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Sulfate reduction behavior in the leachate saturated zone of landfill sites

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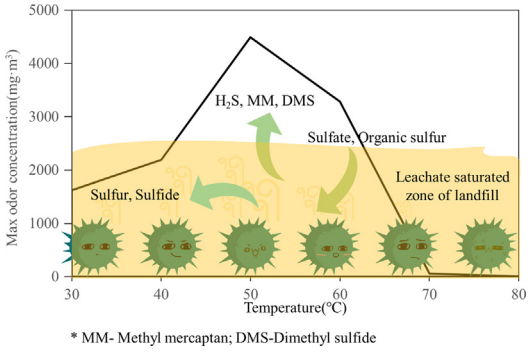
HIGHLIGHTS

- Remarkable sulfate reduction observed in leachate saturated environment.
- H_2S, MM, DMS emissions were most violent at 50 and 60 °C.
- Whole microbial structure and activity were influenced by the temperature.
- dsrA and dsrB quantity decreased under higher temperatures.
- *Dethiobacter* may contribute to higher sulfate reduction rate.

GRAPHICAL ABSTRACT



* MM- Methyl mercaptan; DMS-Dimethyl sulfide

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ABSTRACT

Municipal solid waste landfills are considered one of the most important parts of the sulfur cycle. However, few studies have focused on sulfate reduction in the leachate saturated zone, where the temperature may be variable. In this work, the sulfate reduction behavior was evaluated in a landfill leachate saturated zone under temperatures between 30 and 80 °C. The results show that microbial sulfate reduction is high in the saturated zone, especially when the temperature is at 50–60 °C. The microbial diversity and the abundance of functional genes results reveal that specific sulfate-reducing bacteria such as *Dethiobacter*, the bacteria that offer energy to them, and genes other than dsrA and dsrB may have a close relationship with the variation in the reduction of sulfate. This work may improve the knowledge of sulfate reduction in the landfill sites and therefore offer theoretical support to management strategies.

1. Introduction

Landfill remains one of the most used waste disposal methods because of its relatively low construction and maintenance cost, as well as its high usability (Ying et al., 2019). However, landfill gas emissions

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have elicited great concern because of their strong odors. Hydrogen sulfide (H_2S) is a problematic landfill emission because of its low odor threshold (around 0.5 ppb) and high toxicity (Long et al., 2016). In addition to H_2S, volatile organosulfur compounds (VOSCs) such as methyl mercaptan (MM) and dimethyl sulfide (DMS) are deemed to be the main contributors to landfill odor (Lu et al., 2015). MM has an even lower odor threshold than H_2S (0.07 ppb) (Tamai et al., 2006). A low concentration of MM may cause headaches and nausea, whereas anesthesia occurs at higher concentrations. Sufficiently high concentrations can even cause respiratory paralysis and death (Borras et al., 2016). DMS has the highest odor threshold of the three (5.9 ppb), and may cause skin irritation, respiratory irritation and serious eye damage (Long et al., 2017).

Importantly, H_2S, MM and DMS are generated by the sulfate reducing process. Therefore, the landfill odor arising from H_2S, MM and DMS merits particular attention. However, landfill sites are complicated man-made facilities with a variety of temperature and moisture contents as depth increases. The moisture content shows distinct variation with depth in the landfill. Moisture content is a vital factor controlling microbial metabolisms and activity. Sulfate reducing bacteria (SRB) are bacteria that have the function of sulfate reduction, and are also subject to differences in moisture factors. Our previous research found that the refuse with different moisture contents inside a landfill site showed different H_2S production behaviors (Ying et al., 2019). Moreover, temperature discrepancies inevitably cause major variations in microbial community and activity. Studies have shown that the sulfate reduction rate is higher in sediment of hot springs than in the soil environment (Adams et al., 1981; Fishbain et al., 2003). Fishbain et al. (2003) also found that the sulfate reduction rate could range from undetectable to over 10 mmol $SO_4^{2-}\cdot cm^{-3}\cdot d^{-1}$ in hot springs with different temperatures. However, various odor emission phenomena have been observed due to the temperature discrepancy in landfill environment (Hu et al., 2017; Bhattarai et al., 2018).

