



# Bacterial communities and their association with the bio-drying of sewage sludge

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## ARTICLE INFO

### Article history:

Received 27 September 2015

Received in revised form

10 December 2015

Accepted 16 December 2015

Available online 19 December 2015

### Keywords:

Bacterial communities

Bio-drying

Degradation

Sewage sludge

Water evaporation

Water generation

## ABSTRACT

Bio-drying is a technology that aims to remove water from a material using the microbial heat originating from organic matter degradation. However, the evolution of bacterial communities that are associated with the drying process has not been researched systematically. This study was performed to investigate the variations of bacterial communities and the relationships among bacterial communities, water evaporation, water generation, and organic matter degradation during the bio-drying of sewage sludge. High-throughput pyrosequencing was used to analyze the bacterial communities, while water evaporation and water generation were determined based on an in situ water vapor monitoring device. The values of water evaporation, water generation, and volatile solids degradation were 412.9 g kg<sup>-1</sup> sewage sludge bio-drying material (SSBM), 65.0 g kg<sup>-1</sup> SSBM, and 70.2 g kg<sup>-1</sup> SSBM, respectively. Rarefaction curves and diversity indices showed that bacterial diversity plummeted after the temperature of the bio-drying pile dramatically increased on d 2, which coincided with a remarkable increase of water evaporation on d 2. Bacterial diversity increased when the pile cooled. During the thermophilic phase, in which *Acinetobacter* and *Bacillus* were the dominant genera, the rates of water evaporation, water generation, and VS degradation peaked. These results implied that the elevated temperature reshaped the bacterial communities, which played a key role in water evaporation, and the high temperature also contributed to the effective elimination of pathogens.

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## 1. Introduction

By 2014 in China, approximately 129 million tonnes of municipal wastewater were treated daily in wastewater treatment plants (WWTPs). During this process, over 30 million tonnes of sewage sludge (SS), an unavoidable byproduct, were generated annually (National Bureau of Statistics of the People's Republic of China (2014)). Despite improvements in mechanical dewatering techniques during the past few decades, including centrifugation and the use of vacuum filters or belt filter presses, the moisture contents of sludge cake after mechanical dewatering in most WWTPs in China are still as high as 80–85% (Chen et al., 2011; Shen et al., 2012; Cai et al., 2013, 2015). This is obviously unsuitable for the

final disposal, and therefore an extended dewatering is often required. Thus, the moisture content should be reduced to an appropriate level for the subsequent disposal, and this should become a principal part of SS treatment (Huang et al., 2014). SS management in which dewatering plays a significant part accounts for between 49 and 53% of the total operating costs (Murray et al., 2008; Hospido et al., 2010), and improper treatment may lead to re-pollution because of the large amounts of pollutants in SS (VerBerkmoes et al., 2009; Poulsen and Bester, 2010). Consequently, drying SS poses economic and environmental challenges for WWTPs and municipalities around the globe (Raynaud et al., 2012; Jin et al., 2014).

On the other hand, bio-drying is a technology that aims to remove water from a pile of biodegradable material via aerobic microbial fermentation (Cai et al., 2012; Winkler et al., 2013). The initial mixture of materials for SS bio-drying is characterized by a high diversity of microbial flora; therefore, bio-drying is a highly dynamic process in which bacteria and fungi play a vital role. Because of the biologically-generated heat (bio-heat) and mechanical operations, bio-drying piles progress through mesophilic,

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thermophilic, and cooling phases (Cai et al., 2013), which are accompanied by the evolution of bacterial communities. The composition of bacterial populations during composting, a similar process based on aerobic fermentation, has been reported; e.g., *Pseudomonas* and *Bacillus* are the main decomposers of complex polymers, while *Luteaella thermophila* can metabolize many organic acids and amino acids, and *Ureibacillus* contributes to the degradation of organic compounds, especially cellulosic material (Casacchia et al., 2011; Kuok et al., 2012). Despite the previous studies of the bacterial populations in compost, variations in the bacterial communities that play key roles in the bio-drying process have not been fully investigated.

During bio-drying, bio-heat is crucial for water evaporation, and it is produced via the microbial degradation of compounds, such as complex carbon, cellulose, hemicelluloses, and proteins, in the material (Navaee-Ardeh et al., 2010; Storey et al., 2015). Fundamentally, the generation of bio-heat for water evaporation is dependent on the degradation of organic matter (Villegas and Huiliñir, 2014). Synchronously, the increase in the temperature of the pile that results from the production of bio-heat reshapes the bacterial communities (Su et al., 2015), and the shift in the bacterial communities influences organic matter degradation and bio-heat production, thereby ultimately affecting water evaporation (Cai et al., 2015). Additionally, bio-heat production is accompanied by water generation, which results from microbial metabolism and may impact the drying efficiency (Cai et al., 2012). Although bio-drying is a process that primarily results from the metabolic activities microorganisms (He et al., 2013), few studies have combined the study of water removal with investigations of bacterial communities during bio-drying.

The object of this study was to investigate (1) the variations in the bacterial communities during SS bio-drying, and (2) the relationships among the bacterial communities, water evaporation, and organic matter degradation. For this purpose, pilot-scale bio-drying was performed. The bacterial communities and the organic matter composition were analyzed, and the water evaporation and water generation values were calculated.

## 2. Materials and methods

### 2.1. Materials

The SS and bio-drying product were obtained from a SS treatment plant in Shanghai, China. Bio-drying product was the cured mixture of sewage sludge and bulk agent, which had been bio-dried before this series of experiments. Sawdust, which was used as the bulk agent (BA), was obtained from a timber mill. The SS, bio-drying product, and sawdust were mechanically mixed at a volumetric ratio of 3:2:1 to constitute the SS bio-drying material (SSBM). The moisture contents of the SS, BA, bio-drying product, and initial SSBM were 82.3, 20.6, 40.8, and 66.1%, respectively, the bulk density values were 1.04, 0.19, 0.49 and 0.80 g cm<sup>-3</sup>, respectively, and the volatile solids (VS) contents were 613.0 g kg<sup>-1</sup> SS, 983.3 g kg<sup>-1</sup> BA, 669.2 g kg<sup>-1</sup> SSBM, and 737.5 g kg<sup>-1</sup> SSBM, respectively.

