Effects of intermittent chemical dosing on volatile sulfur compounds in sewer headspace

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ABSTRACT
Volatile sulfur compounds (VSCs), including hydrogen sulfide (H₂S) and volatile organic sulfide compounds (VOSCs), can be produced in sewer systems causing sewer odor problems. In this study, the effects of intermittently dosing ferric iron, hydrogen peroxide, and nitrate on H₂S and VOSCs in sewer headspace were investigated. In order to characterize the composition of VSCs, an HC-3 trace sulfur analyzer and gas chromatograph (GC) equipped with a triple quadrupole-type mass spectrometry (MS) apparatus were used to determine the VSCs. The results indicated that the effect of intermittent addition of 40 mg/L ferric iron or 40 mg/L hydrogen peroxide is limited for VSCs inhibition. However, the intermittent addition of 40 mg N/L nitrate has a relatively stable control effect of H₂S and VOSCs which maintaining 60% removal rate. Moreover, methyl mercaptan (MeSH) was the most abundant compound of the total VOSCs released and the results of the theoretical odor concentration study also indicate MeSH is the main VOSC causing the significant odor problem. Therefore, more attention should be focused on the VOSCs which have extremely low odor threshold.

Keywords: Chemical dosing; Headspace phase; Sewer odor; Theoretical odor concentration; Volatile sulfur compounds

1. Introduction
During the transport of sewage over a long distance, various odorous compounds can be produced in sewer systems cause sewer odor problems and adverse health effects [1]. Hydrogen sulfide (H₂S) is a volatile and poisonous compound with rotten egg smell which is considered the major reason for odor emission in sewer management. Sulfide also can cause the corrosion of sewer infrastructure and has a detrimental effect on human health. Various chemical dosing methods have been extensively used to remove H₂S in sewer networks [2].

The commonly used chemicals to mitigate the negative effects of sulfide are iron salts, oxygen, nitrate, sodium hydroxide, magnesium hydroxide [3]. All these chemicals, except for NaOH, typically require continuous dosing strategy. However, continuous dosing will result in high chemical supply costs. Besides, the intermittent operation of pumping stations makes traditional continuous dosing inadequate. Therefore, the intermittent dosing strategy was proposed as an alternative cost-effective strategy for sulfide elimination, especially in the condition of periodical operation of pump stations. Liu et al. [4] proposed intermittent nitrate dosing for sulfide control in sewer sediments. Jiang et al. [5] investigated the intermittent dosing of free nitrous acid as a measure for controlling sulfide and methane production. Moreover, the sulfide compounds control effect of the intermittent dosing was also assessed in other previous researches [6].

On the other hand, VOSCs such as methyl mercaptan (MeSH), dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), carbon disulfide (CS₂), and ethanethiol (EtSH) with obnoxious odors also can be produced in sewers which can subsequently be transferred to the headspace air by mass transfer [7]. In terms of odor impact, VOSCs may still cause serious odor problems due to their extremely low odor threshold values. However, the bulk of investigations have focused entirely on the variation of sulfide in the liquid phase before and after treatment. The control effect of intermittent dosing on typical VOSCs (MeSH, EtSH, DMS, DMDS, and CS₂) is unclear.

This study aims to understand the typical VOSCs and H₂S concentration changes during the intermittent dosing process and to evaluate the effectiveness of different chemicals for VSCs control. The results of this study can provide valuable insights into the management of odor problems in sewer systems.
eration variation in sewer headspace with intermittently chemical dosing such as ferric iron, hydrogen peroxide, and nitrate. Moreover, the odor control effect of these chemicals was evaluated by theoretical odor concentration. Besides, the changes in the composition of microbial communities in sewage after intermittent chemical dosing were explored through 16S rRNA gene sequencing over the period to discuss the control mechanism.

