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Special issue:
**Sustainable water management
for green infrastructure**

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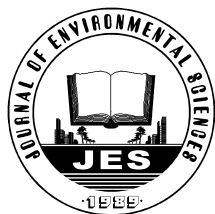


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Comparison of biochemical characteristics between PAO and DPAO sludges

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ABSTRACT

A successful enhanced biological phosphorus removal (EBPR) was observed in both anaerobic-aerobic sequencing batch reactor (An-Ox SBR) to induce growth of phosphorus accumulating organism (PAO) and anaerobic-anoxic (An-Ax) SBR to induce growth of denitrifying PAO (DPAO). Although the EBPR performance of An-Ox SBR was higher by 11.3% than that of An-Ax SBR, specific phosphorus release rates in the An-Ax SBR (22.8 ± 3.5 mg P/(g VSS·hr)) and the An-Ox SBR (22.4 ± 4.8 mg P/(g VSS·hr)) were similar. Specific phosphorus uptake rates under anoxic and aerobic conditions were 26.3 ± 4.8 mg P/(g VSS·hr) (An-Ax SBR) and 25.6 ± 2.8 mg P/(g VSS·hr) (An-Ox SBR), respectively, which were also similar. In addition, an analysis of relationship of poly- β -hydroxyalkanoates (PHA) synthesized under anaerobic conditions with phosphorus release ($P_{\text{released}}/PHA_{\text{synthesized}}$) and of PHA utilized under anoxic and aerobic conditions with phosphorus uptake ($P_{\text{uptaked}}/PHA_{\text{utilized}}$) verified that biological activities of EBPR per unit biomass between DPAO and PAO were similar. An analysis of the specific denitrification rate of DPAO showed that NO_3^- -N can be denitrified at a rate that does not substantially differ from that of an ordinary denitrifier without additional consumption of organic carbon when the PHA stored inside the cell under anaerobic conditions is sufficiently secured.

Introduction

In Korea, the effluent quality criteria of total phosphorus (TP) in wastewater treatment plants (WWTPs) has been strengthened from 2 mg/L to 0.2, 0.3, and 0.5 mg/L as of 2012 to protect the water quality of major rivers (Ministry of Environment in Korea, 2009). The characteristic of Korean sewage with an unfavourable C/N/P ratio due to the lack of organic substrate led to a difficulty to meet a stringent effluent limitation. Because of the tougher regulations and the unfavourable C/N/P ratio for biological nitrogen (N) and P removal, plant modification mostly adopted the chemical P removal. However, increased operating costs and complexity of chemical sludge in biological plants are the drawbacks of the chemical P removal.

The use of denitrifying phosphorus accumulating organism (DPAO) would be an alternative to attain an appreciable removal of both N and P biologically in weak sewage. It had been demonstrated experimentally that genera of PAO could grow and uptake phosphorus in the anoxic zone (Kapagiannidis et al., 2013; Kim et al., 2013; Lanham et al., 2011; Oehmen et al., 2010; Podedworna and Zubrowska-Sudol, 2012; Schuler and Jenkins, 2003). DPAO in the biological nutrient removal (BNR) system is capable to remove N and P simultaneously because DPAO utilizes NO_3^- -N as an electron acceptor, and also do not use organic substances in the anoxic zone (Flowers et al., 2009; Kern-Jespersen and Henze, 1993; Oehmen et al., 2010).

The primary difference between PAO and DPAO is utilization of the electron acceptor. Ordinary PAO uses oxygen in an aerobic condition, while DPAO utilizes nitrate under an anoxic condition. As a result, the system

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utilizing DPAO can not only reduce the oxygen requirement to oxidize poly- β -hydroxyalkanoates (PHA) in the aerobic zone, but to save the chemical oxygen demand (COD) utilization. The maximum aerobic P uptake ability of DPAO is approximately 60% of that of PAO (Hu et al., 2001), although Kuba et al. (1994, 1996) suggested that overall performance of P removal capability of both species in BNR system is similar. DPAO seems a relatively “weaker” microbial group than ordinary heterotrophic PAO. Although the microbiological aspects of DPAO are still not fully understood, DPAO has an advantage for simultaneous removal of both N and P with a minimized COD utilization. According to Brdjanovic et al. (1998), DPAO could remove 4–5 g NO_3^- -N per g P without COD utilization. Further, Murnleitner et al. (1997) and Kuba et al. (1996) suggested that DPAO produced 20%–30% less sludge than PAO. The possible advantages of DPAO in weak sewage are not fully investigated in detail because of the limited understanding on DPAO. Therefore, the objective in this study was to examine biochemical characteristics of PAO and DPAO sludges experimentally with an operation of two identical lab-scale SBRs.