### 2.2. Experimental procedure

The initial SSBM was loaded into a tank to form a pile. The bio-drying pile was 1.6 m in height (Chen et al., 2011), and an unsealed cylinder made of hydrophobic material, with an internal diameter of 1.13 m and a cross-sectional area of 1.0 m<sup>2</sup>, was vertically installed within the pile; the volume and weight of the experimental pile were 1.6 m<sup>3</sup> and 1280 kg, respectively. A water vapor sensor, which consisted of an ultrasonic anemometer (Gill Instruments, Lymington, UK), a temperature sensor, and a humidity

sensor (Rotronic, Bassersdorf, Switzerland), was installed along the central axis of the cylinder 0.5 m above the pile surface to collect the data for the water calculations. A thermal flowmeter (Virvo, Madison, WI, USA) was located in the ventilation duct to measure the air volume aerated into the pile. The pile was subjected to an auto control technology for bio-drying (CTB, GreenTech Environmental Engineering Co., Beijing, China). The experimental equipment is shown in Fig. S1.

During the 20-day bio-drying period, the pile was aerated intermittently by an air blower, and it was mechanically turned four times (on d 9, 12, 15, and 18). The on-line temperature monitoring indicated that the pile experienced a mesophilic phase, an initial thermophilic phase (pile temperature higher than 50 °C), a second thermophilic phase (after the turning on d 9, the pile temperature dropped and then rebounded), and a cooling phase.

### 2.3. Sample collection

Samples that were subjected to analysis consisted of the SS (collected at 22.5 °C), BA (collected at 22.5 °C), and SSBM during the mesophilic phase (collected on d 0 at 25.5 °C and on d 2 at 48.5 °C), the first thermophilic phase on d 6 (collected at 68.3 °C), the second thermophilic phase on d 11 (collected at 50.3 °C), the cooling phase on d 16 (collected at 38.7 °C), and the product on d 20 (which was collected at 21.9 °C).

### 2.4. DNA extraction, polymerase chain reaction, and pyrosequencing

DNA was extracted from the samples (0.5 g wet weight) with the E.Z.N.A. Bacterial DNA Kit for Soil (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. Successful DNA isolation was confirmed by agarose gel electrophoresis. Primers (338F 5'-ACTCTACGGGAGGCAGCA-3' and 806R 5'-GGAC-TACHVGGGTWCTAAT-3') targeting the V1–V3 region of the 16S bacterial rRNA gene were chosen for the amplification and subsequent pyrosequencing of the polymerase chain reaction (PCR) products (Majorbio Bio-Pharm Technology Co., Shanghai, China). The PCR conditions were as follows: 95 °C for 3 min, followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The PCR products were purified using a DNA gel extraction kit (Axygen, Tewksbury, MA, USA), and then subjected to pyrosequencing using the Roche 454 FLX Titanium Sequencer at the Majorbio Bio-Pharm Technology Co., Shanghai, China.

### 2.5. Water calculations

Water evaporation included the water evaporated during the bio-drying and water loss by turning (Zhao et al., 2010; Huiliñir and Villegas, 2015), which can be obtained by integrating the vapor flux as follows. The humidity, temperature, and air velocity of the airflow above the pile surface were measured using the vapor sensor, and the vapor flux was calculated using the following formula:

$$e = q_e \cdot u_e \cdot \rho_e \quad (1)$$

where  $e$  is the vapor flux above the pile surface (kg m<sup>-2</sup> s<sup>-1</sup>);  $q_e$  is the specific humidity of the airflow above the pile surface (kg water kg<sup>-1</sup> air);  $u_e$  is the vertical air velocity of the airflow above the pile surface (m s<sup>-1</sup>); and  $\rho_e$  is the density of the air (kg m<sup>-3</sup>).

Water generation can be calculated according to the water mass balance of the SSBM:

$$M_{H_2O,t-1} + \Delta M_{H_2O,g} + I = M_{H_2O,t} + E \quad (2)$$

where  $M_{H_2O,t-1}$  is the total moisture of the pile on day  $t - 1$  (kg);  $\Delta M_{H_2O,g}$  is the water generation (kg);  $I$  is the water input via aeration (kg);  $M_{H_2O,t}$  is the total moisture of the pile on day  $t$  (kg); and  $E$  is the water evaporation (kg).

The details of the calculations are shown in Table 1.

## 2.6. Organic matter analysis

The organic matter contents in the SSBM were analyzed by determining the VS as described by the US Department of Agriculture and the US Composting Council (2001). Additionally, the organic matter components, including the hydrolysable compounds, lipids, hemicellulose, cellulose, lignin, and humic acid contents, were analyzed by the fractional extraction and gravimetric method as previously described (Bao, 2000). Hydrolysable compounds and lipids are easily degradable organic compounds, while hemicellulose, cellulose, and lignin are more resistant organic substrates that may be partially degraded and slowly transformed. Humic acid is a polymerized compound that is produced from degraded organic matter (Chaari et al., 2015).

**Table 1**  
Details of the water evaporation and water generation calculations.

Equations	
Total weight of moisture in the pile on day $t$ :	
$M_{H_2O,t} = M_0 \frac{(1-MC_0)(1-VS_0)}{(1-MC_t)(1-VS_t)} MC_t$	
Vapor flux above the pile:	
$e = q_e \cdot u_e \cdot \rho_e$	
Specific humidity of the airflow above the pile:	
$q_e = \beta \cdot \frac{M_{water}}{M_{air}} \cdot \frac{10^{8.896 - \frac{2738}{T-273}}}{760 - 10^{8.896 - \frac{2738}{T-273}}}$	
Water evaporation in a period:	
$E = \int_{t_0}^{t_1} f(t) dt$	
Water vapor input per second:	
$i = q_i \cdot Q_i \cdot \rho_i$	
Aeration water input in a period:	
$I = \int_{t_0}^{t_1} g(t) dt$	
Water mass balance of the pile:	
$M_{H_2O,t-1} + \Delta M_{H_2O,g} + I = M_{H_2O,t} + E$	
Water generation:	
$\Delta M_{H_2O,g} = E - I - (M_{H_2O,t-1} - M_{H_2O,t})$	
Nomenclature	
$M_{H_2O,t}$	Total weight of moisture in the pile on day $t$ (kg)
$M_{H_2O,t-1}$	Total weight of moisture in the pile on day $t-1$ (kg)
$M_0$	Initial weight of the pile on day 1 (kg)
$MC_0$	Moisture content of the bio-drying material on day 1 (%)
$VS_0$	Volatile solids content of the bio-drying material on day 1 (%)
$e$	Vapor flux above the pile ( $\text{kg m}^{-2} \text{s}^{-1}$ )
$q_e$	Specific humidity of the airflow above the pile ( $\text{kg water kg}^{-1} \text{air}$ )
$u_e$	Vertical air velocity of the airflow above the pile ( $\text{m s}^{-1}$ )
$\rho_e$	Air density above the pile ( $\text{kg m}^{-3}$ )
$M_{water}$	Molecular mass of water ( $\text{g mol}^{-1}$ )
$M_{air}$	Molecular mass of air ( $\text{g mol}^{-1}$ )
$T$	Air temperature ( $^{\circ}\text{C}$ )
$\beta$	Relative air humidity (%)
$E$	Water evaporation (kg)
$t_0$	Start time (s)
$t_1$	End time (s)
$f(t)$	Function of $e$ ( $\text{kg m}^{-2} \text{s}^{-1}$ )
$i$	Water vapor input per second ( $\text{kg s}^{-1}$ )
$q_i$	Specific humidity of the forced air via aeration ( $\text{kg water kg}^{-1} \text{air}$ )
$Q_i$	Volume of the forced air ( $\text{m}^3 \text{s}^{-1}$ )
$\rho_i$	Air density of the forced air ( $\text{kg m}^{-3}$ )
$I$	Water input via the aeration (kg)
$g(t)$	Function of $i$ ( $\text{kg s}^{-1}$ )
$\Delta M_{H_2O,g}$	Water generation (kg)

