence of an electron acceptor, and anaerobic and aerobic conditions took up phosphate for 4 h.

The average PO_4^{3-}-P removal efficiency in the granular PAO and DPAO SBR remained 94.9% and 91.8%, respectively, consistently (Fig. 2). However, when the granular PAO SBR was immediately changed to 10 °C (phase II) after 87 d of operation at 20 °C (phase I), the concentration of phosphate effluent was retained at approximately 20 mg/L (Fig. 2b). During the 10 d of operation, EBPR ability was restored through temperature

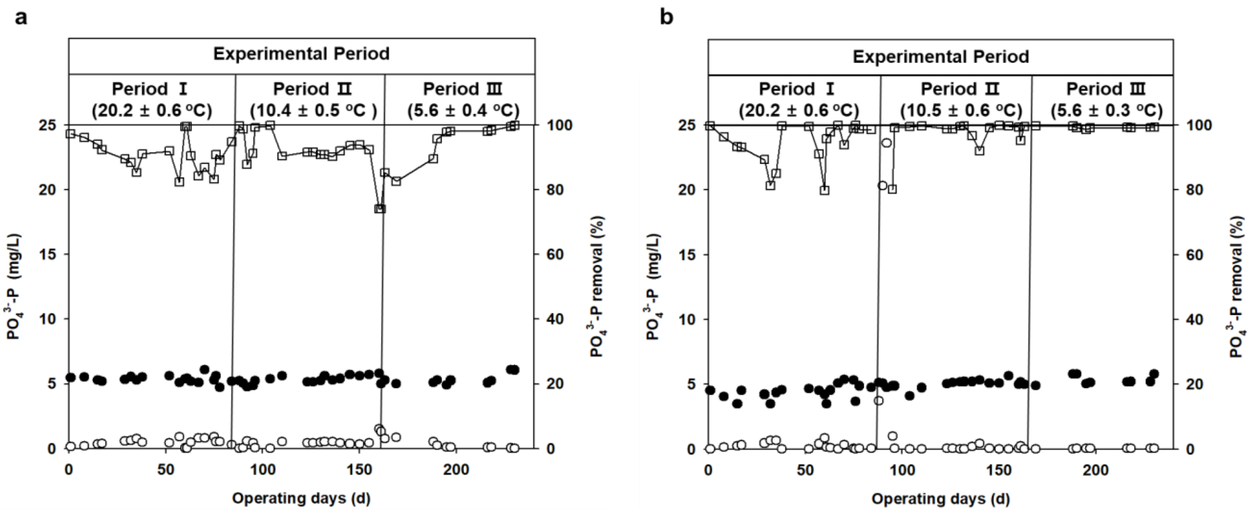

Fig. 2 Phosphate concentrations in the influent (●) and effluent (○) and phosphate removal (□) in the long-term operation of the DPAO (a) and PAO (b) SBRs. DPAO, dinitrifying phosphorus accumulating organisms, and PAO, phosphorus accumulating organisms.