1 Materials and methods

1.1 Reactor configuration and operating conditions

Using a PLC (programmable logic controller), the operation cycle for the anaerobic-anoxic (An-Ax) SBR for DPAO growth was set as follows: fill (0.5 hr); anaerobic stage (2.5 hr); anoxic stage (4 hr); settling (0.5 hr); and decanting (0.5 hr). The operation cycle for the anaerobic-aerobic (An-Ox) SBR for the PAO growth was set similarly: fill (0.5 hr); anaerobic stage (2.5 hr); aerobic stage (4 hr); settling (0.5 hr); and decanting (0.5 hr). The inflow for both reactors was fed to 3.4 L per cycle at every 8-hr interval; thus, the daily total inflow was 10.2 L. In the An-Ax SBR, an external electron acceptor had been added with 15 mg NO_3^- -N/L to the initial anoxic period. For the oxic condition in the An-Ox SBR, aeration was supplied to maintain oxic dissolved oxygen (DO) level of

2–3 mg/L. The solid retention time of the An-Ax SBR was, on average, 27 days, whereas the An-Ox SBR operated for an average of 25 days. Mixed liquor suspended solid of the An-Ax SBR was 2180 mg/L, and the An-Ox SBR had an average of 2580 mg/L.

1.2 Synthetic wastewater

Table 1 presents the influent characteristics of synthetic wastewater. Propionic acid (HPr) was the major substrate. Various forms of nitrogenous components were used as nitrogen sources. P was supplied by KH_2PO_4 . For an effective comparison of the test results through the selective cultivation by DPAO and PAO, the influent with the same C/N/P ratio was supplied into both the An-Ax SBR and the An-Ox SBR. The reason for using HPr was that it could possibly exclude glycogen accumulating organism, which has been known to inhibit enhanced biological phosphorus removal (EBPR), and selectively cultivate PAO and DPAO (Lopez-Vazquez et al., 2009; Oehmen et al., 2006). For the N in the An-Ax SBR, 10 mg/L of NH_4^+ -N was injected into the influent, accounting for microbial synthesis. For NO_3^- -N, the KNO_3 reagent was used to externally supply as a concentration of 15 mg/L so that the DPAO could use as an electron acceptor in the anoxic condition. For the N in the An-Ox SBR, 25 mg/L of NH_4^+ -N was injected into the influent so that the TN concentration of the An-Ax SBR would be equal to that of the An-Ox SBR.

1.3 Chemical analysis

The analysis, which was based on standard methods (APHA et al., 2005), was performed in terms of the COD (total, soluble), NH_4^+ -N, TP, PO_4^{3-} -P, TSS, and VSS. NO_2^- -N and NO_3^- -N were measured by ion chromatography (IC-80, Dionex, USA). The analysis of TN was conducted using a DR4000 (Hach Co., USA). Samples were taken regularly from both SBRs. The P contents of the biomass were also measured to identify the extent of the EBPR.

1.4 Batch experiments

Batch tests were conducted for measuring specific phosphorus release rate (SPRR), specific phosphorus uptake rate (SPUR) and specific denitrification rate (SDNR). The

Table 1 Composition of the influent synthetic wastewater

| | Chemicals | Concentration |
|-----------------------|--------------------------------------|---|
| Carbon source | $\text{CH}_3\text{CH}_2\text{COONa}$ | 0.15 g/L (150 mg COD/L) |
| | KNO_3 (An-Ax SBR) | 7.21 g/L (15 mg NO_3^- -N/L) |
| Nitrogen source | NH_4Cl (An-Ax SBR) | 0.038 g/L (10 mg NH_4^+ -N/L) |
| | NH_4Cl (An-Ox SBR) | 0.095 g/L (25 mg NH_4^+ -N/L) |
| Phosphorus source | KH_2PO_4 | 0.0175 g/L (6 mg PO_4^{3-} -P/L) |
| Trace metal solution* | | 0.1 mL/L of influent |

* $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 1.5 g/L, $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ 1.5 g/L, $\text{ZnSO}_4 \cdot 5\text{H}_2\text{O}$ 0.1 g/L, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 0.1 g/L, and $(\text{NH}_4)_6\text{Mn}_7\text{O}_{24} \cdot 5\text{H}_2\text{O}$ 0.1 g/L.

