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Effects of tetrakis (hydroxymethyl) phosphonium sulfate pretreatment on characteristics of sewage sludge

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ABSTRACT

Recently, tetrakis(hydroxymethyl)phosphonium sulfate (THPS) was found to play an important role in the sludge pretreatment process. However, the effects of THPS pretreatment on the characteristics of sewage sludge are still insufficiently understood. The properties of sludge after pretreatment with different concentrations of THPS were investigated in this study. The results showed that pH, dewatering ability, and particle size of sludge decreased with increase in THPS concentration. The volatile suspended solids (VSS) and total suspended solids (TSS) of sludge also decreased slightly with increase in THPS concentration. The specific oxygen uptake rate (SOUR) results suggested that lower THPS concentrations (≤1.87 mg/g VSS) enhanced the activity of sludge, but higher concentrations (≥1.87 mg/g VSS) inhibited it. Gram-negative bacteria with peritrichous flagellation (such as *Pseudomonas*, *Escherichia*, and *Faecalibacterium*) were extremely sensitive to THPS. The decrease in specific most probable numbers (MPNs) of pathogens (total coliforms and *Escherichia coli*) with the increase in THPS concentration also proved the sterilization ability of THPS in the sludge pretreatment process. Pretreatment of sludge with concentrations of THPS higher than 37.41 mg/g VSS would meet the pathogen requirements for land application of Class A biosolids.

Introduction

Sludge is an important by-product of biological wastewater treatment. In a conventional wastewater treatment plant, the volume of sludge produced is about 1.00% (dewatered sludge is 0.50%) of the volume of influent wastewater to be treated (Foladori et al., 2010). According to Paul and Debellefontaine (2007), more than 1.00×10^7 tons of sewage sludge are produced each year in the 27 European Union member states (Paul and Debellefontaine, 2007), and over 3.00×10^7 tons of dewatered sludge (with 80.00% water content) are produced each year in China (China State EPA, 2014). With stricter environmental regulations and increasing quantities of wastewater, the production of sewage sludge is expected to

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continuously increase (Guo et al., 2013). The sludge needs to be further treated before disposal; otherwise, it may cause serious environmental impacts (Guo et al., 2014; Li et al., 2015).

Many techniques are used for the treatment and disposition of sludge, including physical, chemical, and biological treatment (Andreoli et al., 2007; Piterina et al., 2010; Lowman et al., 2013; Xu et al., 2014a; Yang et al., 2016, 2017; Grobelak et al., 2017). In recent years, more stringent environmental laws have been legislated in many developed and developing nations. Increasing environmental concerns led to biological methods of sludge treatment (including composting, aerobic, and anaerobic digestion), which were widely accepted for the following reasons: (i) achieving sludge reduction targets; (ii) transforming sludge into bio-fertilizer; and (iii) removing pathogens and toxic compounds (Anjum et al., 2016). However, due to the structure and composition of sludge (e.g., extracellular polymeric substances (EPS), cell wall, and inhibitory compounds), the biological treatment of sludge has also encountered a series of obstacles (Ruffino et al., 2015; Serrano et al., 2015).

To overcome these obstacles, sludge pretreatment methods have been developed, including alkaline, acid, thermal, ozonation, ultrasound, and microwave treatments (Chang et al., 2011; Choi and Ahn, 2014; Veluchamy and Kalamdhad, 2017; Zhen et al., 2017). However, these pretreatment methods usually require large chemical inputs, frequent power input, and have potential ecological risks (Wu et al., 2017a). Additionally, as a “biological cocktail” containing a mixture of different organisms, sewage sludge contains a large number of saprophytes and pathogens. The pathogens are potential biohazards and can cause significant biological contamination if they are released to the environment without regulation. Therefore, when the aim is to use sludge for application to land, preventing the spread of these pathogens in the environment and elimination of pathogens is required. Recent studies found that it is possible to use biocides to pretreat sewage sludge because the tolerances of different microorganisms to biocides are different (Morente et al., 2013; Anjum et al., 2016), especially to tetrakis(hydroxymethyl)phosphonium sulfate (THPS) (Wu et al., 2017b).