In the deeper layers of the landfill site, the refuse always stays in a saturated state because of the accumulation of leachate. Moreover, the deeper layer always has a high temperature because it has more active microbial metabolism than other layers. Hao et al. (2017) reported that the highest temperature of the landfill bottom layer can reach over 80 °C. It is reasonable to assume that intense sulfate reduction behavior can occur in the landfill leachate saturated zone, especially in terms of the relative higher temperature. High sulfate reduction rates are frequently observed in the sediment interface where solid and liquid are in close contact, considered a special saturated zone (Jorgensen et al., 2019). The landfill leachate saturated zone is a more complicated scenario than marine or lake sediments, because of the continuous input of electron donors and acceptors from the top refuse layer. Unfortunately, to our best knowledge, no previous studies have considered sulfate reduction in the leachate saturated zone.

In this research, a series of landfill leachate saturated zones were simulated under various temperatures and the generation of H_2S, MM and DMS was tracked. Moreover, the microbial diversity and the functional genes abundance were analyzed. This study aimed to provide accurate landfill management strategies for odor control by determining the sulfate reduction behavior discrepancy and its associated mechanisms.

2. Materials and methods

2.1. Materials

Mineralized waste refuse was collected from a real municipal solid waste landfill in Beijing, China. A drilling machine was employed to gather the waste from 10 points. A certain quantity of waste was gathered from 3 to 15 m beneath the cover. Then, mineralized refuse from 12 m and 15 m was selected and mixed thoroughly. Inert materials including stones, plastics and glasses were removed. The remaining refuse was cut into pieces <1 cm in diameter. A sieve was not used since the moisture content of the waste was extremely high. Then the cut refuse was gathered and intensively mixed again, then kept in an airtight plastic bag in a 0 °C refrigerator.

Since the in-situ leachate from the real landfill site was hard to collect simultaneously with the landfilled refuse, simulated leachate was prepared according the real leachate water quality. The main characteristics of the landfilled refuse and simulated leachate are listed in Table 1.

2.2. Experimental design

To achieve a leachate-saturated environment, 500-mL anaerobic bottles were used as the reactors. One hundred grams of collected refuse and 300 mL of leachate were gathered in 18 bottles, and the headspace volume (V) was recorded. Then the bottles were sealed with butyl rubber stoppers and plastic caps. Two three-way valves, connected with a long and a short needle tube, respectively, were connected to each bottle through the caps, and the long tube was able to reach the leachate surface. Then a Shih's fermenter was connected to the three-way valve with the short needle, to acquire the gas generation volume (V_1). A diagram of the reactor is shown in Fig. S1. Six of the bottles were sterilized with an autoclave and the six water bath kettles were set at 30, 40, 50, 60, 70 and 80 °C, respectively. Two unsterilized reactors and one sterilized reactor were settled into each kettle to simulate the different temperatures.

2.3. Analytical methods of sulfur-containing components, nitrogen-containing components and organic carbon

Samples from gaseous phase in the bottle were collected by an injector through the three-way valve with short needle, and detected directly on a daily basis until no sulfur-containing odor could be detected. The H_2S, MM and DMS concentrations were determined using a gas chromatograph equipped with a flame photometric detector (GC 7890A; Agilent Technologies, Santa Clara, CA, USA) (Zhang et al., 2017). The H_2S, MM and DMS concentrations were recalculated according to Eq. (1).

$$C = C_0 \times \frac{V}{(V + V_1)} \quad (1)$$

where C is the revised concentration of H_2S, MM or DMS, $mg\cdot m^{-3}$; C_0 is the detected concentration of H_2S, MM or DMS, $mg\cdot m^{-3}$; V is the headspace volume in the reactor, m^3; and V_1 is the generated gas volume, m^3.

Sulfate (SO_4^{2-}), sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^{2-}$), sulfur (S_0), sulfide (S^{2-}), nitrate (NO_3^-), nitrite (NO_2^-) and dissolved organic carbon (DOC) concentrations in the solid and liquid phase were determined before and after the experiment. The methods used to determine the concentrations were in accordance with the previous work (Fang et al., 2016; Ying et al., 2019). All analyses mentioned above were performed in triplicate.

2.4. Microbial diversity community analysis

Five grams of sample from each unsterilized reactor were collected after no sulfur-containing odor could be detected, and the samples at

Table 1
The main characteristics of mineralized refuse and leachate.

