



Research Paper

Pilot-scale reactor for removing VOCs from a biowaste treatment plant: removal performance, degrading microorganisms, and their functional genes

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ABSTRACT

The widespread generation and treatment of biowaste contribute to odorous volatile organic compounds (VOCs) emissions and health issues and are a challenge to contemporary society. This study designed equipment that integrated a chemical washing tower (CWT) with a biotrickling filter (BTF) to remove VOCs from a biowaste treatment plant and reduce associated health risks. The total VOC concentrations at the biowaste treatment plant ranged from 700.1 to 1800.0 ppb and aliphatic hydrocarbons and volatile organic sulfur compounds (VOSCs) were the dominant VOCs. The inoculum added to the system contained *Bacillus*, *Burkholderia*, and other effective microorganisms. It removed 97.9 % of VOSCs (5 ppm) within 3 days under laboratory conditions. At the pilot scale, the combined CWT-BTF system removed 84.0 % of VOCs, with the BTF unit demonstrating superior performance compared to the CWT. The health risks of some VOCs decreased after purification, although the non-cancer and cancer risks remained above acceptable levels. After inoculation, the dominant microorganisms in the BTF changed from *Bacillus*, *Klebsiella*, and *Burkholderia pseudomultivoran* to *Polynucleobacter*, *Verrucomicrobia*, and *Planctomycetota*, which were primarily involved in energy metabolism pathways. Additionally, *Polynucleobacter* sp. 16-46-70 was found to be involved in sulfur metabolic pathways. Genes related to sulfur metabolism (*cysK*, *cysJ*, and *metB*) and nitrogen metabolism (*gltB*, *GDH2*, *nirB*, and *niT*) were involved in VOCs removal in BTF. This study indicates that the CWT-BTF technique at suitable loading rates is an effective method for removing VOCs emitted by biowaste and offers insights into the bacteria and genes that hold potential for enhancing removal efficiency.

1. Introduction

Rising global population and consumption has led to approximately one-third of global food production being wasted across the supply chain. This waste poses significant environmental and public health risks and uses up public resources. Incineration and landfilling (Liu et al., 2022; Zhang et al., 2020a) are currently used to decompose the biowaste and address these issues. However, during incineration or landfilling, volatile organic compounds (VOCs), such as dimethyl sulfide, carbon disulfide, benzene, and toluene, are released from food waste (Wang et al., 2023; Wang et al., 2019; Zheng et al., 2020a) leading to unpleasant odors that negatively impact the quality of life in nearby

communities. Additionally, these VOCs can cause discomfort or respiratory issues that potentially impact human health. For instance, dimethyl disulfides (DMS) that have unpleasant odors may cause loss of appetite (Calderon et al., 2012) and benzene exposure may lead to leukemia and cancer (Hajizadeh et al., 2018). Therefore, it is crucial to develop effective ways to remove VOCs during biowaste treatment to mitigate health and environmental concerns.

Various methods, including physicochemical, biochemical, and combined treatment processes, are used to remove VOCs (Huang et al., 2016). Physicochemical methods, such as chemical absorption, photocatalytic oxidation, and membrane separation, effectively remove VOCs in laboratory settings, but their pilot-scale application is limited due to

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high costs and potential secondary pollution (Li et al., 2024; Ozturk and Yilmaz, 2006; Soreanu et al., 2013; Zhou et al., 2024). Physical absorption methods, such as activated carbon absorption, are widely used to remove VOC; however, the costs associated with equipment replacement after saturation and treating the saturated activated carbon are high (Guo et al., 2024; Li et al., 2020). In contrast, biological methods, including biofilters, bioscrubbers, and biotrickling filters (BTFs), are cost-effective and environmentally friendly (Marycz et al., 2022). Among these, BTFs have been widely used in various pilot-scale systems, either independently or in combination with technologies such as photocatalytic oxidation and spray towers, to remove large volumes of VOCs. For instance, Liao et al. combined a BTF with a spray tower to remove VOC emissions from waste produced by a printed circuit board pyrolysis workshop (Liao et al., 2015). Similarly, He et al. effectively removed VOCs at a paint plant by integrating a BTF with photocatalytic oxidation (He et al., 2012). Chemical washing towers (CWTs) have been used prior to BTF treatment to prevent acidification or alkalization of the recycled solution, which improves microbial community stability (Jia et al., 2023; Jia et al., 2022). Nevertheless, combining BTFs with CWTs to treat VOCs from biowaste, which may contain acidic waste gases, remains largely unexplored.