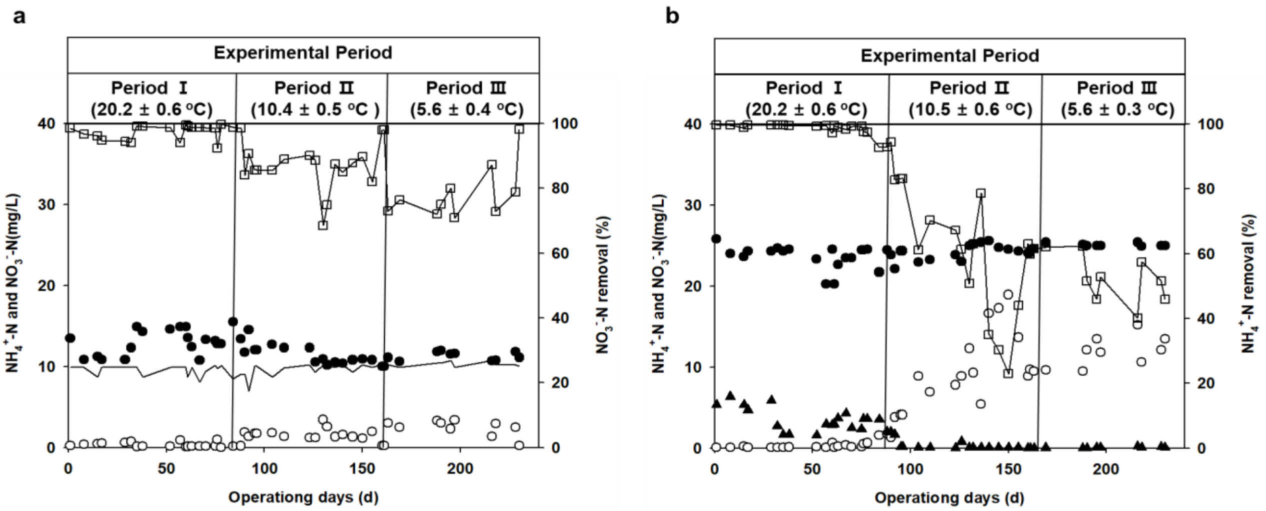

Fig. 3 Ammonium (─), nitrate concentrations in the influent (●) and effluent (○) and nitrate removal (□) in the DPAO SBR (a) and nitrate (▲), ammonium concentrations in the influent (●) and effluent (○) and ammonium removal (□) in PAO SBR (b) long-term operation. DPAO, diritrifying phosphorus accumulating organisms, and PAO, phosphorus accumulading organisms.

adaptation. Erdal *et al*. (2003) reported that EBPR function was lost when the operating temperature was changed from 20 °C to 5 °C in lab-scale UCT process and then recovered through adaptation. Conversely, in this study, even though the granular DPAO SBR was temperature changed, it showed good P removal. Therefore, granular DPAO SBR was less affected by the temperature change than the granular PAO SBR.

Granular sludge can be considered as a special case of biofilm growth with a three-dimensional and more complex structure, in which microbes are attached to each other and embedded in an extracellular matrix, with different functional microbial populations located in different spaces (Adav *et al.* 2008). Generally, aerobic heterotrophic micro- organisms and some autotrophic microorganisms such as nitrifying bacteria reside in the outer layers of granular sludge, while facultative or anaerobic bacteria such as denitrifiers exist in the inner parts (Winkler *et al.* 2013). In millimeter sized granules, aerobic and anoxic/anaerobic microenvironments are maintained due to microbial respiration in the outer region of granule along with diffusion limitation (Nancharaiah and Kiran Kumar Reddy 2018). Presence of different redox conditions in a single granule allow occurrence of simultaneous nitrification and denitrification process even when aeration is on (Nancharaiah *et al*. 2016, Coma *et al*. 2012). The parameters that can influence P release include DO in the bulk liquid, size of granules, electron donor availability and microbial activity. Accordingly, the size of flocs or granules, oxygen concentration and COD/P ratio, are able to lead to substrate-rich aggregates with an oxygen-free zone in the interior where the EBPR process can be activated. So, in granular sludge, the P release would

