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Evaluation of the sludge reduction effectiveness of a metabolic uncoupler-tetrakis (hydroxymethyl) phosphonium sulfate in anaerobic/anoxic/oxic process

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ABSTRACT

A metabolic uncoupler, tetrakis (hydroxymethyl) phosphonium sulfate (THPS), was used to reduce sludge production in a laboratory-scale anaerobic/anoxic/oxic (A^2/O) process. THPS was continuously added into anaerobic, anoxic, and oxic tank of the systems, respectively, along with 3.5 mg/L influent. The effects of THPS additions on sludge reduction, wastewater treatment efficiencies, and sludge characteristics were discussed in about a 99 d operational period. The results showed that the cumulative reduction of waste activated sludge reached about 30.8–31.7% and the decrease of sludge yield (Y_{obs}) reached about 16.1–16.5% with the system added THPS in the anaerobic tank the most. Meanwhile, the wastewater treatment efficiency decreased slightly when THPS was added. Analysis of microbial communities in activated sludge in the oxic tank suggested that adding THPS would result in a new dominant micro-organism. However, the efficiencies of sludge reduction and wastewater treatment and microbial communities of activated sludge were similar when THPS was added into different tanks of the A^2/O process, so the adding site of the metabolic uncoupler had little effect.

Keywords: Adding site; Anaerobic-anoxic-oxic process; Metabolic uncoupler; Sludge reduction; THPS

1. Introduction

Excess sludge is a by-product of biological wastewater treatment. Increases in urban populations across the world, coupled with concomitant growth in wastewater treatment plants (WWTPs), have resulted in huge production of excess sludge. In the United States, it was estimated that 7.1 million tons of sludge was produced for use or disposal in 2000, and this figure grew to 7.6 million tons in 2005 [1]. The European Union produced 10 million tons of dry sludge in 2005 [2], while China produced 1 million tons of dry sludge in the same year [3]. Because it accounts for 25–60% of the total cost of a typical wastewater treatment plant, the treatment and disposal of excess sludge has become a serious issue for many WWTPs [4].

Up to now, sludge reduction methods mainly fall into two categories [5–7]: (1) sludge reduction through post treatment and (2) source sludge reduction (namely *in situ* sludge reduction during the wastewater treatment process). Of these two, the source sludge

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reduction process is more effective. The main advantage of this method is that it can minimize the sludge yield from the source of the wastewater treatment process itself. The source sludge reduction methods include lysis-cryptic growth, uncoupling metabolism, maintenance metabolism, predation on bacteria, and so on. Compared with other methods, the uncoupling metabolism is sometimes preferred because excess sludge can be effectively reduced with little modification to the existing wastewater treatment process. With the advantages of being convenient, highly efficient, and easy to operate, the application of metabolic uncouplers to reduce the excess sludge yield is already of great interest to many researchers. Some metabolic uncouplers, including 2,4-dichlorophenol [8], 3,3′,4′,5-tetrachlorosalicylanilide (TCS) [9,10], 2,4-dinitrophenol (dNP) [11], gramicidin [12], 2,4,6-trichlorophenol (TCP) [13], and tetrakis (hydroxymethyl) phosphonium sulfate (THPS) [14,15], have been used to reduce excess sludge production in wastewater biological treatment. THPS, a type of environmentally friendly biocide, can be used as a metabolic uncoupler to reduce sludge production in biological wastewater treatment [14,15].

Although it is the biological wastewater treatment system used in most studies about sludge reduction with metabolic uncouplers, the conventional activated sludge system is generally not used in isolation in WWTPs [16,17]. Nitrogen (N) and phosphorus (P) concentrations in municipal wastewater have increased significantly along with social and economic development. Meanwhile, excessive N and P in wastewater may lead to serious environmental problems, such as eutrophication, when discharged into receiving waters [18]. Because the A^2/O process can simultaneously remove N and P from wastewater, it has become the most common process used in WWTPs [18]. However, to date, few studies have examined sludge reduction by metabolic uncouplers in the A^2/O process. Additionally, most metabolic uncouplers were added into the oxic tank and few were added into anaerobic/anoxic tank and the effects of metabolic uncouplers in the latter two tanks were not clear.

The objective of this study was therefore to investigate the effect of THPS on sludge reduction and wastewater treatment when added into different A^2/O sites.