As well as selecting the most appropriate combined processes for VOCs removal, the factors influencing VOC removal efficiency also need to be considered, such as packing material type, inoculum source, biofilm formation on the packing material, and inlet gas composition and concentration. Specifically, the packing material primarily influences microbial attachment and biofilm formation, they were used to provide stable and massive location for the microorganisms adhering and forming microbe aggregate (Gutiérrez-Acosta et al., 2012). Previous studies have demonstrated that many packing materials, such as bamboo charcoal and volcanic rock, can be used to remove VOCs in a BTF (Deng et al., 2022b; Zhao et al., 2022). Inoculum source determines which pollutants can be degraded, and the biofilm on the packed bed plays a crucial role in the metabolic conversion of pollutants (Rybarczyk et al., 2019). Commonly used inocula in BTFs, such as activated sludge from domestic sewage treatment plants or commercial deodorizing microbial consortia, have been shown to remove VOCs (Wu et al., 2024; Zhang et al., 2023). Microorganisms such as *Bacillus cereus* GIGAN2 and *Burkholderia fungorum* FLU100 have shown good removal characteristic of DMDS and toluene (Dobslaw and Engesser, 2015; Liang et al., 2015). The addition of specific microorganisms can improve VOC degradation and removal efficiency and reduce the start-up time. However, there has only been limited research into the use of VOC specific degradation cultures as inocula for BTFs and very few studies have investigated microbial community dynamics within BTFs.

To address this knowledge gap, this study designed a pilot-scale integrated technique that combined a BTF with CWT to remove VOCs released by a biowaste treatment plant and mitigate associated health risks. The first objective was to analyze the emission characteristics of the VOCs from the plant. Subsequently, an inoculum was prepared by mixing dominant VOCs degrading bacteria with other effective microorganisms and then the removal efficiencies (REs) of the microorganisms for VOCs were assessed. Following inoculation, the BTF start up requirements, the VOC REs, and the health risks at various stages of the CWT-BTF treatment process were evaluated. Additionally, the structure and functional genes of the microbial communities in the BTF were assessed by metagenomic sequencing. The data provided valuable insights that can be used to develop more efficient removal methods or select the bacteria with high efficiency for removing VOCs from food waste treatment plant.

2. Materials and methods

2.1. Experimental setup and sample collection

The pilot-scale site was at a biowaste treatment plant located in south

China where nearly 710 tons of biowaste are processed daily. The biowaste contains about 150 tons of food waste, 50 tons of kitchen waste, 500 tons of feces, 5 tons of waste oil, and 5 tons of dead livestock. In this study, the biowaste was pretreated (crushing and dehydration) and then subjected to anaerobic digestion, dehydration, and incineration. The high concentrations of VOCs from the anaerobic digestion reactor were incinerated, while odorous gases from the pretreatment plant and waste unloading hall were collected and purified by the pilot CWT-BTF designed by Guangdong Zike Environmental Protection Co., Ltd (Fig. 1). Specifically, a centrifugal fan mounted after the coupling reactor passed the VOCs from the pretreatment plant or waste unloading hall to the CWT1 or CWT2 outlets (CWT dimensions: basal diameter (Φ) \times height (H): 4.7 m \times 8 m) and then into the BTF1 or BTF2 outlets (BTF dimensions: length (L) \times width (W) \times H: 26 m \times 13 m \times 4 m) at a flow rate of 92,000 m³·h⁻¹. In the CWT, two layers of polyhedral hollow balls (DN76 mm, PP-sphere) were used as the packing material and 10 % caustic soda was used to maintain a pH of 9.0. The pH was real-time detected by a pH sensor in the CWT and the caustic soda was automatically added by the machine when the pH was lower than 9.0. The empty tower velocity and effective residence time of the CWT were 1.47 m·s⁻¹ and 2.04 s, respectively. Bamboo charcoal and volcanic rock at a ratio of 1:9 (V/V) were used as the packing material in the BTF and the empty bed residence time was 25 s. They were chosen as the packing material due to their low price, high porosity, void fraction, and surface area (Deng et al., 2022a; Zhu et al., 2023).