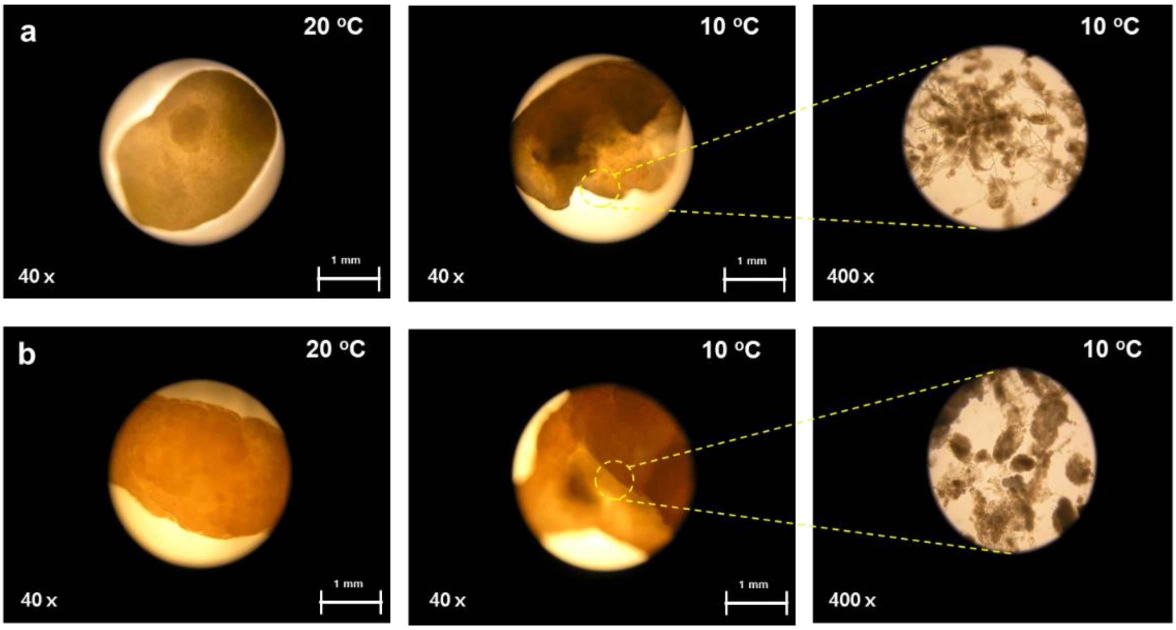

Fig. 4 DPAO and PAO granular sludge at different temperature used in this study. DPAO granular sludge (a) for start-up at 20 °C (×40), granules disintegration when temperature was decreased to 10 °C (×40), and filaments observed at 10 °C (×400). PAO granular sludge (b) for start-up at 20 °C (×40), granule disintegration when temperature was decreased to 10 °C (×40), and filaments observed at 10 °C (×400). DPAO, dinitrifying phosphorus accumulating organisms, and PAO, phosphorus accumulating organisms.

carried on more easily than that can be seen in flocculent activated sludge.

Additionally, removal efficiency of NO_3^--N as an electron acceptor in the granular DPAO SBR was slightly decreased from approximately 95.1% to 81.7%, as temperature decreased from 20 °C to 5 °C. However, the average removal efficiency of NO_3^--N was >80%. This indicated successful removal of NO_3^--N in the granular DPAO SBR (Fig. 3a). However, the average NH_4^+-N removal efficiency in the granular PAO SBR (Fig. 3b) was drastically decreased from 99.1% to 54.5%, as temperature decreased from 20 °C to 5 °C, due to reduction in the amount of converted NH_4^+-N to NO_3^--N by nitrifier at low temperature.

3.2 Effect of temperature change on morphological characteristics

The aerobic granular sludge was observed after approximately 220 d of stable EBPR performance in both SBR reactors. From 20 °C (87 d regular operation), the temperature in SBRs was decreased to 15 °C (72 d stable operation) and 5 °C (58 d stable operation). Morphological characteristics of the DPAO and PAO granules at different temperatures are shown in Table 2.

During the stable operation at 20 °C, the average diameter of the DPAO granules was 2.2 ± 1.7 mm, which was approximately five-fold larger than that of PAO SBR granules (0.4 ± 0.3 mm).

The type of EA (O_2 or NO_3^- in this study) is another key factor that affects the granulation process and morphology. The nitrate solution was used as the sole EA in the DPAO SBR and was added at the beginning of the anoxic condition.