2. Materials and methods

2.1. A^2/O systems

The tests were carried out in four laboratory-scale A^2/O systems and Fig. 1 is the schematic diagram and photograph of the experimental setups. The volumes for each part of the A^2/O systems are summarized in Table 1. The anaerobic tank and anoxic tank were mixed with mechanical stirring and the oxic tank was aerated to maintain dissolved oxygen (DO) levels (1.5–3.0) (Table 1). The design of system was according to the standard of China [19].

2.2. Wastewater and its treatment

Diluted sewage from a residential area in Beijing, China was used in this study. The characteristics of the influent to the A^2/O systems are summarized in Table 2.

Four laboratory-scale A^2/O systems were used in the test: THPS was added into (1) the anaerobic tank of one system (Ana-THPS), (2) the anoxic tank of one system (Ano-THPS), and (3) the oxic tank of one system (Oxi-THPS). No THPS was added in the last system (control). The hydraulic retention time (HRT) and DO of the four systems are also summarized in Table 1. The HRTs for three tanks were designed according to the design standard of China [19] and previous literatures [18,20]. According the design standard of China [19], the internal recycling ratio (mixing liquor recycling) and external recycling ratio (sludge recycling) for the four systems were 200 and 100%, respectively. THPS was continuously added into the corresponding tank of the first three systems at a concentration of 3.5 mg THPS/L influent. The sludge concentrations in the oxic tanks were controlled at 2–4 g/L by regularly discharging sludge from the oxic tanks. The test was carried out at room temperature (20–28℃).

2.3. Analysis

The influent, effluent, and the mixed sludge in the oxic tanks were periodically sampled. Influent and effluent samples were analyzed for a range of water quality parameters including chemical oxygen demand (COD), pH, suspended solid (SS), total nitrogen (TN), and total phosphorus (TP). The sludge concentration (mixed liquor suspended solids (MLSS)) and settleability (sludge volume index (SVI)) of the mixed sludge were determined. Sludge reduction, production of waste activated sludge (WAS), sludge yield (Y_{obs}), and removals for various water parameters were calculated using the same methods as previous studies [7,15,]. COD was analyzed by a COD meter (HACH DR2800, America). The three-dimensional fluorescence spectrums of soluble organic matter in the effluents were analyzed by a fluorescence spectrum analyzer (Cary Eclipse, Varian, Palo Alto, CA, USA). The remaining parameters were analyzed by standard methods [21].

Table 1
Parameters of the A^2/O systems

Item	Anaerobic tank	Anoxic tank	Oxic tank	Settlement tank
Working volume (L)	4	4	16	4
HRT (h)	2	2	8	2
DO (mg/L)	<0.1	0.1–0.2	1.5–3.0	–

Table 2
Characteristics of influent added to the A^2/O systems

Index	Range	Average
COD (mg/L)	64–452	172
BOD_5 (mg/L)	29–204	77
pH	7.4–8.4	7.8
SS (mg/L)	16–551	195
NH_4^+-N (mg/L)	24.4–59.5	46.1
TN (mg/L)	37.6–92.4	56.9
TP (mg/L)	3.0–9.5	5.9

2.4. *DNA extraction, polymerase chain reaction, and denaturing gradient gel electrophoresis*

The activated sludge in the oxic tank of each of the four systems was sampled at 60 d and the microbial populations of these samples were analyzed using polymerase chain reaction–denaturing gradient gel electrophoresis (PCR-DGGE). The total DNA of sludge samples was extracted by a nucleic acid automatic extraction system (TANBead Smart LabAssist-16, Taiwan). The extracted DNA was then used as a template for PCR amplification.

The eubacterial universal primers, 357F (5′-CCTACGGGAGGCAGCAG-3′) and 517R (5′-ATTA-CCGCGGCTGCTGG-3′), were used to amplify a segment of the eubacterial 16S rDNA [22]. A GC-clamp was added to the forward primers to facilitate DGGE. A PTC-200 thermal cycler (BioRad Laboratories, Hercules, CA, USA) was used for PCR amplification at a final volume of 50 μL. The reaction mixture contained 1 μL of both primers (10 μM), 4 μL of each dNTP, 5 μL of 10× buffer, 50 ng DNA template, and 1.5 units of Taq DNA polymerase. The PCR conditions for eubacteria were as follows: 94˚C for 5 min, followed by 30 1 min cycles at 94˚C, 48˚C for 1 min, 72˚C for 1 min, and a final 10 min extension at 72˚C. A 5 μL aliquot of the PCR product was separated on a 0.8% (w/v) agarose gel at 100 V for 30 min to verify amplification prior to DGGE.

