Effects of aeration strategy on the evolution of dissolved organic matter (DOM) and microbial community structure during sludge bio-drying

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Abstract Sludge bio-drying in which sludge is dried by means of the heat generated by the aerobic degradation of its own organic substances has been widely used for sludge treatment. A better understanding of the evolution of dissolved organic matter (DOM) and its degradation drivers during sludge bio-drying could facilitate its control. Aeration is one of the key factors that affect sludge bio-drying performance. In this study, two aeration strategies (pile I—the optimized and pile II—the current) were established to investigate their impacts on the evolution of DOM and the microbial community in a full-scale sludge bio-drying plant. A higher pile temperature in pile I caused pile I to enter the DOM and microbiology stable stage approximately 2 days earlier than pile II. The degradation of easily degradable components in the DOM primarily occurred in the thermophilic phase; after that degradation, the DOM components changed a little. Along with the evolution of the DOM, its main degradation driver,

the microbial community, changed considerably. Phyla *Firmicutes* and *Proteobacteria* were dominant in the thermophilic stage, and genus *Ureibacillus*, which was the primary thermophilic bacteria, was closely associated with the degradation of the DOM. In the mesophilic stage, the microbial community changed significantly at first and subsequently stabilized, and the genus *Parapedobacter*, which belongs to *Bacterioidetes*, became dominant. This study elucidates the interplay between the DOM and microbial community during sludge bio-drying.

Keywords Microbial community · Sludge bio-drying · Dissolved organic matter

Introduction

The treatment and disposal of sewage sludge has become a worldwide environmental challenge due to strict legal regulations, land shortages, rising costs, and public concern (Wei et al. 2003). Sludge reduction, including a reduction of volume and quantity as well as stabilization, is the principal goal of sewage sludge treatment. Sludge bio-drying, which is derived from but quite different from composting, which requires a relatively long residence time (30–50 days) (Winkler et al. 2013), is an innovative approach for biomass energy utilization in which sludge is dried by means of the heat generated by the aerobic degradation of its own organic substances (Zhao et al. 2010). Compared to thermal drying in which sludge was dried at relatively high temperatures at the cost of amounts of non-renewable energy resource, it is cost-effective and environmentally friendly. It can be considered as an effective volume and quantity reduction process and is used as a pre-treatment method that renders the output more suitable for post-treatment by partial bio-stabilization (Zhao et al.

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2010). Depending on the pre-treatment and different bio-drying processes, sludge bio-drying products can be used for incineration, soil amelioration, or landscaping (Yang et al. 2014).

Bio-drying has primarily been studied for municipal solid wastes (MSW) (Velis et al. 2009; Zhang et al. 2009), although a few studies have been reported for sewage sludge bio-drying (Winkler et al. 2013; Zhao et al. 2010). Compared to MSW, there are some problems in sewage sludge bio-drying, including more moisture and lower porosity of biomass matrix, which are disadvantageous to effective aeration and energy recovery (Huiliñir and Villegas 2014). Previous studies had focused on technical parameters and their bio-drying effects, such as initial moisture content and especially aeration strategy. Cai et al. (2013) demonstrated that forced aeration controlled the pile temperature and improved evaporation, making it the key factor that influenced water loss during sewage sludge bio-drying. Sharara et al. (2012) indicated that a high aeration level was superior in terms of both drying energy and time requirements to the other aeration rates that were considered. Additionally, Villegas and Huiliñir (2014) studied initial moisture content (MC) and aeration strategy and proposed kinetics of volatile solid degradation under different initial MC and air-flow rates of sewage sludge bio-drying. However, there is little information about the effects of aeration strategy on the dynamics of organic matter degradation and the microbial mechanisms during sludge bio-drying.