The microorganisms inoculated onto the BTF degrade the VOCs. Based on the pollution profiles of the VOCs from the biowaste treatment plant, two degradation microorganisms (*Bacillus* and *Burkholderia* isolated from waste landfill leachate) were selected and the inoculum was prepared by mixing them with commercially effective microorganisms (Wonobio Biotechnology Co., Ltd, China). First, the VOCs removal ability of these microorganisms was verified in the laboratory according to the detailed procedure in the supporting information (SI). Then, 40 L of VOCs degrading bacteria (>) and 240 L of effective microorganisms were enriched in nutrient broth and brown sugar to 10⁹ CFU·mL⁻¹, respectively, in a 37 °C incubator. Finally, the enriched culture was added to the circulating pool and sprayed into the BTF every 2 h. The CWT-BTF reactor was successfully operated for 150 days with no clogging or breakdown problems. After the first inoculation, the removal performance of the BTF was further improved by performing a second inoculation using the same method used in the first inoculation.

Gas samples were collected using vacuum stainless Summa canisters (2.7 L, Silonite™, Entech Instruments Inc., Simi Valley, CA, USA) after they had been pre-rinsed six times using high-purity nitrogen (99.99 %) and vacuumized. The VOCs were sampled according to the guideline for Ambient air-Determination of volatile organic compounds-Collected by specially-prepared canisters and analyzed by gas chromatography/mass spectrometry (HJ759-2015). The samples were collected from the CWT/BTF inlet and at the BTF outlet prior to inoculation (1st), at 20 days (2nd) and 100 days (3rd) after initial inoculation, and at 50 days (4th) after reinoculation. 20 day was selected since the acclimation time of microorganisms takes two weeks to six months (Cho et al., 1992). Gas samples from the waste unloading hall and pretreatment plant were also collected to investigate the VOC pollution profiles in the biowaste treatment plant. A sample collected upwind of the treatment plant was used as the blank control. Circulation water (500 mL) and packing materials (5 g) in the upper layer of BTF were also simultaneously collected for microbial analysis and the sampling temperature and water quality were measured and are shown in Tables S1 and S2.

2.2. VOCs concentrations, RE analyses, and health risks

A gas chromatography-mass spectrometer (GC-MS) (7890A GC-5975C MS, Agilent Technologies, Santa Clara, CA, USA) coupled with a pre-concentrator (Entech 7200, Entech Instruments Inc. Simi Valley, CA, USA) was used to measure the VOCs concentration and composition

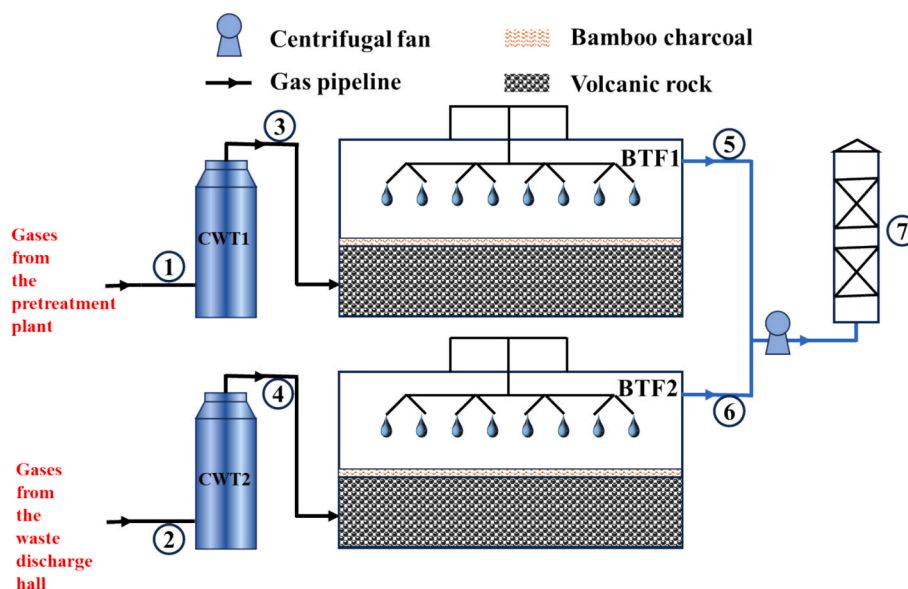


Fig. 1. Flow chart of the CWT-BTF. ① to ⑦ represent the following sampling sites: ① and ②: the inlet of CWT1 and CWT2; ③ and ④: the inlet of BTF1 and BTF2; ⑤ and ⑥: the outlet of BTF1 and BTF2; ⑦: the total outlet.