2. Methods and Materials

2.1. Experimental Setup and Operation

The system consists of three laboratory-scale PersPex™ reactors operated in parallel, namely R1, R2, and R3. Each reactor had a volume of 1.9 L, with a diameter of 90 mm and a height of 300 mm and equipped with a gas sampling port as shown in Fig. 1. The sediment samples were collected using a cleaning device from the main sewer in Hangzhou, China, which were then transported to the lab and kept at 4°C before usage [8, 9]. The sediment samples had the following properties: bulk density 1.22 g/cm³; total solids (TS) 75.2%; and volatile solids (VS) 4.8% [10]. 380 mL sediment was inoculated in each reactor, resulting in an initial sediment depth of 60 mm. Synthetic domestic wastewater was used in this study according to the previous study [11]. Sulfate concentration was prepared with 66 ± 3 mg/L and the chemical oxygen demand (COD) concentration was 300 ± 10 mg/L. The reactors were fed with synthetic sewage through a peristaltic pump intermittently (COD) concentration was 300 ± 10 mg/L. The reactors were fed with synthetic sewage through a peristaltic pump intermittently every 24 h.

The reactors had been operated anaerobically for 30 d at 25 ± 5°C before this study was conducted. This experiment was conducted in two phases. In phase I, the monitoring was performed for 5 d (Day 1 to Day 5) before starting the chemical addition to confirm the steady-state conditions in terms of VSCs production. In phase II (Day 6 to Day 10), the R1, R2, and R3 were intermittently dosed with ferric, H₂O₂, and nitrate, respectively every 24 h. These chemical agents have different mechanisms for VSCs elimination. Nitrate is a thermodynamically favorable electron acceptor, while H₂O₂ is a strong oxidant. Ferric ions (Fe³⁺) oxidize sulfide to elemental sulfur while being reduced into Fe²⁺, which precipitates with sulfide to form ferrous sulfide precipitants.

Throughout the two phases, the gas samples in each reactor were collected each day at 9:00 h for analysis of the sulfur-containing compounds. After sampling, fresh synthetic sewage was pumped to the reactor to thoroughly replace the previous liquid in reactors. Then, the gas-phase in reactors was purged with high purity nitrogen gas (500 mL/min) for 20 min every day after sampling to remove previous gas remaining.

2.2. Chemical Analysis Method

The MeSH and H₂S concentrations were determined using an HC-3 trace sulfur analyzer and gas chromatograph (GC) equipped with a flame ionization detector (FID). The HC-3 trace sulfur analyzer equipped with a polytetrafluoroethylene column and a flame photometric detector was used for the analysis of MeSH, with high-purity nitrogen (10 mL/min) as the carrier gas. H₂S was detected by GC with FID equipped with a DB-624 capillary column (30 m × 0.320 mm × 1.80 μm). Nitrogen was chosen as the carrier gas, with a flow of 12 mL/min. The respective temperature of the chromatographic column, sample injector, and detector was set at 120°C, 250°C, and 220°C [12].

Other sulfur-containing gases such as EtSH, DMS, DMDS, and CS₂ were identified by full-scan using a triple quadrupole-type mass spectrometry (MS) apparatus (Agilent 6890N GC and 5975B MS). The chromatographic column used was DB-624 (60 m × 0.25 mm × 1.40 μm) and the carrier gas flow was maintained at 1.0 mL/min flow rate. The GC oven temperature program was as follows: initial 35°C was held for 3 min, increasing at a rate of 6°C/min to 140°C, then at a rate of 10°C/min to 220°C, held for 3 min. The temperature of the injector and detector was maintained at 140°C and 220°C, respectively. Electron impact ionization (EI) was used as an ionization mode of the MS. Ion source temperature was kept at 230°C. The selected ion monitoring (SIM) mode of EI was also applied in the quantitative analysis.