The microbial metabolism for sludge bio-drying is similar to composting. Both consist of mesophilic and thermophilic stages involving numerous microorganisms, but the former aims at MC removal, while the latter focuses on bio-stabilization and the maturity of the composted materials (Zhang et al. 2009). Overviews of the evolution of the microbial community during sludge composting, including group succession and utilizable substrate for different process stages and temperature ranges, have been reported previously (Nakasaka et al. 2009). Also, evolution of DOM during composting has been widely studied using Fourier transform infrared spectroscopy (FTIR) and three-dimensional excitation-emission matrix (EEM) methods (Said-Pullicino and Gigliotti 2007; Straathof and Comans 2015). However, sludge bio-drying is operated within an MC that is typically lower than optimal composting, and the aeration condition is also managed differently (Velis et al. 2009). Therefore, the behavior of bio-degradation may be atypical compared with composting research results (Adani et al. 2002). In addition, in the past, the composting microbial community was usually studied using traditional methods such as terminal restriction fragment length polymorphism (T-RFLP), PCR denaturing gradient gel electrophoresis (DGGE), clone library, or culturing methods. As a new method for microbial community structure studies, high-throughput sequencing has increased the capacity and rate of the discovery of unknown

microorganisms and given a more comprehensive picture of microbial biodiversity (Petrosino et al. 2009). It has been recently used in a few studies on the composting microbial community (Tkachuk et al. 2014), but those studies have focused on the start and the end of composting. Information on the evolution of the microbial community throughout the composting process is still deficient.

Dissolved organic matter (DOM) is the main active organic fraction and is more easily utilized by microorganisms relative to solid-phase organics (Said-Pullicino and Gigliotti 2007). DOM contains soluble breakdown products, bio-originating compounds, and repolymerized macromolecules, which are direct nutritional sources for the microbes. The insoluble organic substrates have to be hydrolyzed to the DOM before biological utilization (Wang et al. 2014). They represent the most active fraction of the organic matter, both biologically and chemically, and thus greatly impact the microbial community and its activity. Therefore, investigating the transformation of DOM is crucial for optimizing sludge bio-drying operations. The chemical composition and structure of DOM have been widely investigated in composting to evaluate the quality of the composting product (Straathof and Comans 2015). However, to our knowledge, there is little information about the evolution of DOM and its degradation driver (the microbial community) during sludge bio-drying. A better understanding of the evolution of DOM and the microbial community structure during sludge bio-drying could facilitate the optimization of the sludge bio-drying operation and improve its performance. Thus, with the aim of enhancing sludge bio-drying, two aeration strategies (pile I—the optimized and pile II—the current) for sludge bio-drying were established to investigate their effects on the evolution of the DOM and microbial community in full-scale sludge bio-drying. This study elucidates the interaction between the DOM and microbial community structure during sludge bio-drying.

Materials and methods

Process description, experimental design, and sampling

The sludge bio-drying plant located in Shenyang, Liaoning province, in China is designed to treat 1000 t of dewatered sewage sludge at 80 % of MC per day. This plant is the largest sludge bio-drying plant in China. The plant employs a horizontal agitated solid bed process (HASB) with forced aeration and turning. It is designed for reducing the MC of the dewatered sewage sludge from approximately 80 % to less than 40 %. There are 96 tunnels in total, and each tunnel is 90 m long, 3 m wide, and 2.5 m tall. According to the aeration condition, each tunnel was divided into seven parts: one pre-mixed area and six different aeration areas (areas A, B, C, D, E, and F). Their details are shown in Fig. S1. The operational

period of sludge bio-drying was set at 21 days, during which each pile was turned once a day along with the tunnel at a rate of marching of 4 m day^{-1} . The size of each pile was 4 m long, 3 m wide, and 2.5 m tall.

The raw sludge has an average total solid content of 20 % and an organic fraction of 62 % and is mainly from municipal wastewater treatment plants (WWTPs), with a small proportion from industrial WWTPs in Shenyang. Prior to the bio-drying process, the raw sludge is mixed with the bio-dried sludge product to control the initial MC at approximately 60 %. The pre-dried sludge was previously collected through bio-drying the raw sludge and rice husk. The aeration required for water evaporation and the oxygen supply for the microbial activity were monitored and controlled online.

