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Effects of sulfur-metabolizing bacterial community diversity on H_2S emission behavior in landfills with different operation modes

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Abstract Hydrogen sulfide (H_2S) is one of the major contributors to offensive odors from landfills, and its concentration differs under different operation modes. This study examined the distribution of H_2S emission from different landfill depths under different operation modes (anaerobic, semi-aerobic, semi-aerobic transformation, and the three operation modes with additional leachate recirculation). The microbial community (especially the sulfur-metabolizing bacterial community) was investigated using high-throughput sequencing technology. The results showed that the semi-aerobic mode could substantially lower the risks of H_2S pollution in landfills, which might be because of the difference in biological processes related to sulfur metabolism driven by functional microbes. A myriad of factors are responsible for mutually shaping the sulfur-metabolizing bacterial community composition in landfills that might subsequently affect the behavior of H_2S emission in landfills. The differences in abundance of the genera *Acinetobacter* and *Paracoccus* (phylum *Proteobacteria*) caused by environmental factors might explain the differences in H_2S emission. H_2S odor control could be realized if the related functional microbe diversity can be influenced by adjustments to landfill operation.

Keywords H_2S · Landfill · Operation modes · Sulfur-metabolizing bacteria · High-throughput sequencing

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Introduction

Landfills are widely used around the world because of their low cost and ease of management (Long et al. 2008). Conventional landfill (anaerobic landfill) always needs long-term maintenance because of the slow rate of decomposition, while decomposition can be enhanced in bioreactor landfill with the help of leachate recirculation (Long et al. 2010a). However, there is a limited capacity for landfills with increased amounts of municipal solid waste (MSW), and creating new landfills once an old landfill is closed is very difficult because of a shortage of land (Sener et al. 2006). Compared with traditional anaerobic landfill, semi-aerobic landfill has become popular because of its faster degradation rate and higher leachate quality without an obvious increase in cost (Hirata et al. 2012). Regardless of the mode of operation, the risk of odor pollution from landfills still exists. Hydrogen

sulfide (H_2S), with an extremely low odor threshold (around 0.5 ppb) and high toxicity (Firer et al. 2008), is one of the major contributors to odor pollution from landfill and it has been found at concentrations ranging from hundreds of micrograms per cubic meter to thousands of milligrams per cubic meter in air at different landfill sites (Ding et al. 2012; Kim et al. 2005).

H_2S is produced by the conversion of organic and/or inorganic compounds containing sulfur (CCS) during decomposition. Landfills are complex artificial habitats and the microbial communities resident in landfill are responsible for the conversion of CCS. For example, sulfate-reducing bacteria (SRB) can play an instrumental role in biogenic production of H_2S. Meanwhile, biooxidation of sulfide and intermediary sulfur compounds carried out by sulfide-oxidizing bacteria (SOB) are crucial in the removal of H_2S. Sulfur-metabolizing bacteria play an important role in sulfur cycling in landfills. Sulfur-metabolizing bacteria have been the focus of many previous studies. Zhang et al. (2012) found that the bacterial and archaeal communities from a white hydrothermal plume were dominated by sulfur-reducing *Nautilia* and *Thermococcus*, whereas a yellow hydrothermal plume and the surface water were dominated by sulfide-oxidizing *Thiomicrospira* and *Euryarchaeota* Marine Group II, respectively. Lou et al. (2013) concentrated on enrichment, isolation, and identification of SOB from a sulfide removing bioreactor. *Rhodopseudomonas* and *Halothiobacillus* were found to be the main SOB in the sulfide-removing reactor, and were responsible for sulfur oxidation in the treatment system. Unfortunately, studies targeting the composition of sulfur-metabolizing bacteria in landfill refuse are scarce. Differences in H_2S emissions from landfills might be attributed to the differences in activity of some sulfur-metabolizing bacteria. Therefore, it is necessary to focus on the relationship between sulfur-metabolizing bacterial composition and landfill scenarios. This could provide a reference for controlling landfill bacterial community composition and transforming landfill technology based on the identification of H_2S emission behaviors.

In this study, the distribution of H_2S emission at different depths inside landfill was first examined in six lab-scale simulated landfills with different operation modes. The microbial community composition inside different landfills during each designated landfill phase, especially the sulfur-metabolizing bacterial community, was investigated using high-throughput sequencing technology. The study aimed to evaluate the relationship between the H_2S emission behavior and the corresponding sulfur-metabolizing bacterial community in different landfill scenarios such as landfill modes and depths.

Materials and methods

Experimental set-up

Six different sets of simulated bioreactor landfills were designed. R1 and R3 were operated as anaerobic landfill; R2 and R4 were semi-aerobic landfill; R5 and R6 were semi-aerobic landfills switched from anaerobic mode after day 175. The leachate of R1, R2, and R6 were single pass leaching, while the leachate of R3, R4, and R5 were directly recirculated by a peristaltic pump into simulated landfill after collection every two days. Complete configurations of the six landfills are shown in Fig. 1. Each simulated landfill had a diameter of 0.5 m and a height of 2.0 m and was operated at room temperature. The simulated landfills with semi-aerobic operation mode had three inlets at the bottom of each landfill. The three inlets were set in the same horizontal position, and the angle between them was 120°. A center vent pipe with a diameter of 5 cm was set in the middle of each semi-aerobic landfill. The semi-aerobic system achieved through a convection process. Before day 175, the inlets and center vent pipes in R5 and R6 were in a closed state to maintain anaerobic conditions. From top to bottom, each landfill had a 100-mm-thick layer of headspace, a 1600-mm-thick layer of landfill site, and a 300-mm-thick layer of leachate collection. A 100-mm-thick layer of gravel was placed at the bottom of each landfill site to simulate a leachate collection system and to prevent clogging of the leachate withdrawal outlets. The MSW was loaded in 1450-mm layers and compacted using a shovel and a sledge hammer. A 50-mm-thick layer of sand was placed on top of the MSW to simulate intermediate cover and an upper drainage layer and to provide even distribution of the recirculated leachate. Headspace on top of each landfill created a leachate distribution system. The MSW layer was divided into three layers: shallow,