## 2.7. Data processing

The operational taxonomic units (OTUs) were determined following the online instructions of the Quantitative Insights Into Microbial Ecology software (Caporaso et al., 2010). OTUs were defined at the 97% similarity level using UCLUST clustering (Edgar, 2010). The difference between microbial communities was compared using Bray–Curtis distances, followed by principal coordinate analysis (PCoA). To understand the richness of species at different periods of bio-drying, two diversity indices, Shannon and inverse Simpson indices were determined using R3.1.0 (R Development Core Team, 2010). A higher value for the Shannon index or a lower value for the Simpson index indicates greater microbial diversity (Velvizhi and Venkata Mohan, 2015; Su et al., 2015). A heat map was conducted using the pheatmap package (Kolde, 2013). Pearson's correlation test and other tests were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA) and Origin 9.0 (OriginLab, Northampton, MA, USA), respectively.

## 3. Results

### 3.1. Bacterial communities

#### 3.1.1. Decrease in bacterial diversity after bio-drying

High-quality sequences were clustered into 692 OTUs at the 97% similarity level. Rarefaction curves of OTUs became flatter to the right, indicating an effective sampling of community diversity. The curves also presented the species richness of various samples, which indicated that the bacterial diversity decreased after the self-heating of the bio-drying pile (Fig. S2). The high temperature of the pile during the bio-drying altered the microbial communities. Shannon and inverse Simpson indices showed that the highest diversity values were found on d 0, and bacterial diversity decreased after the temperature of the pile increased on d 2, compared with that on d 0 and in the SS. However, when the temperature fell below  $50^{\circ}\text{C}$ , the bio-drying entered the cooling phase, and bacterial diversity recovered, yet it was lower than that of the SS (Table 2).

PCoA showed that samples obtained prior to self-heating (SS and d 0), during self-heating (d 2 and 6), and during the cooling phase (d 16 and 20) clustered into three separate groups (Fig. 1). The sample from d 11, which was obtained during the second thermophilic phase, fell between the d 2 and 6 and d 16 and 20 groups. The first two PCs explained 64.0% of the variance of the bacterial communities. The SSBMs on d 2 and 6 were separated from that of the control SS and other SSBMs along PC2, which explained 25.71% of the variation, implying that the variation of the bacterial communities occurred in part because of the high temperature during the first thermophilic phase.

#### 3.1.2. Shift in bacterial abundance

The compositions of bacterial communities displayed distinct temporal variations during the SS bio-drying at the phylum level, as presented in Fig. 2, and all detected bacterial communities were clustered into four subgroups. For all samples, bacterial communities in group B were highly abundant, while those in groups A and C were of low abundance, and those in group D were moderately abundant. Groups C and D showed that the abundances of the communities decreased after the bio-heating of the pile.

Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria were the four most dominant phyla during the entire bio-drying process, collectively accounting for 91.5% and 99.7% of the total sequences on d 0 and 20, respectively. The percentages of the Bacteroidetes and Actinobacteria decreased from 26.6% to 3.3% on

**Table 2**  
Shannon and inverse Simpson indices for different samples.

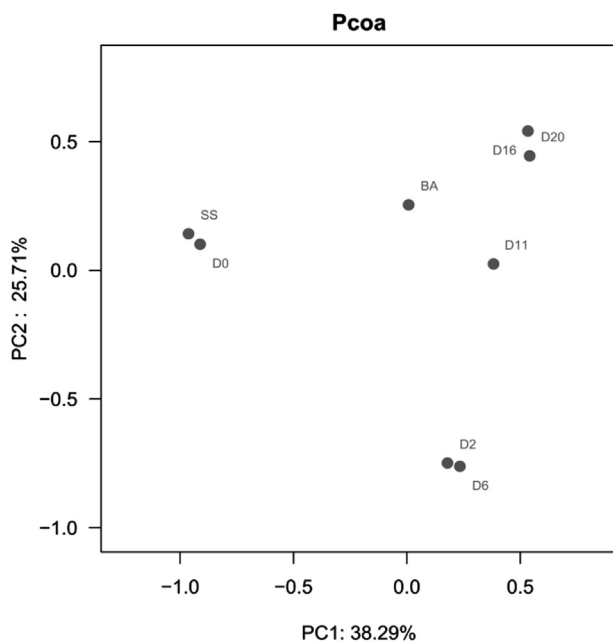
Samples	Phase belonged	Temperature when collected (°C)	Defined at 97% similarity level		
			Shannon	Simpson	Coverage
SS	—	22.5	4.77	0.025	0.997
D0	Mesophilic	25.5	5.16	0.014	0.993
D2	Mesophilic	48.5	3.77	0.060	0.992
D6	First thermophilic	68.3	3.84	0.049	0.994
D11	Second thermophilic	50.3	3.52	0.066	0.993
D16	Cooling	38.7	3.56	0.055	0.994
D20	—	21.9	4.11	0.032	0.995

d 0–1.4% and 1.8% on d 11 during the thermophilic phase, respectively, but rebounded in the cooling phase and reached 39.1% and 6.8% on d 20, respectively. In contrast, the Proteobacteria and Firmicutes were enriched from 46.8% to 14.8% on d 0 to their peak values of 65.3% on d 2 and 82.9% on d 11, respectively, after the temperature of the pile increased. Most of these bacteria have been recognized as thermophiles, such as *Acinetobacter* (phylum Proteobacteria) and *Bacillus* (phylum Firmicutes) (Eichorst et al., 2013; Nakasaki et al., 2013). The abundances of the bacterial communities in groups C and D decreased after entering the thermophilic phase, and showed no increases after the pile cooled, implying that they were killed at temperatures greater than 50 °C.