Two aeration strategies were used for two piles to investigate the effects of aeration on the evolution of DOM and the microbial community; their details are listed in Table 1. One aeration strategy for pile I was designated as the experiment, which was designed according to the functions of aeration in different areas including oxygen supply, heat dissipation, and water evaporation. Another aeration strategy for pile II was designated as the control, which was the aeration process in current use in the plant. In pile I, the aeration in areas A and B was mainly used to supply oxygen for the microbial activity to promote an increase in the pile temperature. In areas C, D, and E, aeration was used to maintain the pile temperature at less than 60°C in the thermophilic stage and to allow the sludge bio-drying to progress effectively. In Area F, aeration was mainly used to evaporate water to meet the requirement that the MC should be less than 40 % of the sludge bio-drying product. Details of the design of the aeration strategy for pile I are described in the Supporting Information.

The sampling in piles I and II throughout the sludge bio-drying process was carried out on days 1, 3, 5, 7, 11, 14, 17, and 21, respectively. Because each pile was turned once per day, each pile can be considered to have been homogeneous. To obtain a representative solid sample, the surface area of each pile ($4 \times 3 \text{ m}$) was diagonally divided into four parts, and five sampling points were chosen on the diagonals.

Samples (100–200 g each) were collected at a pile depth of 10–15 cm. The samples were mixed together, and a representative sample (100 g) was acquired from the mixed samples using the quartering method (Michele et al. 2015). Details of the solid sampling can be found in the Supporting Information (Fig. S2). Meanwhile, the core temperature of each pile was monitored. Each solid sample was divided into two parts; one sample was used for an analysis of physical and chemical parameters, and the other sample was stored at -20°C for further analysis.

Analysis methods for MC, TS, VS, and DOM

The MC (%) was measured by drying the solid sample in a drying oven at 105°C until a constant weight was achieved; the remaining weight fraction ($1 - \text{MC}$) was regarded as total solids (TS (%)). The volatile solid (VS) content (%) was analyzed by heating the dried sample at 600°C for 6 h in an electrical muffle furnace. All tests were conducted in duplicate.

The DOM was extracted following the method previously reported (Said-Pullicino and Gigliotti 2007) with a slight modification. Briefly, the freeze-dried solid samples were ground and passed through a 100 mesh, and then, 20 g of samples was extracted using 200 mL of distilled water (1:10, w/v) for 24 h in a horizontal shaker at 180 rpm at room temperature. The extracts were then centrifuged at 10,000 rpm for 10 min and filtered through $0.45\text{-}\mu\text{m}$ membrane filters. The filtrates that were obtained were used for further analysis. The polysaccharide and protein content in the DOM was measured by the phenol–sulfuric acid method (DuBois et al. 1956), with glucose as the standard, and by a modification of the Bradford method (Peterson 1977), with bovine serum albumin as the standard, respectively.

Prior to the FTIR analysis, the DOM samples were freeze-dried in a vacuum to evaporate the water, and then, the dried samples were used for the FTIR analysis (FT-IR, Nicolet 8700, USA). Before EEM and size exclusion chromatography (SEC) analyses were performed, the DOM was diluted by the

Table 1 The operation parameters used in this study for pile I and pile II during sludge bio-drying

Parameter	Pile I	Pile II
Mixing ratio of dewatered sludge and bio-dried sludge product	1:3 (v/v)	1:3 (v/v)
Pile size (length $\times$ width $\times$ height)	$4 \times 3 \times 2.5 \text{ m}$	$4 \times 3 \times 2.5 \text{ m}$
Aeration strategy	Time control model (min h^{-1}) Temperature feedback control model (start aeration if temperature is above the set temperature) ^b	A 4; B 8; C 15; D 17; E 20; F 30 A 45°C ; B 55°C ; no temperature control model for other areas
Turning frequency	Once a day	Once a day

Letters A–F refer to pile sections

^a If temperature is above the set temperature, temperature feedback control model started, and if not, time control model for A and B would be adopted automatically.

same amount ($\times 50$) for all samples to keep the dissolved organic carbon (DOC) below 20 mg L^{-1} . Fluorescence EEM spectroscopy was performed using a PerkinElmer LS50B fluorescence spectrophotometer (PerkinElmer, USA). Excitation and emission were simultaneously scanned at wavelengths from 200 to 400 nm and from 220 to 550 nm, respectively, at 5-nm intervals. The slit widths were set to 10 nm for both the excitation and emission monochromators, and the scan speed was set to 1200 nm min^{-1} . Molecular weight (MW) distributions were determined by HPSEC, as previously reported (Wang et al. 2010).