The DGGE of the PCR product was determined using a D-Code system (Bio-Rad Laboratories), and 40–70% denaturing gradients were used to separate the amplified 16S rDNA. The gel was electrophoresed in 1× TAE buffer at 70 V and 60˚C for 10 h. The resulting gel was then visualized using Gel Red (Biotium, Hayward, CA, USA). Analysis of the DGGE gel was conducted using the Bio-Rad Software, Quantity One™.

3. Results and discussion

3.1. *Effect of THPS addition on sludge reduction*

The four systems ran in stable conditions for about 99 d. The cumulative WAS and Y_{obs} in the stable stage are summarized in Fig. 2. At the end of the test, the cumulative WAS of the four systems reached 533.1 (control system), 364.1 (Ana-THPS system), 365.1 (Ano-THPS system), and 368.7 g (Oxi-THPS system) (Fig. 1(A)). The Y_{obs} of the four systems was calculated (Fig. 1(B)) and they are 0.330–0.468 mg MLSS/mg $COD_{removal}$ (mean = 0.363 mg MLSS/mg $COD_{removal}$, Control system), 0.260–0.465 mg MLSS/mg $COD_{removal}$ (mean = 0.303 mg MLSS/mg $COD_{removal}$, Ana-THPS system), 0.262–0.466 mg MLSS/mg $COD_{removal}$ (mean = 0.304 mg MLSS/mg $COD_{removal}$, Ano-THPS system), and 0.261–0.467 mg MLSS/mg $COD_{removal}$ (mean = 0.305 mg MLSS/mg $COD_{removal}$, Ano-THPS system), respectively. The values of Y_{obs} of this study were at normal levels [23] and similar to our previous study [15] but higher than some previous studies. For example, Banu et al. and Raj got 0.12 g MLSS/gCOD and 0.22 g MLSS/gCOD in their studies, respectively [24,25].

The cumulative WAS reductions of the systems (comparing the control) were 31.7% (Ana-THPS system), 31.5% (Ano-THPS system), and 30.8% (Oxi-THPS system). Similarly, we were able to calculate the Y_{obs} decrease of the latter three systems following the previous methods [7,15] and they were about 16.5% (Ana-THPS system), 16.3% (Ano-THPS system), and 16.1% (Oxi-THPS system), respectively. The results showed that the cumulative WAS reductions and sludge yield decrease for three THPS added systems were similar, which suggests that the addition

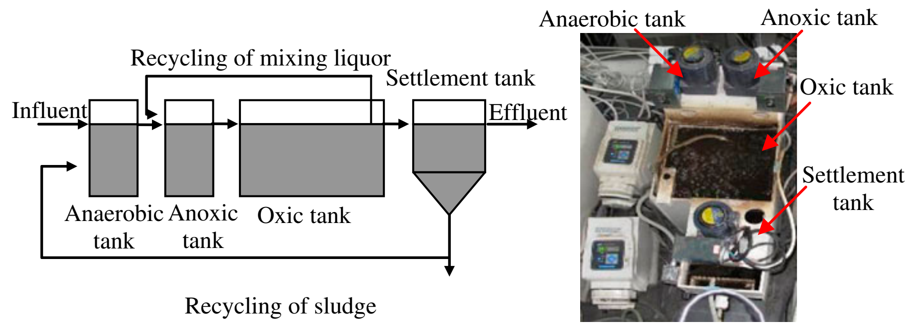

Fig. 1. The schematic diagram and photograph of the experimental setups.