2.3. Theoretical odor Concentration

The odor threshold concentration of each volatile sulfur compound varies widely shown in Table S1 [13]. High volatile compound concentration does not always cause malodor. Therefore, the theoretical odor concentration (TOC) is used to identify the main odorous substances which are based on the ratio of odorous compound concentration and its odor threshold value, as shown in Eq. (1) and Eq. (2) [14].

\[
D_t = \frac{C_t}{C_i}
\] (1)
\[ D_T = \sum_{i=1}^{n} D_i \]  

where \( C_i \) is the analytical concentration of compound \( i \) (\( \mu g/m^3 \)); \( C'_i \) is the odor threshold concentration of compound \( i \) (\( \mu g/m^3 \)); \( D_i \) is defined as “theoretical odor concentration”; \( n \) is the total number of compounds; \( D_T \) is the total theoretical odor concentration of all \( n \) compounds.

2.4. Microbial Community Analysis

On Day 4, Day 6, and Day 10, the biofilm samples in wastewater were collected from each sewer reactor. The total genomic DNA was extracted from collected samples using the PowerMax™ DNA Kit according to manufacturer instructions (MOBIO Laboratories, San Diego, CA, USA) and quantified using the Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted genomic DNA (gDNA) was amplified with the primers 515F/806R (515F: 5’-GTGCCAGCMGCCGCGGTAA-3’; 806R: 5’-GGACTACVSGGGTATCTAAT-3’) targeting the V4 hypervariable region [15]. The PCR was conducted for each sample of the reaction mixture (50 \( \mu L \)) containing 25 \( \mu L \) of Phusion High-Fidelity PCR Master Mix with HF Buffer, 3.0 \( \mu L \) of each primer, 10 \( \mu L \) of template DNA, and nuclease-free water. The PCR conditions were as follows: initial denaturing step at 98°C for 30 s, followed by 25 cycles of 98°C for 15 s, 58°C for 15 s, 72°C for 60 s, and a final extension of 10 min at 72°C. Subsequently, PCR products of each sample were purified by AMPure XP beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). A mixture of the amplicons was then used for high-throughput sequencing on the Illumina MiSeq platform [16].

After sequencing, the primers and spacers were trimmed. To minimize the effects of random sequencing error, both the low quality (lower than 20) and the sequences shorter than 150 bp were removed. The operational taxonomic units (OTUs) analysis was performed using QIIME v2.4.4. The OTUs were picked by using the de novo OTUs picking protocol with a 97% identity threshold. The VENARCH was used to perform species annotation on the representative sequence based on the SILVA128 database and further generated OTU lists.

3. Results and Discussion

3.1. Concentration Changes of VSCs in Headspace Gas Phase

The odor of volatile pollutants that escaped from the sewers is primarily caused by VSCs (i.e., VOSCs and \( \text{H}_2\text{S} \)) released from the liquid phase. Therefore, we evaluate the VSCs concentration variation in headspace 24 h after each dosing. The variation of total VSCs concentration, as well as VOSCs and \( \text{H}_2\text{S} \) percentage in the headspace before (Phase I) and after \( \text{FeCl}_3 \), \( \text{H}_2\text{O}_2 \), and \( \text{KNO}_3 \) intermittent dosing (Phase II) are shown in Fig. 2. In Phase I, the total VSCs concentration in the headspace ranged from 15,000 to 23,000 \( \mu g/m^3 \). VOSCs had much higher concentrations than \( \text{H}_2\text{S} \) in Phase I, accounting for nearly 90% of total VSCs concentration. It might be attributed to the degradation of sulfur-containing amino acids such as methionine and cysteine in wastewater, which was usually decomposed to VOSCs by deamination and demethylation. The result in this study was constant with the previous study which showed VOSCs were the highest concentration of the detected VSCs [14]. In Phase II, the intermittent addition of \( \text{FeCl}_3 \), \( \text{H}_2\text{O}_2 \), and \( \text{KNO}_3 \) contributed to significant decreases in the VSCs concentrations in the first three days. The total VSCs in the headspace decreased from 22,000-24,500 \( \mu g/m^3 \) to 6,200-7,500 \( \mu g/m^3 \) for ferric iron and nitrate intermittent addition, from 20,000 \( \mu g/m^3 \) to 9,800 \( \mu g/m^3 \) for \( \text{H}_2\text{O}_2 \) addition. However, the total VSCs concentration picked up in the last two days and reached around 25,000 \( \mu g/m^3 \) for ferric iron and \( \text{H}_2\text{O}_2 \) addition, while it rebounded slowly and increased to 11,500 \( \mu g/m^3 \) for nitrate addition. In Phase II, VOSCs were still the most abundant, with an average percentage concentration of 80%, 82%, and 86%, respectively, while the proportion of \( \text{H}_2\text{S} \) increased slightly.