	SO_4^{2-}	$S_2O_3^{2-}$	S^0	S^{2-}	TOC	NH_4^+	NO_3^-	NO_2^-
Mineralized refuse (mg/kg)	2746.9	/	/	127.81	3620.05	2326.13	67.77	/
Leachate (mg/L)	1105.3	/	/	/	731.29	5.63	7.63	/

the same temperature were collected together. Microbial DNA was extracted from the samples using the E.Z.N.A.® soil DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The bacterial 16S rRNA genes of the DNA were amplified with the 515FmodF (5′-GTGYCAGCMGCCGCGGTAA-3′) and 806RmodR (5′-GGACTACNVGGGTWTCTAT-3′). The PCR reaction was in accordance with previous work (Liu et al., 2018). The bacterial community was investigated by high-throughput sequencing on an Illumina sequencing system in the laboratory of the Majorbio BioPharm Technology Co. Ltd. (Shanghai, China). Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) with a novel 'greedy' algorithm that performs chimera filtering and OTU clustering simultaneously.

2.5. Functional genes quantity

DNA isolation, detection and PCR amplification were in accordance with Section 2.4. The dsrA and dsrB genes of the bacteria were amplified with primers: dsrA_F (5′-ACSCACTGGAAGCACG-3′) and dsrA_R (5′-CAACATCGTYCAYACCCAGGG-3′); dsrB_F (5′-ACSCACTGGAAGCACG-3′) and dsrB_R (5′-GTGTAGCAGTTACCGCA-3′). The concentration of constructed plasmid was determined with a NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA).

3. Results and discussion

3.1. Release behavior of sulfur-containing odors

While the H_2S, MM and DMS concentrations remained below 10 $mg \cdot m^{-3}$ in the sterilized reactors, the temperature showed a remarkable influence on H_2S, MM and DMS concentrations in the unsterilized reactors. This reveals that almost all the H_2S, MM and DMS detected under the various temperatures in the system were generated by microbial activities after the reactors were loaded, and the minor concentrations detected in the sterilized reactors may be caused by the volatilization in the original refuse sample.

Compared with the reactors at 30 °C, both the generation speed and quantity of the odors were dramatically enhanced when the temperature was at 50 and 60 °C, but was inhibited under higher temperatures. As shown in Fig. 1(a), when the temperature was 30 °C, the maximum H_2S concentration occurred on day 11, which is 1626.7 $mg \cdot m^{-3}$. Although the generation of H_2S was markedly faster at 40 °C, the maximum concentration of 2191.9 $mg \cdot m^{-3}$ was brought forward to Day 5. At 60 °C, the generation speed reached a maximum on Day 3, and the corresponding concentration was 3289.8 $mg \cdot m^{-3}$. Instead of acquiring a faster generation speed, the reactors under 50 °C gave a maximum H_2S concentration of 4493.1 $mg \cdot m^{-3}$ on Day 4. The H_2S generation substantially increased at 50 and 60 °C.

The MM and DMS concentrations were similar to those of H_2S as shown in Fig. 1(b) and (c). At 30 °C, MM appeared on Day 7, reached the peak on Day 10 and was not detected on Day 11, whereas DMS appeared on Day 4, reached the peak on Day 11 and the concentration was below the detection limitation on Day 12. This may be because DMS can be generated through degradation of amino acid, and MM may mutually transform with H_2S and DMS, as shown in Eq. (2) (Bentley and Chasteen, 2004). The MM was detected on Day 1 at 40–60 °C, and reached the peak on Day 3, and the corresponding maximum concentrations were 145.0, 397.0 and 1363.6 $mg \cdot m^{-3}$, respectively. Likewise, DMS was also detected on Day 1 at 40 to 60 °C, the concentration reached the peak on Day 4 at 40 °C, whereas it was on Day 3 at 50 and 60 °C; the corresponding maximum concentrations were 160.4, 338.5 and 314.2 $mg \cdot m^{-3}$ respectively. The H_2S, MM and DMS concentrations decreased sharply after the peak occurred, whereas a gentle decline was observed at 60 °C.