in the gas samples. The system was equipped with a flame ionization detector (FID) and had three columns: a DB-1 capillary column ($60\text{ m} \times 0.250\text{ mm} \times 1.0\text{ }\mu\text{m}$, Agilent Technologies), a HP-AL/S + PT capillary column ($50\text{ m} \times 0.32\text{ mm} \times 8\text{ }\mu\text{m}$, Agilent Technologies), and an inert fused silica column ($4.070\text{ m} \times 0.15\text{ mm}$, Agilent Technologies). A detailed analysis method for VOCs can be found in the SI. Briefly, the VOCs were qualified and quantified based on the retention times and the mass peaks obtained from standard Photochemical Assessment Monitoring Stations (PAMS), TO-15 (Linde Spectra Environment Gases, Branchburg, NJ, USA), and for sulfur-containing compounds (Air Liquide, Paris, France). The data were then compared to those in the National Institute of Standards and Technology (NIST) 05 database.

The performance of the CWT-BTF was evaluated by calculating the REs, lifetime cancer risk (LCR), and non-cancer risk (HR) using the equations outlined in the SI. The reference concentrations for VOCs and the corresponding unit risk data were obtained from the Integrated Risk Information System (IRIS) (<https://www.epa.gov/iris>) (Jia et al., 2019) and are shown in Table S3.

2.3. Microbial community structures and functional genes analysis

A Rapid Soil DNA Isolation Kit (Sangon Biotech, Shanghai, China) was used to extract genomic DNA according to the manufacturer's instructions from the circulation water samples after they had been filtered through a $0.22\text{ }\mu\text{m}$ mixed cellulose esters membrane. After determining the concentration and purity of the isolated DNA using a Nano-Drop 2000 (Thermo Fisher, Tenmecula, CA, USA) it was subjected to metagenomic sequencing by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). BLASTP (BLAST Version 2.2.28+, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used to align the non-redundant gene catalogs against the NCBI NR database. The expected value (e-value) of the BLAST alignment parameter was set to $1\text{e-}5$. The community metabolic pathways and genes were analyzed by searching the Kyoto Encyclopedia of Genes and Genomes database (KEGG) (<https://www.kegg.jp/kegg/>) with an e-value cutoff of $1\text{e-}5$ and the biofilm on the packing material was visualized using a field-emission scanning electron microscope (FESEM) (ZEISS Ultra 55, Carl Zeiss, Oberkochen, Germany). Prior to visualization, the samples were fixed overnight in 2.5 % glutaraldehyde, dehydrated with 30, 50, 70, 90, and 100 % ethanol, and then vacuum freeze-dried for over 3 h.

2.4. Statistical analysis

R program (version 3.3.1) was used to draw the Venn diagrams, bar plots, pie diagrams, and heatmaps. The species contribution analysis was performed by Python (Version 2.7.0) and all the data were analyzed using IBM SPSS Statistics 19.0 (IBM Corp., Armonk, NY, USA) at the $p < 0.05$ significance level.

3. Results and discussion

3.1. Pollution profiles, removal performance, and health risk attenuation of the VOCs

3.1.1. Pollution profiles of the VOCs from the biowaste treatment plant

The gases produced by the different treatment processes were sampled and analyzed by GC-MS to investigate VOCs emission trends in the biowaste treatment plant. Fig. 2a shows that although the VOC composition in the waste treatment hall and the pretreatment plant was the same, the total VOC concentration in the waste unloading hall (900.4 ppb) exceeded that in the pretreatment plant (560.2 ppb). The concentration hierarchy for the VOCs was volatile organic sulfur compounds (VOSCs) > aliphatic hydrocarbons (AIHs) > aromatic hydrocarbons (AHs) > nitrogen and oxygen-containing compounds (NAOCCs) > halogenated hydrocarbons (HHs). The primary AHs, HHs, AIHs, NAOCCs, and VOSCs detected in the waste unloading hall and pretreatment plant were the same in all the samples and were *p*-xylene, dichloromethane, *n*-butane, vinyl acetate, and dimethyl trisulfide (DMTS), respectively (Figs. S1 and S2). Specifically, the DMDS, DMTS, and *n*-butane concentrations were 351.1 and 237.9, 438.5 and 269.8, and 17.0 and 15.6 ppb in the waste unloading hall and pretreatment plant, respectively.