Fig. 3 displays the total VOSCs and \( \text{H}_2\text{S} \) concentration in the headspace in Phase I and II. The initial VOSCs concentrations in R1, R2, and R3 ranged from 14,700-22,000 \( \mu g/m^3 \), 15,200-19,900 \( \mu g/m^3 \), and 13,400-24,600 \( \mu g/m^3 \), respectively, which were much higher than the results of field studies [7, 16, 17] probably because of the optimal lab condition of microorganisms growth and higher sulfur content. In Phase II, \( \text{FeCl}_3 \), \( \text{H}_2\text{O}_2 \), and \( \text{KNO}_3 \) addition caused a substantial decrease of VOSCs.
Variation of the total VOSCs and H$_2$S concentrations in the headspace in Phase I and II (a) VOSCs; (b) H$_2$S.

Fig. 3. Variation of the total VOSCs and H$_2$S concentrations in the headspace in Phase I and II (a) VOSCs; (b) H$_2$S.

on day 6 to day 8, reaching the average concentration of 5,300 μg/m$^3$, 8,400 μg/m$^3$, and 7,100 μg/m$^3$, respectively. However, the VOSCs concentrations in the treatment of FeCl$_3$ and H$_2$O$_2$ increased significantly on day 9 and day 10, even at the end of the experiment around 20% and 30%, respectively higher than the initial average VOSCs concentrations. For H$_2$S, its concentration also decreased from about 2,000 μg/m$^3$ to about 1,000 μg/m$^3$ on the first day of Phase II (Fig. 3(b)). Previous studies have shown that all three chemicals that possess oxidization could oxidize sulfide chemically. Besides, ferric iron could precipitate sulfide while nitrate could oxidize sulfide biologically by stimulating heterotrophic nitrate-reducing bacteria (hNRB) and nitrate-reducing sulfide-oxidizing bacteria (NR-SOB) in sediment. Therefore, a good sulfide control effect after treatment could be achieved. However, a similar rebound trend was also observed, and the H$_2$S increased four-fold and two-fold in the intermittent addition of FeCl$_3$ and H$_2$O$_2$ respectively on the last day. H$_2$O$_2$ has been used to reduce COD and offensive odor in domestic or industrial wastewater for many years [18], but the disadvantage is the short lifetime of H$_2$O$_2$ [19]. Moreover, sulfide oxidation by H$_2$O$_2$ could also involve the formation of elemental sulfur [20]. Ferric ions also could oxidize sulfide to elemental sulfur while being reduced into ferrous ions [21]. The produced elemental sulfur accumulated on the sediment probably attributed to the rebound of VSCs in the later-stage of the experiment. Besides, different chemicals including ferric chloride and hydrogen peroxide showed that all could reduce VOSCs production in biosolids and freshwater sediment, and none were vastly superior to the other [22]. However, the result in this study showed that the concentration of VOSCs rebounded in the late-stage experiment which was inconsistent with the previous study. This result could attribute to the sewer sediment composition which needs further research. On the contrary, the H$_2$S and VOSCs concentrations are relatively stable after the addition of nitrate and increased slightly to 1,700 μg/m$^3$ and 8,000 μg/m$^3$, respectively. Previous studies revealed that syntrophic degradation of VOSCs by methanogens and nitrate-reducing bacteria could exist in freshwater sediments [23]. Therefore, nitrate addition could stimulate nitrate-reducing bacteria to thrive in the sewer sediment which could degrade VOSCs thereby maintain the VOSCs control efficacy. This result indicated that nitrate could be a preferred option among the intermittent dosage for VSCs control.

