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Comparison of conventional and inverted A²/O processes: Phosphorus release and uptake behaviors

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Abstract

Two full-scale systems operated in parallel, a conventional A²/O system consisting of anaerobic, anoxic and oxic compartments in succession and an inverted system consisting of anoxic, anaerobic and oxic compartments without internal recycle, were compared in terms of their phosphorus removal performance, with an emphasis on phosphate (P) release behaviors, using both operational data and simulation results. The inverted system exhibited better long-term phosphorus removal performance (0.2 ± 0.3 vs. 0.7 ± 0.7 mg/L), which should be attributed to the higher P release rate (0.79 vs. 0.60 kg P/(kg MLSS·day)) in the non-aerated compartments. The P release occurred in both the anoxic and anaerobic compartments of the inverted system, resulting in more efficient P release. Although the abundances of the '*Candidatus* Accumulibacter phosphatis' population in the two systems were quite similar ($(19.1 \pm 3.27)\%$ and $(18.4 \pm 4.15)\%$ of the total microbe (DAPI stained particles) population in the inverted and conventional systems, respectively, by fluorescence *in situ* hybridization (FISH)), the high-concentration DAPI staining results show that the abundances of the whole polyphosphate accumulating organisms (PAOs) in the aerobic ends were quite different (the average ratios of the poly-P granules to total microbes (DAPI stained particles) were $(45 \pm 4.18)\%$ and $(35 \pm 5.39)\%$, respectively). Both the operational data and simulation results showed that the inverted system retained more abundant PAO populations due to its special configuration, which permitted efficient P release in the non-aerated compartment and better P removal.

Key words: phosphate release and uptake; A²/O process; inverted A²/O process; activated sludge model

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Introduction

The A²/O process, consisting of anaerobic, anoxic and oxic units in succession, has been widely used because of its capability for simultaneous removal of nitrogen and phosphorus (Barnard et al., 1975, 1976; Rabinowitz and Marais, 1980; Mino et al., 1998), with an internal recycle ratio as high as 100%–300%, and sludge return ratio of approximately 100%. The interference with phosphate (P) release by the carry-over nitrate in the returning sludge, however, has been found to affect phosphorus removal efficiency, because several heterotrophic bacteria may consume the fermentable chemical oxygen demand (COD) for energy and growth using nitrate as an external electron acceptor (Venter et al., 1978; Hascoet and Florentz, 1985). It has been estimated that for 1 mg NO₃⁻-N recycled to an anaerobic reactor, 8.6 mg fermentable COD will be consumed (Henze et al., 2008), which will lead to substrate competition between denitrification and P release. To

avoid this influence and improve the phosphorus removal performance, several modified systems were investigated, i.e., the University of Cape Town (UCT) system, five-stage Bardenpho process and Biological-chemical phosphorus removal (BCFS) system (van Loosdrecht et al., 1998; Tchobanoglous et al., 2002). However, these processes have also been known for their relatively high energy consumption and complex configuration (Baeza et al., 2004). To reduce energy consumption and prevent the potential interference of nitrate with P release, a so-called inverted A²/O process was developed by canceling the internal recycle and reversing the positions of the anaerobic and anoxic units (Zhang and Su, 1999; Zhang and Gao, 2000). Since then, the inverted A²/O process has been applied in several sewage treatment plants in China.

In the inverted A²/O process, the influent is distributed into the anoxic and anaerobic units with a defined ratio (usually 20%/80% or 30%/70%). Under optimal conditions, carry-over nitrate is removed in the anoxic tank, and then P can be released adequately in a subsequent anaerobic tank without nitrate interference. Aerobically,

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the P uptake by the polyphosphate accumulation organisms (PAOs) is enhanced because these microbes are under “starvation” conditions. While the influence of influent distribution on phosphorus and TN removal performance has been discussed (Gao et al., 2005; Liu et al., 2010a, 2010b), studies regarding the P release behavior in inverted systems have been minimal, and the improvement in phosphorus removal performance compared to conventional systems has not yet been verified at the full-scale level.

In this study, parallel full-scale conventional and inverted A²/O systems in a sewage treatment plant located in north China were compared in terms of their P release and uptake behaviors. The fluorescence *in situ* hybridization (FISH) technique was used to quantify PAOs, and the 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) high-concentration staining method was used to quantify the intercellular poly phosphate (poly-P) particles in activated sludge (Wong et al., 2005). At the same time, the activated sludge model (ASM) was employed to simulate the two systems, particularly focusing on the P release and uptake behaviors. This will be the first comprehensive study on the phosphorus removal performance of a full-scale inverted A²/O system.

1 Materials and methods

1.1 Targeted systems and water sampling

The sewage treatment plant studied consists of a conventional and inverted A²/O system, respectively, each of which has a treatment capacity of 200,000 m³/day. The influent distribution ratio of the inverted A²/O was stable (30% to the anoxic tank and 70% to the anaerobic one). The sludge return ratio was approximately 100% for both systems during the sampling period. The detailed descriptions of the two systems are shown in Fig. 1 and Table 1. As shown in Table 1, the conventional system was a little larger than the inverted one. From February to July 2009, mixed liquor samples were taken weekly from the two ends

Table 1 Details of the inverted and conventional A²/O systems

	Flow rate (m ³ /day)	Unit	Volume (m ³)	Depth (m)	HRT (hr)
Inverted	200000	Anoxic	3060	6	1.5
		Anaerobic	5034	6	2.4
		Aerobic	19986	6	9.6
Conventional	200000	Anaerobic	3221	6	1.5
		Anoxic	6441	6	3
		Aerobic	22544	6	10.8

and the middle of each unit for water quality monitoring, and activated sludge was characterized monthly by taking samples from the ends of the aerobic and anaerobic units. After sampling, samples were immediately transported to the laboratory for analysis. The determination of COD, biological oxygen demand (BOD), mixed liquor suspended solids (MLSS) and phosphate species followed standard procedures (APHA, 1998). Routine data during 2007–2009 were kindly provided by the plant. The operating conditions, such as sludge retention time (SRT) and influent distribution were also monitored.