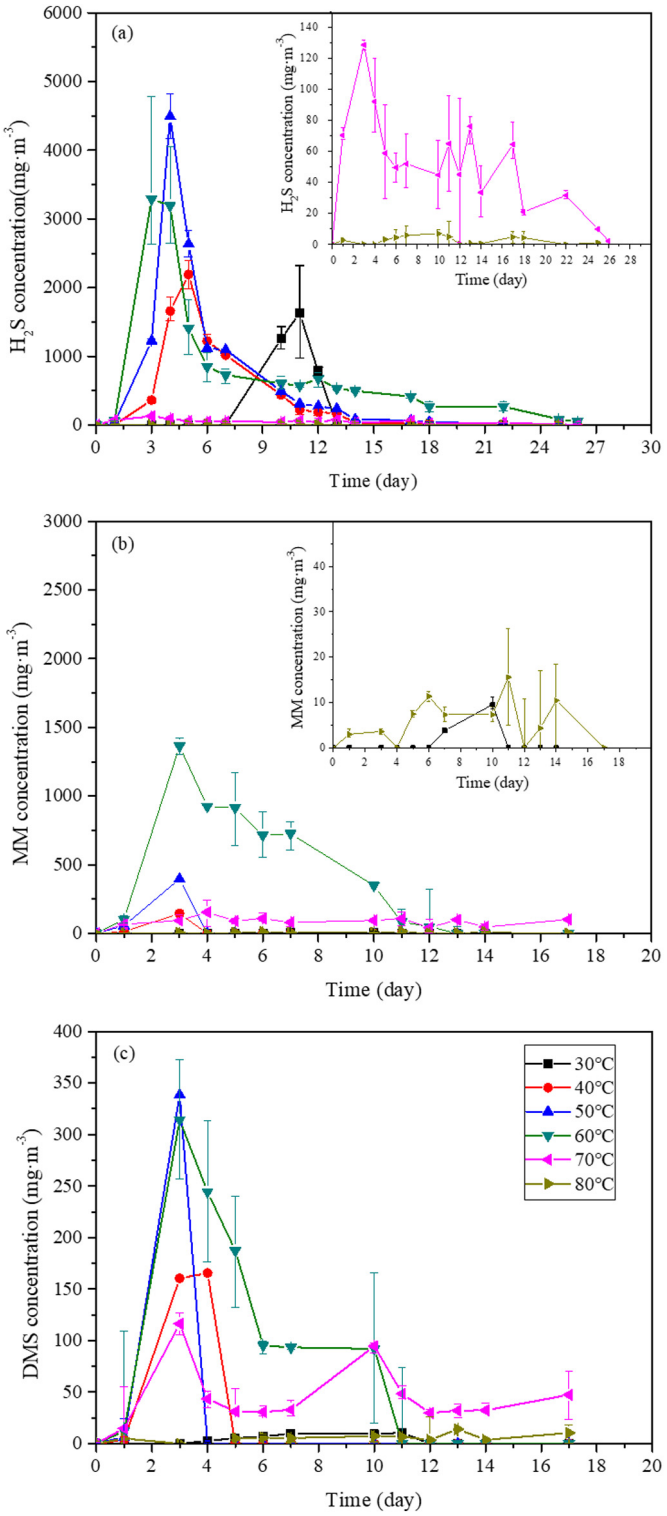

Fig. 1. The emission of hydrogen sulfide (a), methyl mercaptan (b) and dimethyl sulfide (c) under various temperatures, the enlarged view is the emission of them which are not clearly showed in the origin figure.

$$H_2S \underset{\text{demethylation}}{\overset{\text{methylation}}{\rightleftarrows}} MM \underset{\text{demethylation}}{\overset{\text{methylation}}{\rightleftarrows}} DMS \quad (2)$$

The generation speed of H_2S, MM and DMS was relatively low at 70 °C, and there was a slight fluctuation over time, which might be

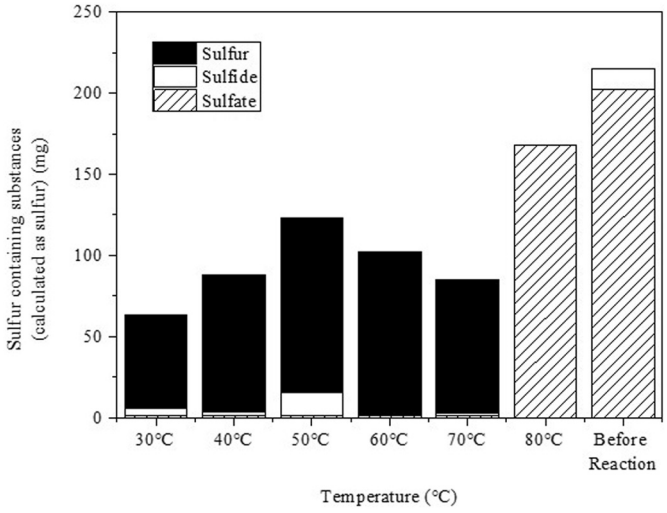

Fig. 2. Sulfate fate (calculated as sulfur) after the reaction.

caused by the constant generation, transformation and dissolution of the odors. Hardly any H_2S, MM and DMS was detected in the reactors at 80 °C, indicating that microbial sulfate reduction activity had completely ceased.