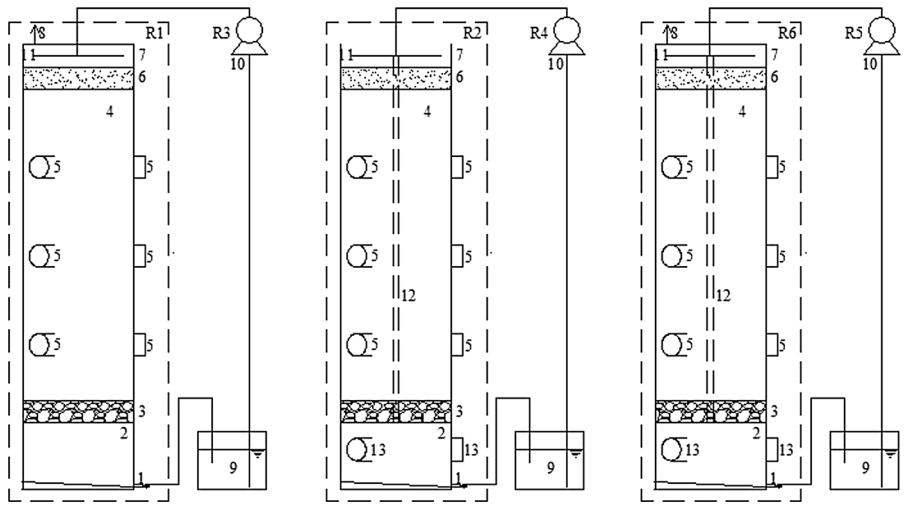

Fig. 1 Schematic diagram of simulated landfill systems (*1* Leachate outlet *2* Leachate collection tank *3* Gravel layer *4* Landfill site *5* Sampling port *6* Sandy layer *7*. Headspace *8* Gas outlet *9* Leachate recirculation tank *10* Peristaltic pump *11* Leachate distribution system *12* Center vent pipe *13* Inlet)

middle, and deep layers and each layer had three sampling ports. Each simulated landfill was packed with fresh refuse collected from the transport station of Hangzhou (Zhejiang, east China), with a wet density of 880 kg m^{-3}. The characteristics of MSW used in this experimental were (by wet weight, w/w): food and fruit waste, 66.8 %; plastic, 3.2 %; paper, 9.2 %; dirt, 1.8 %; glass, 0.4 %; cellulose textile, 0.5 %; metal, 0.2 %; timber, 1.6 %; residue, 16.5 %. The moisture content of MSW was 68 %.

Sample collection

Based on our previous landfill studies (Fang et al. 2009a, b; Long et al. 2009, 2010b), the decomposition of simulated landfills approached stabilization after about 200 days' operation. In this study, we observed that the cover layer of six simulated landfills significantly settled down after 175 days. This meant that the rapid degradation phase finished by this time. To evaluate the further stabilization behavior of landfill, we turned on the ventilation system of R5 and R6 to switch them from anaerobic to semi-aerobic. Therefore, after 175 days operation, the six landfill reactors were anaerobic landfill (R1 and R3), semi-aerobic landfill (R2 and R4), and semi-aerobic landfills switched from anaerobic (R5 and R6).

At day 200, gas and refuse were sampled from three layers of the sampling ports around the side of each simulated landfill. Gas samples in the positions of 5, 12, and 20 cm from the center vent were collected. Approximately 100 g refuse samples were collected from each refuse sampling port and the refuse samples from all three ports in one layer were mixed as a sample ($\sim$300 g), of which 50-g refuse samples were stored at room temperature to dry and 20-g refuse samples were stored at −80 °C for the following bacterial community analysis. Because of the landfill settlement caused by the rapid degradation of refuse, the refuse samples in the shallow layer of R2 and R4 could not be collected.

Chemical analysis

Gas samples were analyzed for H_2S and CO_2. The H_2S in the gas samples was analyzed using a gas chromatograph equipped with a flame photometric detector (GC 7890A; Agilent Technologies, Santa Clara, CA, USA) (Fang et al. 2015). A gas chromatograph equipped with a thermal conductivity detector (GC 7890II; Shanghai Tianmei Scientific Instruments Co., Ltd., Shanghai, China) (Fang et al. 2015) was used to determine the CO_2 concentrations in the gas samples.

pH, dissolved organic carbon (DOC), sulfate (SO_4^{2-}), sulfide (H_2S, HS^-, S^{2-}), ferrous (Fe^{2+}), nitrate (NO_3^-), and nitrite (NO_2^-) concentrations of the refuse samples were analyzed on the day the samples were collected. Distilled water was used as the extraction solution (solid–liquid ratio of 1:10) for analysis of pH, DOC, and SO_4^{2-}. The pH was determined using a pH meter (SevenEasy, Mettler-Toledo, Switzerland). The DOC was determined using a