1.2 FISH analysis and high-concentration DAPI staining

The FISH analysis was conducted according to Amann et al. (1995). Briefly, activated sludge samples were fixed for 3 hr with 4% paraformaldehyde at 4°C and stored in a 1:1 (V/V) mixture of phosphate-buffered saline (PBS, pH 7.4) and ethanol at –20°C. Following sonication on ice for about 3 min, a 3-μL sample was placed in the wells of a slide and immobilized for 3 hr. The slides were then dehydrated for 5 min using 50%, 80% and 98% ethanol, respectively. PAOmix probes (PAO462: CCGTCATCTACWCAGGGTATTAAC 5'–3', PAO651: CCCTCTGCCAAACTCCAG 5'–3', PAO846: GTTAGCTACGGCACTAAAAGG 5'–3', Crocetti et al., 2000) were commercially synthesized and 5' labelled with fluorescein isothiocyanate (FITC) for hybridization. Following the hybridization and washing procedures,

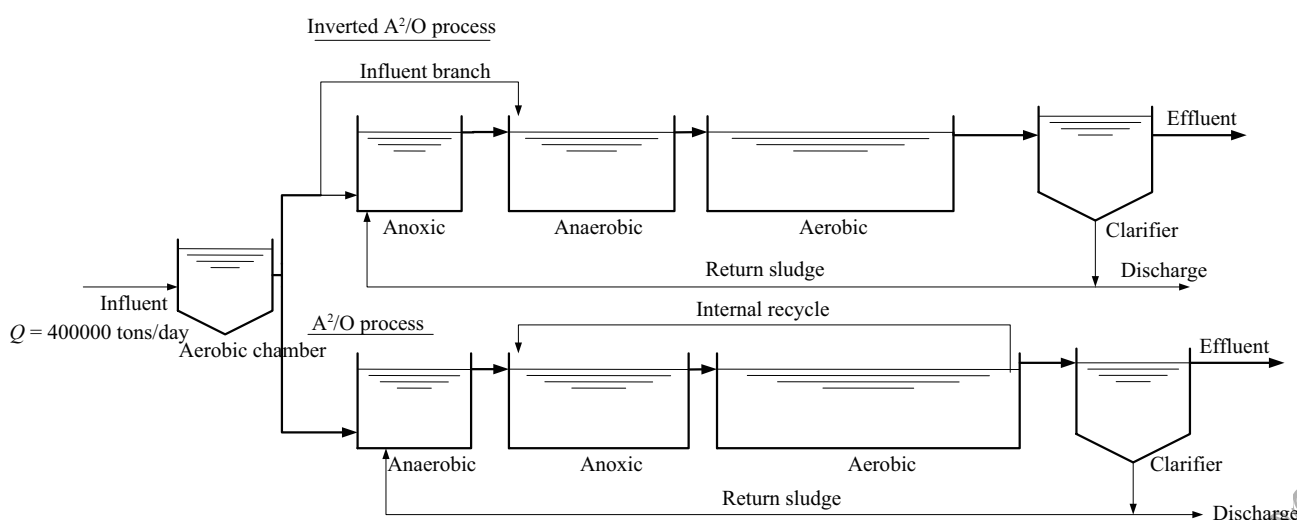


Fig. 1 Configuration of the inverted A²/O and conventional A²/O systems.

Table 2 COD fractions relative to the total COD in the effluent of grit chambers

Name	Symbol	Percentage (%)
Total COD	TCOD	100
Inert soluble COD	S_I	1.0 ± 0.2
Readily biodegradable soluble COD	S_S	–
Volatile fatty acids, VFA, in COD	S_A	20 ± 3.3
Fermentable, readily biodegradable COD	S_F	12 ± 1.2
Slowly biodegradable particulate COD	X_S	55 ± 6.8
Inert particulate COD	X_I	12 ± 1.4

Table 3 Major parameters of the inverted and conventional A²/O systems

	Feb 2009–Jul 2009	
	Inverted	Conventional
Q (ton/day)	200000	
COD _{in} (mg/L)	440 ± 130	
COD _{eff} (mg/L)	59 ± 17*	37 ± 12
BOD _{5in} (mg/L)	236 ± 65	
BOD _{5eff} (mg/L)	7 ± 1	6 ± 1
Total phosphorusin (mg/L)	5.47 ± 0.46	
Total phosphoruseff (mg/L)	0.29 ± 0.25	0.71 ± 0.64
NO ₂ ⁻ -N(mg/L)	10.4 ± 3.7	3.48 ± 0.57
NO ₃ ⁻ -N (mg/L)	6.36 ± 2.0	10.39 ± 2.36
MLSS (mg/L)	2881 ± 356	3803 ± 350
SRT (day)	8.65 ± 2.48	13.56 ± 3.20
Influent distribution	3:7	/

* Value was affected by residual nitrite (10.4 ± 3.7 mg N/L).

the fluorescence-hybridized cells were analyzed with an epifluorescence microscope (Axioskop2 mot plus, Zeiss, Germany) equipped with a cooled CCD camera. The proportion of PAOs was calculated with the software provided by Zeiss (Axio Vision 4.1). At least 40 views were obtained for each sample and averaged to obtain the final result. The ratio of PAOs to total bacteria was then given by dividing the FISH result with the DAPI count.

At the same time, the DAPI high-concentration staining method described in a previous study (Wong et al., 2005) was used to determine the abundance of poly-P granules in the activated sludge. Cells were fixed onto a glass slide, stained with a freshly prepared DAPI solution (50 µg/mL) at room temperature for 10 min, rinsed thoroughly with water, and then air-dried. Observation of poly-P particles was performed using the same method as FISH. The ratio of poly-P particles was then given by dividing the DAPI high-concentration staining result with the DAPI count obtained during FISH analysis.

1.3 Activated sludge model

The full-scale plant mathematic model was constructed by AQUASIM software (EAWAG, license number: 251) based on ASM2d (Henze et al., 2000). Whole biological tanks were considered as CSTR, while the settling compartment was set as an ideal location for solid-liquid separation without biochemical reaction. ASM2d without chemical precipitation of phosphate was employed to model the biochemical reactions in both systems. Sensitivity analysis was used for parameters estimation.

The effluent from the aerated grit chamber was analyzed according to the standard Dutch STOWA guidelines for

wastewater characterization (Roeleveld and van Loosdrecht, 2002). The soluble COD fraction was measured after flocculation with Zn(OH)₂ and filtration with a 0.45 µm filter membrane (Millipore, USA) as recommended in the STOWA protocol. The weekly measured total COD was fractionated into model components as shown in Table 2.

2 Results and discussion

2.1 Long term performance of the two systems

The average soluble COD, TN and phosphate in the effluents of the inverted and conventional A²/O systems in 2007–2008 were (42.7 ± 8.3) and (41.5 ± 7.91) mg/L, (21.7 ± 4.3) N and (16.5 ± 3.5) N mg/L, (0.2 ± 0.3) and (0.7 ± 0.7) PO₄³⁻-P mg/L, respectively. The two systems showed similar COD removal performance, but different TN and phosphorus removals. The above data were in accordance with previous lab-scale studies (Zhou, 2001; Wang, 2008).