THPS (CAS No. 55566–30-8) is an environmentally friendly biocide. Its discoverer was awarded the Designing Greener Chemicals Award in 1997 by the United States Environmental Protection Agency (USEPA). In recent decades, THPS has been applied in processes concerned with reducing sludge production in wastewater treatment plants (Guo et al., 2014; Xiao et al., 2016; Li et al., 2016; Zuriaga-Agustí et al., 2016; Han et al., 2017) and improving the biodegradability of sludge (Wu et al., 2017b). Studies have also found that THPS can affect the bacterial population of activated sludge (Guo et al., 2014; Xiao et al., 2016). As a fully water soluble, non-oxidizing, and antimicrobial biocide, THPS targets a wide range of bacteria (Enning et al., 2015; Okoro, 2015; Okoro et al., 2016). Zhao et al. (2009) found that the bactericidal ability of THPS is high at concentrations above 15 mg/L. Based on these studies, THPS could be used to eliminate pathogens and sanitize sewage sludge before sludge application to land. However, very little research has been conducted on the effects of THPS on sludge characteristics.

Thus, the present research focused on the characteristics of sludge after adding THPS. The objectives of this research were as follows: (i) to analyze the change in physicochemical properties of sludge after adding THPS; (ii) to apply the Illumina MiSeq high-throughput sequencing technique to identify the change in bacterial population after adding THPS; and (iii) to clarify the effects of different THPS concentrations on sludge pathogens.

1. Materials and methods

1.1. *Sewage sludge*

The sewage sludge used in this study was obtained from the aeration tank of a municipal wastewater treatment plant in Beijing, China, which uses an activated sludge process and handles 4.00×10^5 tons of wastewater daily. First, the sludge samples were filtered using a 40-mesh sieve to remove larger particles. Then, samples were washed three times with deionized water (Xiao et al., 2018). The sludge samples were concentrated and then stored at 4 °C before testing. The characteristics of the sewage sludge are summarized as: pH 7.41; volatile suspended solids (VSS) 4.62 g/L; total suspended solids (TSS) 6.51 g/L; VSS/TSS 0.71; soluble chemical oxygen demand (SCOD) 22.80 mg/L; total chemical oxygen demand (TCOD) 4430 mg/L.

1.2. *Tetrakis(hydroxymerhyl)phosphonium sulfate*

THPS (CAS No. 55566–30-8) was obtained from Solvay-Hengchang (Zhangjiagang, China) Fine Chemical Co., Ltd. It is a colorless, transparent liquid. The characteristics of THPS are summarized as: pH 4.34; molecular formula $C_8H_{24}O_{12}P_2S$; molecular weight 406.28; density 1.38 g/L.

1.3. *THPS pretreatment of sewage sludge*

Pretreatment tests were conducted in thirteen 1000 mL flasks, in which 500 mL of sewage sludge was added. The blank control (T1) contained sewage sludge without any pretreatment. T2–T12 contained sewage sludge with pretreatment using different THPS concentrations. The concentrations of THPS are summarized in Table 1. The other control (T13) contained sewage sludge with acid pretreatment; 4 mol/L HCl was added until the pH of T13 was the same as that of T12. After a pretreatment time of 30 min, the sludge samples were analyzed.

The analyzed parameters included pH, particle size, dewatering ability, soluble chemical oxygen demand (SCOD), soluble proteins (SPN), soluble polysaccharides (SPS), specific oxygen uptake rate (SOUR), pathogens content, and microbial communities.

1.4. *Analysis methods*

The pH of the sludge samples was measured using a pH meter (PB-10, Sartorius, Germany). TCOD and SCOD of the sludge samples were analyzed using a COD detector (D2800, HACH, USA) (Guo et al., 2014). PN and bound extracellular polymeric substances (BEPS-PN) were measured according to the Lowry method using bovine serum albumin as a standard solution (Liu and Fang, 2003). Carbohydrates (SPS and BEPS-PS) were measured according to the phenol-sulfuric acid method using

Table 1 – Summary of sludge pretreatments.

Number	Pretreatment	Concentration of THPS (mg/g VSS)
T1	Control	0
T2	THPS pretreatment	0.75
T3		1.87
T4		3.74
T5		5.61
T6		7.48
T7		17.70
T8		37.41
T9		56.11
T10		74.81
T11		187.03
T12		374.05
T13	HCl pretreatment	0

T1: raw sludge (blank control); T2–T12: sludge pretreated with different tetrakis(hydroxymethyl)phosphonium sulfate (THPS) concentration; T13: sludge pretreated with 4 mol/L HCl (acid pretreatment control); VSS: volatile suspended solids.

glucose as a standard solution (Zhou et al., 2014). The samples were filtered through a 0.45 μm membrane before determining the concentrations of SCOD, SPN, and SPS. BEPS were extracted using the method of Chen et al. (2001). The concentrations of TSS and VSS were measured using standard methods (APHA, 1998). Particle size of the sludge samples was determined using a laser particle size analyzer (Mastersizer 2000, Malvern, UK) (Houghton et al., 2002). The SOUR was measured using the method described by Awong et al. (1985). Capillary suction time (CST), used as an index of dewatering ability, was measured using a CST tester (304 M, Triton Electronics Ltd., UK). Total coliform and *Escherichia coli* were selected as indicators of pathogens, and they were also measured using standard methods (APHA, 1998).