These phenomena were also similar to those recorded under similar circumstances in other environments. For example, Frank et al. (2015) found that sulfate reduction was enhanced when the temperature was 50 °C on the seafloor. In addition, in composting reactors, VOSCs including MM and DMS were more easily generated when the composting temperature was over 55 °C (Maulini-Duran et al., 2013; Zang et al., 2016). Overall, it is clear that under certain temperatures (around 50–60 °C), the microbial activity can be dramatically enhanced, leading to an elevated generation speed and volatile concentrations of H_2S, MM and DMS. In conclusion, a considerable difference in odor generation phenomena may exist in the leachate saturated zone of landfill sites.

3.2. Sulfate reduction behavior discrepancy

Various sulfur-containing substances can be formed during the sulfate reduction process. The fractions of each sulfur-containing substance are shown in Fig. 2. Sulfite and thiosulfate were not detected at any temperature. In the reactors below 80 °C, sulfate was almost exhausted after no odors could be detected. Sulfur was the dominant fraction, occupying over 85% of all the sulfur-containing substances. At the same time, the sulfide content in all the reactors except 50 °C showed a decline compared with the original sample. Since the maximum concentration of H_2S (4493.1 $mg \cdot m^{-3}$) may generate around 1268 mg of sulfur, sulfide might have been be oxidized to sulfur through simultaneous desulfurization and denitrification. Hardly any nitrogen forms were detected after the reaction (see Table S1), which supports this finding. The insufficient sulfur for odor generation also reveals that sulfate was not the only electron acceptor, and organic sulfur might be another important acceptor, which is in accordance with previous work (Zhang et al., 2017) and other forms of sulfur, such as trisulfide, should exist after the reaction in the system.

To evaluate the temperature effect on the sulfate reduction, the contribution of sulfate to sulfide and sulfur generation was listed in Table 2 according to the theoretical generation equations (Eqs. (3) and (4)).

$$SO_4^{2-} + 2(CH_2O) + 2H^+ \overset{SRB}{\rightarrow} H_2S + 2CO_2 + 2H_2O, \quad (3)$$

$$SO_4^{2-} \overset{SRB}{\rightarrow} S. \quad (4)$$

The temperature clearly affected the transform of sulfur-containing substances. Although some indictable sulfur containing components were present, the contribution rate was 28.9% at 30 °C and it reached over 50% at around 50–60 °C, indicating that sulfate reduction was greatly enhanced. Interestingly, the contribution rate was similar at 40 °C and 70 °C. This was consistent with the constant detection of MM and DMS under 70 °C, sulfate reduction should be as intense as that under lower temperatures, and may cause problems in the long run.

The temperature effect on microbes can also be revealed through DOC consumption, since it can be considered as the electron donor for microbial processes. Fig. 3 shows that microbial activity was inhibited at 70 and 80 °C and almost no DOC was utilized when the reactor temperature was 80 °C. This might be the main reason that sulfate reduction was low at higher temperatures; the microbial activity was weakened. However, the DOC consumption was similar in the reactors below 60 °C, which indicates that the total microbial activity was similar at 60 °C and thus the difference of sulfate reduction may be that the microbial structure was reshaped. Therefore, the microbial community structure was then investigated.

3.3. Functional microbial community structure at various temperatures

The principal co-ordinates analysis (PcoA) (see Fig. S2) result shows that temperature exerted a strong influence, whereas at each temperature, microbial composition was distinguished from the others, which may cause variable generation of odors under different temperatures. The composition was relatively similar for reactors at 50 and 60 °C, and also between those at 30 and 40 °C. As shown in Fig. 4a, *Methanosarcina*, *Caldicoprobacter* and *Norank_f_Lentimicrobiaceae*, were dominant at 30 to 50 °C, whereas at 60 °C, the abundance of *Bacillus* and *Halocella* reached over 0.4. *Unclassfied_f_Bacillaceae* and *Atopostipes* were dominant at 70 and 80 °C. However, to our knowledge, no present studies have reported sulfate reduction ability of these

Table 2
Contribution rate of sulfate to sulfide and sulfur generation.