The water quality parameters during February to July, 2009 are shown in Table 3. The average soluble COD in the effluent of the inverted A²/O systems was higher than the conventional system, which was due to the accumulation of NO₂⁻-N in the inverted system. Considering the oxygen demand of NO₂⁻-N, the calibrated COD removal performances of two systems were 89% and 92%, respectively, which was in accordance with the result of 2007–2008. P removal performance of each system was not inhibited by the nitrite accumulation during the sampling period with the maximum concentration 12 mg/L, in accordance with phenomena reported in a previous study (Zeng et al., 2011). Again, the inverted system exhibited better phosphorus removal performance (0.29 ± 0.25 vs. 0.71 ± 0.64 mg/L).

2.2 P release and uptake behaviours

Variations of P concentrations along the reactor lengths were determined from February to July, 2009, and the results are shown in Fig. 2. Because the two systems are different in sizes, the length percentage (the ratio of the distance between the inlet and the sampling point to the total length of the system) was used to represent the reactor length.

As shown in Fig. 2, the P release occurred in the anoxic compartment (average, 21.9 ± 8.2) P mg/L, and continued in the anaerobic one (average, 20.2 ± 9.1) P mg/L) of the inverted A²/O system taking into consideration the dilution ratios in the two reactors (dilution ratio: 1.3 in the anoxic compartment and 2.0 in the anaerobic one). Then the P uptake occurred along the aerobic compartment. On the other hand, the P release occurred mainly in the anaerobic compartment in the conventional system, with a P concentration of (19.02 ± 0.96) P mg/L. The P concentration in the anoxic unit was almost constant at (4.61 ± 0.96) P mg/L, which was lower than that in the anaerobic compartment taking into consideration the dilution factor of 2.5. Thus the P release stopped in this reactor. Therefore

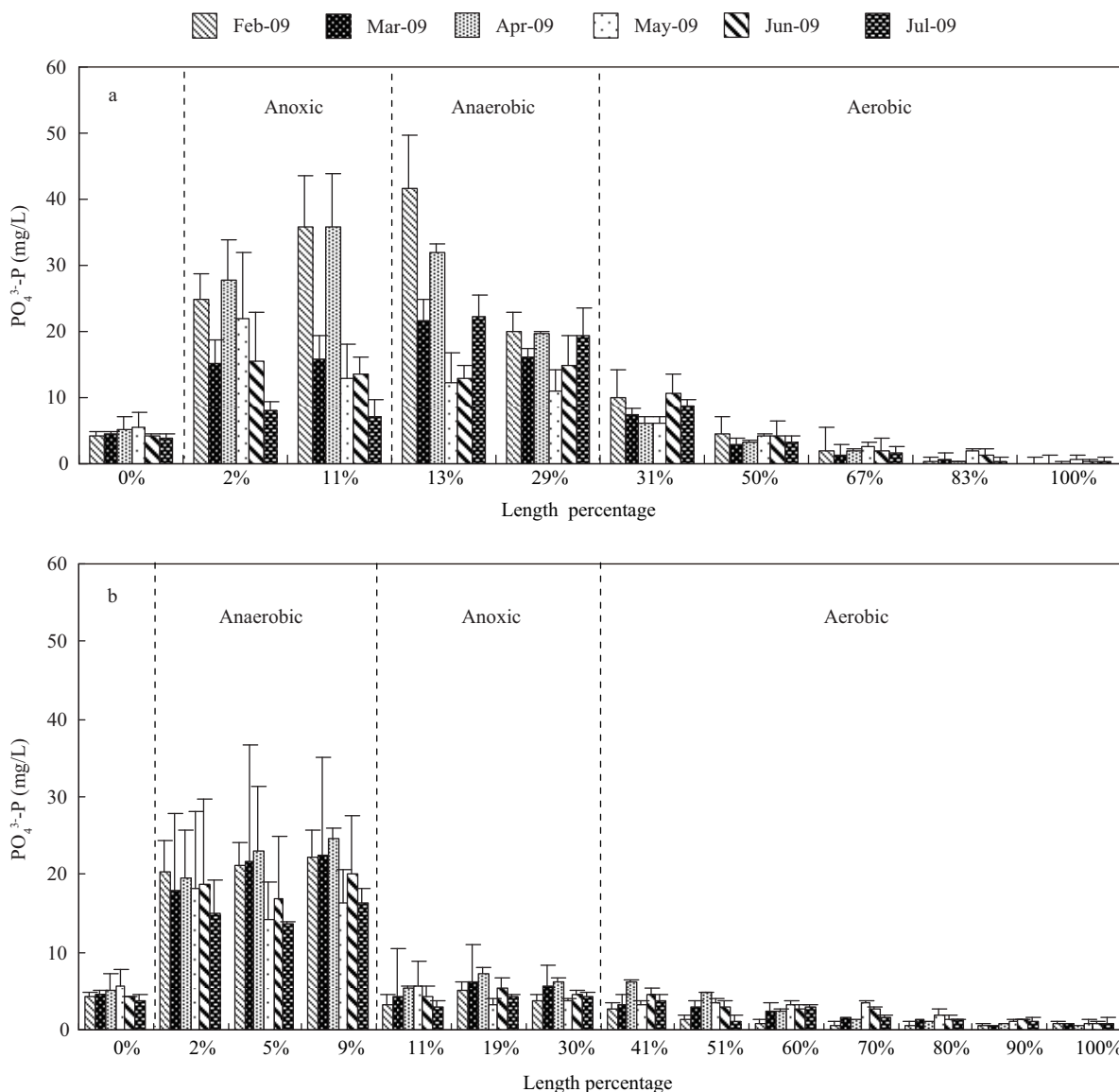


Fig. 2 Variations of phosphorus concentrations along the reactor channels in the two systems. (a) inverted A²/O system, (b) conventional A²/O system.

P release occurred in the whole non-aeration compartment (3 hr) of the inverted A²/O system, but only occurred in the anaerobic compartment (1.5 hr) of the conventional one. This might have led to the difference of the P release rate (the P release amount in a day divided by the amount of activated sludge) of the two systems (0.79 and 0.60 kg P/(kg MLSS·day) for the inverted and conventional systems, respectively). Thus the inverted system exhibited better phosphorus removal performance because of the higher P release rate of activated sludge in the system (Lie et al., 1997; Thomas et al., 2003; Oehmen et al., 2007).

During the sampling period, the COD consumption in the anoxic and anaerobic compartments of the inverted system was 2000 and 7000 kg/day, respectively, while in the conventional system, the COD consumption in the anoxic and anaerobic compartments was 7000 and 4000 kg/day, respectively. It is clear that the anaerobic compartment in the inverted system consumed much higher COD, which

might be an important reason for the more efficient P release.

