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Phosphorus accumulation by bacteria isolated from a continuous-flow two-sludge system

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Abstract

In this article, polyphosphate-accumulating organisms (PAOs) from a lab-scale continuous-flow two-sludge system was isolated and identified, the different phosphorus accumulation characteristics of the isolates under anoxic and aerobic conditions were investigated. Two kinds of PAOs were both found in the anoxic zones of the two-sludge system, one of them utilized only oxygen as electron acceptor, and the other one utilized either nitrate or oxygen as electron acceptor. Of the total eight isolates, five isolates were capable of utilizing both nitrate and oxygen as electron acceptors to uptake phosphorus to some extent. And three of the five isolates showed good phosphorus accumulative capacities both under anoxic or aerobic conditions, two identified as *Alcaligenes* and one identified as *Pseudomonas*. *Streptococcus* was observed weak anoxic phosphorus accumulation because of its weak denitrification capacity, but it showed good phosphorus accumulation capacity under aerobic conditions. One isolates identified as *Enterobacteriaceae* was proved to be a special species of PAOs, which could only uptake small amounts of phosphorus under anoxic conditions, although its denitrification capacity and aerobic phosphorus accumulation capacity were excellent.

Key words: aerobic phosphorus accumulation; anoxic phosphorus accumulation; continuous-flow two-sludge system; polyphosphate-accumulating organisms (PAOs)

Introduction

Polyphosphate accumulation and denitrification are important biochemical processes in biological nutrient removal (BNR) system. Enhanced biological phosphorus removal (EBPR) process is based on the ability of certain bacteria, such as polyphosphate-accumulating organisms (PAOs), to take up excess orthophosphate and store it as polyphosphate. Then phosphorus is removed with the biomass from the wastewater treatment plant.

In the early studies of EBPR processes, it was assumed that PAOs could only grow and accumulate phosphorus under aerobic conditions. Several early studies mainly on the aerobic heterotrophic bacteria have shown that the removal and release of phosphorus within sludge are the results of the dominance of a single genus of bacteria known as *Acinetobacter* spp. (Buchan, 1980, 1983; Horan, 1991; Van Starkenburg *et al*., 1993). *Acinetobacter* spp. is deemed to have a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor (Juni, 1984).

However, from basic microbiological viewpoint, there is no reason why nitrate or nitrite could not be used as an electron acceptor for phosphorus removal. In fact, several recent publications have reported the occurrence of phosphorus removal with the presence of nitrate in activated sludge system (Vlekke *et al*., 1988; Pokethitiyook *et al*., 1990; Shin *et al*., 1992; Wanner *et al*., 1992; Kerrn-Jespersen and Henze, 1993; Kerrn-Jespersen *et al*., 1994; Kuba *et al*., 1993; Ng *et al*., 2001; Peng *et al*., 2004). It was hypothesized that biological phosphorus removal population comprises at least two groups: one group capable of utilizing either oxygen or nitrate as electron acceptor (denitrifying PAOs), and the other group capable of utilizing only oxygen as electron acceptor (aerobic PAOs) (Gerber *et al*., 1987; Kerrn-Jesperson and Henze, 1993; Meinhold *et al*., 1998).

With the development of phosphorus and nitrogen removal techniques in microbiological field, PAOs have been investigated more and more. *Acinetobacter* spp. are the most common isolates from EBPR process, but the community analyses using molecular techniques do not support their predominance in the EBPR process (Cloete and Steyn, 1987; Wagner *et al*., 1993, 1994). In addition to *Acinetobacter*, bacteria like *Aeromonas*, *Pseudomonas*, *Alcaligenes*, *Comamonas-Pseudomonas* group, *Flavobacterium-Cytophaga* group, *Moraxella*, *Xanthomonas*, *Paracoccus*, *Bacillus*, *Corynebacterium*