Temperature (°C)	SO_4^{2-} decrement (mg)	S^0 increment (mg)	S^{2-} increment (mg)	SO_4^{2-} contribution rate (%)
30	200.7	57.9	−8.45	28.9
40	200.8	84.6	−10.4	42.1
50	201.0	107.8	1.6	54.4
60	201.0	101.3	−12.7	50.4
70	200.9	82.6	−11.5	41.1
80	34.3	0	−12.5	0.0

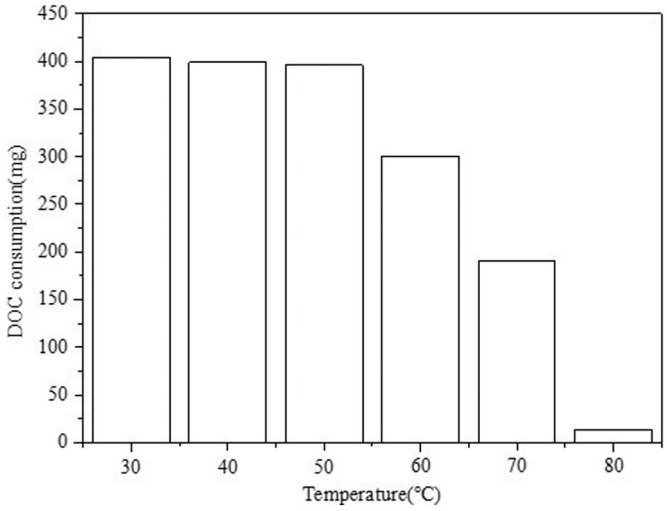

Fig. 3. Consumption of DOC in the reactors.

bacteria or other species with high relative abundances. Therefore, the relative abundances of SRB at various temperatures were acquired.

As shown in Fig. 4b. *Uncultured_prokaryote_g__norank_Peptococcaceae* and *Unclassified_g_norank_f_Peptoccaceae* were predominant in the reactors at 30 and 40 °C. *Uncultured_bacterium_g_Dethiobacter* was the dominant sulfate-reducing bacteria at 50 and 60 °C. The abundance of *uncultured_bacterium_g_desulfitibacter* was high at 70 °C, whereas several different bacteria were observed at 80 °C. The dependency of environmental factors is shown in Fig. 5. It was found that among the SRB, *Uncultured_bacterium_g_Dethiobacter* had a close correlation with S_0 ($P < 0.001$) and H_2S ($P < 0.01$) generation. This indicates that the increase in *Uncultured_bacterium_g_Dethiobacter* might enhance the sulfate reduction degree.

In an attempt to ensure the enhanced sulfate reducing activity by *Uncultured_bacterium_g_Dethiobacter*, functional genes were analyzed. DsrA and dsrB are two basic functional genes involved in sulfate reduction to adenosine phosphosulfate (APS). However, research has shown that more functional genes can be involved in the sulfate reducing process (Florentino et al., 2019). For example, *Uncultured_bacterium_g_Dethiobacter* has no dsrA and dsrB genes, and can only use sulfite or thiosulfate as the electron acceptor (Sorokin et al., 2008). Interestingly, the quantity of dsrA and dsrB did not show any dependency with the generation of sulfur-containing odors, but was only relevant to the temperature (Fig. S3). This indicates that sulfate reduction to APS by dsrAB may not be rate limiting and temperature increase might speed up the reduction of other intermediates through *Uncultured_bacterium_g_Dethiobacter*. Moreover, Sim et al. (2019) found that the activity of APr, a reductant of APS, was more activated under elevated temperature. Therefore, further study