It has been reported that the P release depends on nitrate concentrations (Simpkins and McLaren, 1978; Hascoet and Florentz, 1985). To compare the influence of nitrate and nitrite (NO_x-N) on P release in the two systems, the P and NO_x-N concentrations in the non-aerated compartments were analyzed, and their relationship is shown in Fig. 3 and Table 4. The NO_x-N and P concentrations

Table 4 Slope, correlation coefficient and relevance of P- NO_x-N in each compartment

Unit	Statistical significance	Slope, <i>k</i>	Correlation coefficient, <i>r</i>
I-Ano	<i>P</i> < 0.05	-5.6	-0.84
I-Ana	<i>P</i> < 0.05	-4.8	-0.69
C-Ano	<i>P</i> < 0.05	-0.4	-0.48
C-Ana	<i>P</i> < 0.05	-1.1	-0.88

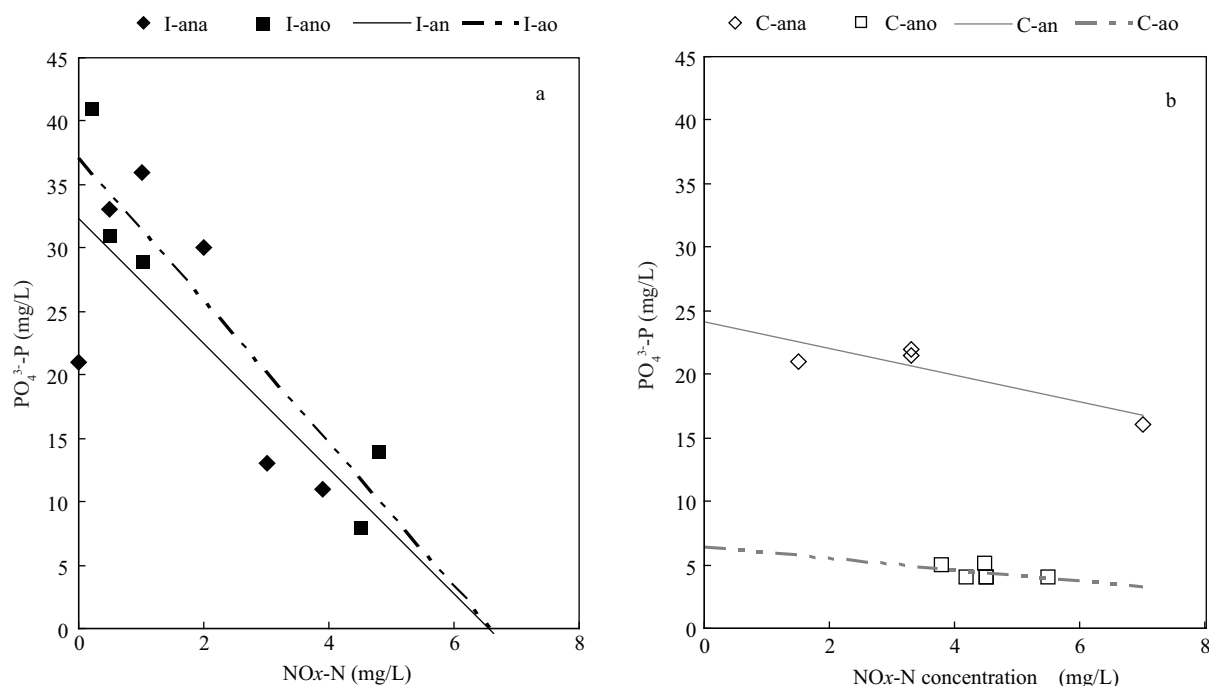


Fig. 3 Phosphorus and NO_x-N concentrations in the non-aerated compartments. (a) inverted system; (b) conventional system. I-ano: anoxic unit of inverted system; I-ana: anaerobic unit of inverted system; C-ana: Anaerobic unit of conventional system; C-ano: anoxic unit unit of conventional system.

had high relevancies in both anaerobic and anoxic compartments of the inverted system ($r_{I-ano}, r_{I-ana} > 0.8$), and the impacts of NO_x-N on P release were significant as indicated by the high k values ($k_{I-ano} = -5.6$, $k_{I-ana} = -4.8$), suggesting that the denitrification in the anoxic unit is important. In the conventional system, however, high relevance ($r_{C-ana} > 0.8$) was still observed between NO_x-N and P, while the impact of NO_x-N on P release was less significant ($k_{C-ana} = -1.1$) in the anaerobic compartment. The low relevance between NO_x-N and P in the anoxic compartment was because P release stopped in this compartment. Thus it is clear that the impact of NO_x-N on P release was more significant in the inverted system.

2.3 Abundance of PAOs and intercellular poly-P granules

The different responses of activated sludge to NO_x-N concentration changes in the aspect of P release suggested that the PAOs populations might be quite different in the two systems. The dominant *Rhodocyclus* species of PAOs, namely '*Candidatus Accumulibacter phosphatis*', were quantified by FISH using PAO mix as the probe. The average ratios of PAOs to total microbes (DAPI stained particles) were $(19.1 \pm 3.27)\%$ and $(18.4 \pm 4.15)\%$, respectively, for the inverted and conventional A²/O systems, showing that the abundances of the *Candidatus Accumulibacter phosphatis* population in the two systems were quite similar and in accordance with previously reported data (Saito et al., 2004). However, other PAO populations might co-exist with '*Candidatus Accumulibacter phosphatis*' (Hesselmann et al., 1997; Liu et al., 2001). The high-concentration DAPI staining method has been used

to quantify the intercellular poly-P granules, i.e., bacteria accumulating phosphate in the cells as poly-P granules in the aerobic compartments (Kawaharasaki et al., 1999, 2002). The average ratios of the poly-P granules in the aerobic ends to total microbes (DAPI stained particles) were $(45 \pm 4.18)\%$ and $(35 \pm 5.39)\%$, respectively, for the inverted and conventional A²/O systems, showing that the difference of the ratios of poly-P granule to DAPI positive cell in the two processes was statistically significant ($p < 0.05$). The above results indicated: (1) PAO populations other than '*Candidatus Accumulibacter phosphatis*' existed extensively in the two systems; (2) the inverted system possessed more abundant PAO populations. Thus it is possible that the special configuration of the inverted system permitted more abundant PAO populations, resulting in better P removal performance.

2.4 Modeling of the two systems

The phosphorus removal performance of the two systems in July 2009 was simulated by employing ASM2d (Henze et al., 1999), and the results are shown in Fig. 4. The parameter values estimated by the model were validated with the analytical data. Key factors were sought by sensitivity analysis.

Stable simulation was performed first for a period of at least 3 times SRTs for the initial calibration of kinetic and stoichiometric parameters with pre-months average data. The calibrated values of the parameters were then used as defaults for the following dynamic simulation (Fig. S3a, S4a).