An-Ax SBR: anaerobic-anoxic sequence batch reactor; An-Ox SBR: anaerobic-aerobic SBR.

mixture of anoxic and aerobic sludge was used for the batch experiment. After 120 days when stable EBPR performance was observed in the An-Ax SBR and the An-Ox SBR, sludge was collected from the both SBRs. 200 mg/L of HPr was added as a sole carbon source. For 2.5 hr, anaerobic condition was maintained for P release. Subsequent to anaerobic condition, air and nitrate were injected for anoxic and aerobic conditions, respectively, to determine the anoxic and oxic P uptake rate. DO was maintained to 2–3 mg/L. 20 mg/L of nitrate was added as electron acceptor for DPAO.

SDNR test was performed to evaluate denitrification ability of DPAO. As a control experiment, a conventional SDNR test of the activated sludge (from Jungnang wastewater treatment plant in Seoul, Korea) was conducted with the injection of 200 mg/L of HPr and 30 mg/L of nitrate in anoxic condition. For SDNR test of DPAO sludge, 500 mg/L of the An-Ax sludge was collected from the An-Ax SBR. For PHA utilization as internal carbon source, P release was induced under anaerobic condition for 2 hr. Subsequent to anaerobic condition, about 30 mg/L of nitrate was added as electron acceptor for DPAO without external COD addition. SCOD and nitrate concentration at every 30 min were measured.

1.5 PHA and glycogen

The PHA analysis was conducted according to the method proposed by Comeau et al. (1988). The PHA was calculated from the sum of poly- β -hydroxybutyric acid (PHB) and poly- β -hydroxyvaleric acid (PHV). For the pretreatment of the sample, 50 mL of sample was first centrifuged and, then the sample was dried at room temperature and then was frozen and stored. Thereafter, 2 mL of 3% H_2SO_4 , 2 mL of chloroform, and 0.75 g of 0.3% benzoic acid (internal standard: benzoic acid) were mixed with 20 mg of sludge. The prepared sample was transferred to a Pyrex test tube (15 mL) with a Teflon-lined cap, and after heating for 3.5 hr at 100°C, 2 mL of a denser chloroform layer was transferred to a Pyrex test tube (10 mL), and 0.5 mL of distilled water was added. Finally, after 3 min of centrifugation (1500 $\times g$), the chloroform layer was transferred to the gas chromatography (GC) vial and measured using the GC equipment. The GC analysis used a DB-WAX column (length of 15 m, diameter of 0.52 mm) (Mega bore J&W Scientific Co. capillary column, USA).

The glycogen analysis was conducted according to the method proposed by Smolders et al. (1994). After 50 mL of sample was centrifuged and lyophilized, 5 mL of 0.6 mol/L HCl was added to 20 mg of sludge, transferred to a Pyrex test tube (10 mL) with a Teflon-lined cap, and was heated for 6 hr at 100°C. The heated sample was air-cooled and centrifuged again. From the centrifuged sample, 1 mL of supernatant from the Pyrex test tube was transferred to a high performance liquid chromatography (HPLC) vial and processed. A Bio-Rad Aminex ion-exclusion column

was used; the injection volume was 50 μL , and the carrier solution used 0.008 mol/L H_2SO_4 and helium gas. The flow rate was adjusted to 0.6 mL/min, and the detector temperature was set to 35°C.

2 Results and discussion

2.1 EBPR performance

Figure 1 shows the behaviors of the SCOD, $\text{PO}_4^{3-}\text{-P}$, and $\text{NO}_3^- \text{-N}$ concentrations, and the changes in PHA and glycogen after the stabilization (100 days of operation). Average MLVSS in the An-Ax SBR and the An-Ox SBR was 2600 and 3280 mg/L, respectively. For the changes in SCOD, there was almost no difference between the two reactors. The removal efficiencies of TP in the An-Ox SBR and the An-Ax SBR were 86.5% and 75.2%, respectively (**Table 2**). Better EBPR performance was observed in the An-Ox SBR.