High-throughput sequencing

Each solid sample (0.5 g) was collected for DNA extraction in duplicate with the FAST DNA Spin Kit for Soil (MP Biomedicals, USA). The duplicate DNA extracts were then merged together for further PCR amplification. PCR primers 515F/806R targeting the bacteria and archaea 16S V4 region were selected for the microbial community structure analysis (Caporaso et al. 2010). The reverse primer contained a 6-bp error-correcting barcode that was unique to each sample. The barcode was permuted for each sample and allowed the identification of individual samples in a mixture in a single Illumina MiSeq sequencing run. DNA was amplified in triplicate for each sample following the protocol described previously (Caporaso et al. 2010). PCR amplicons were further purified with a DNA purification kit (BioFlux, Japan), and the concentrations were determined using spectrometry (NanoDrop-1000, USA). Amplicons from different samples were then mixed to achieve equal mass concentrations in the final mixture, which were sent out to Novogene Co., Ltd., in Beijing for small-fragment library construction and pair-end sequencing using the Illumina MiSeq sequencing system (Illumina, USA).

Bioinformatics analysis

Sequencing reads were assigned to each sample according to the unique 6-bp barcode of each sample. The barcode and primers then were removed. Pairs of reads from the original DNA fragments were merged using FLASH (Magoč and Salzberg 2011). The overlapped reads are called “raw reads.” The raw reads then were filtered using QIIME quality filters. Default settings for the Illumina processing in QIIME were used. PCR chimeras were filtered out using UCHIME (Edgar et al. 2011). The reads are then “clean reads.” The average length of all of the clean reads was 253 bp. After the above filtration, the minimum number of selected sequences in the 16 samples was 24,515. To fairly compare the 16 samples at the same sequencing depth, the sequence number was normalized by extracting the first 24,515 sequences in each sample for all of the following

analyses. All of the normalized sequences were uploaded to MG-RAST (<http://metagenomics.anl.gov/linkin.cgi?project=9439>).

The taxonomic classification of the sequences of each sample was carried out individually using the Ribosomal Database Project (RDP) Classifier. The sequences to different taxonomy levels were assigned at the bootstrap cutoff of 50 % suggested by the RDP. In addition, the sequences in each set were assigned to phylotype clusters at cutoff levels of 3 and 6 % as described previously (Zhang et al. 2012), and on the basis of these clusters, rarefaction curves, H' , E , varH , and Chao1 richness indices were calculated using the relevant RDP pipeline modules.

Data analysis

The results of MC, VS, and EEM were analyzed through Origin 9.0 (OriginLab, USA). A heat map of the top 10 genera in each sample was built using the R. Circos graph that was produced by the Circos software (<http://circos.ca/>). According to the relative content of each genus from the classification, a principal component analysis (PCA) was performed using Canoco 5.0 (Microcomputer Power, USA). The data were statistically analyzed and the significance of variance was examined by SPSS 16.0 (Microsoft, USA).

Results

Sludge bio-drying performance

The pile temperature was a significant factor that affected water evaporation and organic degradation in the sludge bio-drying. This study compared two different aeration strategies that led to two different pile temperature profiles in the thermophilic stage for pile I and pile II, as shown in Fig. 1a. Pile I reached the highest temperature of 78°C in area A (day 1), but the highest temperature of 56°C in pile II was reached in area B (day 3). During bio-drying, aeration has three effects (water evaporation, oxygen supply for microbial activity, and heat dissipation), and the time for the aeration of the oxygen supply was shorter than those for heat dissipation and water evaporation in this study (Table 1). In area A (day 1), the aeration rate (6 min h^{-1}) for pile I was greater than that (4 min h^{-1}) for pile II and resulted in a higher pile temperature in pile I (78°C), which may indicate that the aeration rate of 4 min h^{-1} (pile II) in area A was insufficient for microbial activity. In area B (days 3 and 5), the aeration rate was increased to 8 min h^{-1} for pile II, the microbial activity increased, and thus, its pile temperature reached a maximum (56°C). Along with the increasing aeration time, the heat dissipation by aeration was dominant, and thus, the changes in the pile temperature were almost the same for both piles after day 3 (Fig. 1a).