Sensitivity analysis using AQUASIM software showed that the most sensitive parameters for phosphorus removal

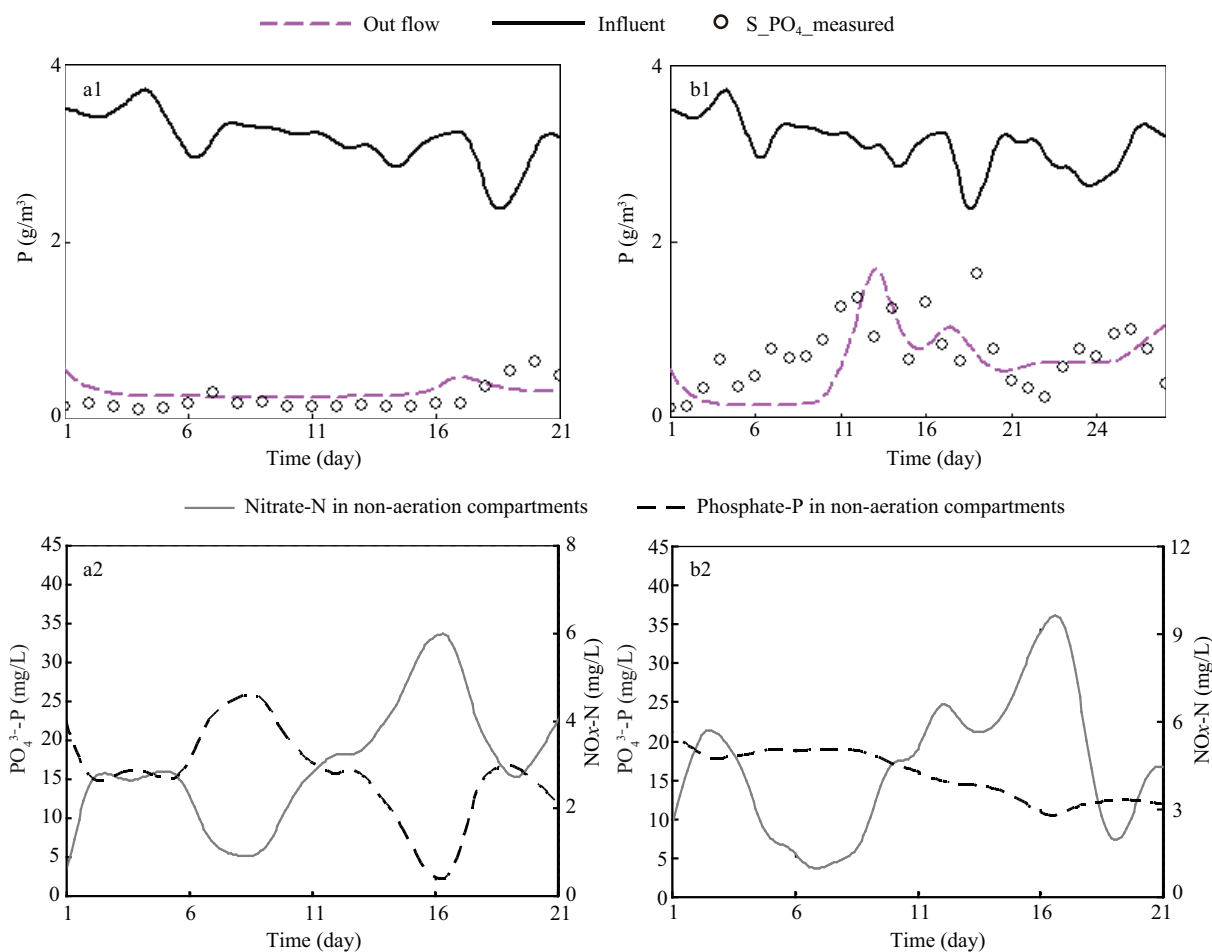


Fig. 4 P concentrations in different compartments of the two systems. (a) inverted A²/O system; (b) conventional A²/O system.

were polyhydroxyalkanoates (PHA) storage rate constants (q_{PHA}) and poly-P storage rate constants (q_{PP}) (shown in Fig. S2). Therefore these two parameters were calibrated using the measured data, while the other parameters were employed as default values (Brun et al., 1997; Gernaey et al., 2004). It is well known that q_{PHA} and q_{PP} , determining the P release and uptake behaviours, respectively, could be acquired via ASM calculation (van Veldhuizen et al., 1999). Figure 4a1 and b1 show that the simulated results could capture variations of daily measured effluent P concentrations. Based on the agreement between the simulated P concentrations and the measured data, the values of q_{PHA} for the inverted and conventional A²/O systems were calibrated to be 6.19 and 4.13 g $X_{PHA}/(g X_{PAO}\cdot day)$, respectively, and the values of q_{PP} were 2.22 g $X_{PP}/(g X_{PAO}\cdot day)$ and 1.54 g $X_{PP}/(g X_{PAO}\cdot day)$; these values were in accordance with the previous studies (6 to 3 g $X_{PHA}/(g X_{PAO}\cdot day)$ for q_{PHA} and 3 to 1.5 g $X_{PP}/(g X_{PAO}\cdot day)$ for q_{PP} ; Smolders et al., 1994; Rieger et al., 2001). The values of q_{PHA} and q_{PP} in the inverted system were much higher than those in the conventional system, which might be related to the higher PAO abundance.

The P and NO_x-N concentrations in the non-aerated compartments in the inverted and conventional A²/O sys-

tems were simulated continuously as shown in Fig. 4 a2 and b2, respectively. It is clear that the P release behaviour responded sensitively to the variations of NO_x-N concentration in the inverted system, particularly when the NO_x-N concentration was over 3 mg N/L. For the conventional system, on the other hand, the response of P release to the variations of NO_x-N concentration was not so significant, and the P release began to be affected to some extent only when the NO_x-N concentration was over 6 mg N/L. Thus the simulated results also supported the hypothesis that the PAO populations in the inverted system were more sensitive to the fluctuations of the NO_x-N concentrations as we observed in Section 2.2. In addition to the difference in PAO abundances, the difference in PAO populations may have also contributed to the different phosphorus removal performance, which requires further experimental proof.

3 Conclusions

The phosphorus removal performance of a full-scale inverted and a conventional A²/O system receiving the same municipal wastewater was compared using both operational data and simulated results, and the inverted system

exhibited better phosphorus removal performance. By canceling the internal recycle and reversing the positions of the anaerobic and anoxic compartments, the inverted system retained more abundant PAO populations, which permitted efficient P release.

Acknowledgments

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Appendix A. Supplementary data

Supplementary figures and table associated with this article can be found in the online version.