Meanwhile, the analysis of PHA and glycogen can be considered as an important factor to verify the EBPR performance of the PAO and DPAO. PHA is used as the critical energy source that accumulates phosphate under anoxic or aerobic conditions after the PAO or DPAO converts volatile fatty acid (VFA) into PHA under anaerobic conditions (Mino et al., 1994; Smolders et al., 1994). Additionally, because glycogen provides reducing power when the PAO or DPAO synthesizes VFA into PHA under anaerobic conditions (Smolders et al., 1994), it was also analyzed and illustrated along with PHA (**Fig. 1**). PHA increased with the release of phosphate under anaerobic conditions. As PHA decreased in the anoxic or aerobic condition, only phosphate accumulation occurred in the An-Ox SBR (nitrification here, but it is not relevant to denitrifying EBPR), whereas the P uptake as well as the denitrification occurred simultaneously in the An-Ax SBR. In both reactors, glycogen, as the reducing power for PHA synthesis, tended to decrease under anaerobic conditions and recover under the anoxic or the aerobic conditions. This result means that the process continues to repeat when glycogen is used as the reducing agent to convert the VFA to PHA through the PAO or DPAO during the anaerobic period, and glycogen is recovered during the anoxic or aerobic conditions.

Table 2 outlines the mass balance of the An-Ax SBR and the An-Ox SBR after stabilization at each reaction step. In the An-Ax SBR operating under alternating anaerobic-aerobic conditions, 408.1 mg soluble COD (SCOD) was consumed under anaerobic condition, accounting for approximately 70% of the entire SCOD removed. This means that most carbon energy was consumed under anaerobic condition for P release, and then a part of it was used for the denitrification. Influent P (21.4 mg $\text{PO}_4^{3-}\text{-P}$) released up to 152.8 mg at the end of anaerobic

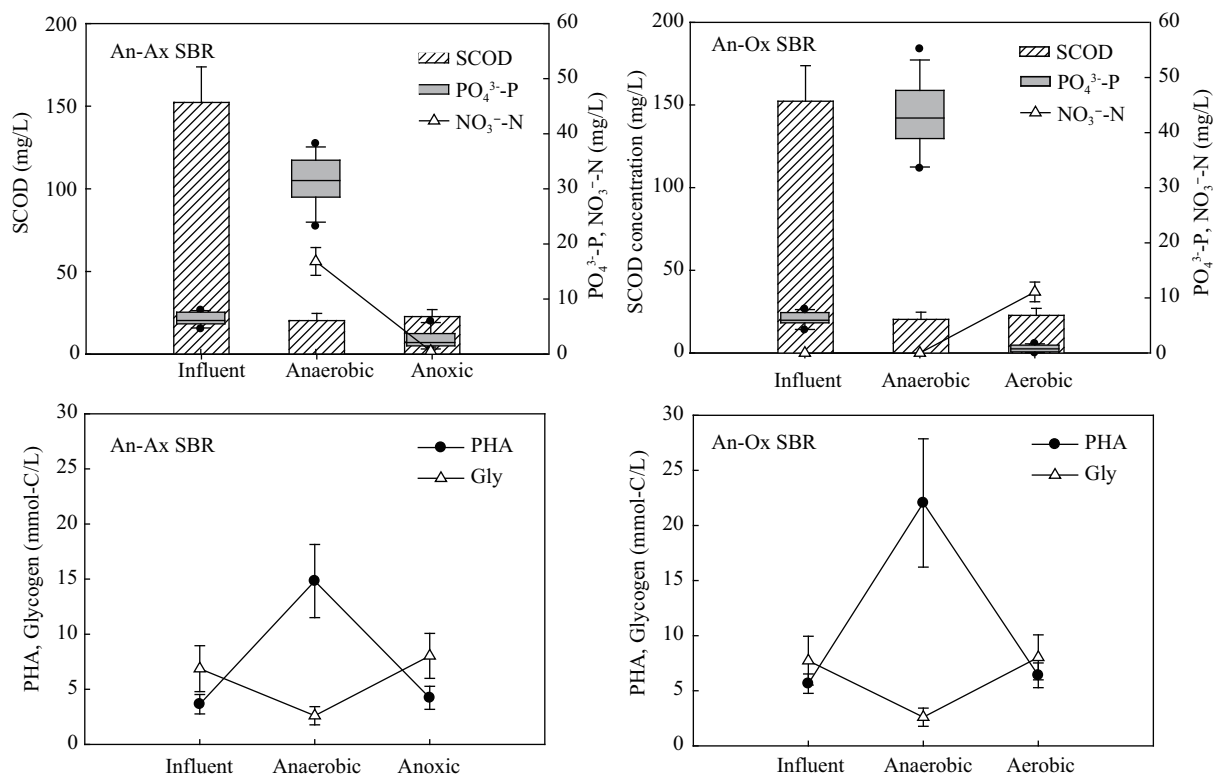


Fig. 1 Enhanced biological phosphorus removal (EBPR) under the influent-anaerobic-anoxic conditions of An-Ax SBR and the influent-anaerobic-aerobic conditions of An-Ox SBR.