Table 2 Steady-state characteristics of granular sludge in the denitrifying phosphorus accumulating organisms (DPAO) and phosphorus accumulating organisms (PAO) sequencing batch reactors (SBRs) at different temperatures

	Granular DPAO SBR			Granular PAO SBR		
	20 °C (87 d)	10 °C (72 d)	5 °C (58 d)	20 °C (87 d)	10 °C (72 d)	5 °C (58 d)
Average diameter (mm)	2.2 ± 1.7	1.0 ± 0.2	0.6 ± 0.3	0.4 ± 0.3	0.2 ± 0.1	-[a]
Eff. TSS (mg/L)	11.0	25.7	8.5	15.4	30.7	7.4
VSS (mg/L)	10.2	22.2	7.7	14.5	25.5	6.2
Granule/Flocculant MLSS conc. ratio	0.59 ± 0.07	0.51 ± 0.09	0.43 ± 0.08	7.76 ± 0.64	3.92 ± 2.66	1.46 ± 0.30
SVI_{30} (mL/g)	134	146	153	86	120	172

The nitrate, injected at the initial 15 min of the anoxic period, was rapidly consumed during denitrification of denitrifying PAO in the DPAO SBR. The granulation environment in the DPAO SBR more closely resembled that in a UASB, where the firm and dense core are loosely covered with an anaerobic biofilm. Based on these results, we propose that both the availability and types of EA are crucial for granulation.

After 87 d, the temperature was lowered from 20 °C to 10 °C. Fig. 4 shows images of the granules as observed under a 40× electron microscope in DPAO and PAO SBRs after approximately 160 d of operation. The granule in DPAO and PAO SBRs was observed to be unstable and disintegrated at 10 °C. Moreover, granules obtained using an optical microscope (magnification, ×400) contained filamentous microorganisms leading to biomass washout. Owing to this, in the case of granular PAO SBR, the TSS

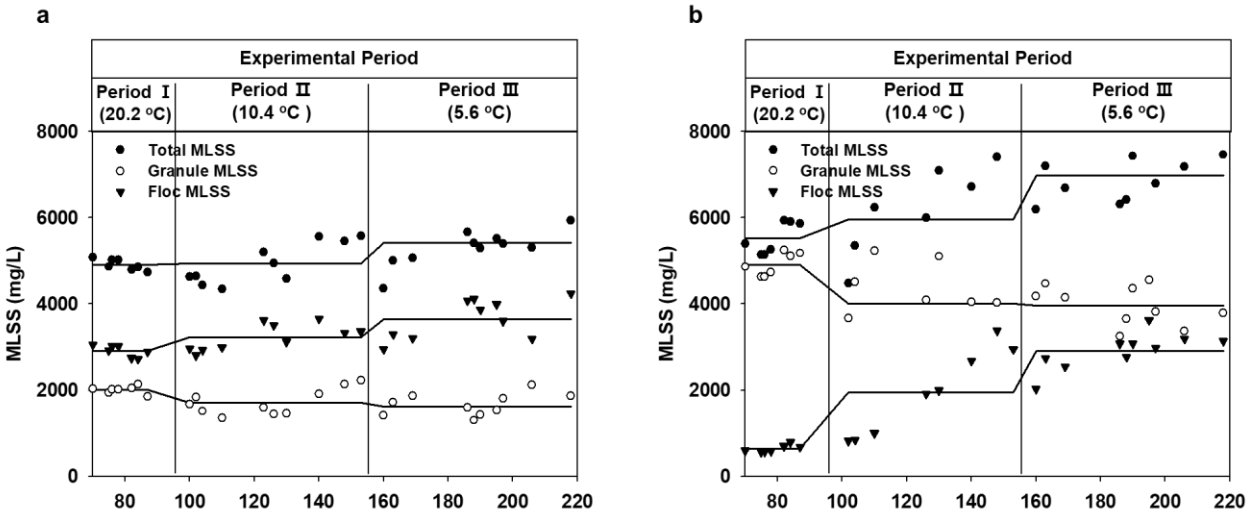

Fig. 5 Flocculent and granular sludge MLSS in the granular DPAO (a) and PAO (b) SBRs at different temperatures. MLSS, mixed liquor suspended solids, DPAO, diritrifying phosphorus accumulating organisms, and PAO, phosphorus accumulading organisms.

concentration of the effluent increased from 15.4 mg TSS/L to 30.7 mg TSS/L when the operating temperature was changed from 20 °C to 10 °C. DPAO SBR granule increased from 11.0–25.7 mg TSS/L.