References

- Amann R I, Ludwin W, Schleifer K H, 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiology Review*, 59(1): 143–169.
- American Public Health Association/American Water Works Association/Water Environment Federation, 1998. Standard Methods for the Examination of Water and Wastewater (20th ed.). Washington DC, USA.
- Baeza J A, Gabriel D, Lafuente J, 2004. Effect of internal recycle on the nitrogen removal efficiency of an anaerobic/anoxic/oxic (A²/O) wastewater treatment plant (WWTP). *Process Biochemistry*, 39(11): 1615–1624.
- Barnard J L, 1975. Biological nutrient removal without the addition of chemicals. *Water Research*, 9(5-6): 485–490.
- Barnard J L, 1976. A review of biological phosphorus removal in the activated sludge process. *Water South African*, 2(3): 136–144.
- Brun R, Kühni M, Siegrist H, Gujer W, Reichert P, 2002. Practical identifiability of ASM2d parameters-systematic selection and tuning of parameter subsets. *Water Research*, 36(16): 4113–4127.
- Crocetti G R, Hugenholtz P, Bond P L, Schuler A, Keller J, Jenkins D et al., 2000. Identification of polyphosphate-accumulating organisms and design of 16S rRNA-directed probes for their detection and quantitation. *Applied Environmental and Microbiology*, 66(3): 1175–1182.
- Gao J F, Zhou Y, Lü P H, Gao W, Tian H Y, 2005. Research and analysis of nitrogen removal on modified and reversed A²/O process. *Applied Chemical Industry*, 34(8): 487–489.
- Gernaey K V, van Loosdrecht M C M, Henze M, Lind M, Jørgensen S B, 2004. Activated sludge wastewater treatment plant modelling and simulation: state of the art. *Environmental Modelling & Software*, 19(9): 763–783.
- Hascoet M C, Florentz M, 1985. Influence of nitrates on biological phosphorus removal from wastewater. *Water South African*, 11(1): 1–8.
- Henze M, Gujer W, Mino T, Matsuo T, Wentzel M C, Marais G V R et al., 1999. Activated sludge model No.2D, ASM2D. *Water Science & Technology*, 39(1): 165–182.
- Henze M, Gujer W, Mino T, van Loosdrecht M C M, 2000. Activated Sludge Models ASM1, ASM2, ASM2d and ASM3: Scientific and Technical Report No. 9. IWA Task Group on Mathematical Modelling for Design and Operation of Biological Wastewater Treatment. IWA Publishing, London.
- Henze M, van Loosdrecht M C M, Ekama G A, Brdjanovic D, 2008. Biological Wastewater Treatment: Principles, Modelling and Design. IWA Publishing, London. 162.
- Hesselmann P P X, Werlen C, Hahn D, van der Meer J R, Zehnder A J B, 1999. Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge. *Systematic and Applied Microbiology*, 22(3): 454–465.
- Kawaharasaki M, Manome A, Kanagawa T, Nakamura K, 2002. Flow cytometric sorting and RFLP analysis of phosphate accumulating bacteria in an enhanced biological phosphorus removal system. *Water Science & Technology*, 46(1-2): 139–144.
- Kawaharasaki M, Tanaka H, Kanagawa T, Nakamura K, 1999. In situ identification of polyphosphate-accumulating bacteria in activated sludge by dual staining with rRNA-targeted oligonucleotide probes and 4',6-diamidino-2-phenylindol (DAPI) at a polyphosphate-probing concentration. *Water Research*, 33(1): 257–265.
- Lie E, Christensson M, Jönsson K, Ostgaard K, Johansson P, Welander T, 1997. Carbon and phosphorus transformations in a full-scale enhanced biological phosphorus removal process. *Water Research*, 31(11): 2693–2698.
- Liu H, An H J, Mou R Z, 2010a. Design and operation of second-phase project of Qingdao Licunhe WWTP. *China Water & Wastewater*, 26(20): 76–80.
- Liu W T, Nielsen A T, Wu J H, Tsai C S, Matsuo Y, Molin S, 2001. In situ identification of polyphosphate- and polyhydroxyalkanoate-accumulating traits for microbial populations in a biological phosphorus removal process. *Environmental Microbiology*, 3(2): 110–122.
- Liu X F, Yang Y L, Zhang X B, 2010b. On the optimization of A²O process. *Shanxi Architecture*, 36(8): 193–194.
- Mino T, van Loosdrecht M C M, Heijnen J J, 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research*, 32(11): 3193–3207.
- Oehmen A, Lemos P C, Carvalho G, Yuan Z G, Keller J, Blackall L L et al., 2007. Advances in enhanced biological phosphorus removal: From micro to macro scale. *Water Research*, 41(11): 2271–2300.
- Rabinowita B, Marais G V R, 1980. Chemical and biological phosphorus removal in the activated sludge process. M.A.Sc. thesis, Univ. Capertown, S.A., Res. Rep. No.W32.
- Rieger L, Koch G, Kühni M, Gujer W, Siegrist H, 2001. The eawag bio-p module for activated sludge model no. 3. *Water Research*, 35(16): 3887–3903.
- Roeleveld P J, van Loosdrecht M C M, 2002. Experience with guidelines for wastewater characterisation in The Netherlands. *Water Sciences & Technology*, 45(6): 77–87.
- Saito T, Brdjanovic D, van Loosdrecht M C M, 2004. Effect of nitrite on phosphate uptake by phosphate accumulating organisms. *Water Research*, 38(17): 3760–3768.
- Simpkins M J, McLaren A R, 1978. Consistent biological phosphate and nitrate removal in an activated sludge plant. *Progress in Water Technology*, 10(5-6): 433–442.
- Smolders G J F, van der Meij J, van Loosdrecht M C M, Heijnen J J, 1994. Stoichiometric model of the aerobic metabolism of the biological phosphorus process. *Biotechnology and Bioengineering*, 44(7): 837–848.
- Tchobanoglous G, Burton F L, Stensel H D, 2002. Wastewater Engineering: Treatment and Reuse (4th ed.). Metcalf & Eddy Inc., McGraw-Hill Science Engineering, NY, USA.
- Thomas E C, Ehlers M M, van Heerden J, Atkinson B, 2003. The microbiology of phosphorus removal in activated sludge. In: Handbook of Water and Wastewater Microbiology. 373.