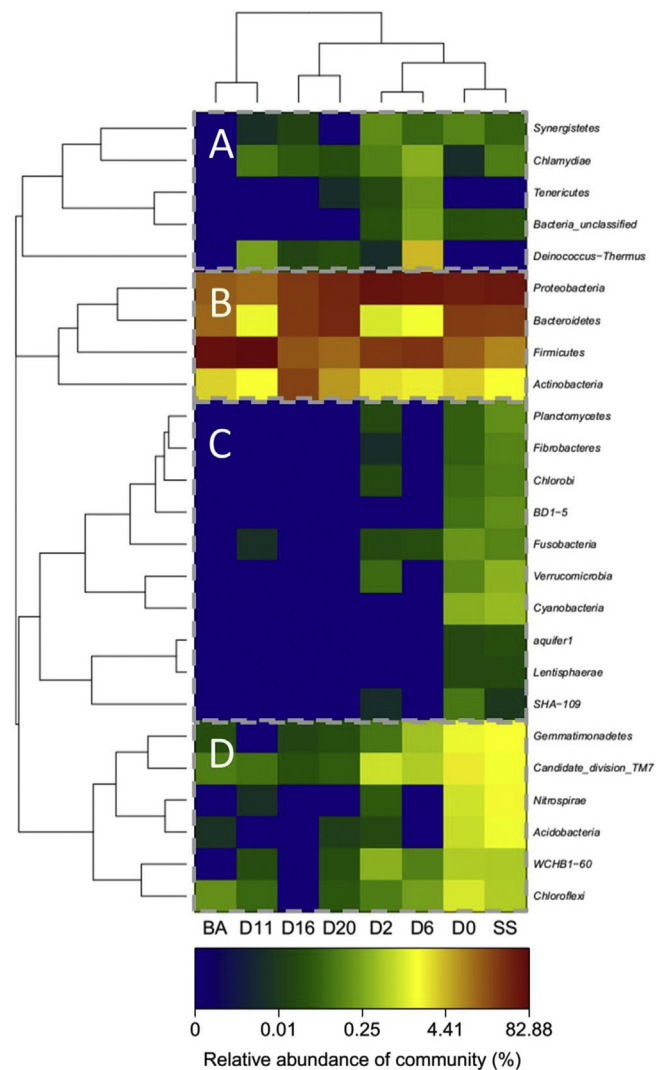
The dominant bacteria (at the genus level) during the different phases were as follows.

**Mesophilic phase:** At the beginning of this phase, no genera were present in high proportions in the SSBM (Fig. S3). *Ferribacterium* (6.8%, phylum Proteobacteria), *Arcobacter* (5.7%, phylum Proteobacteria), *Flavobacterium* (4.5%, phylum Bacteroidetes), and a non-culturable genus of *Chitinophagaceae* (1.6%, phylum

Bacteroidetes) were dominant in the initial mesophilic phase on d 0. As the temperature of the pile increased, the abundances of these genera were significantly ( $P < 0.05$ ) lower on d 2, as well as in the bio-drying product, except that the abundance of



**Fig. 1.** Principal coordinate analysis (PCoA) showing the potentially correlated variables of the bacterial distribution pattern. Sewage sludge (SS) and the bulk agent (BA) were collected at 22.5 °C, and the d 0 (D0) samples were collected at 25.5 °C at the beginning of the mesophilic phase; d 2 (D2) samples were collected at 48.5 °C at the end of the mesophilic phase, as well as at the beginning of the first thermophilic phase, and d 6 (D6) samples were collected at 68.3 °C during the first thermophilic phase; d 11 (D11) samples were collected at 50.3 °C during the second thermophilic phase; d 16 (D16) samples were collected at 38.7 °C during the cooling phase, and d 20 (D20) samples were collected at 21.9 °C at the end of the bio-drying process.



**Fig. 2.** Heat map analysis of bacterial communities showing their distribution at the phylum level. The bulk agent (BA) and sewage sludge (SS) were collected at 22.5 °C; d 0 (D0) samples were collected at 25.5 °C and d 2 (D2) samples were collected at 48.5 °C during the mesophilic phase; d 6 (D6) samples were collected at 68.3 °C during the first thermophilic phase, and d 11 (D11) samples were collected at 50.3 °C during the second thermophilic phase; d 16 (D16) samples were collected at 38.7 °C during the cooling phase; d 20 (D20) samples were collected at 21.9 °C at the end of the bio-drying process.



*Flavobacterium* rebounded during the cooling phase, becoming the second most dominant genus at the end of the bio-drying process (10.9% on d 20). At the later stage of the mesophilic phase, *Acinetobacter* (phylum Proteobacteria) was enriched from 0.8% on d 0–47.2% on d 2, becoming the most abundant thermophilic genus, and its abundance decreased to 1.6% on d 20 when the bio-drying ended. The abundances of *Tepidimicrobium* (phylum Firmicutes) and *Comamonas* (phylum Proteobacteria) increased from 0.03% to 0.5% on d 0–7.4% and 5.5% on d 2, respectively, and they were the second and third most dominant genera, respectively, in the later stage of the mesophilic phase.

**Thermophilic phase:** In the first thermophilic phase, *Acinetobacter* (phylum Proteobacteria) maintained its predominance, accounting for 42.3% of the bacteria on d 6, followed by the genus *Bacillus* (phylum Firmicutes) (5.8% on d 6). In the second thermophilic phase, *Bacillus* (phylum Firmicutes) became the most abundant genus (51.6% on d 11), although its abundance decreased to 5.9% on d 20, making it the fourth most abundant bacteria when the bio-drying ended. *Pseudomonas* (phylum Proteobacteria) was the third most abundant genus in the BA (7.2%), and its abundance decreased during the first thermophilic phase, but increased after the second thermophilic phase, reaching a proportion of 3.5% on d 11.

**End of bio-drying:** *Spingobacterium* (phylum Bacteroidetes) was the most dominant genus when the bio-drying ended (23.8%), followed by *Flavobacterium* (10.9%).

### 3.2. Water variation

The moisture content of the SSBM decreased from 66.1 to 54.7% after the 20-day bio-drying process. The water evaporation rate per day is presented in Fig. 3, and it totaled  $412.9 \text{ g kg}^{-1}$  SSBM during the entire process. From d 2, the daily evaporation rate drastically increased, peaking at  $37.3 \text{ g kg}^{-1}$  SSBM  $\text{d}^{-1}$  on d 4. Then, the water evaporation rate gradually decreased until the pile was mechanically turned on d 9. Either after the first turning on d 9 or after the second turning on d 12, the water evaporation rate increased, which was mainly attributed to the elimination of pile compaction and the biodegradation of some undegraded material (Cai et al., 2015). After d 14, the water evaporation rate declined to  $4.1 \text{ g kg}^{-1}$  SSBM  $\text{d}^{-1}$ .