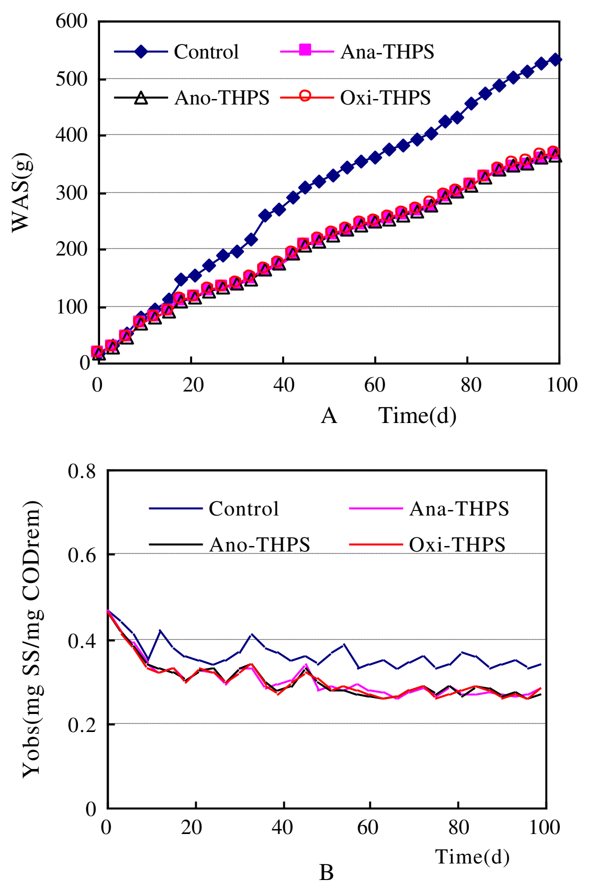

Fig. 2. Cumulative WAS and sludge yield (Y_{obs}) in the test.

site in A^2/O process had little effect on the sludge reduction effectiveness of THPS. In fact, the sludge reduction due to THPS in this study was comparable to that reported in previous studies with chemical couplers (Table 3) and with other sludge reduction methods (like recycling pretreated sludge) [26,27]. For example, Chen et al. [29], when using TCS as an uncoupler in activated sludge culture, only reported a 27.8% reduction in sludge, Wang et al. [31] used TCP in A^2/O and achieved a reduction of 67.1%, while Do et al. [26] and Uan et al. [27] got 33 and 52% of sludge reduction by recycling thermochemically pretreated sludge, respectively. The sludge reduction obtained in this study, however, is higher than that has been reported in previous studies also used THPS [14,15]. Differences in the sludge reduction may be due to the different uncoupler type, uncoupler quantity, scale, wastewater treatment system and sludge reduction technology, and so on. Additionally, although THPS was added into anaerobic or anoxic tank of system, it flowed rapidly into the oxic tank, which caused the differences between different THPS adding sites were little.

3.2. Effects of THPS addition on wastewater treatment

Ideally, the addition of a metabolic uncoupler should have little effect on the efficiency of the wastewater treatment system [34–36]. Therefore, we examined the effects of THPS additions on wastewater treatment in the different tanks. The characteristics of the effluents from the four systems are summarized in Table 4. The removals of COD, TN, and TP were calculated following the methods in our previous study [15].

The removals of COD for the four systems are summarized in Fig. 3. It could found that the removals of COD were 76.7–97.6% (mean = 87.5%) in the control system, 67.4–94.8% (mean = 79.9%) in the Ana-THPS system, 70.4–93.4% (mean = 80.5%) in the Ano-THPS system, and 70.8–95.1% (mean = 80.60%) in the Oxi-THPS system, respectively. These results suggest that THPS additions would lead to slight decreases in COD removal (about 6.9–7.6%), and that there was little difference between the three THPS addition systems. The lower COD removals as a result of THPS additions were comparable to results of previous studies (Table 3). Three-dimensional

Table 3
Sludge reduction, COD removal, and SVI change reported in other studies

Uncoupler	System	Scale (L)	Sludge reduction (%)	COD removal (%)	SVI change (%)	Ref.
TCS	CAS[a]	17.3	30.0	−[c]2.8	+[e]15.0	[28]
TCS	CAS	25	27.8	−0.1	–	[29]
TCP	SBR[b]	2	47.0	−8.0	+20.0	[13]
TCP	SBR	10	49.8	−7.0	–	[30]
TCP	A^2/O	60	67.1	−26.8	+9.3	[31]
DNP	SBR	7	21.0	*[d]	−5.0	[11]
pNP	SBR	20	57.0	−22.5	−48.0	[32]
Malonic acid	SBR	2	53.8	−9.8	–	[33]
THPS	A^2/O	5,000	22.5	−6.8	+31.0	[15]
THPS	SBR	35	30.0	−13.0	*	[14]
THPS	A^2/O	28	30.8–31.7	−(6.9–7.6)	+(12.4–13.0)	This study

Note: [a]CAS: conventional activated sludge; [b]SBR: sequencing batch reactor; [c]−: decrease; [d]+: increase; [e]*: not mentioned.