1.5. *Microbial community analysis*

The DNA of microbial communities in the sludge samples was extracted by a nucleic acid automatic extraction system (Smart LabAssist-16, TANBead, Chinese Taipei). The concentration of the extracted DNA was measured using a NanoDrop 1000 (NanoDrop 1000, Thermo, USA), and the DNA was then used as a template for polymerase chain reaction (PCR) amplification. The methods of PCR amplification and high-throughput sequencing were described in a previous study (Han et al., 2018).

1.6. *Data analysis*

Averages and standard errors of results were reported based on triplicates for each analysis or measurement.

2. Results and discussion

2.1. *Effects of THPS pretreatment on the characteristics of sludge*

After pretreatment, the sludge characteristics changed. The effects of pretreatment on the characteristics of sludge (pH, dewatering ability, particle size, specific surface area, VSS, TSS, soluble organic matter, bound EPS, and SOUR) are summarized in Fig. 1.

Because THPS is a weak acid (pH = 4.34), the pH of the sludge samples slowly decreased from 7.41 ± 0.22 to 5.43 ± 0.23 as the concentration of THPS increased from 0 to 374.05 mg/g VSS (Fig. 1a). The initial pH of the HCl-pretreated sludge (T13) was the same as that of the THPS pretreated (T12), and the pH of the HCl-pretreated sludge (T13) was 5.78 ± 0.23 after 30 min of treatment, which was also same as the pH of the sludge pretreated with 187.03 mg THPS/g VSS (T11). Under the same pH (T13 and T12) condition, the difference in pH after THPS pretreatment was not due to their initial pH but probably due to sludge microorganisms consuming H^+ because the activities of the sludge samples were different (Seviour and Nielsen, 2010; Sfaelou et al., 2015).

CST was used as an index of dewatering ability of sludge. The effects of the pretreatments on sludge CST are summarized in Fig. 1a. The results showed that the sludge CST decreased from 8.40 to 7.53 sec as the THPS concentration increased from 0 to 56.11 mg/g VSS (T9). The sludge CST was stable at 7.52–7.54 sec when the THPS concentration was higher than 56.11 mg/g VSS (T9–T12). HCl pretreatment (T13) caused the sludge CST to decrease to 7.56 sec. Fig. 1a shows that the changes in pH and CST were consistent for the samples with low THPS concentrations (<56.11 mg/g VSS), similar to the results of previous research (Devlin et al., 2011; Raynaud et al., 2012). However, the changes in pH and CST were not consistent for the samples with high THPS concentrations (>56.11 mg/g VSS). The subsequent result showed that the change trend of CST and EPS was consistent. Thus the main reason for the change of sludge CST might be THPS damaged bacteria, which led to the change of EPS content, and then the change of CST.

The results of the sludge particle size analysis are summarized in Fig. 1b. The sludge particle size ranged from 65.40 ± 1.60 to 70.50 ± 2.90 μm, which is normal, as the diameter of most activated sludge flocs ranged between 68 and 183 μm in real wastewater treatment plants (Han et al., 2012). The results showed that sludge particle size decreased with the increase in THPS concentration, and HCl pretreatment (T13) had little effect on sludge particle size (Fig. 1b). The slight effect of HCl pretreatment may have been due to the pH, because the sludge pH was only 5.30 ± 0.12 after pretreatment (Devlin et al., 2011). Corresponding to the changes in sludge particle size, the specific surface area of the sludge particles increased as the THPS concentration increased (Fig. 1b). Because the initial pH of T13 was identical to that of T12, the change in pH due to pretreatment may not be the reason for the changes in sludge particle size and specific surface area. The main reason for the change of particle size and specific surface area of sludge is THPS itself with the characteristics of disintegrating floc structure (Wu et al., 2017b).