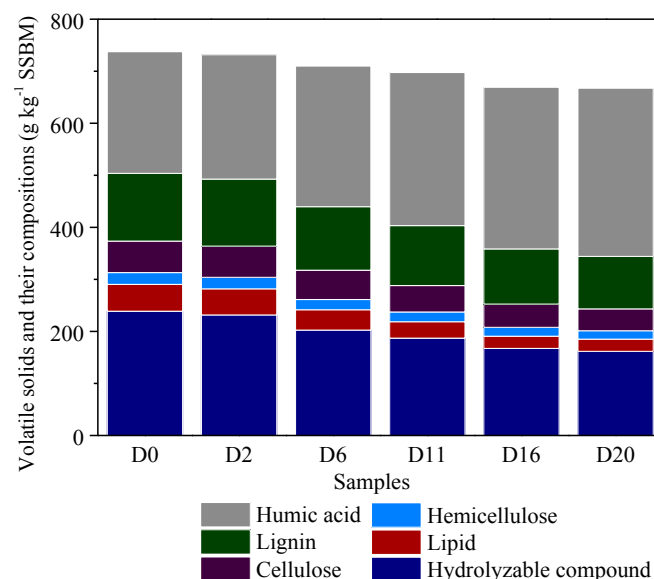
The water generation, which is also shown in Fig. 3, totaled  $65.0 \text{ g kg}^{-1}$  SSBM during the entire process, and the ratio of water generation to water evaporation was about 1:6.3. The water

generation rate increased after the mesophilic phase, and it peaked at  $9.40 \text{ g kg}^{-1}$  SSBM  $\text{d}^{-1}$  on d 3, and then decreased. After the first turning, the water generation rate rebounded to  $8.50 \text{ g kg}^{-1}$  SSBM  $\text{d}^{-1}$ , and then gradually decreased, reaching  $0.16 \text{ g kg}^{-1}$  SSBM  $\text{d}^{-1}$  on day 20.

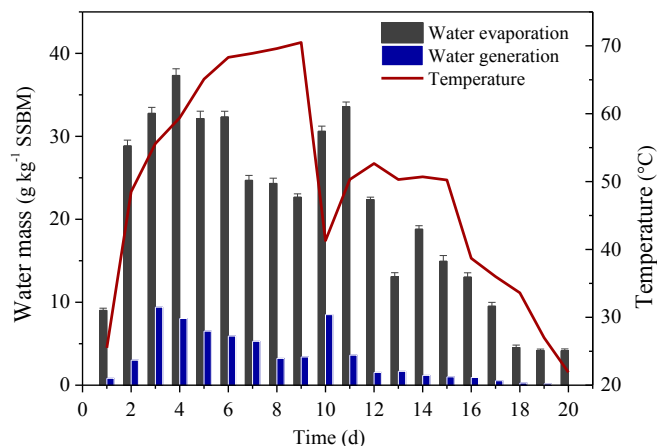
### 3.3. Organic matter

The organic matter content, which is represented by the VS and the compositions of different samples, is presented in Fig. 4. The VS decreased from  $737.5 \text{ g kg}^{-1}$  SSBM to  $667.3 \text{ g kg}^{-1}$  SSBM after the bio-drying process, and the total degradation of organic matter was  $70.2 \text{ g kg}^{-1}$  SSBM. The stage between d 2 and 6, as well as the stage between d 11 and 16, were the top two periods for the degradation of organic matter, with degradation values of  $21.8 \text{ g kg}^{-1}$  SSBM and  $21.5 \text{ g kg}^{-1}$  SSBM, respectively. On d 0, the contents of hydrolysable compounds and lipids were  $238.8 \text{ g kg}^{-1}$  SSBM and  $51.5 \text{ g kg}^{-1}$  SSBM, accounting for 32.4 and 7.0% of the VS, respectively, and the humic acid content was  $233.8 \text{ g kg}^{-1}$  SSBM, accounting for 31.7% of the VS. After the 20-day bio-drying process, the contents of the hydrolysable compounds and lipids decreased to  $161.7 \text{ g kg}^{-1}$  SSBM and  $23.4 \text{ g kg}^{-1}$  SSBM, accounting for 24.2 and 3.5% of the VS, respectively, while the humic acid content increased to  $323.2 \text{ g kg}^{-1}$  SSBM, accounting for 48.4% of the VS. Additionally, the hemicellulose, cellulose, and lignin contents decreased from  $22.7 \text{ g kg}^{-1}$  SSBM,  $60.5 \text{ g kg}^{-1}$  SSBM, and  $130.2 \text{ g kg}^{-1}$  SSBM to  $16.2 \text{ g kg}^{-1}$  SSBM,  $42.0 \text{ g kg}^{-1}$  SSBM, and  $100.8 \text{ g kg}^{-1}$  SSBM, respectively.

In general, for the overall bio-drying process, the degradation of hydrolysable compounds was the greatest, with a degradation value of  $77.1 \text{ g kg}^{-1}$  SSBM, representing 32.3% of the total degradation. The degradation values of lipids, hemicellulose, cellulose, and lignin were  $28.2 \text{ g kg}^{-1}$  SSBM,  $6.5 \text{ g kg}^{-1}$  SSBM,  $18.4 \text{ g kg}^{-1}$  SSBM, and  $29.4 \text{ g kg}^{-1}$  SSBM, and their corresponding degradation proportions were 54.7, 28.6, 30.5, and 22.6%, respectively.



**Fig. 4.** Volatile solids and their compositions in different samples of sewage sludge bio-drying material. Samples on d 0 (D0) were collected at  $25.5^\circ\text{C}$ , and d 2 (D2) samples were collected at  $48.5^\circ\text{C}$  during the mesophilic phase; d 6 (D6) samples were collected at  $68.3^\circ\text{C}$  during the first thermophilic phase, and d 11 (D11) samples were collected at  $50.3^\circ\text{C}$  during the second thermophilic phase; d 16 (D16) samples were collected at  $38.7^\circ\text{C}$  during the cooling phase; d 20 (D20) samples were collected at  $21.9^\circ\text{C}$  at the end of the bio-drying process.



**Fig. 3.** Water evaporation, water generation, and pile temperature during the bio-drying process. The pile was mechanically turned four times (on d 9, 12, 15, and 18). After the turning on d 9, the pile temperature plummeted and then rebounded.