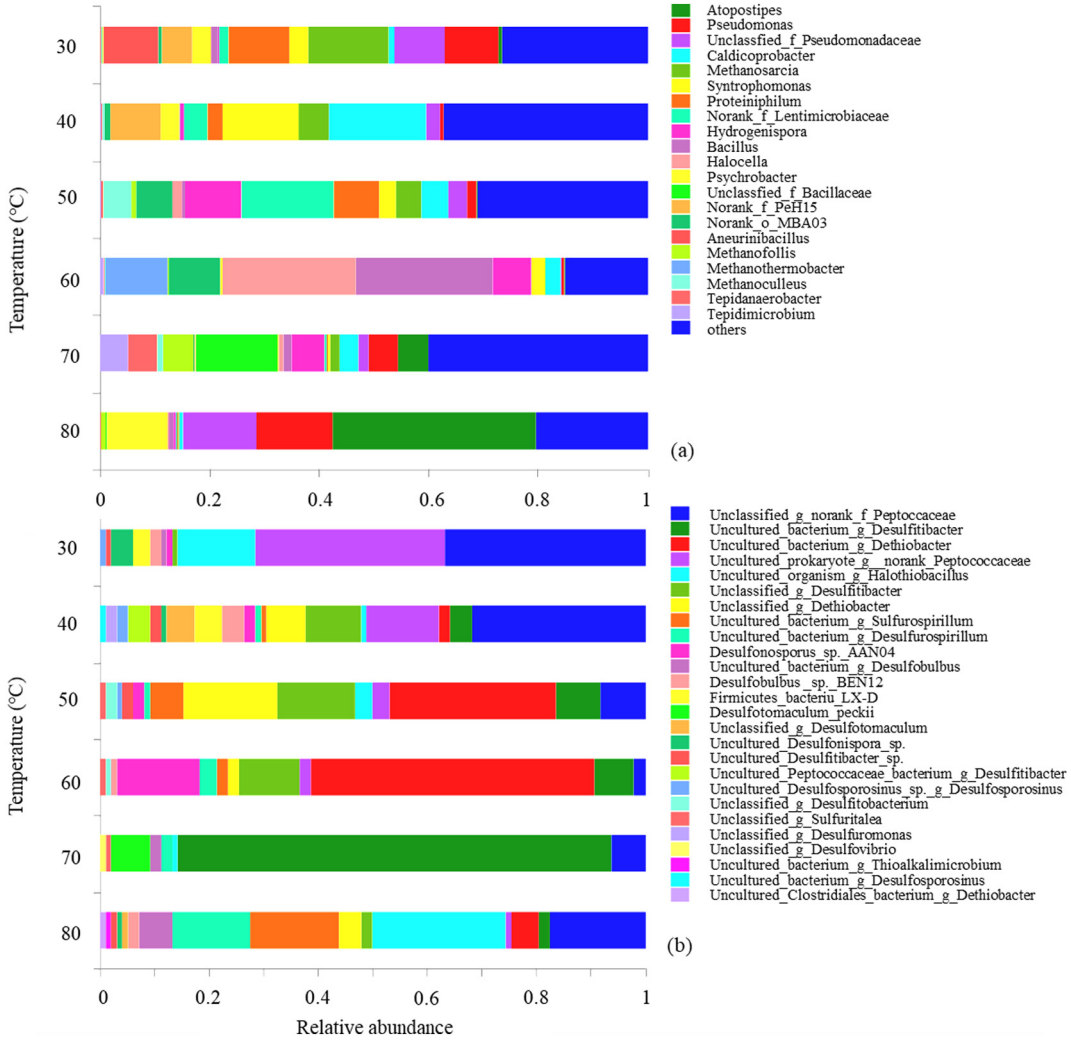

Fig. 4. Microbial community of all the species at genus level (a) and SRBs at species level (b).