The VSS and TSS of sludge also slightly decreased with the addition of THPS (Fig. 1c). When the concentration of THPS increased from 0 to 374.05 mg/g VSS, the VSS and TSS of the sludge decreased from 4.62 ± 0.24 and 6.51 ± 0.33 to 4.37 ± 0.15 and 6.15 ± 0.21 mg/L, respectively. The VSS and TSS of the acid-pretreated sludge (T13) were 4.44 ± 0.16 and 6.29 ± 0.23 mg/L,

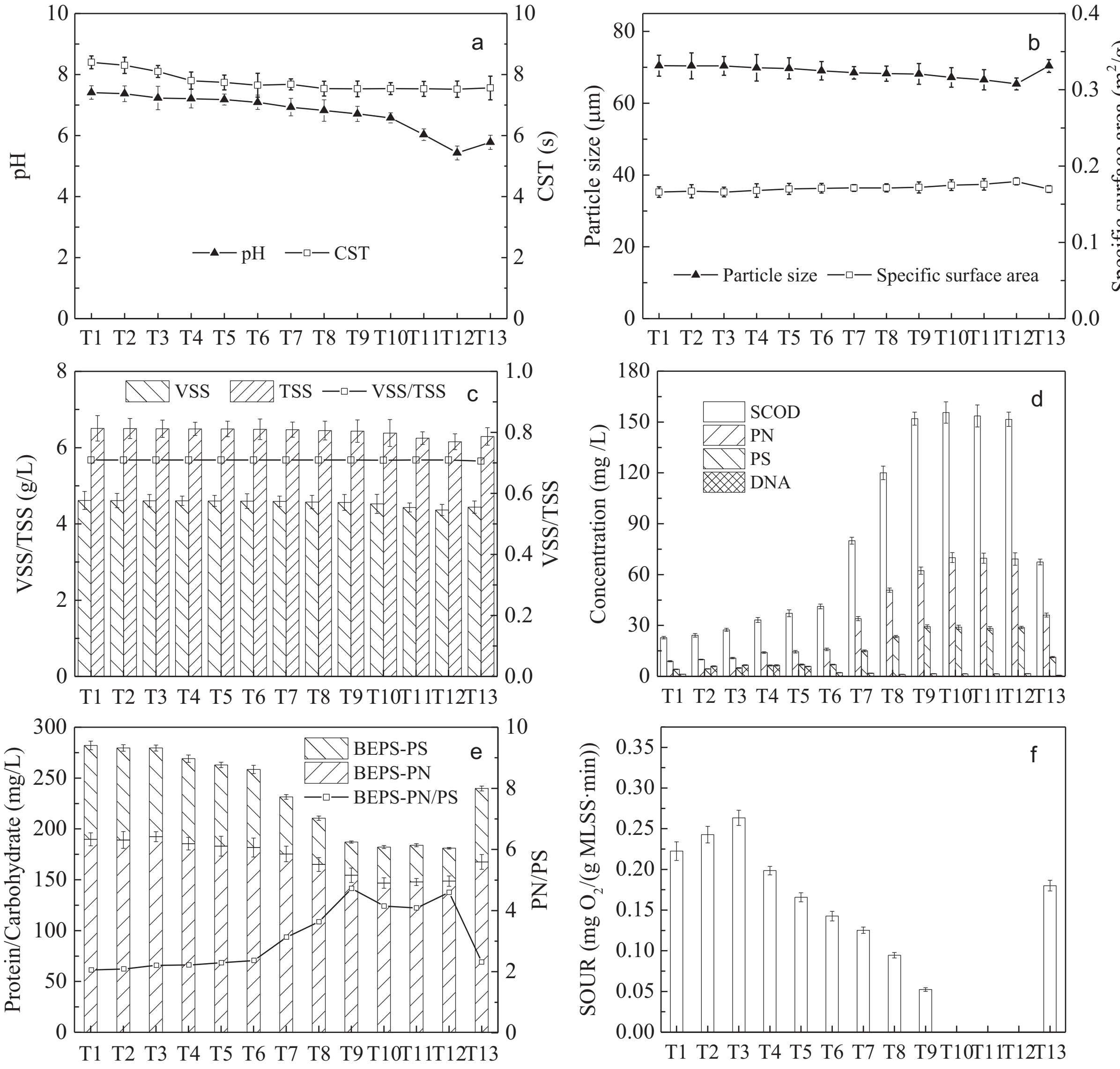

Fig. 1 – Effects of various pretreatments on sludge characteristics, including (a) pH and capillary suction time (CST), (b) particle and specific surface area, (c) volatile suspended solids (VSS), total suspended solids (TSS) and VSS/TSS, (d) organic compositions of soluble chemical oxygen demand (SCOD), (e) protein (PN) and carbohydrate (PS) in bound extracellular polymeric substances (BEPS) and their ratio, and (f) specific oxygen uptake rate (SOUR). MLSS: mixed liquor suspended solid.