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and many other Gram-positive bacteria have been reported to accumulate polyphosphate in activated sludge (Hiraishi and morishima, 1990; Streichan *et al*., 1990; Van Groenestijn, 1988). The most common genera with denitrifying species are *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Paracoccus* and *Bacillus* (Tiedje, 1988). It is validated that both the abilities to accumulate polyphosphate and to denitrify are widespread among bacteria. Using culture-dependent techniques JØrgensen and Paulii (1995) isolated 15 strains of bacteria which can accumulate phosphorus and denitrify at the same time and pointed out that polyphosphate accumulation and denitrification in activated sludge can be carried out by the same organisms. Furthermore, a molecular approach using PCR-DGGE analysis was adopted in to study community structure of their PAOs community (Ahn *et al*., 2002). Marked differences in DGGE profiles of 16S rDNA fragments in biomasses from denitrifying PAOs and aerobic PAOs communities were revealed. The presence of common bands was taken to imply that some populations were capable of both aerobic and anoxic polyphosphate accumulation in EBPR, and two of these bands were recovered and sequenced (Loy *et al*., 2002; Amann and Ludwig, 2000). Consequently, two groups of PAOs are now recognized (Seviour *et al*., 2003).

Recently, to consider the proportion of PAOs capable of anoxic phosphorus accumulation by using nitrate instead of oxygen as electron acceptor, the denitrifying activity of denitrifying PAOs were introduced into the activated sludge model (ASM) by the IAWQ task group (Henze *et al*., 1999). Nevertheless, still less is known about the community structure and identity of denitrifying PAOs. Foregone studies mainly focused on the microbiology of anaerobic/aerobic EBPR systems, and very few studies have been reported on denitrifying EBPR systems.

The aims of this study were, therefore, to isolate and identify PAOs from a lab-scale denitrifying EBPR systems, to investigate the different characteristics of phosphorus accumulation under anoxic and aerobic conditions.

1 Materials and methods

1.1 Isolation and identification of potential PAOs

Acetate mineral media (AMM) was used for the initial enrichment and isolation of potential PAOs (JØrgensen and Paulii, 1995). Samples of activated sludge were obtained from the anoxic zones of one lab-scale continuous-flow two-sludge system situated at Harbin, China (Li *et al*., 2006). Potential PAOs were isolated according to the following procedure: ten milliliters of sludge was mixed with 90 ml of 0.9% NaCl. Then the mixture were homogenized using glass beads for floc break up, and serial dilutions (10^{-1}–10^{-7}) were prepared and plated onto AMM agar using the spread plate technique. The composition of the AMM was as follows (per liter): 3.68 g $CH_3COONa{\cdot}3H_2O$, 28.73 mg $Na_2HPO_4{\cdot}2H_2O$, 57.27 mg NH_4Cl, 131.82 mg $MgSO_4{\cdot}7H_2O$, 26.74 mg K_2SO_4, 17.2 mg $CaCl_2{\cdot}2H_2O$, 12 g HEPES buffer (Sigma, Australia), 15 g agar and 2 ml trace mineral solution. The trace mineral solution was: 50 g EDTA, 5 g $FeSO_4{\cdot}7H_2O$, 1.6 g $CuSO_4{\cdot}5H_2O$, 5 g $MnCl_2{\cdot}4H_2O$, 1.1 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 50 mg H_3BO_3, 10 mg KI and 50 mg $CoCl_2{\cdot}6H_2O$. The pH value was adjusted to 7.0 with 1 mol/L NaOH. The plates were incubated at 30°C. After 3 to 5 d of incubation, well-separated colonies were randomly isolated from the plates, restreaked thrice on the AMM. Subsequent to P uptake screening, positive PAOs were identified to generic level using Gram stains, key differential biochemical tests, cellular and colonial morphological characteristics (Buchanan and Gibbons, 1974).