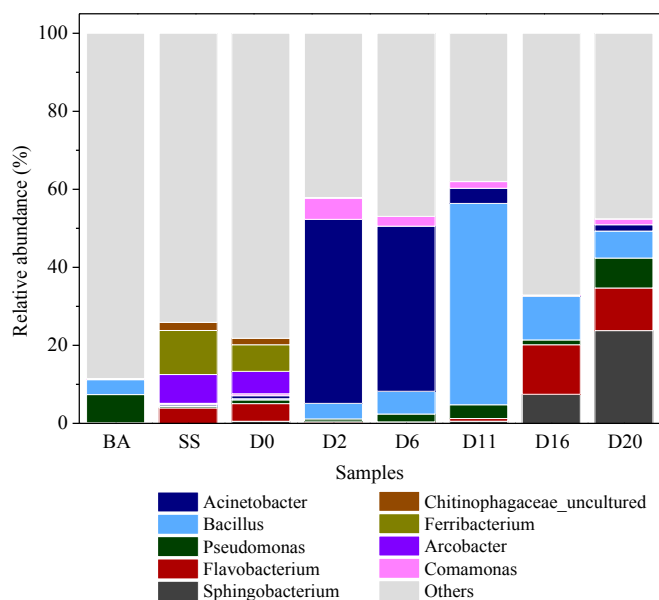
Conversely, the amount of humic acid increased by 38.2%–89.4 g kg<sup>-1</sup> SSBM. In the stage between d 2 and 6 (the early and middle stage of the first thermophilic phase), the daily degradation rates of the hydrolysable compounds, lipids, and hemicellulose, which can be seen in Fig. 4, reached their maxima. In the stage between d 11 and 16 (the second thermophilic phase), the daily degradation rates of the hydrolysable compounds, lipids, and hemicellulose recovered, and those of cellulose and lignin reached their maxima.

## 4. Discussion

### 4.1. Decomposition of the dominant bacteria

The dominant bacteria in different phases (Fig. 5) and their target decompositions, which were collected from the literature, were used to specify the bio-drying in terms of microbial degradation.

Mesophilic phase: *Ferribacterium*, *Arcobacter*, *Flavobacterium*, and a non-culturable genus of *Chitinophagaceae* were the dominant genera during the initial mesophilic phase. *Ferribacterium* are Fe (III)-reducing bacteria (Cummings et al., 1999), while *Flavobacterium* has been reported to degrade lignin and pentachlorophenol (Scelza et al., 2008; Aarthi et al., 2004), and *Chitinophagaceae\_uncultured* is capable of degrading cellulose (Eichorst et al., 2013). *Arcobacter*, identified as a pathogenic genus (Merga et al., 2014), was inactivated during the thermophilic phase, and it was not detected on d 16 and 20, which was beneficial to the utilization of the bio-drying product. At the later stage of the mesophilic phase, *Acinetobacter*, *Tepidimicrobium*, and *Comamonas* became the dominant genera. *Acinetobacter* can degrade lignin and diesel fuel, as well as reduce nitrate to ammonia (Trois et al., 2010; Mara et al., 2012). *Comamonas* can metabolize lignin and other complex organic compounds (Young et al., 2008).



**Fig. 5.** Percentages of dominant bacteria and the total percentages of non-dominant bacteria at the genus level after the heating of the pile. Samples from d 2 (D2), 6 (D6), and 11 (D11) were collected at 48.5 °C, 68.3 °C, and 50.3 °C, respectively, after the remarkable heating of the pile; the bulk agent (BA), sewage sludge (SS), and d 0 (D0) samples were collected at 22.5 °C, 22.5 °C, and 25.5 °C, respectively, before the remarkable heating of the pile; d 16 (D16) samples were collected at 38.7 °C during the cooling phase, and the d 20 (D20) samples were collected at 21.9 °C at the end of the bio-drying process.

Thermophilic phase: *Acinetobacter* was the most abundant thermophilic genus during the first thermophilic phase, and it can degrade lignin and diesel fuel, as well as reduce nitrate to ammonia (Trois et al., 2010; Mara et al., 2012). *Bacillus* was the most abundant genus during the second thermophilic phase, and *Pseudomonas* was also one of the dominant genera. *Bacillus* can degrade protein and starch, while *Pseudomonas* can degrade lipids and lignin, and are also known as competent cellulose-degrading bacteria (Ten et al., 2005; Prabhakaran et al., 2015).

End of the bio-drying process: *Sphingobacterium*, which can degrade fatty acids and some other complex organic compounds (Kuo et al., 1999), was the dominant genus when the bio-drying ended.

### 4.2. Water evaporation was increased by bacterial degradation

Bio-heat was produced when various microorganisms degraded various substances, which caused the temperature of the bio-drying pile to increase and resulted in distinct phases that were defined based on the pile temperature. The changes in temperature reshaped the bacterial communities that played important roles in substance degradation and heat production. During this process, the microorganisms that produced bio-heat to increase the water evaporation generated metabolic water during the degradation of organic matter. To illustrate the relationships among VS degradation, water generation, and water evaporation, their variations in each phase were calculated as follows (Table 3).

In the mesophilic phase, bacterial diversity was the highest, and even the most dominant genus accounted for only 6.8% of the bacteria. During this period, the VS degradation value was 5.9 g kg<sup>-1</sup> SSBM, the water generation value was 3.8 g kg<sup>-1</sup> SSBM, and the water evaporation value was 37.9 g kg<sup>-1</sup> SSBM.

However, the bacterial diversity plummeted after the self-heating of the pile. Simultaneously, there was a dramatic increase in water evaporation on d 2. The bacterial diversity reached its lowest level on d 11 according to the Shannon and inverse Simpson indices (Table 2), indicating a selective effect of high temperature. During the thermophilic phase, in which *Acinetobacter* and *Bacillus* were the dominant genera, the VS degradation, water generation, and water evaporation all reached their peak values of 62.5 g kg<sup>-1</sup> SSBM, 59.3 g kg<sup>-1</sup> SSBM, and 337.7 g kg<sup>-1</sup> SSBM, respectively. The remarkable evaporation value could be due to the large amount of bio-heat produced by the microorganisms and the convection promoted by the mechanical aeration. The elevated temperature also contributed to the effective elimination of pathogens, such as *Arcobacter* and *Prevotella*, which existed in the SS and were present on d 0, but which were not detected in the bio-drying product.

In the early and middle stage of the first thermophilic phase (from d 2 to 6), the daily degradation rates of hydrolysable compounds, lipids, and hemicellulose all reached their maxima, while the daily water evaporation peaked, as presented in Fig. 3. Nevertheless, the daily degradation rates of these organic matters decreased after d 6 (the late stage of the first thermophilic phase) because of the high temperature and the compaction effects on the pile, which may kill most bacteria. Consequently, a decrease in water evaporation was also observed after d 6. Thus, the decline in both the compound degradation and water evaporation rates indicated that sufficient aeration and mechanical turning were required to prevent the pile from overheating, as well as to provide a porous environment for aerobic degradation.