TOC analyzer (TOC-V CPN, Shimadzu, Japan). The SO_4^{2-} was determined using an ion chromatograph described by Fang et al. (2015). The sulfide was determined by the method described by Qiu et al. (1992). The ferrous concentration was determined by the o-phenanthroline method after extraction with 1 mol L^{-1} HCl (solid-to-liquid ratio of 1:100) (Li et al. 2010). Then, 2 mol L^{-1} KCl solution was used as the extraction solution (solid–liquid ratio of 1:10) for analysis of NO_3^- and NO_2^-. NO_3^- and NO_2^- were determined by ultraviolet spectrophotometry and *N*-(1-naphthyl)-ethylenediamine dihydrochloride, respectively (APHA 1999). The dry samples were milled for the subsequent determination of TN by the H_2SO_4–H_2O_2 digestion indophenol-blue colorimetric method (Lu 1999).

All the analyses were carried out in triplicate from three samples to ensure the validity of the results, and all the results of chemical analyses were calculated on a dry-weight basis.

Bacterial community analysis

The genomic DNA of the sample was extracted using an extraction kit (DR4011; Bioteke Corporation, Beijing, China) according to the manufacturer's instructions. The bacterial 16S rRNA gene of the extracted DNA was amplified using the primer pair 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACATCGACGGGTATTCTAAT-3′) (Masoud et al. 2011). The bacterial community was investigated by Illumina high-throughput sequencing, which was conducted by Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China) (Zhang et al. 2015). Sequences were clustered into operational taxonomic units by setting a 0.03 distance limit (equivalent to 97 % similarity) using the Usearch program. Sequences were then phylogenetically assigned to taxonomic classifications using an RDP classifier and were allocated to different levels (Zhang et al. 2015). Raw sequence files for the 16 samples analyzed here have been deposited in the NCBI Sequence Read Archive.

Statistical analysis

Statistical analyses were conducted using surfer 10, R Statistics Program, and SPSS 19.0. Relative abundance heat maps were created from phylum-level bacterial annotations. Prior to statistical analysis, relative abundance was log-transformed to achieve a more normal distribution. Linear regressions were performed using SPSS software to relate environmental factors to community composition. Multivariate analysis was conducted using canonical correspondence analysis (CCA) of genus-level bacterial taxa and refuse property data. Calculations were performed using the CCA function of the vegan package in R Statistics Program using refuse property data as environmental parameters. Data were transformed and scaled prior to analysis as follows: taxa abundances were log(x + 1)-transformed to dampen the contribution of abundant taxa and each environmental parameter was converted to its z-score.

Results and discussion

Distribution of H_2S emission in landfill

The H_2S concentration varied in different landfills, with relatively higher concentrations under anaerobic conditions (R1 and R3) and lower concentrations under semi-aerobic conditions (R2 and R4) (Fig. 2). These results corresponded to the fact that during the degradation of sulfur-containing substrates under anaerobic conditions in landfill, H_2S will be produced and released (Mescia et al. 2011). For R1 and R3, the highest H_2S concentrations reached 4.46 ± 0.68 and 6.13 ± 0.32 mg m^{-3}, respectively, under anaerobic conditions, while in R2 and R4, the highest H_2S concentrations reached 3.70 ± 0.13 and 3.17 ± 0.07 mg m^{-3}, respectively, under semi-aerobic conditions. The difference in H_2S emission behavior between anaerobic and aerobic conditions can be further verified by the special landfill mode, namely semi-aerobic switched from anaerobic. After transformation from anaerobic to semi-aerobic conditions (R5 and R6), the H_2S concentrations substantially decreased with air exposure. The highest H_2S concentrations in R5 and R6 decreased to 2.92 ± 0.13 and 2.73 ± 0.08 mg m^{-3}, respectively, after exposure to air. However, the lowest H_2S concentrations in R5 and R6 were 1.4 and 1.5 times higher than in R2 and R4. The H_2S emissions were lowest in R2 and R4 of all the landfill configurations tested. The difference in H_2S emission behavior was mainly attributed to the different environment for biodegradation. Under