respectively. These results indicated that the addition of THPS slightly decreased the concentration of sludge solids, and that both THPS and HCl could solubilize organic and inorganic matter and decrease the VSS and TSS of the sludge. The sludge solids reduction via THPS pretreatment (T12) was higher than that of the acid pretreatment control (T13), which suggested that the effects of THPS on sludge solids concentration were not solely caused by acidic pH (Devlin et al., 2011). In fact, THPS could have disintegrated the sludge, which could have released organic and inorganic matter from the sludge to the water (Xu et al., 2014b; Li et al., 2016; Wu et al., 2017b). This would have resulted in the decrease in sludge concentration. Meanwhile, the sludge VSS/TSS ratio was calculated; the results are summarized in Fig. 1c. The VSS/TSS ratios of the pretreated sludge were similar, which suggested that the solubilization of organic and inorganic matter at different THPS concentrations was similar.

Sludge pretreatment released microbial matter (Carrère et al., 2010; Zhen et al., 2017) and resulted in changes in SCOD, PN, and PS (Fig. 1d). The SCOD, PN, and PS in the pretreated sludge increased with the increase in THPS concentration when the concentration of THPS was lower than 74.81 mg/g VSS (T10). When the concentration of THPS was 74.81 mg/g VSS, the SCOD, PN, and PS in the pretreated sludge reached their maximum concentrations (155.60 ± 6.30, 70.00 ± 2.90, and 29.30 ± 1.20 mg/L, respectively), which were 6.80, 7.80, and 7.10 times that of the control (T1), respectively. When the concentration of THPS was higher than 74.81 mg/g VSS, the SCOD, PN, and PS in the pretreated sludge remained stable. These results indicated that the magnitude of

Table 2 – Concentration and purity of extracted DNA.

No.	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
DNA (ng/μL)	77.21	101.38	94.40	67.23	69.41	63.02	64.19	35.49	28.62	22.03	19.71	27.69	77.04
Ratio of absorbance at 260nm and 280nm (A260/A280)	1.77	1.72	1.68	1.81	1.82	1.76	1.63	1.75	1.64	1.74	1.70	1.67	1.78

increase in sludge SCOD, PN, and PS following THPS pretreatment was higher than that in a previous study that used THPS pretreatment (Wu et al., 2017b). One of the possible reasons for this is that the sludge was washed in this study, which lowered the SCOD, PN, and PS in raw sludge; raw sludge was used in the study conducted by Wu et al. (2017b). Even so, the effects of THPS pretreatment in this study were lesser than those of other pretreatments (such as ultrasound, thermal hydrolysis, alkaline, etc.) used in previous studies (Khanal et al., 2007; Li et al., 2012; Zhen et al., 2017). In addition, the concentrations of SCOD, PN, and PS in the acid-pretreated sludge (T13) were 67.40 ± 1.70, 36.10 ± 1.20, and 11.30 ± 0.40 mg/L, respectively, which were higher than those in the control (T1) but much lower than those in the THPS-pretreated sludge with the same pH (T12). This result suggested that the effect of THPS on the sludge did not depend solely on the acidic conditions.

The BEPS in the pretreated sludge were detected (Fig. 1e). EPS were presented as the sum of proteins and carbohydrates because they are the main components of EPS. The proteins and carbohydrates of BEPS in the THPS-pretreated sludge decreased with the increase in THPS concentration when the THPS concentration was lower than 56.11 mg/g VSS, and they remained stable when the THPS concentration was higher than 56.11 mg/g VSS. The concentrations of proteins and carbohydrates of BEPS in the HCl-pretreated sludge (T13) were 167.50 ± 7.30 and 81.28 ± 2.40 mg/L, respectively, which were lower than those in the control sample (T1) (Chen et al., 2001) and much higher than those in the THPS-pretreated sludge with the same pH (T12). These results suggested that THPS pretreatment could decrease the concentration of BEPS, and that acidic pH was not the only reason for this phenomenon. A previous study proved that the decrease in EPS can improve the dewaterability of sludge (He et al., 2015). Thus, the changes in CST and BEPS in the sludge were consistent (Fig. 1a and e) in this research.