1.2 Phosphorus uptake assay

Potential PAO isolates were initially screened for denitrification using a colorimetric biochemical reduction test (Drysdale *et al*., 1999). This step identified the denitrifying heterotrophic bacteria from the isolates. Positive denitrifying isolates were then tested for their phosphorus uptake efficiency under anoxic condition as follows (JØrgensen and Paulii, 1995; Lacko *et al*., 2003): the bacteria were pregrown anaerobically under phosphorus limitation (4 mg PO_4-P/L) in liquid acetate mineral media to deplete resident poly-P granules. The composition of the medium was (per liter): 3.23 g $CH_3COONa{\cdot}3H_2O$, 22.98 mg $Na_2HPO_4{\cdot}2H_2O$, 152.76 mg NH_4Cl, 81.12 mg $MgSO_4{\cdot}7H_2O$, 17.83 mg K_2SO_4, 11.00 mg $CaCl_2{\cdot}2H_2O$, 7 g HEPES buffer. The trace mineral solution was added as described in Section 1.1. The pH value was adjusted to 7.0 with 1 mol/L NaOH. The cultures were incubated at 30°C for 24 h on a shaker (80 r/min, Desk type oscillator registration THZ-92B, Shanghai, China). After incubation the biomass was centrifuged (4000 r/min, 20 min, 10°C, Anke TDL-40B, China) and washed with sterile distilled water. Cells were re-centrifuged and then suspended in phosphorus uptake media (JØrgensen and Paulii, 1995) and incubated for a further 20 h. Anoxic conditions were created in the uptake media via the addition of KNO_3 coupled with N_2 sparging prior to inoculation. Samples for analysis were withdrawn during the incubation and filtered through a syringe filtering unit with a 0.2-μm final filter. NO_3-N concentration, P concentration and OD600 were monitored using a UV754 spectrophotometer while uninoculated media were used as controls for the experiments. All the isolates were tested for their phosphorus uptake efficiency under aerobic conditions. The procedure was similar to the anoxic phosphorus uptake test except for creating aerobic conditions by shaking the flasks on a shaker.

2 Results and discussion

2.1 Identification of the isolates

Totally there were eight strains isolated from the acetate mineral media. They were identified as species of *Staphylococcus*, *Streptococcus*, *Alcaligenes*, *Bacillus*, *Pseudomonas* and *Enterobacteriaceae* by using various biochemical tests. Five strains of isolates did show den-

itrification capacities, they were identified as species of *Streptococcus*, *Pseudomonas*, *Alcaligenes* and *Enterobacteriaceae*.

2.2 Anoxic phosphorus uptake assay

It was found that the five strains which showed denitrification activities could accumulate phosphorus in different extent by doing anoxic phosphorus uptake test. Results of anoxic P uptake studies conducted on the monoculture of the five denitrifying isolates are shown in Table 1 and Fig.1. Of five isolates, *Alcaligenes* (PA4) demonstrated the best anoxic phosphorus accumulation with 5.55×10^{-11} mg PO_4-P/cell removal after 8 h and 5.01×10^{-11} mg PO_4-P/cell release 20 h later. *Pseudomonas* (PA3) and *Alcaligenes* (PA2) accumulated 3.98×10^{-11} mg PO_4-P/cell and 3.56×10^{-11} mg PO_4-P/cell after 20 h, respectively, and were also classified as good phosphorus accumulators. The remaining two isolates were regarded as weak anoxic phosphorus accumulators because they accumulated less than 10^{-11} mg PO_4-P/cell after 20 h.