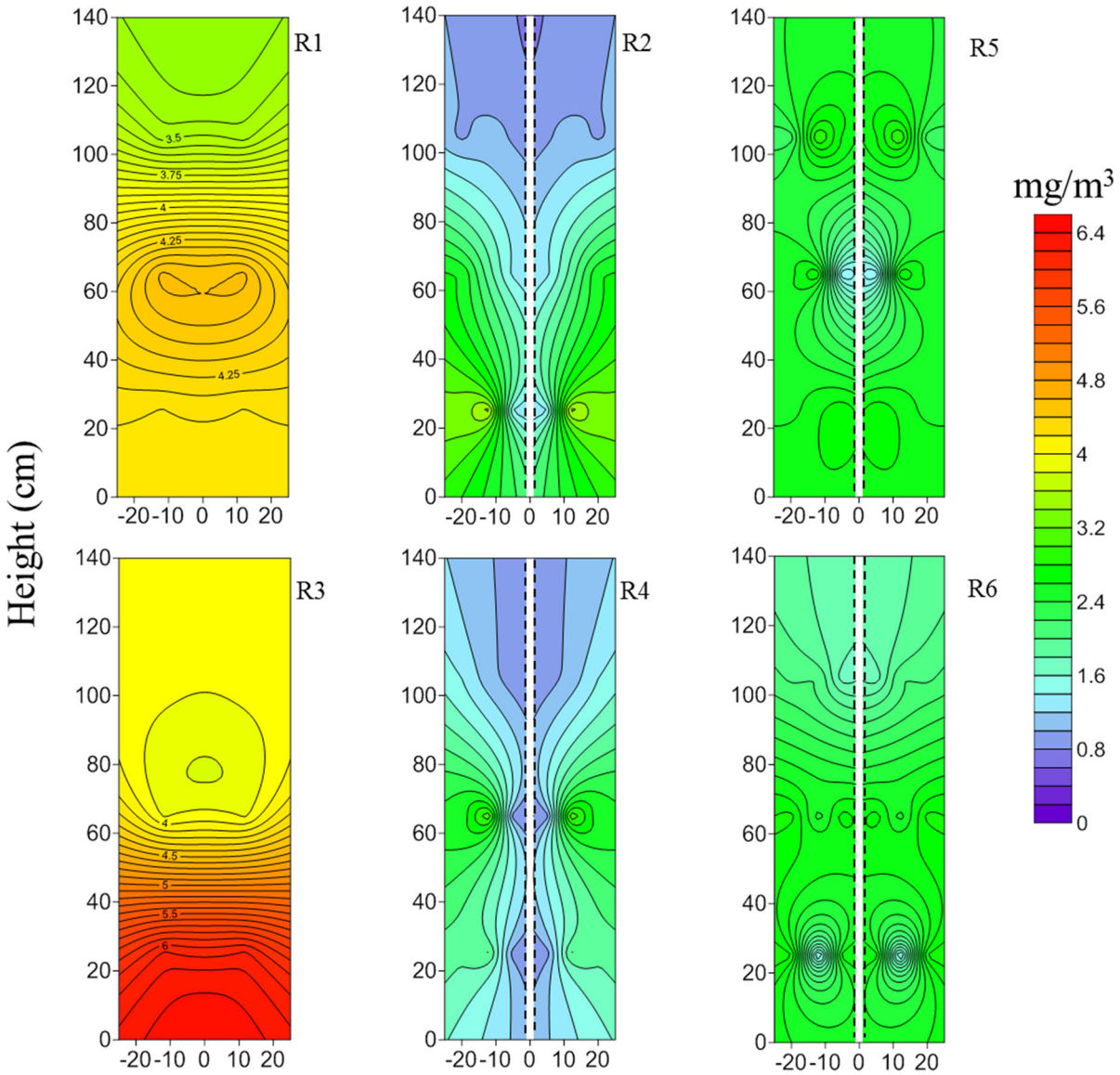

Fig. 2 Distribution curve of H_2S emission in simulated landfill

anoxic and anaerobic conditions, some SRB can grow with archaea and use SO_4^{2-} as an electron acceptor to oxidize CH_4 (Boetius et al. 2000; Michaelis et al. 2002; Caldwell et al. 2008), whereas under oxic conditions SOB oxidize H_2S to S^0 and SO_4^{2-} (Kelly et al. 1997). The semi-aerobic mode of landfill operation could substantially attenuate the risk of H_2S pollution in landfill. One of the main reasons for this might be the difference in biological processes related to sulfur metabolism driven by functional microbes.

Diversity of sulfur-metabolizing bacteria in landfill

To investigate the differences in H_2S emission behavior described above, refuse samples at day 200 were sampled to analyze the microbial diversity. High-throughput sequencing results showed that the microbial community structures in landfill at the phylum level varied with operation modes and sampling depths (Fig. 3). Five major phyla accounted for more than 94 % of the total bacterial abundance in the refuse

samples investigated here, including *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Cyanobacteria*. Some members of the *Firmicutes* and *Proteobacteria* are known to be involved in the reductive and oxidative pathway of the sulfur cycle (Köchling et al. 2015; Luo et al. 2013). Schauer et al. (2011) found that *Bacteroidetes* was highly abundant in sulfur-rich sediments. Phylum *Firmicutes* was predominant in the deep layer of R1 (55 %) and R3 (49 %), and also accounted for relatively high proportions in the middle layer of R2 (50 %) and R4 (52 %). After semi-aerobic transformation, the abundance of phylum *Firmicutes* increased to 66 and 73 % in the top layer of R5 and the middle layer of R6, respectively. Phylum *Proteobacteria* was most abundant in the middle layer of R1 (51 %) and R3 (50 %) and accounted for 44 and 26 % in the deep layer of R2 and R4, respectively. With air exposure, a large shift in the proportion of *Proteobacteria* was observed in R5 and R6, showing the largest relative abundance in the deep layer of R5 (35 %) and in the shallow layer of R6 (41 %). The proportion of *Bacteroidetes* varied greatly in the refuse samples (0.5–21 %). *Actinobacteria* and *Cyanobacteria* occurred in the shallow layer of R6 with a relative abundance of 3.8 and 0.05 %, respectively, but they were detected at higher proportions in other refuse samples. Comparison among the five major phyla abundances showed that *Proteobacteria* was most consistent with the H_2S emission behaviors. Based on the relative abundance of bacterial phyla, cluster analysis showed that the refuse samples from the landfills under the same oxygen conditions grouped together. Samples from the anaerobic landfill, semi-aerobic landfill, and the semi-aerobic transformation landfills were separated from each other, which was consistent with the distribution of H_2S emission in landfills. These results indicated that the oxygen condition might affect the composition of sulfur-metabolizing bacterial communities and then cause the final H_2S emission behavior difference, of