The SOUR of the sludge was affected by the addition of THPS (Fig. 1f). When the concentration of THPS increased from 0 to 1.87 mg/g VSS, the SOUR increased from 0.22 ± 0.01 to 0.26 ± 0.01 mg O_2/(g MLSS (mixed liquor suspended solid)·min). However, as the concentration of THPS increased, the SOUR started to decrease and eventually reached 0 when the THPS concentration was higher than 74.81 mg/g VSS. These results suggested that lower concentrations of THPS (≤1.87 mg/g VSS) could enhance the activity of sludge, and higher concentrations of THPS (≥1.87 mg/g VSS) could inhibit its activity. At low concentrations of THPS, the sludge activity was enhanced because the slight increase in outer membrane permeability increased the matter exchange of the microbial cells and improved the microbial activity (Guo et al., 2014). However, at high concentrations of THPS the microbial cells were killed because of the high permeability of their outer membranes and the deactivation of the proteins in their cell walls; this reduced the sludge activity (Wen et al., 2012; Okoro et al., 2016; Wu et al., 2017b). The SOUR of the HCl-pretreated sludge (T13) decreased to 0.18 ± 0.01 mg O_2/(g MLSS·min), which occurred because the acidic pH partly inhibited the sludge activity. However, the effect of THPS on the SOUR was not dependent on pH, as the SOUR of the HCl-pretreated sludge (T13) was much higher than that of the THPS-pretreated sludge with the same pH (T12) (Fig. 1f).

2.2. Effect of THPS pretreatment on the microbial community of the sludge

To investigate the effects of THPS pretreatment on microorganisms in the sludge, the sludge microbial communities were analyzed using Illumina MiSeq sequencing (Illumina MiSeq platform, Majorbio Bio-Pharm Technology, China). The total DNA in the sludge was extracted, and the extracted DNA concentration was measured (Table 2). The extracted DNA concentration of the HCl-pretreated sludge (T13) was 77.00 ng/μL, which was equal to that of the control sludge (77.20 ng/μL) (T1). The DNA concentration in the sludge pretreated with low concentrations of THPS (≤ 1.87 mg/g VSS) increased relative to that in the control (T1). As shown in Table 2, the DNA concentrations were 101.40 and 94.40 ng/μL when THPS of 0.75 mg/g VSS (T2) and 1.87 mg/g VSS (T3) were added, respectively. As the concentration of THPS increased, the DNA concentration in the THPS-pretreated sludge showed a decreasing trend. When the concentration of THPS was 37.41 mg/g VSS (T8), the extracted DNA concentration of the THPS-pretreated sludge was reduced by 54.02% compared to that of the control (T1). When the concentration of THPS increased to 187.03 mg/g VSS (T9), the extracted DNA concentration of THPS-pretreated sludge was 19.70 ng/μL. These results indicated that low concentrations of THPS could increase the sludge DNA concentration. This result was consistent with the change in SCOD (Fig. 1d), but the trend was the opposite of that observed for bound EPS (Fig. 1e). EPS play an important role in protecting sludge structure (Zhao et al., 2015). Hence, it was revealed that a THPS concentration of 0.75 mg/g VSS disrupted the cell walls or membranes after destroying EPS. The effect of THPS on sludge microbial cell structure was lesser than that in a previous study (Wu et al., 2017b).

Meanwhile, the ratio of absorbance at 260 nm and 280 nm (A260/A280) was used to analyze the purity of the extracted DNA (Table 2). The results showed that the A260/A280 ratios of the extracted DNA for all samples were near 1.8, which suggested that the purity of the extracted DNA was acceptable; thus, the DNA could be used for PCR amplification and high-throughput sequencing. The rarefaction analysis is summarized in Appendix A Table S1. The results indicated that a dependable inventory of bacterial gene sequences was present in all samples.

Bacteria in the phyla *Proteobacteria* and *Bacteroidetes* were dominant in all sludge samples (Fig. 2). The percentage of

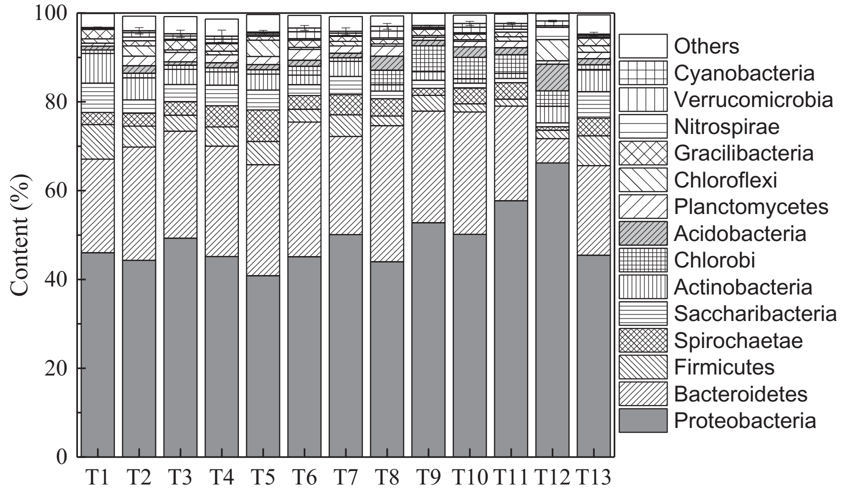

Fig. 2 – Percentages of bacterial phyla in all samples.