Fig. 1 Evolution of temperature (a), moisture content (b), and volatile solids (c) during sludge bio-drying

The difference in pile temperature in the first three days resulted in different sludge bio-drying performances (Fig. 1b), and pile I reached the designed bio-drying requirement (MC, 40 %) much earlier (approximately 2 days) than pile II. It was observed that at the beginning of the sludge bio-drying, the reduction of the MC in the sludge was markedly heterogeneous (day 1 to day 5, Fig. 1b), which is in accordance with previous studies (Sugni et al. 2005). The air carried the moisture from the bottom and reached the saturation point as it passed through the matrix. Thus, on reaching the top of the pile, the air could not absorb any more water. As the sludge bio-drying progressed, the dried layer extended to the top gradually after every turning, which drove the reduction of the moisture content. Although the moisture content of both piles at the end of experiments reached the design requirement (40 %), the bio-drying performance of pile I was much better than that of pile II; i.e., not only did pile I reach the designed moisture content faster (about 2 days ahead) than pile II (Fig. 1b) but also the final moisture content of pile I (33.1 %) was also lower than that of pile II (37.8 %). These results clearly show that the aeration strategy for pile I was more effective for sludge bio-drying. Jewell et al. (1984) reported maximum moisture removal rates at 46 °C but maximum organic degradation at 60 °C during bio-drying of dairy manure. Adani et al. (2002) also confirmed that maximum moisture removal was achieved at 45 °C and that maximum organic degradation was achieved at 70 °C during the bio-drying of municipal solid waste. In this study, it was also confirmed that the maximum moisture removal rate was achieved between 40 and 50 °C, and the maximum organic degradation rate was achieved at 78 °C (Fig. 1c). All of these results suggest that at higher pile temperatures, organic

degradation dominated, and along with turning, the temperature began to decrease, and moisture removal began to dominate.

Evolution of DOM in sludge bio-drying

Aeration strategy had an important impact on the evolution of the DOM during sludge bio-drying, and the EEM spectra reflected the evolution of the sludge DOM during bio-drying very well in this study (Fig. 2). According to the EEM spectra analysis, the component of DOM in pile I (after day 5) reached its stable stage earlier (also by approximately 2 days) than pile II (after day 7). The intensity of regions II and IV decreased much on day 5 and day 7 for pile I and pile II, respectively, and after day 3, the intensity of region II in pile II was much higher than that in pile I. Following Chen et al. (2003), the EEM spectra were divided into five regions depending on the wavelengths of the organics. The comparative evolution of region II in this study indicated that protein-like substances degraded more thoroughly in pile I due to the optimized aeration strategy. In area A, the main function of aeration was to supply oxygen for microbial activity, and the fluorescence intensity of the sludge DOM on day 1 was mainly concentrated in regions II and IV, which suggests that the microbial by-products and aromatic proteins were the major fluorescent DOM. After the thermophilic stage, along with the increasing aeration rate, the fluorescence intensity was greatly reduced in regions II and IV. This change demonstrated the rapid biological transformation of the microbial by-products and proteins in the liquid phase (DOM) during the thermophilic stage of sludge bio-drying. The region IV fluorescence intensity of pile I was higher than that of pile II, which suggests higher microbial