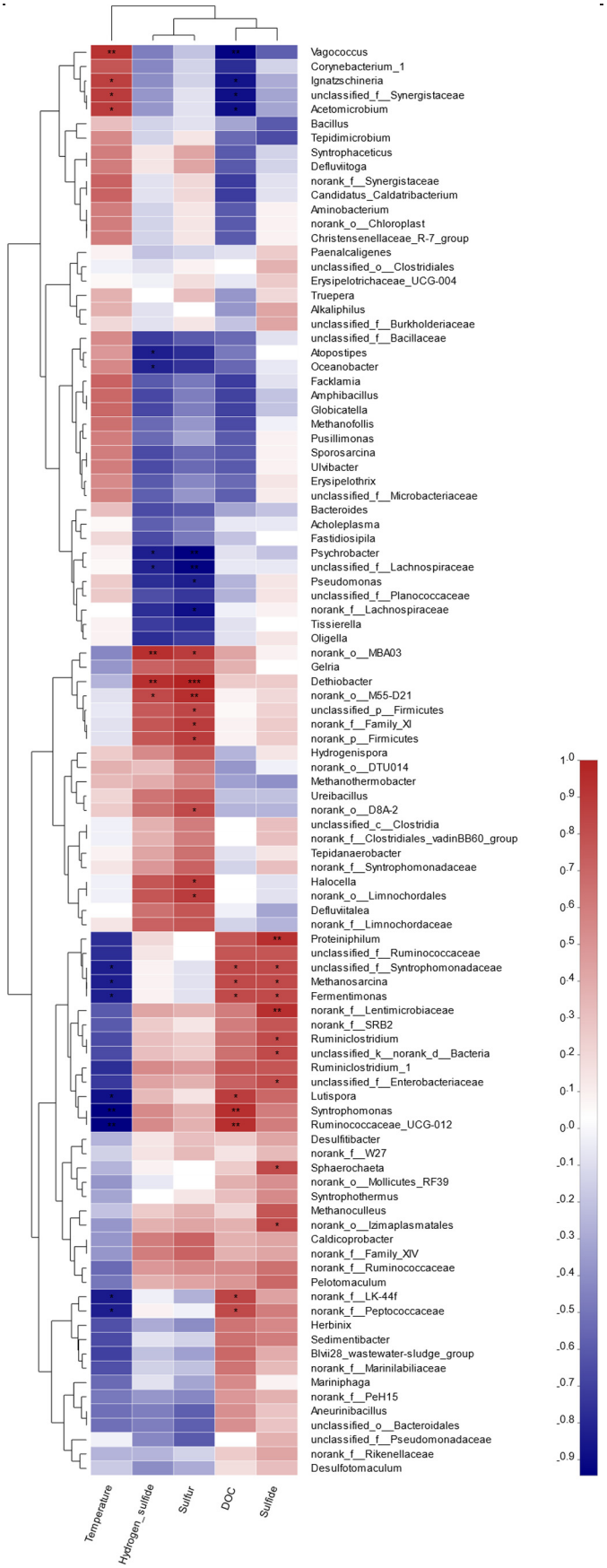

Fig. 5. Correlation analysis of environmental factors.

should focus on the thorough mechanism of temperature impact on sulfate reduction.

Other bacteria from *Clostridia* such as *MBA03* and *M55-D21* also showed a correlation ($P < 0.05$) with H_2S. It has been reported that MBA03 has the ability to degrade polysaccharide, and this may offer sufficient electron donors, as mentioned above. The whole community abundance at genus level as shown in Fig. 4(a) also revealed similar results. *Lentimicrobium* was the dominant genus at 50 °C, whereas *Bacillus* and *Halocella* were dominant at 60 °C. It was found that *Lentimicrobium* and *Halocella* were able to utilize complex organic materials, and the degradation product might be more easily absorbed for sulfate reduction (Zheng et al., 2019; Wang et al., 2019). It should be noted that the abundance of *Pseudomonas* and *Pseudomonadaceae*, which are known to inhibit the activity of SRB through nitrate reduction, was high at lower temperatures, as mentioned in Section 3.2. In addition, these bacteria can cause the oxidation of sulfide through nitrate reduction, and this might explain why sulfide was hardly detected in the system.

4. Conclusion

Our research showed that remarkable differences in sulfate reduction behavior may exist in the landfill leachate saturated zone. The generation pattern of sulfur-containing odors was disparate, being enhanced at 40 °C compared with that at 30 °C, and it considerably higher at 50–60 °C. An extended time may be required to diminish these gases at 60 °C. At 70 °C, the generation may be sustained for a long time, whereas it was inhibited at 80 °C. The transformation of sulfur, nitrogen and organic carbon showed that temperature can affect the sulfate reduction process, but other microbial activities may not be completely terminated. Finally, the microbial community and functional genes results showed that SRB abundance varies with temperature, and higher quantities of dsrA and dsrB were found at lower temperatures, indicating that sulfate reduction behavior may be controlled by specific SRB such as *Dethiobacter* in the leachate saturated zone. Other bacteria that can offer energy to SRB may also exert an influence. Further research is needed to focus on the specific mechanism of this influence.

CRediT authorship contribution statement

Zhiyuan Jin: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Manting Ci:** Writing - review & editing, Investigation. **Wenyi Yang:** Writing - review & editing, Investigation. **Dongsheng Shen:** Methodology, Supervision, Project administration, Funding acquisition. **Lifang Hu:** Methodology, Resources. **Chengran Fang:** Methodology, Resources. **Yuyang Long:** Conceptualization, Methodology, Validation, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138946.

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