The reasons that the phosphorus accumulation capacities were largely different from these denitrifying bacteria were discussed. It was observed that the high anoxic phosphorus accumulation simultaneously gone with the high NO_3-N reduction, but the ratio of phosphorus uptake per cell was larger than of the NO_3-N reduction. *Streptococcus* (PA1) was regarded as weak phosphorus accumulator, this could possibly be due to the weak denitrification capacity with only 2.22×10^{-12} mg NO_3-N/cell reduction after 20 h. Although the *Enterobacteriaceae* (PA5) demonstrated the best efficient denitrification with 1.37×10^{-10} mg NO_3-N/cell reduction, it can only be capable of accumulating a small amount of phosphorus with 5.69×10^{-12} mg PO_4-P/cell. *Enterobacteriaceae* (PA5) may be denitrifying bacteria with non phosphorus accumulation capacity. At the end of the test anoxic phosphorus release by the *Alcaligenes* (PA4) was also observed, which released as much as 5.01×10^{-11} mg PO_4-P/cell with concurrent reduction of 1.02×10^{-10} mg NO_3-N/cell. It is difficult to explain why phosphorus release occurred simultaneously with NO_3-N reduction, as the presence of NO_3-N is known to inhibit phosphorus release under anaerobic conditions (Kuba *et al*., 1996; Muyima *et al*., 1997). However, since acetate was present in the phosphorus uptake media it was possible that phosphorus release was acetate induced even though NO_3-N was available as an electron acceptor (Lacko *et al*., 2003; Muyima *et al*., 1997). Researchers have shown that phosphorus release is directly dependent on the presence of acetate and not necessarily anaerobic conditions which only stimulate fermentation of substrates to acetate and other volatile fatty acids (Lacko *et al*., 2003; Muyima *et al*., 1997).

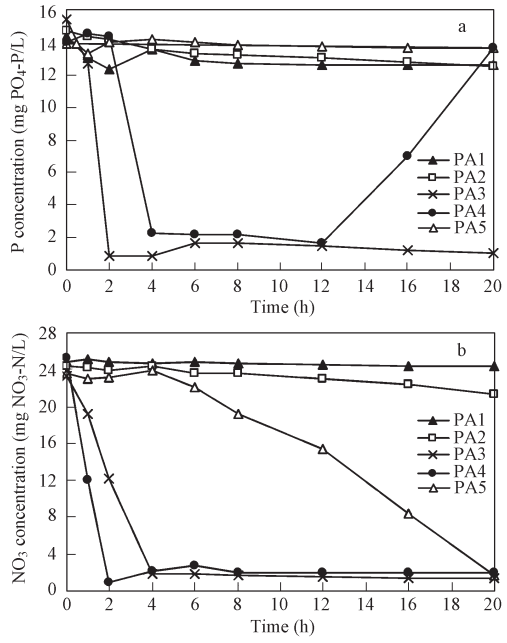

Fig. 1 Phosphorus uptake profiles (a) and NO_3-N reduction profiles (b) by isolates PA1–PA5 when cultivated in phosphorus uptake media under anoxic conditions.

2.3 Aerobic phosphorus uptake assay

Through anoxic phosphorus uptake test, three of five denitrifying bacteria isolates were proved to be capable of good phosphorus accumulation under anoxic conditions. However, the remaining two isolates could not be regarded as weak phosphorus accumulators since these organisms might performance well under aerobic conditions. All the eight isolates were tested for their phosphorus uptake efficiency under aerobic conditions to investigate the different characteristics of the phosphorus accumulation and evaluate the removal capacities under anoxic and aerobic conditions. Results are shown in Table 2 and Fig.2.

Except *Bacillus* (PA7), seven isolates were proved to be good aerobic phosphorus accumulation, while *Enterobacteriaceae* (PA5) demonstrated the most efficient phosphorus accumulation with 5.94×10^{-11} mg PO_4-P/cell removal after 20 h.

Compared with the results of phosphorus accumulation

Table 1 Anoxic phosphorus accumulation capacities shown by the isolated bacteria

Organism	Viable count (cfu/ml)	P uptake[a] (20 h) (mg PO_4-P/L)	P uptake[b] per cell (mg PO_4-P/cell)	NO_3-N reduction[c] (20 h) (mg NO_3-N/L)	NO_3-N reduction[d] per cell (mg NO_3-N/cell)
Streptococcus (PA1)	3.3×10^8	1.74	5.28×10^{-12}	0.73	2.22×10^{-12}
Alcaligenes (PA2)	6.3×10^7	2.24	3.56×10^{-11}	2.94	4.67×10^{-11}
Pseudomonas (PA3)	3.6×10^8	14.34	3.98×10^{-11}	20.69	5.75×10^{-11}
Alcaligenes (PA4)	2.3×10^8	Release 11.52	Release 5.01×10^{-11}	23.41	1.02×10^{-10}
Enterobacteriaceae (PA5)	1.6×10^8	0.91	5.69×10^{-12}	21.91	1.37×10^{-10}