Although DPAO and PAO granules showed relatively high TSS concentration at 10 °C, both sludges recovered normal TSS concentration at 5 °C (8.5 mg and 7.4 TSS/L in DPAO and PAO granules) (Table 2). The SVI_{30} of the DPAO and PAO granules was 153 and 172 mL/g at the operating temperature 5 °C. Although DPAO and PAO granules showed relatively poor SVI values, both sludges showed a straightforward liquid–sludge interface. As the temperature decreased, the suspended solids in the effluent increased due to the flocculent ratio in the SBRs.

In the case of granular DPAO SBR, MLSS concentration ratios of granule and flocculent were 0.59 ± 0.07, 0.51 ± 0.09, and 0.43 ± 0.08, respectively, and the proportion of flocculent in the reactor increased with decreasing operating temperature (Fig. 5a). For this reason, the average diameter of the granules at 5 °C was slightly smaller than that at 20 °C (0.6 ± 0.3 mm at 5 °C vs 2.2 ± 1.7 mm at 20 °C in DPAO granule and not measurable because of flocculation at 5 °C vs 0.4 ± 0.3 mm at 20 °C in PAO granule). This was presumably the lower loading applied at the lowest temperature. The MLSS concentration ratio of granule and flocculent of granular PAO SBR was 7.76 ± 0.64, 3.92 ± 2.66, and 1.46 ± 0.30 at operating temperatures of 20, 10, and 5 °C, respectively. The proportion of flocculent in the reactor increased as the operating temperature decreased. Significantly, when the temperature was changed from 20 °C to 10 °C, the ratio of flocculent abruptly increased to about 2.2-fold (Fig. 5b).

3.3 Temperature dependency

The rates of most biological processes depend on temperature. This dependency can be described with a simplified Arrhenius equation (Eq. (1)). Decreasing the temperature of a stable operating reactor (DPAO and PAO SBR) from 20 to 10 or 5 °C during long-term operation (230 d), showed a significant decrease in conversion rates (Figs. 1-5). Temperature coefficients were derived from these experiments (Table 3, Fig. 6), showing temperature dependency for substrate utilization, phosphate release, phosphate uptake, nitrification, and denitrification.

Table 3 Steady-state characteristics of granular sludge in the denitrifying phosphorus accumulating organisms (DPAO) and phosphorus accumulating organisms (PAO) sequencing batch reactors (SBRs) at different temperatures

	Granular DPAO SBR	Granular PAO SBR	Literature (activated sludge system)
Substrate utilization	1.032 ± 0.026	1.080 ± 0.029	1.095 ± 0.012[a]
Phosphate release	1.017 ± 0.014	1.027 ± 0.013	1.075 ± 0.012[a]
Phosphate uptake	1.032 ± 0.013	1.014 ± 0.013	1.031 ± 0.017[a] 1.06[b]
Nitrification	–	1.051 ± 0.015	1.12[c]
Denitrification	1.065 ± 0.018	–	1.11/1.12[d] 1.13[e]

[a]Brdjanovic *et al*. 1998
[b]de Kreuk *et al*. 2005
[c]ASM2d, Henze *et al*. 2006
[d]Christensson *et al*. 1994
[e]Timmermans and Van Haute 1983