Fig. 2 EEM spectra of dissolved organic matter (DOM) for different samples from piles I and II. Regions I and II, aromatic proteins, III, fulvic acid-like materials, IV, soluble microbial by-product-like materials, and V, humic acid-like materials

activity that resulted in more microbial by-products and a higher temperature. These results were consistent with the evolution of the pile temperature (Fig. 1a). Meanwhile, the fluorescent signal shifted to regions III and V, which indicates that the constituents of the DOM were very well transformed during sludge bio-drying. This is in accordance with previous studies on composting (Zhang et al. 2014). However, along with bio-drying, the fluorescent intensity of regions III and V was gradually reduced and then remained constant, which is relatively different from composting because regions III and V were generally used to represent the maturity of composting (Marhuenda-Egea et al. 2007). Although the aeration time was much higher in the latter area, the components of the DOM changed little (Fig. 2) due to the low moisture content (Fig. 1b). This also demonstrates that sludge bio-drying, unlike sludge composting, is not a stabilization process. A very low moisture content in the sludge would cause early dehydration, which would inhibit the biological process and thus produce physically stable but biologically unstable products (Liang et al. 2003). Sludge bio-drying is designed to remove more MC, and a consequence of this is the loss of DOM. However, this lower moisture content leads to restrained microbial activity, and the remaining DOM is therefore less susceptible to biodegradation. Higher organic content leads to its biological instability, which can be observed from the EEM

spectra (Fig. 2) that show that the humic-like acids were decreasing rather than increasing. In addition, the FTIR analysis (Fig. 3e, f) showed that the absorbance at a wavelength of 1140 cm^{-1} changed a little and even decreased for pile I, while in composting, it should increase and is used as the maturity mark. This further suggests that sludge bio-drying is quite different from composting.

Two peaks were identified, and the MWs of the resolved peaks were calculated to be 2.3 kDa (peak 1) and 44.7 kDa (peak 2) according to the HPSEC chromatographs (Fig. 3c, d). Peak 1 reached its peak value on day 3 and day 1 for piles I and II, respectively. This suggests that after the higher pile temperature, pile I released more small molecular substances, and thus, concentrations of proteins and polysaccharide in the DOM reached their peaks on day 3 in pile I (Fig. 3a). Likewise from the EEM results, after day 5 for pile I and day 7 for pile II, the concentrations of the proteins and polysaccharides and the MW distributions changed a little. Pile I reached the DOM stable stage more quickly (approximately 2 days earlier), and subsequently, the MC removal was mainly caused by physical aeration rather than microbial activity. To elucidate this notion, we further studied the temporal evolution of the microbial community structures in piles I and II.

Fig. 3 Temporal evolution of the concentrations of proteins and polysaccharides (a, b), molecular weight (c, d), and Fourier transform infrared spectroscopy (FTIR) analysis for pile I and pile II

Evolution of microbial community structure

A total of 793,234 high-quality sequences were obtained from 16 solid samples, ranging from 24,515 reads to 88,255 reads; details of the post-run analysis are listed in Tables S1 and S2. For an intercomparison at the same sequencing depth, all of the sample sequences were normalized to 24,515 reads for the downstream analysis.

The numbers of operational taxonomic units (OTUs), Chao 1, H', E, and varH at two cutoff levels (3 and 6 %) are summarized in Table S3. According to the rarefaction curves (Fig. S3) and the OTU numbers, the diversity of the microbial community in sludge bio-drying was decreasing in both piles. For sludge composting, a previous study has indicated that although the initial pile temperature decreased, the bacterial numbers may have decreased by one to two orders of magnitude, and the taxonomic and metabolic diversity increased (Ryckeboer et al. 2003). This suggests that a low moisture content for sludge bio-drying may not only affect the microbial activity but also affect the microbial diversity. The differences in the aeration strategies did not significantly ($P>0.05$) affect the evolution of the diversity of the microbial

community in either pile I or pile II. The profiles of Chao 1, H', E, and varH values were very similar to rarefaction curves and OTU numbers.