Proteobacteria increased from 46.02% to 66.23% when the concentration of THPS increased from 0 (T1) to 374.05 mg/g VSS (T12). The percentage of *Bacteroidetes* present increased at first and then decreased. The highest percentage of *Bacteroidetes* was 30.64%, which was found in the sample with THPS pretreatment of 37.41 mg/g VSS (T8), and the lowest was 5.48%, which was found in the sample with THPS pretreatment of 374.05 mg/g VSS (T12). However, the effect of HCl pretreatment on bacterial communities in the sludge was small; the percentages of *Proteobacteria* and *Bacteroidetes* decreased from 46.00% and 21.10% (T1) to 45.50% and 20.20% (T13), respectively. The difference in bacterial relative content in the phyla suggested that the effects of THPS on sludge microorganisms were not only due to the acidic pH, but more importantly, THPS can damage and even kill bacterial cells, leading to the bacterial population changes.

The contents of 13 genera of bacteria decreased with addition of THPS. The effects of THPS on different bacteria varied significantly. As shown in Fig. 3, the relative contents of *Pseudomonas*, *Escherichia*, and *Faecalibacterium* decreased sharply from 9.17%, 2.88, and 1.31% to 0.28%, 0.09%, and 0.29%, respectively, when 0.75 mg THPS/g VSS was added (T2). The rates of decrease in the relative contents of these bacteria slowed with increasing THPS concentration. When 374.05 mg THPS/g VSS was added (T12), *Pseudomonas* was no longer detected. The relative contents of eight bacteria, including *Arcobacter*, *Aquabacterium*, and *Woodsholea*, gradually decreased with the increase in THPS concentration. For example, the relative contents of *Arcobacter* decreased from 2.52% (T1) to 1.80% (T12), and those of *Aquabacterium* decreased from 0.76% (T1) to 0.34% (T12). In addition, some bacteria (such as *Halomonas* and *Bdellovibrio*) were only affected by high concentrations of THPS. THPS may have killed some bacteria by dissolving lipids, which are one of the components of bacterial cell walls (Downward et al., 1997). Meanwhile, THPS, which is positively charged after ionization in water, can be adsorbed onto the surfaces of negatively charged bacterial cells, changing the primary nature of the membrane. When this change occurs, bacterial movement becomes limited. *Pseudomonas*, *Escherichia*, and *Faecalibacterium* are gram-negative bacteria with peritrichous flagellation (Madigan and Martinko, 2005; Palleroni, 2010; Lopez-Siles et al., 2012); *Arcobacter*, *Aquabacterium*, and *Woodsholea* are gram-negative

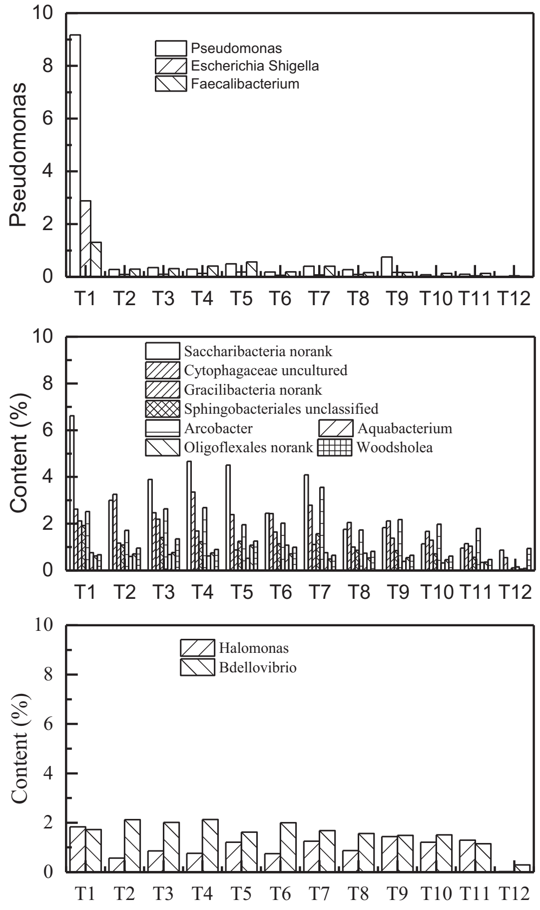

Fig. 3 – Relative contents of bacterial species in all samples.