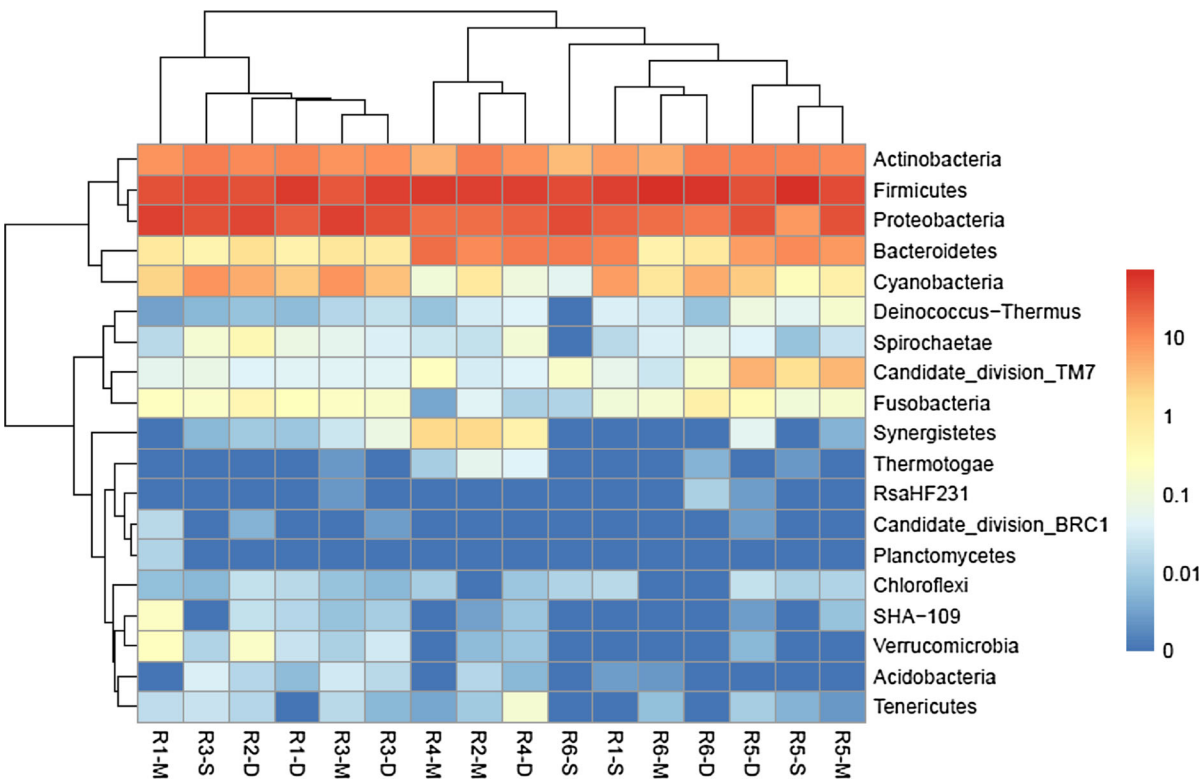

Fig. 3 Relative abundance heatmap of phylum-level bacterial communities. *Dendrogram* of sample sites (average clustering technique) based on community similarity along top axis*; Left dendrogram* depicts clustering of phyla by co-occurrence (average clustering technique)

which *Proteobacteria* abundance might have a greater influence on H_2S emission.

Typical sulfur-metabolizing bacteria being reported were detected in these six landfills (Table 1), including *Acinetobacter*, *Bacillus*, *Comamonas*, *Ochrobactrum*, *Paracoccus*, *Pseudomonas*, and *Rhodococcus*. Among them, *Bacillus* affiliated with the phylum *Firmicutes*, *Rhodococcus* affiliated with the phylum *Actinobacteria*, and the remaining five genera affiliated with the phylum *Proteobacteria*. In these six landfills, the relative abundances of the known sulfur-metabolizing bacteria ranged from 1.1 to 12 %. *Acinetobacter* was the most abundant known sulfur-metabolizing genus. *Acinetobacter* can use dimethyl sulfide as the sole sulfur source (Horinouchi et al. 1997) and participates in thiosulfate oxidation (Luo et al. 2013). It was detected with the highest abundance in R3 and with the lowest abundance in R4. Correspondingly, it was observed that the H_2S emission was the highest in R3 and the lowest in R4. Similarly, the abundance of *Paracoccus* was related to the H_2S emissions. *Paracoccus* are facultative aerobes that can grow heterotrophically with various carbon sources or chemoautotrophically with thiosulfate and sulfide as electron donors under aerobic conditions (Frierich et al. 2008). This suggested that *Acinetobacter* and *Paracoccus* might play important roles in the metabolism of H_2S in landfills. The abundances of *Ochrobactrum* and *Pseudomonas* were relatively low in R4. They can not only use sulfide and thiosulfate for growth under aerobic conditions, but can also use them as a source of electrons to reduce nitrite and/or nitrate anaerobically (Euzeby 1997; Sorokin et al. 1999; Zhang et al. 2008; Mahmood et al. 2009). The abundances of *Bacillus*, *Comamonas*, and *Rhodocccus* were low in all landfills. The differences in abundance of *Acinetobacter* and *Paracoccus* might be the main reason for the observed differences in H_2S emission.