The 24,515 selected effective bacterial sequences in each sample were assigned to different taxa levels (from genus to phylum) using the RDP Classifier at a 50 % threshold. As shown in Fig. 4, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* were the dominant phyla in the bacterial communities of the 16 samples in this study, which is similar to previous studies (Yadav et al. 2014; Ibarbalz et al 2013; Xia et al. 2010). Although the types of the main taxa did not differ much among the samples between the different sludge bio-drying stages, the proportions were quite different (Fig. 4). During sludge bio-drying, the abundance of phylum *Actinobacteria* changed a little. However, phyla *Firmicutes* and *Proteobacteria* obviously decreased. They dominated in the thermophilic stage, but as the pile temperature and moisture content decreased, their abundances changed much, from maxima of 39.7 % (*Firmicutes* in pile I), 27.5 % (*Proteobacteria* in pile I) 42.1 % (*Firmicutes* in pile II), and 32.1 % (*Proteobacteria* in pile II) to minima of 8.0 % (*Firmicutes* in pile I), 10.3 % (*Proteobacteria* in pile I),

Fig. 4 Evolution of the bacterial community of piles I and II at the phylum level. The width of the bars from each phylum indicate the relative abundance of that phylum in the sample

10.6 % (*Firmicutes* in pile II), and 12.7 % (*Proteobacteria* in pile II). Meanwhile, the abundance of phylum *Bacteroidetes* increased by up to eight times, from minima of 6.9 and 5.16 % to 52.2 and 48.7 % for piles I and II, respectively.

For a detailed analysis of the microbial community structure, the 10 most abundant genera in each sample were selected (Fig. 5). The genus *Actinomadura* was abundant (3.0–10.3 %) in all of the samples, and their abundances in the

mesophilic stage were slightly higher. As proposed by Lechevalier and Lechevalier (1970), *Actinomadura* belongs to family *Thermomonosporaceae*, phylum *Actinomycetes*. Members of the genus are aerobic, Gram-positive, non-acid-fast actinomycetes that form non-fragmenting branched substrate mycelia and aerial hyphae bearing chains of up to 15 spores. The primary reservoir of *Actinomadura* is soil, and a few have been isolated from clinical materials (Yassin et al. 2010).

Fig. 5 Heat map of the 10 most prevalent genera in piles I and II (up). The 10 most abundant genera in each sample were selected (a total of 18 and 17 genera for piles I and II, respectively). Principal component analysis (PCA) for the bacterial communities of piles I and II (down)

The dominant genus in the thermophilic stage of sludge bio-drying was *Ureibacillus* (maximum, 8.6 %). Nakasaki et al. (2009) compared the microbial community during thermophilic composting of two different types of anaerobic sludge using PCR-DGGE, and *Ureibacillus* sp. was the observed dominant band during the composting of S-sludge (a high concentration of proteins). Jang et al. (2014) also used PCR-DGGE to study the microbial community of sludge thermophilic aerobic digestion as a sludge pre-treatment for mesophilic anaerobic digestion, and they found that *Ureibacillus thermophiles* and *Bacterium thermus* were the major contributors to the increase in soluble organic matter. *Ureibacillus* sp. is often detected in composting processes and is well known because of its good ability to degrade organic matter. Typical members of *Ureibacillus*, a genus comprised of thermophilic, aerobic, endospore-forming bacteria, are very active endospore-forming bacteria at temperatures near 50–60 °C. These microorganisms can produce thermoactive

hydrolytic extracellular enzymes (e.g., protease, lipase and cellulase) (Liu et al. 2010) and rapidly break down soluble and easily degradable carbon sources. The relatively high protein-like compound degradation rate (Fig. 2 region II) in the thermophilic stage was consistent with the appearance of *Ureibacillus*. These results suggest that *Ureibacillus* may be the dominant potential thermophilic bacteria in sludge bio-drying. As the sludge bio-drying progressed, the moisture content and pile temperature decreased, and easily degradable organic matter, such as proteins and microbial by-products in DOM (Fig. 2 regions II and IV), was exhausted, but fulvic and humic-like acids, such as hydrophobic acid (Fig. 2 regions III and V), increased. In the mesophilic stage, *Ureibacillus* abundance was much reduced to 0.9 % (minimum) from 8.6 % (maximum) for both piles I and II, and other genera, such as the moderately thermophilic and anaerobic genus *Tepidimicroblum*, were abundant in the thermophilic stage (a maximum of 2.7 and 2.1 % for piles I and II, respectively) but