An analysis of the differences among the VOCs taken at the four sampling times (prior to inoculation (1st), at 20 days (2nd) and 100 days (3rd) after initial inoculation, and at 50 days (4th) after reinoculation) from the CWT-BTF showed that there were 103 types of VOCs, which consisted of 28 HHs, 35 AIHs, 23 AHs, 6 NAOCCs, and 11 VOSCs (Table S4). Among them, 40 VOCs were shared by all four samples, which suggested they were common pollutants in biowaste treatment plants (Fig. S3). The total VOCs (TVOCs) concentration at the BTF inlet increased over the sampling period. This may be due to variations in the quantity of waste substrates processed. Differences in waste substrate

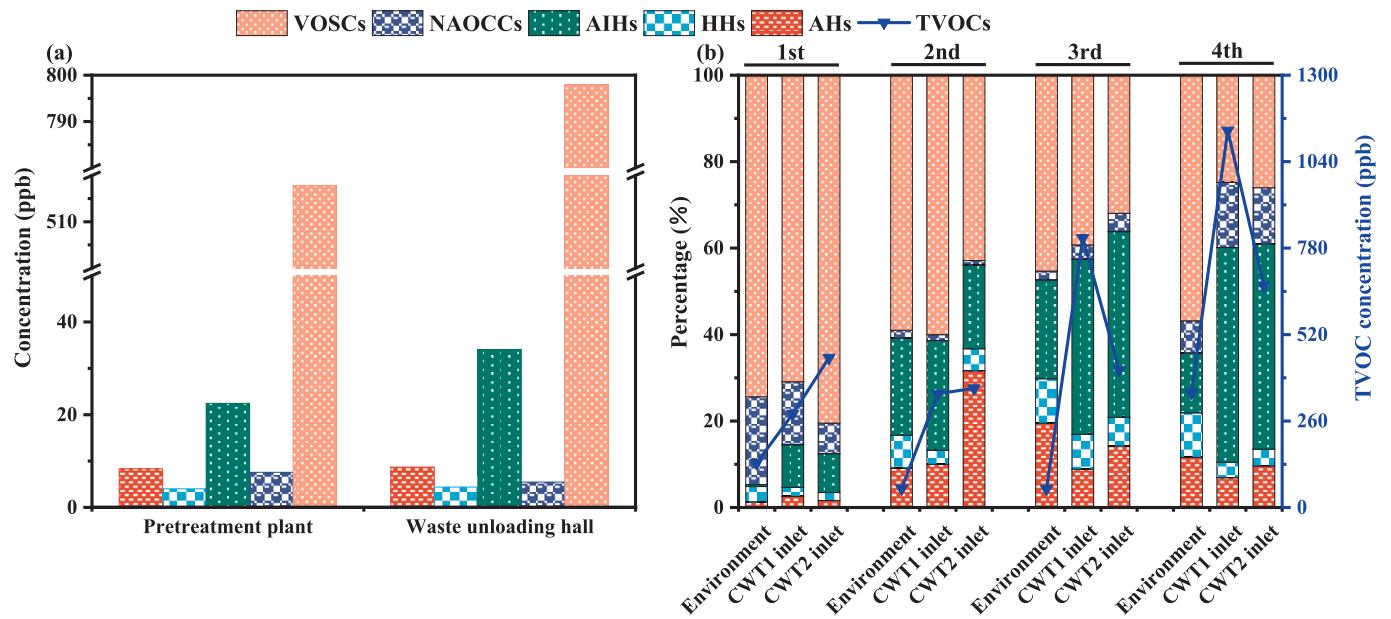


Fig. 2. VOC concentrations in waste pretreatment plant and unloading hall (a). Percentage of various VOCs and TVOCs concentration in the environment, the inlet of CWT1 and CWT2 at the 1st (before inoculation), 2nd (20 days after inoculation), 3rd (100 days after inoculation), and 4th (50 days after reinoculation) sampling events (b). VOSCs, NAOCCs, AIHs, HHs, AHs and TVOCs respectively stand for volatile organic sulfur compounds, nitrogen and oxygen-containing compounds, aliphatic hydrocarbons, halogenated hydrocarbons, aromatic hydrocarbons and total VOCs.