Effect of environmental parameters on bacterial community composition

CCA was performed to investigate the possible relationship between microbial community composition and the detailed surrounding environment of different landfill scenarios (Fig. 4). Based on variance inflation factors, 10 significant environmental variables (Fe^{2+}, SO_4^{2-}, sulfide, total nitrogen (TN), NO_3^-, NO_2^-, pH, DOC, H_2S, and CO_2) were selected for inclusion in the

Table 1 The relative abundances (% of total reads) of the known sulfur-metabolizing bacteria in the simulated landfills, based on the abundances of 16S rRNA gene sequences detected in the DNA by use of pyrosequencing

	R1			R2		R3			R4			R5			R6		
	Shallow	Middle	Deep	Middle	Deep	Shallow	Middle	Deep	Middle	Deep	Shallow	Middle	Deep	Shallow	Middle	Deep
Acinetobacter	3.16	2.91	2.14	1.59	3.10	1.74	6.38	2.22	0.90	0.01	0.79	2.27	3.33	0.82	0.85	2.14
Bacillus	0.06	0.07	0.09	0.03	0.02	0.05	0.03	0.07	0.05	0.04	0.01	0.05	0.01	2.86	0.07	0.05
Comamonas	0.01	0.04	0.08	0.00	0.03	0.01	0.01	0.01	0.00	0.00	0.10	0.02	0.01	0.51	0.01	0.01
Ochrobactrum	0.23	1.39	0.64	0.22	3.05	0.76	0.95	0.70	0.04	0.04	0.15	0.81	0.78	0.03	0.29	0.19
Paracoccus	0.17	1.66	0.90	0.17	0.71	0.43	1.08	1.69	0.11	0.10	0.14	0.82	0.68	0.16	0.42	0.55
Pseudomonas	2.02	2.79	3.74	1.07	1.58	0.37	1.95	0.62	0.03	0.89	0.19	0.75	1.26	7.77	0.93	0.65
Rhodococcus	0.01	0.03	0.02	0.01	0.02	0.02	0.03	0.01	0.00	0.01	0.01	0.04	0.01	0.01	0.02	0.01
Total	5.66	8.89	7.61	3.09	8.51	3.38	10.43	5.32	1.13	1.09	1.39	4.76	6.08	12.16	2.59	3.6

CCA biplot. The first canonical axis explained 24.65 % of the detected microbial diversity and was positively correlated with SO_4^{2-}, CO_2, H_2S, TN, NO_2^-, pH, and DOC. The second axis represented 13.66 % of variance and was positively correlated with Fe^{2+}, sulfide, and NO_3^-. The length of an environmental parameter arrow in the ordination plot indicates the strength of the relationship of that parameter to community composition. As such, pH, NO_2^-, and sulfide appeared to be the most important environmental factors. The stress of pH has a significant effect on the overall diversity and composition of microbial communities in a range of terrestrial and aquatic environments (Fierer and Jackson 2006; Hornstrom 2002). In addition to pH, NO_2^-, sulfide, and DOC were strongly and significantly linked to bacterial community variance in the CCA. Microbial community structure was also influenced by Fe^{2+}, NO_3^-, H_2S, and CO_2. The relatively small magnitude of SO_4^{2-} and TN vectors indicated that SO_4^{2-} and TN were not as strongly correlated to community composition as other environmental parameters. Sulfide was positively related to Fe^{2+} and negatively related to NO_3^- whilst Fe^{2+} was also positively correlated to H_2S. There was no significant relationship between NO_3^- and H_2S. In general, R1 and R3 had a positive relationship with H_2S, while the opposite relationship appeared in R2, R4, R5, and R6. These results showed that H_2S concentration and microbial community composition greatly differed with the operation modes. The landfills with different oxygen distribution could lead to different degradation rates and biological processes. In contrast, the semi-aerobic mode might be more favorable for controlling H_2S emission in landfill.

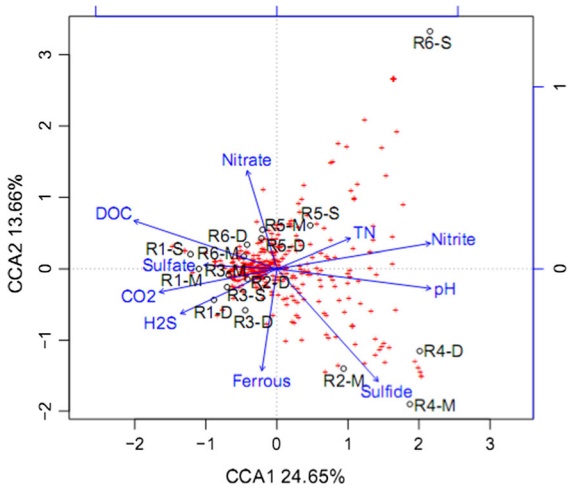

Fig. 4 Canonical correspondence analysis of log (x+1)-normalized genus-level bacterial communities, constrained by the independent, normalized refuse properties. Samples are labeled in *black*. *Blue vectors* indicate the effect of refuse properties on sample community outcomes. *Red taxa points* indicate the average CCA location of the genus-level bacterial taxa. The percentage of variation explained by each axis is shown. The CCA model is significant (P = 0.007)

Pairwise linear regressions were further performed to assess the potential influence of environmental attributes on the sulfur-metabolizing bacterial community or environmental outcomes (Table 2). Any pairwise relationships not listed had a regression p value greater than 0.1. The higher pH levels observed were correlated with higher NO_2^- and sulfide levels, lower SO_4^{2-} level, and decreased relative abundance of *Acinetobacter*, *Ochrobactrum*, and *Rhodococcus* (p values of 0.000, 0.003, 0.017, 0.086, 0.071, and 0.048, respectively). The relative abundance of *Paracoccus* and *Rhodococcus* were found to be positively correlated with the levels of DOC ($p < 0.05$). The relative abundance of *Acinetobacter* and *Rhodococcus* showed a negative correlation with the level of NO_2^-, while the relative abundance of *Bacillus* and *Comamonas* showed a positive correlation with the level of NO_2^-. *Paracoccus* was positively correlated with the levels of H_2S and CO_2, while *Ochrobactrum* was positively correlated with the level of SO_4^{2-}. *Pseudomonas* showed no significant correlation with the determined physicochemical parameters. *Paracoccus* had the most direct relationship with H_2S emission behavior. Relatively higher H_2S concentration in landfill was associated with increased relative abundance of *Paracoccus*, the genus commonly associated with the microbial sulfur cycle.