- 390.
- van Loosdrecht M C M, Brandse F A, de Vries A C, 1998. Upgrading of wastewater treatment processes for integrated nutrient removal-the BCFS (R) process. *Water Science & Technology*, 37: 209–217.
- van Veldhuizen H M, van Loosdrecht M C M, Heijnen J J, 1999. Modelling biological phosphorus and nitrogen removal in a full scale activated sludge process. *Water Research*, 33(16): 3459–3468.
- Venter S L, Halliday J, Pitman A R, 1978. Optimisation of the Johannesburg Olifantsvlei extended aeration plant for phosphorus removal. *Progress in Water Technology*, 10(1-2): 279–292.
- Wang L, 2008. Test run of improved A²O process. *Environmental Science Survey*, 27(2): 52–53.
- Wong M T, Mino T, Seviour R J, Onuki M, Liu W T, 2005. In situ identification and characterization of the microbial community structure of full-scale enhanced biological phosphorous removal plants in Japan. *Water Research*, 39(13): 2901–2914.
- Zeng W, Yang Y Y, Li L, Wang X D, Peng Y Z, 2011. Effect of nitrite from nitrification on biological phosphorus removal in a sequencing batch reactor treating domestic wastewater. *Bioresource Technology*, 102(12): 6657–6664.
- Zhang B, Gao T Y, 2000. Principle and characteristics of reversed A²/O process. *China Water & Wastewater*, 16(7): 11–15.
- Zhang B, Su Y M, 1999. Removal of nitrogen and phosphorus by the reversed A²/O process. *Environmental Engineering*, 17(2): 7–10.
- Zhou B, 2001. Phosphorus and Nitrogen removal performances in reversed A²O process. *China Water & Wastewater*, 17(7): 46–48.

Appendix A. Supplementary data

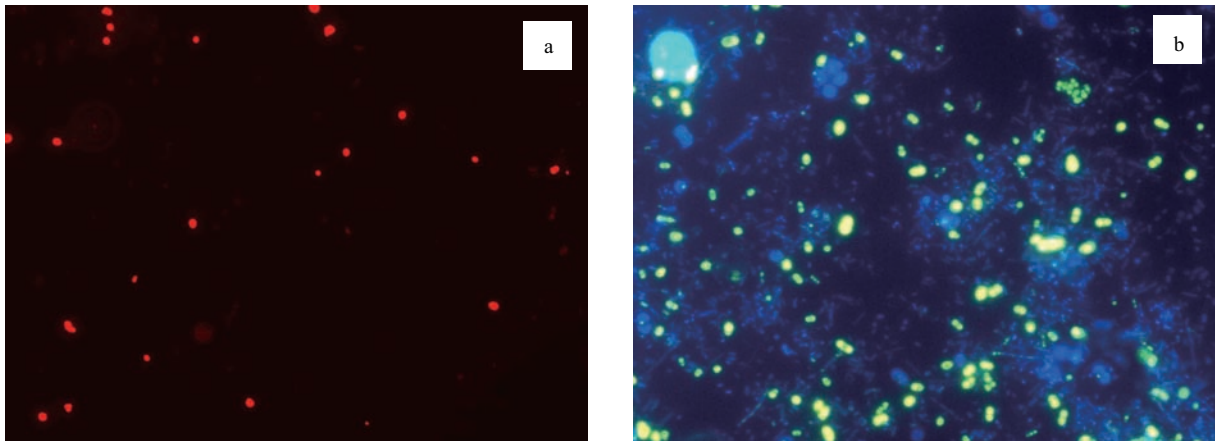


Fig. S1 FISH and High concentration DAPI staining images; (a) *in situ* hybridization of activated sludge samples with probes PAOmix; (b) polyphosphate (DAPI) staining images of activated sludge sample taken from end of aerobic unit.

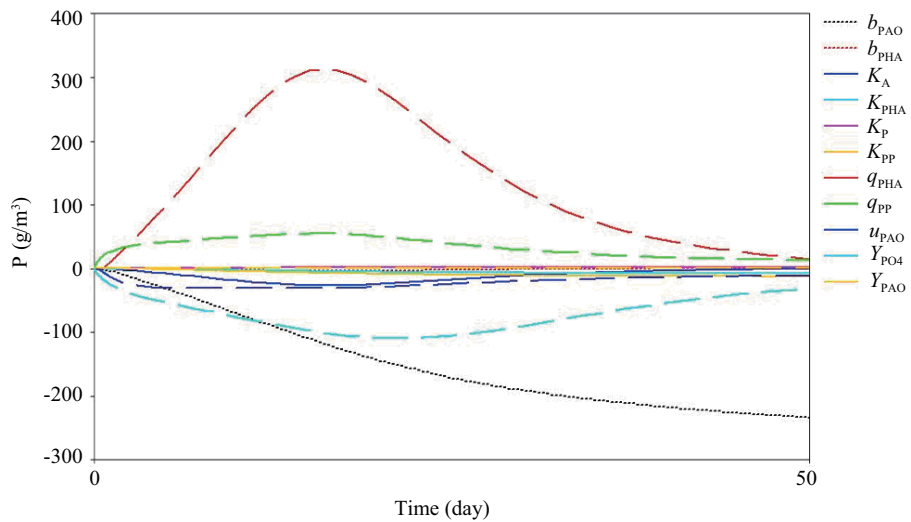


Fig. S2 sensitive analysis for P removal procedure based on AQUASIM.

Table S1 Multi regression analysis for factors in P removal procedure of inverted and conventional A²/O processes during 3 years monitoring

Factor	Inverted A ² /O	Conventional A ² /O
BOD _{5in}	/	/
COD _{in}	/	/
TN _{in}	-0.13	-0.26
TP _{in}	/	/
B/C	/	/
C/N	/	/
C/P	/	/
DO	/	/
SRT	/	/
Sludge Reflux ratio	/	/