Table 2 Mass balance of An-Ax SBR and An-Ox SBR

| | | Influent | Anaerobic | | Anoxic | | Effluent | ΔM | | | g SCOD/g (N+P) |
|-----------|--|----------|-----------|-------|---------|-------|----------|------------|--------|--------|----------------|
| | | | Start* | End | Start** | End | | Anaerobic | Anoxic | Total | |
| An-Ax SBR | Q (L/cycle) | 3.4 | 5.9 | 5.9 | 6.0 | 6.0 | 3.5 | – | – | – | |
| | SCOD (mg/cycle) | 502.5 | 578.0 | 169.9 | 169.9 | 168.0 | 105.7 | –408.1 | –1.9 | –410.0 | |
| | $\text{NH}_4^+\text{-N}$ (mg/cycle) | 32.0 | 42.5 | 36.6 | 36.6 | 27.6 | 14.7 | –5.9 | –9.0 | –14.9 | 3.42 |
| | $\text{NO}_3^-\text{-N}$ (mg/cycle) | 0.0 | 4.0 | 0.0 | 95.0 | 10.2 | 5.6 | –4.0 | –84.8 | –88.8 | |
| | $\text{PO}_4^{3-}\text{-P}$ (mg/cycle) | 21.4 | 27.5 | 152.8 | 152.8 | 11.4 | 5.3 | +125.3 | –141.4 | –16.1 | |
| | | Influent | Anaerobic | | Aerobic | | Effluent | ΔM | | | g SCOD/g (N+P) |
| | | | Start* | End | Start | End | | Anaerobic | Oxic | Total | |
| An-Ox SBR | Q (L/cycle) | 3.4 | 5.9 | 5.9 | 5.9 | 5.9 | 3.4 | – | – | – | |
| | SCOD (mg/cycle) | 511.0 | 585.5 | 180.0 | 180.0 | 175.8 | 104.3 | –405.5 | –4.2 | –409.7 | |
| | $\text{NH}_4^+\text{-N}$ (mg/cycle) | 79.6 | 80.3 | 62.5 | 62.5 | 1.8 | 1.1 | –17.8 | –60.7 | –78.5 | 5.96 |
| | $\text{NO}_3^-\text{-N}$ (mg/cycle) | 0.0 | 18.8 | 0.0 | 0.0 | 46.6 | 26.3 | –18.8 | +46.6 | +27.8 | |
| | $\text{PO}_4^{3-}\text{-P}$ (mg/cycle) | 20.8 | 28.0 | 191.8 | 191.8 | 10.0 | 2.8 | +163.8 | –181.8 | –18.0 | |

The mass balance was analyzed with 15 data sets and calculated on average.

* Influent with recycled Q to the next cycle, ** including external $\text{NO}_3^-\text{-N}$ feed (95.0 mg $\text{NO}_3^-\text{-N}/0.1$ L).

condition. After anaerobic condition, 100 mL of stock nitrate solution added as an electron acceptor for DPAO. At the end of anoxic conditions, $\text{PO}_4^{3-}\text{-P}$ decreased drastically from 152.8 to 11.4 mg. Approximately 94% of $\text{NO}_3^-\text{-N}$ was also removed, clearly demonstrating the phenomenon of denitrifying EBPR through DPAO.

In the An-Ox SBR, 405.5 mg SCOD was removed under

anaerobic condition, whereby a portion of SCOD was used for the removal of residual $\text{NO}_3^-\text{-N}$ left behind from a previous cycle, while the majority of SCOD was used for P release. P release under anaerobic condition increased up to 191.8 mg, which was 8.8-fold higher compared to the influent. Under aerobic condition, P uptake occurred with nitrification, and a typical PAO-related EBPR phenomenon

was observed. According to the calculation using mass balance, the amount of carbon source used by the An-Ox SBR to remove 1 g phosphorus was 174% higher than that used by the An-Ax SBR. We attribute this to the increased consumption of carbon source during the process of denitrification of NO_3^- -N that is formed through the nitrification of PAO.

2.2 Stoichiometry and operating parameters

According to Smolders et al. (1994) and Filipe et al. (2001), PHV, rather than PHB, is synthesized inside the cell when HPr used as a carbon source because propionyl-CoA produced by degradation of HPr is mainly used to synthesize PHV. In this study, the ratio of PHV/PHA at the end of anaerobic condition (150 min from an initiation of the batch experiment) was 78.5%, and as a result, the synthesis and utilization of PHV were higher than that of PHB in the DPAO system using HPr as a sole substrate. Such a result, combined with the results of research showing that growth of glycogen accumulating organism can be excluded when HPr is used as a substrate (Mino et al., 1994; Hood and Randall, 2001), led to an assumption that although PAO or DPAO can synthesize PHV inside the cell during the anaerobic process using HPr, glycogen accumulating organism cannot grow because it is not capable of PHV synthesis despite its ability to store PHB.