Table 4
Characteristics of effluent discharged from the four systems

	Ana-THPS system		Ano-THPS system		Oxi-THPS system		Control system	
Index	Range	Average	Range	Average	Range	Average	Range	Average
COD (mg/L)	6–58	31	7–59	30	7–58	30	5–39	19
pH	7.1–7.5	7.3	7.1–7.6	7.3	7.2–7.6	7.4	7.0–7.5	7.3
SS (mg/L)	10–54	19	9–65	19	9–47	19	9–49	17
NH_4^+-N (mg/L)	0.4–8.6	3.0	0.4–8.4	2.3	0.3–7.6	2.0	0.4–4.3	2.0
TN (mg/L)	7.4–45.3	24.0	6.9–42.7	23.5	6.2–40.0	23.0	4.1–33.5	18.0
TP (mg/L)	1.8–3.9	3.0	1.7–3.8	3.0	1.6–3.9	3.0	0.7–3.6	2.0

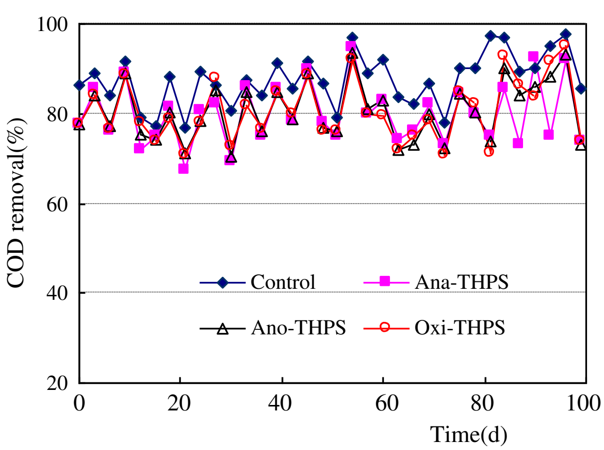

Fig. 3. COD removals in the test.

fluorescence spectroscopy analysis of the effluents (sample time: 60 d) showed that dissolved organic matter in the effluents were similar for the four systems, and that there was only one fluorescence peak ($\lambda_{Ex}/\lambda_{Em}$ = 220–230 nm/320–350 nm) (Fig. 4), which was the peak of fulvic acid [37]. This result suggests that the main fluorescent organic components of effluent were not affected by either THPS additions or where it was added into the system, further suggesting that THPS additions have little effect on organic matter degradation.

The removals of TN for the four systems were 41.2–92.9% (mean = 68.0%) for the control system, 29.0–86.9% (mean = 58.5%) for the Ana-THPS system, 32.6–85.4% (mean = 59.6%) for the Ano-THPS system, and 33.6–89.3% (mean = 59.2%) for the Oxi-THPS system, respectively (Fig. 5(A)). Results indicate that TN removals were very obviously lower (about 8.4–9.4%), when THPS was added. However, THPS additions in different sites of the A^2/O process had similar effects on TN removals. The removals of TN were different to our previous study, which found that THPS additions led to increases in TN removal (about 5%). The difference in the results may be due to the different scales of the two studies and the different THPS adding site, as the other parameters of the two studies were similar. Additionally, the removals of TN