were reduced in the mesophilic stage (a minimum of 0.2 and 0.3 % for piles I and II, respectively). The abundances of *Bacillus*, *Geobacillus*, and *Thauera* also showed the same trend (Fig. 5). However, some genera increased in the mesophilic stage of sludge bio-drying. For example, *Parapedobacter*, belonging to family *Sphingobacteriaceae*, increased sharply from 1.4 and 1.1 % (the minimum) to 19.1 and 14.2 % (the maximum) for piles I and II, respectively. Members of the genus *Parapedobacter*, which are Gram-reaction-negative, aerobic, non-spore-forming, rod-shaped, non-motile bacteria and oxidase and catalase positive, have often been isolated in soil and compost (Kim et al. 2010). Some species of the genus have been reported to be able to hydrolyze polycyclic aromatic hydrocarbon and use pyrene as a sole carbon source for growth (Zhao et al. 2013). The fluorescence intensity decrease in regions III and V in the mesophilic stage may be associated with genus *Parapedobacter*. Other genera, such as *Streptomyces*, *Pedobacter*, and *Olivibacter*, also increased much in the mesophilic stage (Fig. 5).

Discussion

DOM and its transformation drivers in sludge bio-drying

Three separate drying stages previously were identified during sludge bio-drying (Roy et al. 2006): (1) increasing drying rate due to acclimatization of microbes, (2) exponential decrease of the drying rate due to insufficient available nutrients for microbe consumption, and (3) constant drying rate due to the fluctuations of the aeration. This indicates that after some point, the sludge bio-drying was less dependent on the microbial activity and became instead only a physical process (air convection) (Velis et al. 2009). In this study, this point may have been day 5 and day 7 for piles I and II, respectively, because after that, MC removal was still increasing, but the components of the DOM changed a little for either pile (Figs. 2 and 3). In addition, according to the interplay of the DOM and microbial community structure, the sludge bio-drying can also be divided into three separate drying stages in this study (Fig. 5): in stage (I) (days 1, 3, and days 1, 3, 5 for pile I and pile II, respectively), the thermophilic bacteria-like genus *Ureibacillus* dominated, and the easily degradable organics (mainly protein-like substances and microbial metabolic by-products) in the DOM were degraded; in stage (II) (days 5 and 7 and day 7 for pile I and pile II, respectively), the moisture content and pile temperature decreased sharply, the abundance of thermophilic bacteria, such as *Ureibacillus*, decreased and other mesophilic bacteria, such as *Parapedobacter*, increased much, but the fulvic and humic-like acids began to decrease; in stage (III), bio-drying entered into a steady physical process promoted by aeration, and the

components of the DOM and the microbial community structure changed a little. Although both piles I and II experienced three separate stages, the timing of the stages was different. Pile I entered stage II on day 5, but pile II entered stage II on day 7 (Fig. 5). This suggests that a higher pile temperature due to optimized aeration strategy causes the bio-drying to enter into the microbiology stable stage more quickly (approximately 2 days ahead).

This study investigated the evolution of the degradation of organic matter and its microbial mechanisms in detail during sludge bio-drying. The study not only provides a practical basis but also presents chemical and microbial mechanisms to optimize the operation of sludge bio-drying and improve its performance. The following conclusions are drawn:

1. According to the EEM, SEC, FTIR, and concentrations of proteins and polysaccharide analysis, the degradation of the DOM mainly occurred in the thermophilic stage; after that, the DOM component was a little changed.
2. High-throughput sequencing results demonstrated that during sludge bio-drying, the main microbes that contributed to the degradation of DOM in the thermophilic stage belonged to genus *Ureibacillus*, and the main mesophilic microbes after the thermophilic stage belonged to genus *Parapedobacter*. As the sludge bio-drying progressed, the diversity of the microbial community decreased gradually.
3. The optimized aeration strategy caused the DOM and microbial community to enter into a stable stage much faster (approximately 2 days earlier). Not only did pile I (optimized) reach the designed moisture content (40 %) more quickly than pile II but also the final moisture content of pile I (33.1 %) was also lower than that of pile II (37.8 %).

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