Figure 2 shows the behaviors of PHB, PHV, and glycogen in conjunction with changes in the concentrations of SCOD, PO_4^{3-} -P, and NO_3^- -N under alternating An-Ax conditions. The batch test took for 150 min under anaerobic conditions; then, NO_3^- -N was injected to induce anoxic conditions, and the operation lasted for 200 min. As 17 mg/L of NO_3^- -N is injected from the outside and denitrification occurs simultaneously, PO_4^{3-} -P is accumulated at a high rate; therefore, it was confirmed that PHV synthesized as an energy source inside the cell is consumed at a rate similar to that of adsorption of P. Glycogen was recovered

under anoxic conditions.

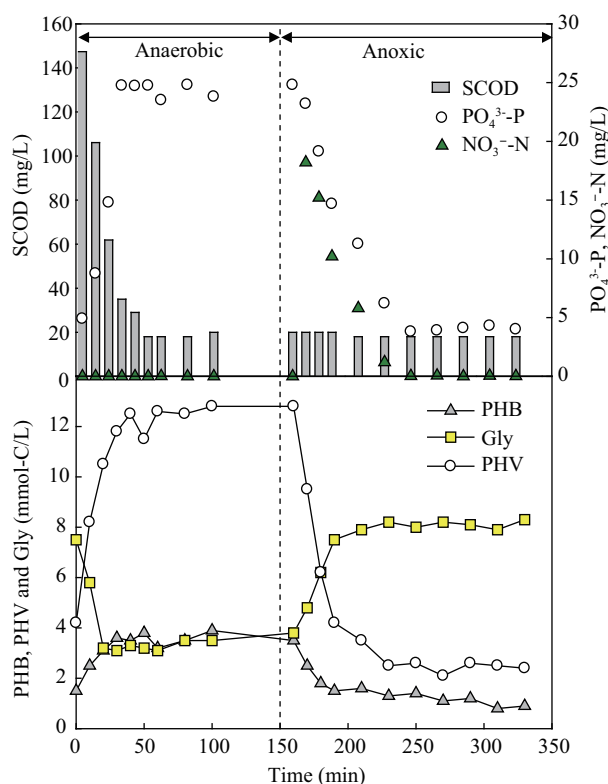


Fig. 2 Batch test result for simultaneous N and P removal under anaerobic-anoxic condition.

The comparative-analytical methods based on the concentration behaviors in two SBR reactors are prone to error to lead a decisive conclusion that a PAO microorganism group has a higher EBPR efficiency than a DPAO microorganism group (**Fig. 1**). Accordingly, as shown in **Table 3**, various operating parameters were estimated and dynamic values per unit biomass were compared to investigate

Table 3 Stoichiometry and operating parameters in An-Ax SBR and An-Ox SBR

| | This study ^a | | Data cited from references | |
|--|-------------------------|-------------|----------------------------|-----------------------|
| | An-Ax SBR | An-Ox SBR | An-Ax SBR | An-Ox SBR |
| SPRR (mg P/(g VSS·hr)) | 22.8 ± 3.5 | 22.4 ± 4.8 | 7.0–30.0 ^b | 5–32.5 ^b |
| SPUR (mg P/(g VSS·hr)) | 26.3 ± 4.8 | 25.6 ± 2.8 | 6.0–18.5 ^b | 5.7–20.8 ^b |
| PHA synthesis in anaerobic condition (mmol-C/g VSS) | 5.1 ± 1.5 | 5.0 ± 0.9 | – | – |
| PHA degradation in anoxic or aerobic condition (mmol-C/g VSS) | 4.9 ± 1.2 | 4.8 ± 1.5 | – | – |
| Glycogen degradation in anaerobic condition (mmol-C/g VSS) | 2.0 ± 0.9 | 1.5 ± 0.7 | – | – |
| Glycogen synthesis in anoxic or aerobic condition (mmol-C/g VSS) | 2.5 ± 0.5 | 2.0 ± 0.2 | – | – |
| Released/PHA _{synthesized} (mmol-P/mmol-C) | 0.07 ± 0.02 | 0.07 ± 0.03 | 0.18 ^c | 0.21 ^c |
| P _{uptaked} /PHA _{utilized} (mmol-P/mmol-C) | 0.09 ± 0.03 | 0.09 ± 0.01 | 0.10 ^c | 0.09 ^c |
| PHA _{synthesized} /Glycogen _{utilized} (mmol-C/mmol-C) | 2.6 ± 0.8 | 3.3 ± 0.5 | 3.8 ^c | 5.2 ^c |
| Cell yield (g VSS/g SCOD) | 0.18 ± 0.05 | 0.23 ± 0.07 | 0.18 ^d | 0.38 ^e |