The temperature dependency of substrate utilization in granular DPAO and PAO SBRs was 1.032 ± 0.026 and 1.080 ± 0.029, respectively. The acetate uptake rate of PAO is strongly dependent on temperature (θ = 1.095, Brdjanovic *et al*. 1998) and therefore, the temperature dependency in DPAO SBR is lower (Fig. 6a). The results of the start-up period at low temperatures indicated that great care has to be taken by the start-up of full-scale or pilot plants to ensure that filamentous organisms and irregular structures in biofilms are prevented (Van Loosdrecht *et al.* 1997, de Kreuk and Van Loosdrecht 2004, McSwain *et al*. 2004,

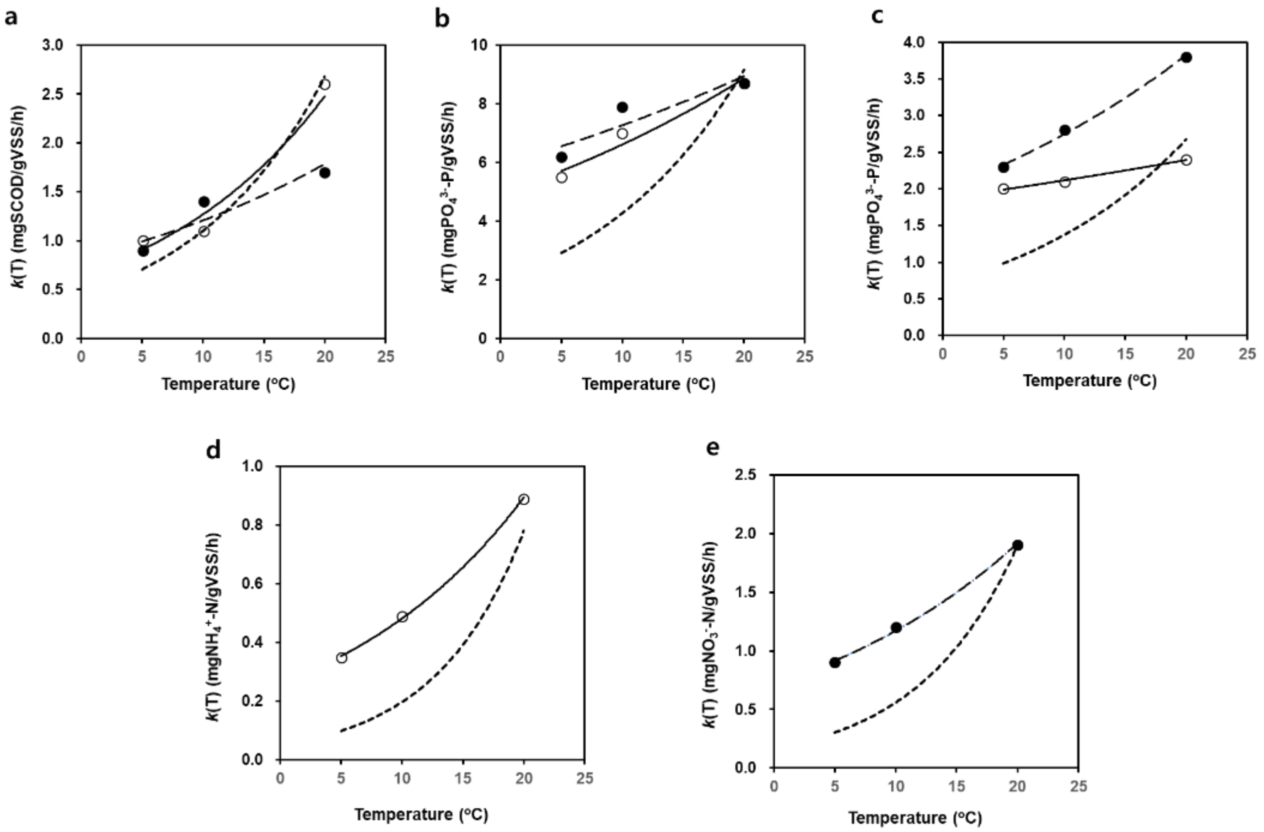

Fig. 6 The maximum measured conversion rates k(T) in mg/g VSS/h, at different temperatures for substrate utilization (a), phosphate release (b), phosphate uptake (c), nitrification rate (d), and denitrification rate (e) for granular DPAO and PAO SBRs, adapted to the three different temperatures (20, 10, 5 °C). The lines are fitted according to Eq. (1) with the temperature coefficients (θ) for granular PAO SBR ((○), solid lines), granular DPAO SBR ((●), dashed lines), and reported values for activated sludge (Table 3, dotted lines).