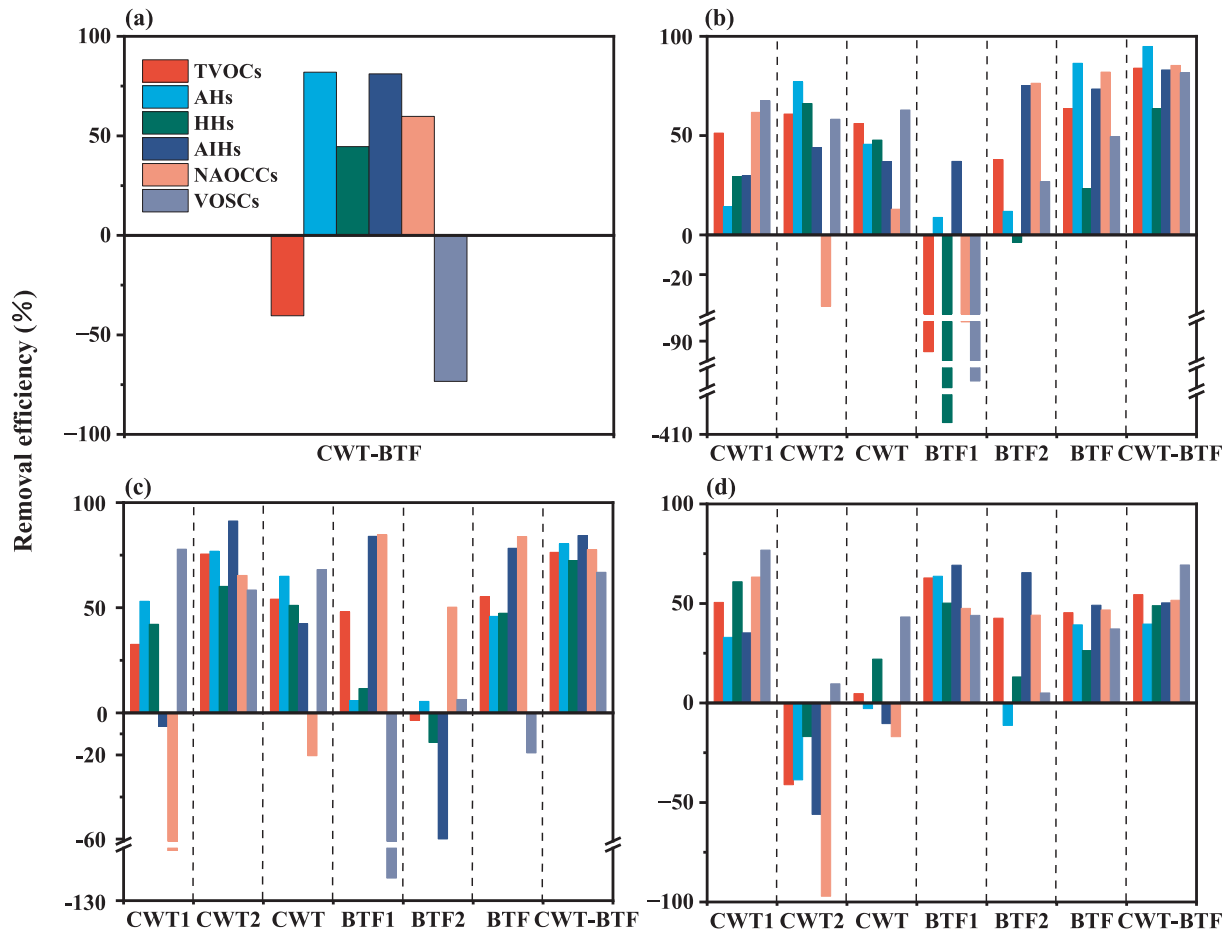


Fig. 3. The removal efficiencies of VOCs by CWT-BTF at the 1st (before inoculation) (a), 2nd (20 days after inoculation) (b), 3rd (100 days after inoculation) (c), and 4th (50 days after reinoculation) (d) sampling events.