SPRR: Specific P release rate; SPUR: specific P uptake rate.

^aEach value indicates average ± standard deviation; ^bMamais and Jenkins, 1992; Kuba et al., 1997; Brdjanovic et al., 1998; Petersen et al., 1998; Monti et al., 2007; ^cAhn et al., 2002; ^dKuba et al., 1996; ^eSmolders et al., 1995.

EBPR activities between the two actual microorganism groups. The calculation of SPRR and SPUR, representative parameters to characterize the EBPR process, revealed that there were no significant differences between the An-Ax SBR and the An-Ox SBR. This means that EBPR activity of DPAO is not significantly different from that of PAO, even though the removal efficiency of TP was higher in the An-Ox system than in the An-Ax system.

DPAO activity was not critically considered in European sewage because relatively sufficient COD existed within sewage for biological N and P removal. Since not only DPAO portion in PAO was small, but also the DPAO activity was less than 60% of ordinary PAO, the first IWA model (ASM2d) which included DPAO contribution in BNR had adopted a correction factor (η) of 0.6 to account a reduction factor for anoxic activity (Henze et al., 1999). Similar results for synthesis and consumption of PHA per unit biomass and the decomposition and recovery of glycogen were obtained in both reactors. This means that EBPR capability is not largely different between PAO and DPAO as demonstrated by the results of SPRR and SPUR. On the other hand, the amount of PHA synthesized with the consumption of 1 mmol-C glycogen showed a slightly higher result in the PAO operating system than DPAO.

2.3 Denitrification by DPAO

From a process design point of view, the major advantage of DPAO is anoxic reduction of nitrate without an addition of external carbon energy. In the reactor system, it looks like autotrophic denitrification because no wastewater COD is needed in the reaction zone, but in reality it more resembles heterotrophic endogenous denitrification. Since anaerobically synthesized PHA is used for the internal carbon source, DPAO do not need external carbon energy for denitrification. **Figure 3a** shows the batch test result to evaluate the denitrification ability of DPAO. In order to determine the SDNR of DPAO sludge in organic-limiting condition, phosphate in the sludge was induced to release completely under the anaerobic condition for 2.5 hr. As a control experiment, SDNR measurement with activated

sludge taken from Jungnang wastewater treatment plant in Seoul, Korea, performed with a sufficient SCOD for denitrification. **Figure 3b** presents the plot of the control test.

SDNR of DPAO sludge and activated sludge was 4.6 and 6.7 mg NO_3^- -N/(g VSS·hr), respectively. Typical heterotrophic denitrification rate in full-scale pre-denitrification process have widely ranged from 1.7 to 17.5 mg N/(g VSS·hr) (Bradstreet and Johnson, 1994; Burdick et al., 1982; Henze, 1991), and autotrophic denitrification rates using elemental sulfur have range from 4.2 to 8.4 mg N/(g VSS·hr) (Batchelor and Lawrence, 1978). Moreover, the denitrification rate with endogenous carbon was reported in the range from 0.63 to 2.5 mg N/(g VSS·hr) (US EPA, 1993). The result shows that the value of SDNR of DPAO was higher than the endogenous denitrification rate, but within the lower range of heterotrophic denitrification. It means that denitrification ability of DPAO is excellent.

2.4 Settling characteristics of DPAO sludge

The settling characteristic of mixed liquor solids is the important parameter when designing the secondary settling tank for liquid-solids separation. **Figure 4a** shows the sludge interface of 30-min settling in the An-Ax and the An-Ox sludges. SVI of the An-Ax sludge was 134 mL/g, while the An-Ox sludge showed relatively low 86 mL/g. Although DPAO sludge showed relatively poor SVI values, both sludge showed a clear liquid-sludge interface (**Fig. 4b**).