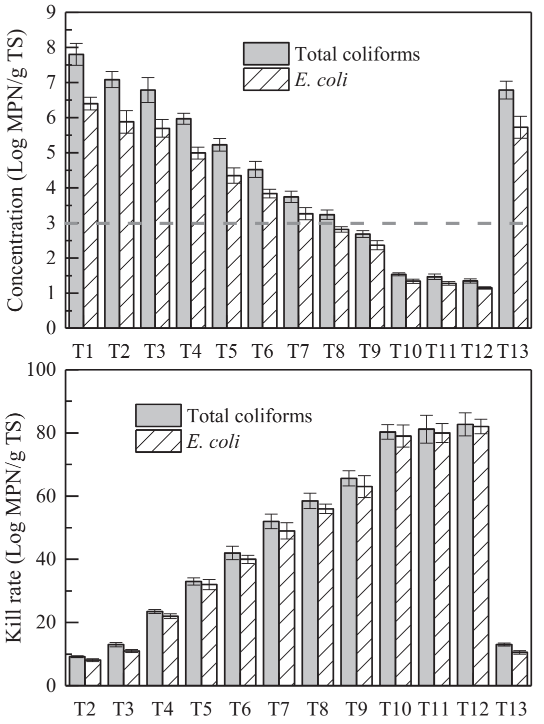

Fig. 4 – Effects of various pretreatments on pathogens. MPNs: specific most probable numbers; *E. coli*: *Escherichia coli*.

bacteria with bipolar flagellation (Kalmbach et al., 1999; Carlström et al., 2013); and *Halomonas* are gram-positive bacteria with bipolar flagellation (Stevens et al., 2009). These analyses indicated that gram-negative bacteria with peritrichous flagellation were extremely sensitive to THPS, and were quickly killed by THPS relative to gram-positive bacteria with bipolar flagellation. One of the main reasons for this is that gram-negative bacteria have a thinner and more loosely adherent layer of peptidoglycan than gram-positive bacteria, which makes the gram-negative bacteria more vulnerable than gram-positive bacteria. A study on the effect of ultrasound on bacteria in municipal wastewater also drew the same conclusion (Drakopoulou et al., 2009).

2.3. Effects of THPS pretreatment on sludge pathogens

To determine whether the THPS-pretreated sludge met the pathogen requirements for land application or landfilling, the effects of THPS pretreatment on sludge pathogens were studied using total coliforms and *E. coli* as indexes (Fig. 4).

The specific most probable numbers (MPNs) of total coliforms and *E. coli* decreased with the increase in THPS concentration (Fig. 4). When the THPS concentration was higher than 56.11 mg/g VSS (T9), the populations of total coliforms and *E. coli* in the pretreated sludge were both lower than 3.00 log MPNs/g TSS, which is required for Class A biosolids as specified by the USEPA. The results showed that the sludge pretreated with high THPS concentrations (>56.11 mg/g VSS) met the pathogen requirement for Class A biosolids, and could be disposed of by land application or landfilling if other quality parameters also met the requirements. The kill rates of total coliforms and *E. coli* from THPS pretreatment were calculated, and the results are also summarized in Fig. 4. When the concentration of THPS was 374.05 mg/g VSS (T13), the maximal kill rates for total coliforms and *E. coli* reached 82.70% ± 1.30% and 82.00% ± 2.50%, respectively. The pathogen populations and their kill rates for the HCl-pretreated sludge (T13) were 6.78 ± 0.26 log MPNs/g TSS and 13.00% ± 0.80% for total coliforms, respectively, and 5.73 ± 0.09 log MPNs/g TSS and 10.50% ± 0.40% for *E. coli*, respectively, which did not meet the pathogen requirement for Class A biosolids and were far lower than those of THPS-pretreated sludge with the same pH (T12). These results suggested that THPS caused changes in bacterial population and the content of pathogenic bacteria by dissolving the cell wall of bacteria, destroying or even killing bacteria.

3. Conclusions

The effects of different concentrations of THPS on physical and chemical properties, microbial communities, and sludge pathogens were studied. The results showed that THPS disintegrated the sludge, which led to decreasing particle size and decreasing CST of the sludge with increasing THPS concentration. Meanwhile, THPS also dissolved bacterial cell walls and damaged the bacteria in the sludge. The results indicated that the changes in the microbial communities and number of pathogenic bacteria in the sludge were closely related to the concentration of THPS. Sludge pretreatment with moderate concentrations of THPS contributed to meet the pathogen requirement for land application of Class A biosolids.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.09.013.

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