After the mechanical turning on d 9, the bio-drying pile entered its second thermophilic phase, in which the daily degradation rate of organic matter increased as a result of the vigorous metabolism activities of thermophilic bacteria such as *Bacillus* and *Pseudomonas*. Similarly, the water evaporation rate increased and reached

**Table 3**Volatile solids degradation, water generation, and water evaporation during each phase (unit: g kg<sup>-1</sup> sewage sludge bio-drying material (SSBM)).

Phase	Volatile solids degradation	Water generation	Water evaporation
Mesophilic phase (2 days)	5.9	3.8	37.9
Thermophilic phase (13 days)	62.5	59.3	337.7
Cooling phase (5 days)	1.8	1.9	37.3

another peak on d 11, as shown in Fig. 3.

During the cooling phase, bacterial diversity recovered, although it was less than that before bio-drying, which was also observed in the study by Caracciolo et al. (2015) during the maturation phase of composting. The VS degradation value was 1.8 g kg<sup>-1</sup> SSBM, the water generation value was 1.9 g kg<sup>-1</sup> SSBM, and the water evaporation value was 37.3 g kg<sup>-1</sup> SSBM. The low VS degradation and water generation rates implied that there was a decline in microbial metabolism despite the extremely large water evaporation value, which was mainly due to sufficient aeration.

In the initial stage of the mesophilic phase, no bacterial genera were present in high proportions in the SSBM. After entering the thermophilic phase, *Acinetobacter*, *Bacillus*, and *Pseudomonas*, which can degrade readily biodegradable substances, such as hydrolysable compounds, lipids, and complex polymers, such as proteins, lignin, and cellulose, became the dominant genera. Followed by the abrupt increase in the abundances of the dominant bacteria, the water evaporation rate increased significantly, which was attributed to the efficient degradation of organic matter after d 2. During the cooling phase, the water evaporation rate declined, as did the bacterial degradation of organic matter.

## 5. Conclusions

- The moisture content of the SSBM decreased from 66.1 to 54.7% after the 20-day bio-drying process, at which time the water evaporation, water generation, and VS degradation values were 412.9 g kg<sup>-1</sup> SSBM, 65.0 g kg<sup>-1</sup> SSBM, and 70.2 g kg<sup>-1</sup> SSBM, respectively, which is approximately equivalent to a 6:1:1 ratio.
- Rarefaction curves and diversity indices implied that the pile temperature shaped the bacterial communities, and that bacterial diversity plummeted after entering the thermophilic phase and rebounded during the cooling phase. PCoA implied that the cluster distribution of the bacterial communities from different samples may be due to the evolution of the pile temperature.
- During the thermophilic phase, in which the dominant genera were *Acinetobacter* (42.3% during the first thermophilic phase) and *Bacillus* (51.6% during the second thermophilic phase), the daily water evaporation, water generation, and VS degradation rates all peaked. Especially during the early and middle stage of the first thermophilic phase, the daily degradation rates of hydrolysable compounds, lipids, and hemicellulose all reached their maxima, thereby propelling the daily water evaporation rate to its maximum value, while the degradation rates of cellulose and lignin reached their maxima during the second thermophilic phase.

## Acknowledgments

This study was funded by the National Natural Science Funds of China (Grant No. 41401538 and No.51408325) and the Zhejiang Provincial Natural Science Foundation of China (Grant No. LQ14D010001), and it was sponsored by the K. C. Wong Magna Fund at Ningbo University.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2015.12.026>.

## References

- Aarthi, T., Leelapriya, T., Kalaichelvan, P.T., Dhilip, K.S., Sanker Narayan, P.V., 2004. Application of weak sinusoidal magnetic field on *Flavobacterium* species in the treatment of paper mill effluent. *Electromagn. Biol. Med.* 23 (3), 215–227.
- Bao, S.D., 2000. *Soil Assay on Properties of Agro-chemistry*, third ed. China Agriculture Press, Beijing (in Chinese).
- Cai, L., Chen, T.B., Gao, D., Zheng, G.D., Liu, H.T., Pan, T.H., 2013. Influence of forced air volume on water evaporation during sewage sludge bio-drying. *Water Res.* 47 (13), 4767–4773.
- Cai, L., Gao, D., Chen, T., Liu, H., Zheng, G., Yang, Q., 2012. Moisture variation associated with water input and evaporation during sewage sludge bio-drying. *Bioresour. Technol.* 117, 13–19.
- Cai, L., Gao, D., Hong, N., 2015. The effects of different mechanical turning regimes on heat changes and evaporation during sewage sludge biodrying. *Dry. Technol.* 33 (10), 1151–1158.
- Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7 (5), 335–336.
- Caracciolo, A.B., Bustamante, M.A., Nogues, I., Lenola, M.D., Luprano, M.L., Grenni, P., 2015. Changes in microbial community structure and functioning of a semiarid soil due to the use of anaerobic digestate derived composts and rosemary plants. *Geoderma* 245–246, 89–97.
- Casacchia, T., Toscano, P., Sofo, A., Perri, E., 2011. Assessment of microbial pools by an innovative microbiological technique during the co-composting of olive mill by-products. *Agric. Sci.* 2 (2), 104–110.
- Chaari, L., Elloumi, N., Mseddi, S., Gargouri, K., Rouina, B.B., Mechichi, T., Kallel, M., 2015. Changes in soil macronutrients after a long-term application of olive mill wastewater. *J. Agric. Chem. Environ.* 4 (1), 1–13.
- Chen, J., Chen, T.B., Gao, D., Lei, M., Zheng, G.D., Liu, H.T., Guo, S.L., Cai, L., 2011. Reducing H<sub>2</sub>S production by O<sub>2</sub> feedback control during large-scale sewage sludge composting. *Waste Manag.* 31 (1), 65–70.
- Cummings, D.E., Caccavo Jr., F., Spring, S., Rosenzweig, R.F., 1999. *Ferritribacterium limneticum*, gen. nov., sp. nov., an Fe(III)-reducing microorganism isolated from mining-impacted freshwater lake sediments. *Arch. Microbiol.* 171 (3), 183–188.
- Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26 (19), 2460–2461.
- Eichorst, S.A., Varanasi, P., Stavila, V., Zemla, M., Auer, M., Singh, S., Simmons, B.A., Singer, S.W., 2013. Community dynamics of cellulose-adapted thermophilic bacterial consortia. *Environ. Microbiol.* 15 (9), 2573–2587.
- He, P., Zhao, L., Zheng, W., Wu, D., Shao, L.M., 2013. Energy balance of a biodrying process for organic wastes of high moisture content: a review. *Dry. Technol.* 31 (2), 132–145.
- Hospido, A., Carballa, M., Moreira, M., Omil, F., Lema, J.M., Feijoo, G., 2010. Environmental assessment of anaerobically digested sludge reuse in agriculture: potential impacts of emerging micropollutants. *Water Res.* 44 (10), 3225–3233.
- Huang, J., Yang, Z.H., Zeng, G.M., Wang, H.L., Yan, J.W., Xu, H.Y., Gou, C.L., 2014. A novel approach for improving the drying behavior of sludge by the appropriate foaming pretreatment. *Water Res.* 68, 667–679.
- Huilinir, C., Villegas, M., 2015. Simultaneous effect of initial moisture content and airflow rate on biodrying of sewage sludge. *Water Res.* 82, 118–128.
- Jin, L., Zhang, G., Tian, H., 2014. Current state of sewage treatment in china. *Water Res.* 66, 85–98.
- Kolde, R., 2013. *Heatmap: Pretty Heatmaps*. R Package Version 0.7.7. <http://CRAN.R-project.org/package=heatmap>.
- Kuo, T.M., Lanser, A.C., Kaneshiro, T., Hou, C.T., 1999. Conversion of oleic acid to 10-ketostearic acid by *Sphingobacterium* sp. strain O22. *J. Am. Oil Chem. Soc.* 76 (6), 709–712.
- Kuok, F., Mimoto, H., Nakasaki, K., 2012. Effects of turning on the microbial consortia and the in situ temperature preferences of microorganisms in a laboratory-scale swine manure composting. *Bioresour. Technol.* 116 (4), 421–427.
- Mara, K., Decorosi, F., Viti, C., Giovannetti, L., Papaleo, M.C., Maida, I., et al., 2012. Molecular and phenotypic characterization of *Acinetobacter* strains able to degrade diesel fuel. *Res. Microbiol.* 163 (3), 161–172.