Average effluent TSS concentration in the An-Ax SBR and the An-Ox SBR was 0.9 mg/L and 0.7 mg/L, respectively. In general, extended non-aerated condition such as anaerobic and/or anoxic condition could deteriorate the sludge settling property, hence Henze et al. (2008) suggested that An and/or Ax portion in BNR system should be lower than 60% of total reactor volume. However, the SVI along with effluent TSS data suggested that the An-Ax sludge exhibited tolerable sludge settleability. Although it seems logical to speculate the deterioration of sludge settleability in the An-Ax sludge without aeration, a further

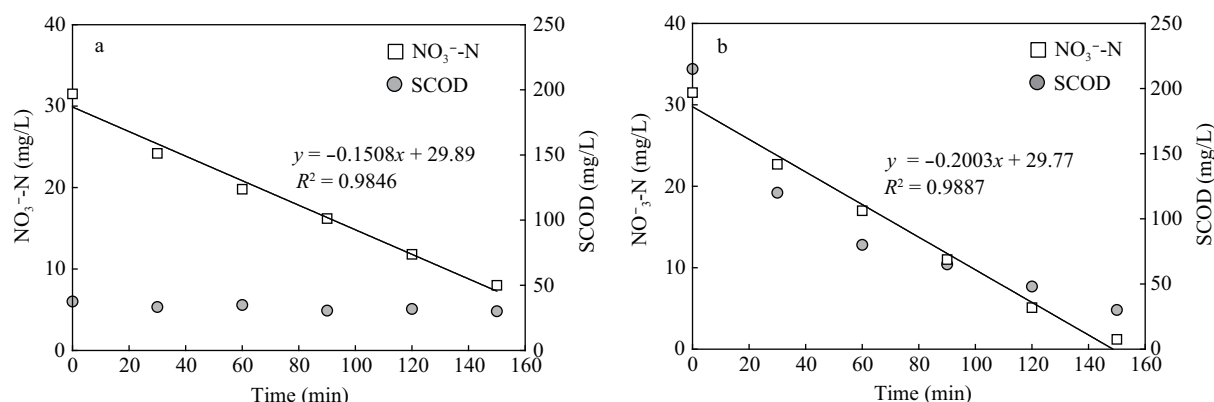


Fig. 3 (a) Specific denitrification rate (SDNR) result of denitrifying phosphorus accumulating organism (DPAO) sludge without COD addition after 2-hr anaerobic condition for PHA synthesis, and (b) SDNR result of activated sludge with 200 mg/L of COD addition in the anoxic sludge.

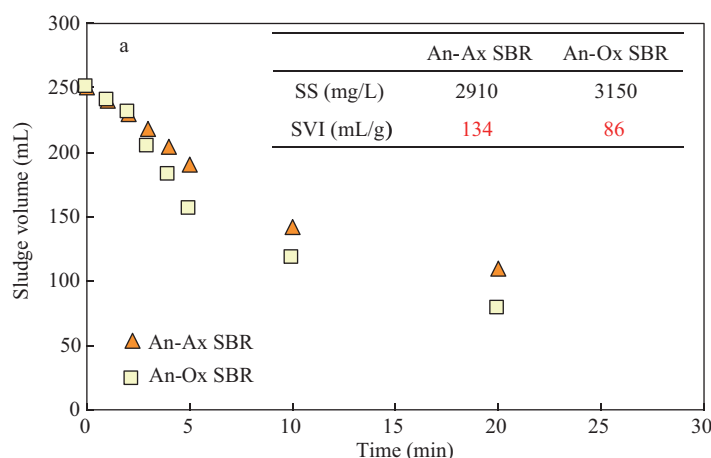


Fig. 4 Sludge settling curve (a) and picture (b) after 30 min in An-Ax SBR and An-Ox SBR.

investigation to characterize the anoxic sludge settling properties will be needed.

3 Conclusions

The comparative study between PAO and DPAO was conducted to operate two lab-scale SBR units with propionate-based synthetic wastewater. For entire operating days, the An-Ax SBR for the growth of DPAOs and the An-Ox SBR for the growth of PAOs exhibited successful EBPR performance. Although EBPR performance was better in the An-Ox sludge, SPRR and SPUR of both SBRs was similar, clearly demonstrating that biological activity per unit biomass was little difference each other. The SDNR test to investigate the denitrification ability of DPAO shows that DPAO significantly contributed to nitrate reduction at the anoxic condition. From the mass balance, the significant amount of anoxic P uptake with denitrification was successfully accomplished in the An-Ax SBR. Although the SVI value of An-Ax sludge was about 35% higher than An-Ox sludge, DPAO sludge showed a clear liquid-solids interface.

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