composition and fermentation temperature may also contribute to the observed differences in VOCs. Previous studies also suggested that variations in the food waste substrates and waste fermentation temperatures may affect VOC emissions (Cui et al., 2022; Komilis et al., 2004; Zhang et al., 2020b). Specifically, Fig. 2b shows that the TVOC concentration at the CWT1 inlet was lower than that at CWT2 at the first two sampling times, whereas the trend reversed at the 3rd and 4th sampling times. The predominant VOCs at the 1st and 2nd sampling times were VOSCs, but there was a shift to AIHs at the 3rd and 4th sampling times (Figs. S4a–11a). The large fat content in food waste may account for the higher proportion of AIHs because alkanes can be released after the incomplete decomposition of fats derived from meats (Wang et al., 2018). The dominant VOSCs detected at the 2nd, 3rd, and 4th sampling times were DMTS, whereas DMDS predominated at the 1st sampling time (Figs. S4f–11f). The dominant AIHs varied across the sampling events: isobutane and *n*-butane at the 1st sampling time, isopentane and cyclopentane at the 2nd, isopentane and isobutane at the 3rd, and isobutane and 1-hexene at the 4th (Figs. S4d–11d). Wu et al. reported that VOSCs tended to be emitted during the microbial degradation of general food waste (Wu et al., 2010), whereas DMDS and DMTS were emitted during the pretreatment of leeks and sprouts (Delbaere et al., 2023) and during the microbial metabolism of kitchen waste (He et al., 2023). Toluene, *p*-xylene, and 1,3-diethylbenzene were the most frequently detected AHs (Figs. S4b–11b); dichloromethane, *cis*-1,2-dichloroethylene, and 1,1,2-trichloroethane were the most prominent HHs (Figs. S4c–11c); and methyl *tert*-butyl ether and vinyl acetate were most common NAOCCs detected (Figs. S4e–11e).

3.1.2. Biofilter start-up

The VOCs concentrations before and after passing through the CWT-BTF at the 1st sampling event (before inoculation) showed that the TVOCs concentration increased from 731.8 to 1027.4 ppb with an RE of −40.4 % (Fig. 3a and S12a). Notably, the RE for VOSCs reached −73.4 %. These results indicated that the reactor had a limited degradation ability for VOCs without the inoculum, particularly for VOSCs, which

comprised over 70.9 % of TVOCs at the treatment plant. DMDSs were the dominant VOSCs and many AHs, such as toluene, are toxic. Therefore, an inoculum containing VOSCs degrading bacteria *Bacillus* and BTEX degrading strain *Burkholderia* mixed with commercially effective microorganisms was selected to enhance TVOCs removal. The VOSCs removal performance in mixed culture in laboratory simulated experiments showed that the REs for DMDS and DMTS reached 97.9 % and 99.7 %, respectively, without any rebound, after 3 days (Fig. S13). These findings indicated that the prepared mixed culture had high removal efficiencies for VOSCs and could be used as an inoculum in the BTF system. The mixed culture was subsequently inoculated into the BTF and the VOCs levels were measured. The results showed that at 20 days post-inoculation (2nd sample event), the VOCs concentration decreased from 700.1 to 112.0 with an RE of 84.0 %, which confirmed that the CWT-BTF effectively removed VOCs (Fig. S12b). Additionally, a biofilm had formed on the packing material. Specifically, single, ovoid, and elliptic microorganisms had densely adhered to the surface of the bamboo charcoal, whereas a reticulate structure was observed on the volcanic rock (Fig. 4). This difference may be due to the distinct characteristics of the packing materials. Previous studies have also reported that packing materials can influence the development of microbial consortia (Anet et al., 2013).

3.1.3. VOCs removal efficiency of the CWT-BTF

A further evaluation of the CWT-BTF removal abilities showed that there was a significant decrease in TVOCs concentration with an RE of 84.0 % at 20 days after inoculation (Fig. S12b). Specifically, the AH, HH, AIH, and VOSC REs for the CWT component reached 45.6 %, 47.8 %, 252.36.9 %, and 62.9 %, respectively (Fig. 3b). These results indicated that the CWT could be used as an effective pretreatment method for removing VOCs by acid-base neutralization reaction prior to BTF treatment. It reduced VOCs (except for NAOCCs) shock loading of the BTF by removing particles (Liang et al., 2020) and hydrophilic pollutants (Chen et al., 2017). In the BTF, the VOCs were serving as the carbon and nitrogen sources of the degrading microorganisms (Wu et al.,