- Merga, J.Y., Royden, A., Pandey, A.K., Williams, N.J., 2014. *Arcobacter* spp. isolated from untreated domestic effluent. *Lett. Appl. Microbiol.* 59 (1), 122–126.
- Murray, A., Horvath, A., Nelson, K.L., 2008. Hybrid life-cycle environmental and cost inventory of sewage sludge treatment and end-use scenarios: a case study from China. *Environ. Sci. Technol.* 42 (9), 3163–3169.
- Nakasaki, K., Araya, S., Mimoto, H., 2013. Inoculation of *Pichia kudriavzevii* RB1 degrades the organic acids present in raw compost material and accelerates composting. *Bioresour. Technol.* 144 (6), 521–528.
- National Bureau of Statistics of the People's Republic of China, 2014. <http://www.stats.gov.cn/>.
- Navaee-Ardeh, S., Bertrand, F., Stuart, P.R., 2010. Key variables analysis of a novel continuous biodrying process for drying mixed sludge. *Bioresour. Technol.* 101 (10), 3379–3387.
- Poulsen, T.G., Bester, K., 2010. Organic micropollutant degradation in sewage sludge during composting under thermophilic conditions. *Environ. Sci. Technol.* 44 (13), 5086–5091.
- Prabhakaran, M., Couger, M.B., Jackson, C.A., Weirick, T., Fathepure, B.Z., 2015. Genome sequences of the lignin-degrading *Pseudomonas* sp. strain YS-1p and *Rhizobium* sp. strain YS-1r isolated from decaying wood. *Genome Announc.* 3 (2), e00019–15.
- R Development Core Team, 2010. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <http://www.R-project.org>.
- Raynaud, M., Vaxelaire, J., Olivier, J., Dieudé-Fauvel, E., Baudez, J.C., 2012. Compression dewatering of municipal activated sludge: effects of salt and pH. *Water Res.* 46 (14), 4448–4456.
- Scelza, R., Rao, M.A., Gianfreda, L., 2008. Response of an agricultural soil to pentachlorophenol (PCP) contamination and the addition of compost or dissolved organic matter. *Soil Biol. Biochem.* 40 (9), 2162–2169.
- Shen, Y.J., Chen, T.B., Gao, D., Zheng, G.D., Liu, H.T., Yang, Q.W., 2012. Online monitoring of volatile organic compound production and emission during sewage sludge composting. *Bioresour. Technol.* 123 (4), 463–470.
- Storey, S., Chualain, D.N., Doyle, O., Clipson, N., Doyle, E., 2015. Comparison of bacterial succession in green waste composts amended with inorganic fertiliser and wastewater treatment plant sludge. *Bioresour. Technol.* 179, 71–77.
- Su, J.Q., Wei, B., Ou-Yang, W.Y., Huang, F.Y., Zhao, Y., Xu, H.J., Zhu, Y.G., 2015. Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ. Sci. Technol.* 49 (12), 7356–7363.
- Ten, L.N., Im, W.T., Kim, M.K., Lee, S.T., 2005. A plate assay for simultaneous screening of polysaccharide- and protein-degrading micro-organisms. *Lett. Appl. Microbiol.* 40 (2), 92–98.
- Trois, C., Coulon, F., Combret, C.P.D., Martins, J.M.F., Oxarango, L., 2010. Effect of pine bark and compost on the biological denitrification process of non-hazardous landfill leachate: focus on the microbiology. *J. Hazard. Mater.* 181 (1–3), 1163–1169.
- US Department of Agriculture and US Composting Council, 2001. Test Methods for the Examination of Composting and Compost. Edaphos International, Houston, TX.
- Velvizhi, G., Venkata Mohan, S., 2015. Bioelectrogenic role of anoxic microbial anode in the treatment of chemical wastewater: microbial dynamics with bioelectro-characterization. *Water Res.* 70 (1), 52–63.
- VerBerkmoes, N.C., Deneff, V.J., Hettich, R.L., Banfield, J.F., 2009. Functional analysis of natural microbial consortia using community proteomics. *Nat. Rev. Microbiol.* 7 (7), 196–205.
- Villegas, M., Huiliniir, C., 2014. Biodrying of sewage sludge: kinetics of volatile solids degradation under different initial moisture contents and air-flow rates. *Bioresour. Technol.* 174, 33–41.
- Winkler, M.K.H., Bennenbroek, M.H., Horstink, F.H., Loosdrecht, M.C.M.V., Pol, G.J.V.D., 2013. The biodrying concept: an innovative technology creating energy from sewage sludge. *Bioresour. Technol.* 147 (7), 124–129.
- Young, C.C., Chou, J.H., Arun, A.B., Yen, W.S., Sheu, S.Y., Shen, F.T., Lai, W.A., Rekha, P.D., Chen, W.M., 2008. *Comamonas composti* sp. nov., isolated from food waste compost. *Int. J. Syst. Evol. Microbiol.* 58, 251–256.
- Zhao, L., Gu, W.-M., He, P.-J., Shao, L.-M., 2010. Effect of air-flow rate and turning frequency on bio-drying of dewatered sludge. *Water Res.* 44 (20), 6144–6152.