3.2. Variation of VOSCs Concentrations in Sewer Headspace Air

MeSH, EtSH, DMS, CS$_2$, and DMDS were detected in the headspace phase. The variation of total VOSCs and the proportions of five components in the headspace are shown in Fig. 4. MeSH was the most abundant compound in the total VOSCs, accounting for around 95% of the VOSCs in Phase I and II. This result agreed with the previous reports that VOSCs were generally dominated by MeSH [7, 16]. In Phase II, the proportion of MeSH was reduced slightly by 12%, 20%, and 5% on day 6 by the addition of FeCl$_3$, H$_2$O$_2$, and KNO$_3$, respectively. However, it was observed that the proportion of DMDS increased, especially with the treatment of H$_2$O$_2$. It is likely due to DMDS was formed by oxidation of MeSH [24]. The levels of EtSH, DMS, and CS$_2$ were relatively low, constituting less than 3% of the initial total VOSCs, even changed little after chemical intermittent dosing.

Fig. 5 illustrated the concentration variation of each VOSCs (MeSH, EtSH, DMS, CS$_2$, and DMDS) in the headspace during Phase I and Phase II. The initial concentration of MeSH fluctuated between 12,100-19,100 μg/m$^3$, 13,000-17,200 μg/m$^3$ and 11,300-21,900 μg/m$^3$, respectively in Phase I. In Phase II, the emission of MeSH was reduced slightly by 74%, 54%, and 50% by the addition of FeCl$_3$, H$_2$O$_2$, and KNO$_3$, respectively on day 6. However, it was also observed that there was a marked increase of MeSH concentration in the treatment with FeCl$_3$ and H$_2$O$_2$ on day 9, even jumped to 20,200 μg/m$^3$ and 16,200 μg/m$^3$, respectively on day 10. The DMDS concentration tended to increase after all three chemicals addition on day 6, it even rose from around 280 μg/m$^3$ to 2,400 μg/m$^3$ with H$_2$O$_2$ treatment, but it then decreased sharply to around 500 μg/m$^3$ on day 7. The results indicate that a greater amount of DMDS can originate from the chemical oxidation of methyl mercaptan when dosing strong oxidants such as hydrogen peroxide [25, 26]. The other three VOSCs (EtSH, DMS, and CS$_2$) showed no significant differences among the three chemical treat-
ments since the average concentrations detected (< 350 μg/m³) were 1-2 orders of magnitudes lower than DMDS and MeSH (Fig. 5(c)-(e)).

3.3. Variation of Theoretical Odor Concentration in Headspace Gas

The theoretical odor concentration (ThOC), which was widely used to determine the main odorous substances [14], was used in this study to assess volatile compounds’ pollution potential and to identify the main odorous compounds in the sewer system. The sulfur compounds include H₂S, MeSH, EtSH, DMS, CS₂, and DMDS, which have all been reported as the main constituents of sewer odor. In terms of the ThOC of sulfur compounds, the contribution of 6 sulfur compounds to the total ThOC of sulfur compounds are shown in Fig. 6. The total ThOC ranged from 8.7 × 10⁴ to 1.3 × 10⁵ for R1 (ferric), from 9.3 × 10⁴ to 1.2 × 10⁵ for R2 (hydrogen peroxide), and from 8.2 × 10⁴ to 1.5 × 10⁵ for R3 (nitrate) in the Phase I. After intermittent dosing chemicals, the total ThOC reduced to 40,000-60,000 in the first 3 days of Phase II, and then rebounded again for the Fe³⁺ and H₂O₂ addition. On the other hand, The ThOC of MeSH was nearly thirty times higher than that of H₂S or EtSH. Even with intermittent chemical dosing, MeSH was still the main sulfur odor constituent emitted from the liquid phase. These results indicate that MeSH is the main substance causing the significant odor problem in this study, with an average percentage concentration of 93.8%, followed by EtSH (3.4%) and H₂S (2.8%). So particular attention needs to be paid to the MeSH which has an extremely low odor threshold (0.15 μg/m³).
3.4. Microbial Communities