In summary, high-throughput sequencing was employed to examine the bacterial communities of refuse samples from landfills with different operation modes and sampling depths. In addition to certain unique genera of bacterial populations in each sample, the known sulfur-metabolizing genera were commonly shared by all samples. The CCA analysis and pairwise linear regressions results showed that a myriad of factors might mutually shape the microbial community composition in landfills. The sulfur-metabolizing bacterial community composition influenced by the operation modes had a strong relationship

Table 2 Significant pairwise linear regressions for community and environmental parameters (Community included *Acinetobacter*, *Bacillus*, *Comamonas*, *Ochrobactrum*, *Paracoccus*, *Pseudomonas*, *Rhodococcus* richness; Environmental parameters included ferrous, sulfate, sulfide, total nitrogen, nitrate, nitrite, pH, DOC, H_2S and CO_2 content)

Outcome	Co-variate	*p* value	Significance threshold[a]	Pearson's correlation coefficient
Acinetobacter	pH	0.086	*	−0.443
Ochrobactrum	pH	0.071	*	−0.463
Rhodococcus	pH	0.048	**	−0.501
Sulfate	pH	0.017	**	−0.584
Sulfide	pH	0.003	***	0.697
Nitrite	pH	0.000	***	0.835
Paracoccus	DOC	0.013	**	0.607
Rhodococcus	DOC	0.036	**	0.527
pH	DOC	0.002	***	−0.715
Sulfate	DOC	0.005	***	0.668
Sulfide	DOC	0.036	**	−0.526
Nitrite	DOC	0.015	**	−0.597
CO_2	DOC	0.070	*	0.464
Acinetobacter	Nitrite	0.050	*	-0.497
Bacillus	Nitrite	0.074	*	0.458
Comamonas	Nitrite	0.058	*	0.483
Rhodococcus	Nitrite	0.032	**	−0.536
Sulfate	Nitrite	0.079	*	−0.452
Sulfide	Nitrite	0.006	***	0.655
Sulfide	Nitrite	0.083	*	−0.447
Sulfide	Ferrous	0.066	*	0.470
Ochrobactrum	Sulfate	0.041	**	0.517
Paracoccus	H_2S	0.001	***	0.731
Paracoccus	CO_2	0.086	*	0.442
H_2S	CO_2	0.017	**	0.588

Asterisk represent the significance: one asterisk means $p \geq 0.05$; two asterisks mean $0.01 < p < 0.05$; three asterisks mean $p \leq 0.01$

with H_2S concentration. The semi-aerobic mode with relatively low abundance of *Paracoccus* might reduce the risk of H_2S pollution in landfills. Further work is necessary to elucidate the factors regulating the landfill bacterial community.

Conclusions

H_2S concentration and microbial community composition might vary depending on the landfill operation modes. The semi-aerobic mode can substantially attenuate the risks of H_2S pollution in landfill, which might be because of the difference in biological processes related to sulfur metabolism driven by functional microbes. The difference in abundance of the genera *Acinetobacter* and *Paracoccus* (phylum *Proteobacteria*) caused by environmental factors might be the main reason for differences in H_2S emission in landfill.

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References

APHA (1999) Standard methods for the examination of water and wastewater. APHA, Washington, DC

Boetius A, Ravenschlag K, Schubert CJ, Rickert D, Widdel F, Gieseke A, Amann R, Jorgensen BB, Witte U, Pfannkuche O (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407:623–626

Caldwell SL, Laidler JR, Brewer EA, Eberly JO, Sandborgh SC, Colwell FS (2008) Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ Sci Technol 42:6791–6799

Ding Y, Cai CY, Hu B, Xu Y, Zheng XJ, Chen YX, Wu WX (2012) Characterization and control of odorous gases at a landfill site: a case study in Hangzhou, China. Waste Manage 32:317–326

Euzeby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol 47:590–592

Fang CR, Long YY, Lu YY, Shen DS (2009a) Behavior of dimethyl phthalate (DMP) in simulated landfill bioreactors with different operation modes. Int Biodeter Biodegr 63:732–738

Fang CR, Long YY, Shen DS (2009b) Comparison on the removal of phthalic acid diesters in a bioreactor landfill and a conventional landfill. Bioresour Technol 100:5664–5670

Fang Y, Du Y, Feng H, Hu LF, Shen DS, Long YY (2015) Sulfide oxidation and nitrate reduction for potential mitigation of H_2S in landfills. Biodegradation 26:115–126

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci USA 103:626–631

Firer D, Friedler E, Lahav O (2008) Control of sulfide in sewer systems by dosage of iron salts: comparison between theoretical and experimental results, and practical implications. Sci Total Environ 392:145–156