[a] P uptake=(P uninoculated control) – (P sample); [b] P uptake per cell=(P uptake)/(viable count×1000); [c] NO_3-N reduction=(NO_3-N uninoculated control) – (NO_3-N sample); [d] NO_3-N reduction per cell=(NO_3-N reduction)/viable count×1000); control: uninoculated sterilized mixed liquor media.

Table 2 Aerobic phosphorus accumulation capacities by the isolated bacteria

Organism	Viable count (cfu/ml)	P uptake[a] (20 h) (mg PO_4-P/L)	P uptake[b] per cell (mg PO_4-P/cell)
Streptococcus (PA1)	4.2×10^8	4.52	1.08×10^{-11}
Alcaligenes (PA2)	2.2×10^8	6.43	2.92×10^{-11}
Pseudomonas (PA3)	3.5×10^8	Release 1.74	Release 4.97×10^{-12}
Alcaligenes (PA4)	2.5×10^8	Release 1.74	Release 6.96×10^{-12}
Enterobacteriaceae (PA5)	1.5×10^8	8.91	5.94×10^{-11}
Staphylococcus (PA6)	2.7×10^8	6.23	2.31×10^{-11}
Bacillus (PA7)	8.2×10^8	4.41	5.38×10^{-12}
Bacillus (PA8)	3.8×10^8	Release 1.15	Release 3.03×10^{-12}

[a], [b], and control are the same as Table 1.

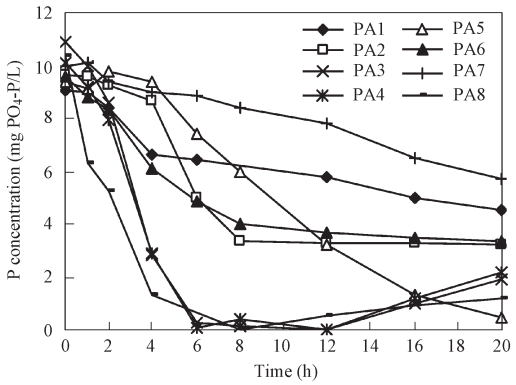

Fig. 2 Phosphorus uptake profiles by isolates PA1–PA8 when cultivated in phosphorus uptake media under aerobic conditions.

tests, it was found that although *Streptococcus* (PA1) and *Enterobacteriaceae* (PA5) were weak phosphorus accumulators under anoxic conditions, they demonstrated excellent phosphorus accumulation capacities under aerobic conditions. *Streptococcus* (PA1) was weak anoxic phosphorus accumulator because of its weak denitrification capacity. *Enterobacteriaceae* (PA5) was hypothesized to be a special species of PAOs that disobey the current theory. Enterobacteriaceae could only uptake small amounts of phosphorus under anoxic conditions, although its denitrifying ability and aerobic phosphorus accumulation ability were excellent. The similar phenomenon had been reported by Lacko *et al*. (2003). This, however, remains uncertain and a more detailed understanding of this species is required. Two strains of *Alcaligenes* (PA2, PA4) and *Pseudomonas* (PA3) demonstrated good phosphorus accumulation both under anoxic and aerobic conditions, while the phosphorus accumulation efficiency under anoxic conditions was higher than that under aerobic conditions. It can be concluded through phosphorus uptake test that phosphorus uptake profiles under anoxic conditions showed similar character and biological metabolism to the aerobic conditions.