Fig. 7 shows the microbial communities before and after intermittent dosing of Fe³⁺, H₂O₂, and NO₃⁻. The relative abundance of bacterial genera related to sulfate reduction remained around 6.5% before chemicals addition (Fig. 7(a)), where Desulfovibrio, Sulfurosirillum, Desulfobulbus, and Pseudomonas dominated in the sulfate-reducing bacteria (SRB). No significant relative abundance change in the sulfate-reducing community was observed with all three chemicals intermittent addition, where Desulfovibrio, Sulfurosirillum, Desulfobulbus, and Pseudomonas still dominated in the SRB, which agreed with the results of previous studies that nitrate and ferric addition did not alter the major SRB populations [27, 28]. The addition of H₂O₂ also did not affect the SRB activity greatly due to its short lifetime. Therefore, the intermittent dosing of Fe³⁺, H₂O₂ and NO₃⁻ in this study had not altered the relative abundance of SRB. Moreover, the sulfate respiration functional group exhibited a slight increase after chemical intermittent dosing, with a two-fold increase in relative abundance after continuing intermittent dosing (Fig. 8). The sulfate respiration functional group responds to the sulfate reduction, which probably leads to the rebound of VSCs in the later stage of this experiment (Fig. 2 and Fig. 4).

On the other hand, the genera related to sulfide oxidizing bacteria (SOB) comprised approximately 3.6%, 3.7%, and 4.6% of all sequences before Fe³⁺, H₂O₂, and KNO₃ dosing, respectively (Fig. 7(b)). Sulfuricurvum, Novispirillum, Pseudomonas, and Cloacibacterium were the dominant genera among detected 16 genera. SOB sequences increased to around 6% in relative abundance with 24h H₂O₂ and nitrate treatment, which may be responsible for the decrease of VSCs.
Further investigation is recommended in the fieldwork.

Actors may vary considerably which could affect the control effect. Wastewater. Besides, the composition of sediment in different reactors, which cannot be assessed with the use of synthetic wastewater contains microorganisms, which continuously inoculate the reactor. This will influence the microbial community in the reactor, which cannot be assessed with the use of synthetic wastewater. Besides, the composition of sediment in different reactors may vary considerably which could affect the control effect. Further investigation is recommended in the fieldwork.

4. Conclusions

In this study, the VSCs (H2S, MeSH, EtSH, DMS, DMDS, and CS2) concentration variation in the sewer headspace gas phase when intermittently dosing ferric iron, hydrogen peroxide, and nitrate were investigated. During intermittent dosing of ferric iron (40 mg/L) and hydrogen peroxide (40 mg/L), the control effect of VSCs in the sewer gas phase was found to be limited. The VSC concentrations increased significantly in the late-stage experiments, even at the end of the experiment around 20% and 30%, respectively higher than the initial average VSCs concentrations. This result is probably attributed to the element sulfur accumulation and the increase of the sulfur-oxidizing bacteria. However, the intermittent addition of 40 mg N/L nitrate has a relatively stable control effect of H2S and VOSCs which maintaining 60% removal rate, which is probably attributed to the enrichment of nitrate-reducing bacteria that degrade VOSCs. Besides, MeSH was found to be the most abundant compound of the total VOSCs released, accounting for nearly 95% of the VOSCs before and after chemicals addition. The results of the theoretical odor concentration study indicated that MeSH accounted for around 94% of the total ThOC, which is the main compound causing the odor problem in this study. More attention should be therefore focused on the MeSH due to its extremely low odor threshold.

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Author Contributions

G.Z. (Ph.D student) analyzed data and wrote the manuscript. T.G. (Ph.D student) conducted experiments. Y.C.Z. (Professor) provided research ideas and revised the manuscript. D.Z. (Professor) and Y.P.Z. (Professor) revised the manuscript.

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