Beun *et al*. 1999, Eikelboom *et al.* 1998, Kruit *et al*, 2002). The temperature dependency of the phosphate release rate in granular DPAO and PAO SBRs was 1.017 ± 0.014 and 1.027 ± 0.013, respectively. The phosphate release rate of PAO is strongly dependent on temperature (θ = 1.075, Brdjanovic *et al*. 1998) and therefore, its temperature dependency in DPAO and PAO SBR, lower (Fig. 6b).

Additionally, comparing the temperature dependency for phosphate consumption to data of flocculated systems (Brdjanovic *et al*. 1998), showed comparable dependencies for both PAO and DPAO SBRs (Fig.6c). PAOs could use oxygen and nitrate for phosphate uptake, therefore, a change in the anoxic and aerobic volumes of the granule did not influence the phosphate uptake rate. Thus, there was no observed difference between the flocculated system (θ = 1.031 ± 0.017) and granular DPAO SBR (θ = 1.032 ± 0.013). This difference might suggest a higher adaptation ability of DPAO towards their phosphate uptake.

The temperature dependency of nitrification in granular PAO SBR (θ =1.051 ± 0.015) was lower than that in activated sludge system (θ =1.12). Moreover, the temperature dependency of denitrification in granular DPAO SBR (θ =1.065 ± 0.018) was lower than that in the activated sludge system (θ =1.11) (Table 3, Fig. 6d and e).

The aerobic granular sludge consists of a layered structure, an aerobic outer layer, containing a mixture of heterotrophic and autotrophic organisms, and an anoxic or anaerobic core, in which denitrifying and anaerobic organisms are present (Winkler *et al*. 2013). A lower temperature leads to lower activity in the aerobic layers and, thus, to a higher oxygen penetration depth. In this situation, the autotrophic organisms existing in the deeper layers of the granule have oxygen at their disposal for nitrification. This extra aerobic volume in the granules compensates for the decreased specific conversion rates. This leads to the overall reduced effect of temperature change on the ammonium oxidation rate. The increased aerobic volume in the granules, leads to a lower anoxic volume. This results in the observed lower denitrification. The volume of anoxic core, in which denitrification takes place, decreases, resulting in higher nitrate concentrations in the effluent. The increased aerobic layer at lower temperatures negatively affects the total nitrogen removal efficiency.

4. Conclusions

This study aimed to determine the temperature dependency of DPAO and PAO granular sludge through temperature operational parameters and evaluate their nutrient removal performance to improve the BNR system. Owing to reduction in the amount of converted NH_4^+-N to

NO_3^--N by nitrifier as the temperature decreased, removal efficiency of NH_4^+-N was drastically decreased from 99.1% to 54.5% in PAO SBR. Due to an increased oxygen penetration depth at low temperatures, nitrification rates were only limitedly influenced. The granule in DPAO and PAO SBRs was observed to be unstable and disintegrated at temperature lowered to 10 °C. Changed conversion rates at low temperatures not only changed nutrient removal efficiencies in aerobic granular sludge but also impacted granule formation. The results of the lower efficacy at low temperatures indicated that the start-up of full-scale plants or pilot plants using this technique requires careful attention. However, this method was found to have a lower temperature dependence than the activated sludge system, confirming that the granular-based BNR process is efficient. Further studies are required to examine whether nitrification efficiency and the morphological characteristics of granular sludge are recovered through continuous long-term operation.

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