3 Conclusions

Using plating technique eight strains of heterotrophic bacteria were isolated and identified. Results showed that two kinds of PAOs were both found in the anoxic zones of a lab-scale continuous-flow two-sludge system. Five strains of the isolates were found to have the abilities of both anoxic phosphorus accumulation (utilizing nitrate as electron acceptor) and aerobic phosphorus accumulation (utilizing oxygen as electron acceptor) at the same time to some extent. Because of the weak denitrification capacity, some isolated organisms were observed weak anoxic phosphorus accumulation, though their aerobic phosphorus accumulation capacities were excellent. A strain identified as *Enterobacteriaceae* was proved to be a special species of PAOs, which could only uptake small amounts of phosphorus under anoxic conditions, although its denitrification capacity and aerobic phosphorus accumulation capacity were excellent.

References

Ahn J A, Daidou T, Tsuneda S *et al*., 2002. Characterization of denitrifying phosphate-accumulating organisms cultivated under different electron acceptor conditions using polymerase chain reaction-denaturing gradient gel electrophoresis assay[J]. Water Res, 36: 403–412.

Amann R, Ludwig W, 2000. Ribosomal RNA-targeted nucleicacid probes for studies in microbial ecology[J]. FEMS Microbiol Rev, 24: 555–565.

Buchan L, 1980. The location and nature of accumulated phosphorous in activated sludge[J]. D. Sc. Thesis, University of Pretoria, South Africa.

Buchan L, 1983. Possible biological mechanism of phosphorus removal[J]. Water Sci Tech, 15: 87–103.

Buchanan R E, Gibbons N E, 1974. Berge's manual of determinative bacteriology[M]. 8th ed. Baltimore: The Williams & Wilkins Company.

Cloete T E, Steyn P L, 1987. A combined fluorescent antibody-membrane filter technique for enumerating *Acinetobacter* in activated sludge[M]. In: Advances in water pollution control, biological phosphate removal from wastewaters (Ramadori R., ed.). Oxford: Pergamon Press. 335–338.

Drysdale G D, BUX F, Kasan H C, 1999. Denitrification by heterotrophic bacteria during activated sludge treatment[J]. Wat SA, 25(3): 357–362.

Gerber A, de Villiers R H, Mostert E S *et al*., 1987. The phenomenon of simultaneous phosphate uptake and release, and its importance in biological nutrient removal[M]. In: Biological phosphate removal from wastewaters (Ramadori R., ed.). Oxford: Pergamon Press.

Henze M, Gujer W, Mino T *et al*., 1999. Activated sludge model no. 2d, ASM2D[J]. Water Sci Tech, 39(1): 165–182.

Hiraishi A, Morishima Y, 1990. Capacity for polyphosphate accumulation of predominant bacteria in activated sludge showing enhanced phosphate removal[J]. J Ferment Bioeng, 69: 368–371.

Horan N J, 1991. Biological waste water treatment systems:

Theory and operation[M]. England: John Wiley & Sons Ltd. 223–230.

JØrgensen K, Paulii A, 1995. Polyphosphate accumulation among denitrifying bacteria in activated sludge[J]. Anaerobe, 1: 161–168.

Juni E, 1984. Genus III: Acinetobacter[M]. In: Bergey's manual of systematic bacteriology (Kreig N. R., Holt J. G., ed.). Baltimore: The Williams and Wilkins Co. 1: 303–307.

Kerrn-Jespersen J P, Henze M, 1993. Biological phosphorus uptake under anoxic and aerobic conditions[J]. Water Res, 27(4): 617–624.

Kerrn-Jespersen J P, Henze M, Strube R, 1994. Biological phosphorus release and uptake under alternating anaerobic and anoxic conditions in a fixed-film reactor[J]. Water Res, 28: 1253–1255.

Kuba T, Smolders G J F, van Loosdrecht M C M *et al*., 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor[J]. Water Sci Tech, 27: 241–252.

Kuba T, van Loosdrecht M C M, Heijnen J J, 1996. Effect of cyclic oxygen esposure on activity of denitrifying phosphorus removing bacteria[J]. Water Sci Tech, 34(1/2): 33–40.