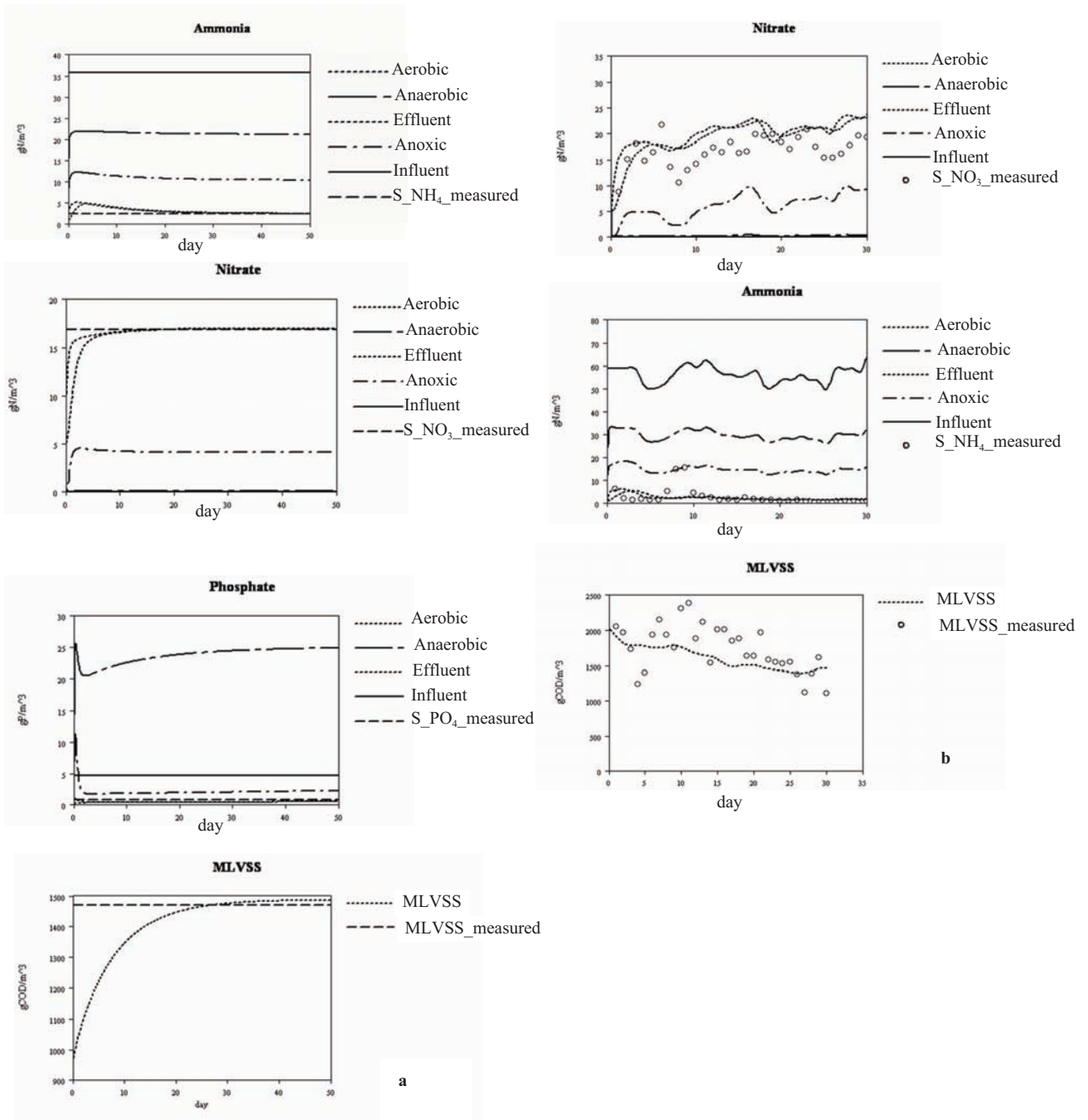


Fig. S3 Results of stable and dynamic simulation for inverted A²/O process: (a) stable simulation results, (b) dynamic simulation results.

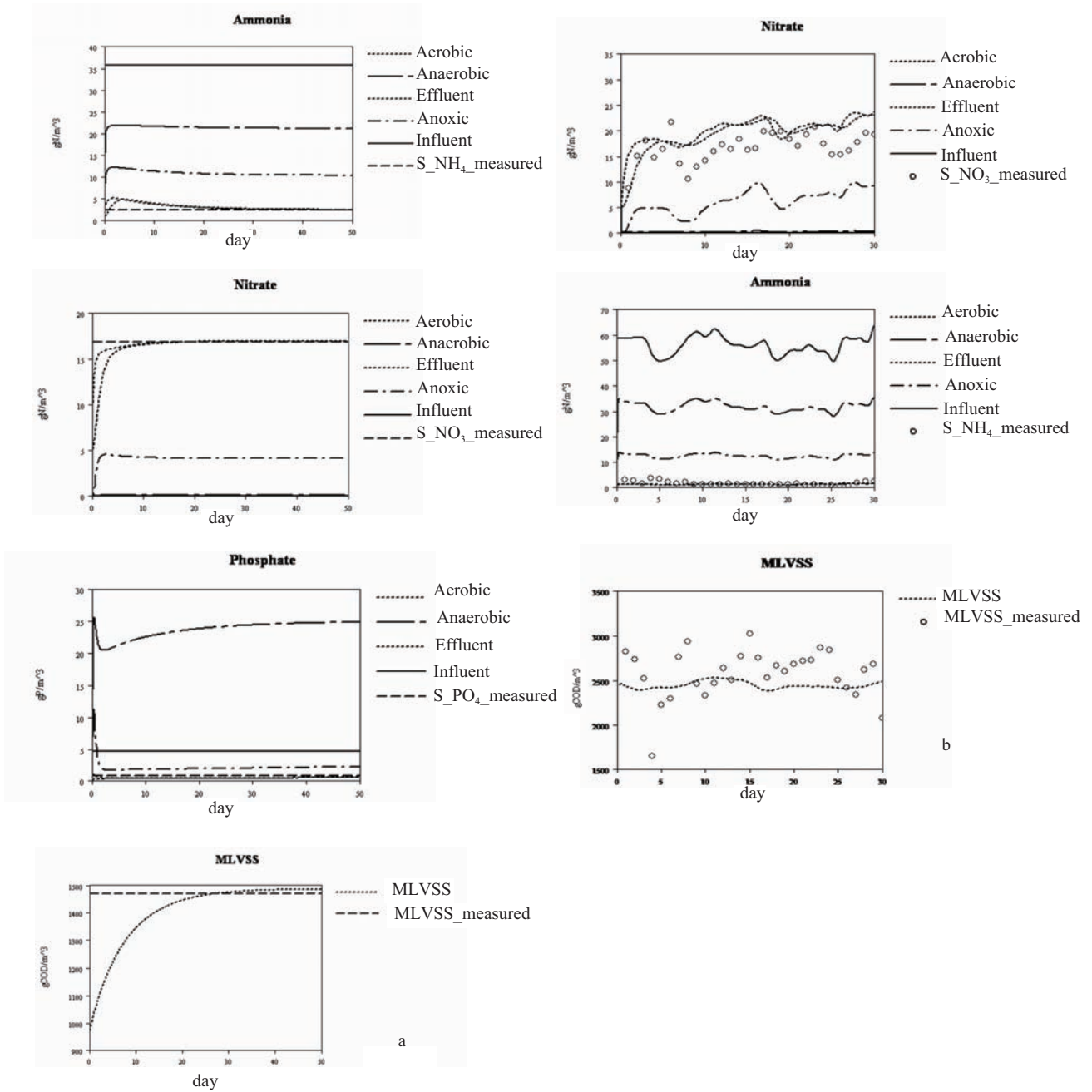


Fig. S4 Results of stable and dynamic simulation for conventional A²/O process: (a) stable simulation results, (b) dynamic simulation results.

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