Frierich CG, Quentmeier A, Bardischewsky F, Rother D, Orawski G, Hellwig P, Fischer J (2008) Redox control of chemotrophic sulfur oxidation of *Paracoccus pantotrophus*. In: Dahl C, Frierich CG (eds) Microbial Sulfur Metabolism. Springer, Heidelberg, Berlin, pp 139–150

Hirata O, Matsufuji Y, Tachifuji A, Yanase R (2012) Waste stabilization mechanism by a recirculatory semi-aerobic landfill with the aeration system. J Mater Cycles Waste Manage 14:47–51

Horinouchi M, Kasuga K, Nojiri H, Yamane H, Omori T (1997) Cloning and characterization of genes encoding an enzyme which oxidizes dimethyl sulfide in *Acinetobacter* sp. strain 20B. FEMS Microbiol Lett 155:99–105

Hornstrom E (2002) Phytoplankton in 63 limed lakes in comparison with the distribution in 500 untreated lakes with varying pH. Hydrobiologia 47:115–126

Kelly DP, Shergill JK, Lu WP, Wood AP (1997) Oxidative metabolism of inorganic sulfur compounds by bacteria. Antonie Van Leeuwenhoek 71:95–107

Kim KH, Choi YJ, Jeon EC, Sunwoo Y (2005) Characterization of malodorous sulfur compounds in landfill gas. Atmos Environ 39:1103–1112

Köchling T, Sanz JL, Gavazza S, Florencio L (2015) Analysis of microbial community structure and composition in leachates from a young landfill by 454 pyrosequencing. Appl Microbiol Biotechnol 99:5657–5668

Li WB, Yao J, Tao PP, Guo MT, Feng XY, He YN, Fang CR, Shen DS (2010) A comparative study on two extraction procedures in speciation of iron in municipal solid waste. J Hazard Mater 182:640–648

Long Y, Guo QW, Fang CR, Zhu YM, Shen DS (2008) In situ nitrogen removal in phase-separate bioreactor landfill. Bioresour Technol 99:5352–5361

Long YY, Hu LF, Fang CR, He R, Shen DS (2009) Releasing behavior of zinc in recirculated bioreactor landfill. Sci Total Environ 407:4110–4116

Long YY, Hu LF, Wang J, Fang CR, He R, Shen DS (2010a) Bio-immobilization of Cu and Zn in recirculated bioreactor landfill. Environ Sci Pollut R 17:1539–1546

Long YY, Shen DS, Wang HT, Lu WJ (2010b) Migration behavior of Cu and Zn in landfill with different operation modes. J Hazard Mater 179:883–890

Lu RK (1999) Agriculture chemical analysis of soil. China Agricultural Scientech Press, Beijing (in Chinese)

Luo JF, Tian GL, Lin WT (2013) Enrichment, isolation and identification of sulfur-oxidizing bacteria from sulfide removing bioreactor. J Environ Sci 25:1393–1399

Mahmood Q, Hu BL, Cai J, Zheng P, Azim MR, Jilani G, Islam E (2009) Isolation of *Ochrobactrum* sp. QZ2 from sulfide and nitrite treatment system. J Hazard Mater 165:558–565

Masoud W, Takamiya M, Vogensen FK, Lillevang S, Al-Soud WA, Sørensen SJ, Jakobsen M (2011) Characterization of bacterial populations in Danish raw milk cheeses made with different starter cultures by denaturating gradient gel electrophoresis and pyrosequencing. Int Dairy J 21:142–148

Mescia D, Hernández SP, Conoci A, Russo N (2011) MSW landfill biogas desulfurization. Int J Hydrogen Energy 36:7884–7890

Michaelis W, Seifert R, Nauhaus K, Treude T, Thiel V, Blumenberg M, Knittel K, Gieseke A, Peterknecht K, Pape T, Boetius A, Amann R, Jorgensen BB, Widdel F, Peckmann J, Pimenov NV, Gulin MB (2002) Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. Science 297:1013–1015

Qiu CL, Liu JX, Peng ML (1992) Amendment method for sulfide content of soil. Public Heath 8:549–550

Schauer R, Røy H, Augustin N, Gennerich HH, Peters M, Wenzhoefer F, Amann R, Meyerdierks A (2011) Bacterial sulfur cycling shapes microbial communities in surface sediments of an ultramafic hydrothermal vent field. Environ Microbiol 13:2633–2648

Sener B, Süzen ML, Doyuran V (2006) Landfill site selection by using geographic information systems. Environ Geol 49:376–388

Sorokin DY, Teske A, Robertson LA, Kuenen JG (1999) Anaerobic oxidation of thiosulfate to tetrathionate by obligately heterotrophic bacteria, belonging to the *Pseudomonas stutzeri* group. FEMS Microbiol Ecol 30:113–123

Zhang JB, Zhang T, Ma K, Chen GH, Zhang DY, Wei XH (2008) Isolation and identification of the thermophilic alkaline desulphuricant strain. Sci China, Ser B 51:158–165

Zhang Y, Zhao ZH, Chen CTA, Tang K, Su JQ, Jiao NZ (2012) Sulfur metabolizing microbes dominate microbial communities in andesite-hosted shallow-sea hydrothermal systems. PLoS One 7:e44593

Zhang XQ, Feng HJ, Liang YX, Zhao ZQ, Long YY, Fang Y, Wang MZ, Yin J, Shen DS (2015) The relief of microtherm inhibition for p-fluoronitrobenzene mineralization using electrical stimulation at low temperatures. Appl Microbiol Biotechnol 99(10):4485–4494