Lacko N, Drysdale G D, Bux F, 2003. Anoxic phosphorus removal by denitrifying heterotrophic bacteria[J]. Water Sci Tech, 47(11): 17–22.

Li X K, Huang R X, Bao L L *et al*., 2006. Simultaneous phosphorus and nitrogen removal in a continuous-flow two-sludge system[J]. Journal of Environmental Sciences, 18(1): 52–57.

Loy A, Daims H, Wagner, 2002. Activated sludge-molecular techniques for determining community composition[M]. In: Encyclopaedia of environmental microbiology (Bitton G., ed.). New York: Wiley Scientific. 26–43.

Meinhold J, Pedersen H, Arnold E *et al*., 1998. Effect of continuous addition of an organic substrate to the anoxic phase on biological phosphorus removal[J]. Water Sci Tech, 38(1): 97–105.

Muyima N Y O, Momba M N B, Colete T E, 1997. Biological methods for the treatment of wastewaters[R]. In: Microbial community analysis: The key to the design of biological wastewater treatment systems (Cloete T. E., Muyima N. Y. O., ed.). IAWQ Scientific and Technical Report, 5: 1–24.

Ng W J, Ong S L, Hu J Y, 2001. Denitrifying phosphorus removal by anaerobic/anoxic sequencing batch reactor[J]. Water Sci Tech, 43(3): 139–146.

Peng Y Z, Wang Y Y, Ozaki M *et al*., 2004. Denitrifying phosphorus removal in a continuous-flow A2N two-sludge process[J]. Journal of Environmental Science and Health Part A–Toxic/Hazardous Substance & Environmental Engineering, 39 (3): 703–715.

Pokethitiyook P, McClintock S A, Randall C W, 1990. The role of nitrate in biological phosphorus removal[C]. Environmental Engineering, Proceedings of the 1990 Speciality Conference, Arlington, Virginia, July 8–11. 330–336.

Seviour J R, Mino T, Onuki M, 2003. The microbiology of biological phosphorus removal in activated sludge systems[J]. FEMS Microbiology Reviews, 27: 99–127.

Shin H S, Jun H B, Park H S, 1992. Simultaneous removal of phosphorus and nitrogen in sequencing batch reactor[J]. Biodegradation, 3: 105–111.

Streichan M, Golecki J R, Schön G, 1990. Polyphosphate-accumulating bacteria from sewage plants with different processes for biological phosphorus[J]. FEMS Microbiol Ecol, 73: 113–124.

Tiedje M J, 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium[M]. In: Biology of anaerobic microorganisms (Zehnder A. J. B., ed.). New York: John Wiley and Sons, Inc. 179–243.

Van Groenestijn J W, 1988. Accumulation and degradation of polyphosphate in *Acinetobacter* sp.[D]. Ph.D Thesis. Wageningen, University of Wageningen.

Van Starkenburg W, Rensink J H, Rijs G B J, 1993. Biological P-removal: State of the art in The Netherlands[J]. Water Sci Tech, 27(5/6): 317–328.

Vlekke G J F M, Comeau Y, Oldham W K, 1988. Biological phosphate removal from wastewater with oxygen or nitrate in sequencing batch reactors[J]. Environ Technol Lett, 9: 791–796.

Wanner J, Cech J S, Kos M, 1992. New process design for biological nutrient removal[J]. Water Sci Tech, 25(4/5): 445–448.

Wagner M, Amann R, Lemmer H *et al*., 1993. Probing activated sludge with oligonucleotides specific for *Proteobacteria*: inadequacy of culture-dependent methods for describing microbial community structure[J]. Appl Environ Microbiol, 59: 1520–1525.

Wagner M, Erhart R, Manz W *et al*., 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus *Acinetobacter* and its application for *in situ* monitoring in activated sludge[J]. Appl Environ Microbiol, 60: 792–800.