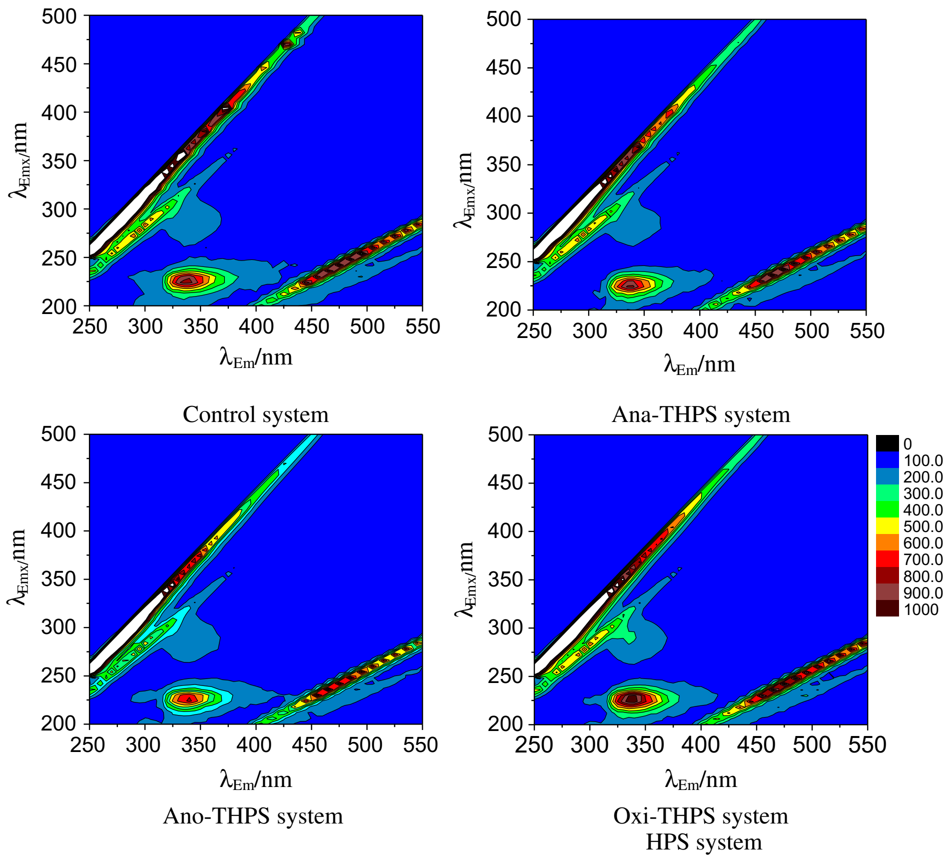

Fig. 4. Fluorescence excitation emission matrices for dissolved organic matter in the effluents from the four systems.

in this study were similar to the studies about sludge reduction. For example, the TN removals were 58.4–65.1% in the study of Do et al. [38].

The removals of TP in the four systems were also studied in the test (Fig. 5(B)), which ranged from 41.9 to 78.5% (mean = 59.7%) in the control system, from 33.8 to 73.5% (mean = 53.7%) in the Ana-THPS system, from 34.0 to 72.2% (mean = 52.9%) in the Ano-THPS system, and from 32.5 to 69.8% (mean = 51.6%) in the Oxi-THPS system. In agreement with other studies [14,30], THPS additions resulted in slightly reduced TP removal efficiency (about 6.0–8.1%). However, there was little difference in TP removal efficiency when THPS was added into different tank in the A^2/O process. Unlike TN, the removals of TP were lower than previous studies about sludge reduction. For example, the TP removal gotten reached 80–83% and 74–84% by Do et al. [26] and Banu et al. [39] in their studies, respectively. Because the TP of effluents (about 1.3–1.8 mg/L at the end of test) were higher than the discharge standard for pollutants in municipal WWTPs in China (GB18918-2002) [40], further treatment of the effluents or adjustment of the process (like adding ferrous sulfate [38] or alum [41]) were needed.

3.3. Effect of THPS addition on sludge characteristics

We examined the effect of THPS on the settleability of activated sludge (SVI) in the oxic tanks. The SVI of the sludge increased because of THPS additions: after THPS addition, SVI values ranged from 84.3 to 115.1 mL/g MLSS (mean = 98.7 mL/g MLSS) in the Ana-THPS system, from 83.0 to 117.2 mL/g MLSS (mean = 99.0 mL/g MLSS) in the Ano-THPS system, and from 76.6 to 118.9 mL/g MLSS (mean = 98.5 mL/g MLSS) in the Oxi-THPS system, respectively (date were not shown). The SVI of sludge in the control system ranged from 72 to 106.2 mL/g SS (mean = 87.6 mL/g MLSS). The SVI of activated sludge for the four systems ranged from 72 to 118.9 mL/g MLSS which suggests that the settling ability of the sludge was good [42] and the changes in the SVI of activated sludge in the test were comparable to those in previous studies (Table 3). Additionally, the addition of THPS would increase the SVI of activated sludge and

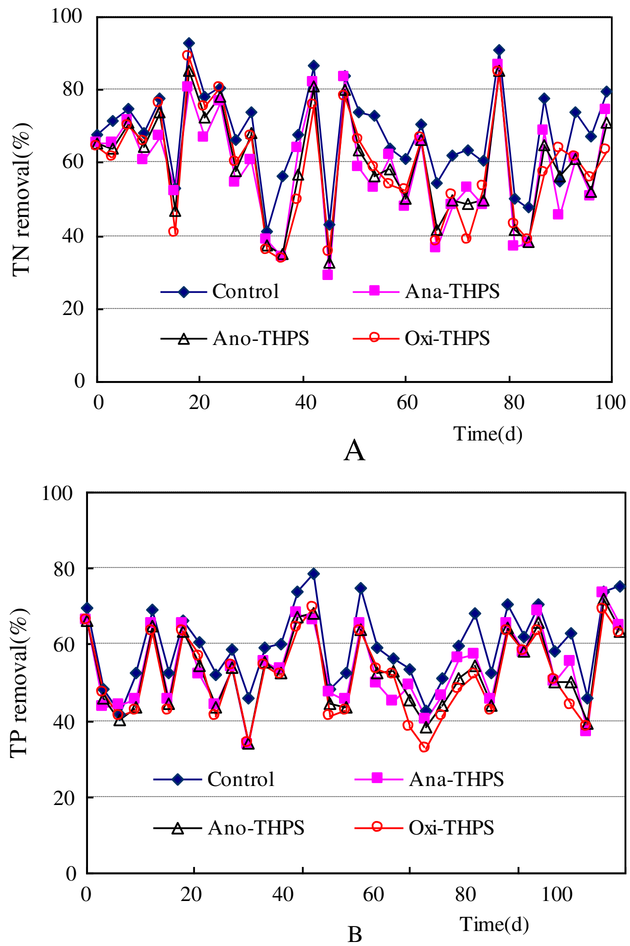

Fig. 5. TN and TP removals in the test.

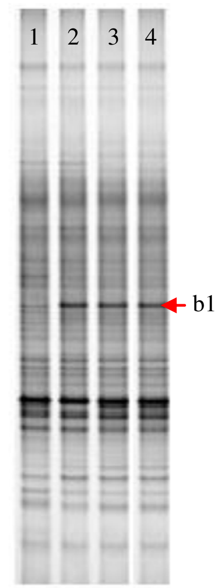

Fig. 6. DGGE profiles for sludge samples in the four systems.

the increases of mean SVI were 12.7 (Ana-THPS system), 13.0 (Ano-THPS system), and 12.4% (Oxi-THPS system). However, the THPS adding site did not impact the effect of THPS on the SVI of activated sludge. Our previous studies [15] also find that the addition of THPS increased the SVI of activated sludge. The increase of SVI for activated sludge may be due THPS would change the EPS content and compositions of activated sludge which affect the settling ability of activated sludge.

The microbial populations of activated sludge from different systems were analyzed by PCR-DGGE (Fig. 6). The DGGE profile indicates that samples 2, 3, and 4 were similar, while sample 1 was different. DGGE band 1 was not only detected in sample 1. In addition, the other dominant bands of all the samples were very similar. Results suggest that additions of THPS resulted in changes in the microbial populations in activated sludge, but that there was little difference due to the THPS adding site. In order to further understand the effects of THPS adding sites, further studies about band 1 and sequence analysis would need.

4. Conclusion

THPS, a metabolic uncoupler, was added to a laboratory-scale A^2/O process at different sites to study its effects on sludge reduction. Experimental results show that the cumulative reductions of WAS reached 30.8–31.7% by the end of the test when THPS was added at a concentration of 3.5 mg THPS/L influent. Meanwhile, the addition of THPS resulted in about 16.1–16.5% of Y_{obs} decrease. The THPS adding sites (anaerobic tank, anoxic tank and oxic tank) slightly affected the sludge reduction effectiveness of THPS. At the same time, THPS additions may have a slight effect on the effectiveness of the wastewater treatment process, as COD, NH_4^+-N TN and TP removals decreased slightly after THPS addition. However, differences due to the different THPS adding sites were minimal. Analysis of microbial communities in activated sludge in the oxic tank suggested that THPS additions would result in a new dominant DGGE band, but that there was little effect on microbial communities due to the different adding sites.

Acknowledgments

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