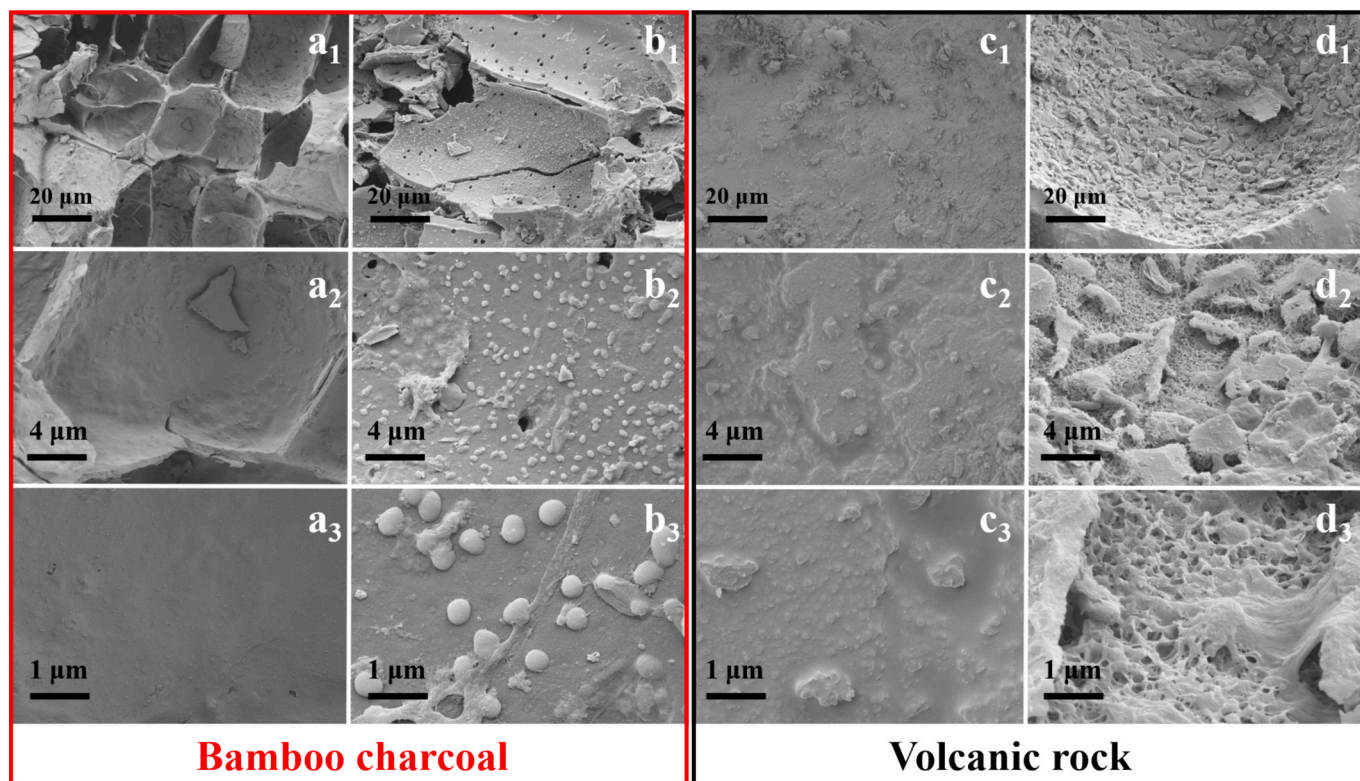


Fig. 4. FESEM images of bamboo charcoal and volcanic rock in the BTF collected before (a1–a3, c1–c3) and after 20 days of inoculation (b1–b3, d1–d3).

2018a). The AH, NAOCC, AIH, VOSC, and HH REs for the BTF were 86.5 %, 82.0 %, 73.5 %, 49.5 %, and 23.3 %, respectively. Therefore, the BTF (63.5 %) had a higher overall treatment effectiveness for VOCs (except for HHs) than the CWT (56.1 %). Notably, the BTF1 samples showed negative REs for VOSCs, HHs, and NAOCCs, which was probably due to lower the concentration of TVOCs in the BTF1 samples than in the BTF2 samples, which may have hindered biofilm formation. Previous studies have also reported that poor microorganism activities can be due to the inadequate availability of carbon sources during BTF start-up (Yang et al., 2019).

After 100 days of CWT-BTF treatment (3rd sampling event), the TVOC REs dropped to 76.35 % (Fig. 3c). This decline may be attributed to the sudden increase in inlet TVOCs (from 700.1 to 1226.3 ppb) (Fig. S12c). Such elevated concentrations can be toxic to microorganisms and influence the mass transfer rate within the biofilm (Tu et al., 2017). Previous studies have also reported that shock loading with VOCs can adversely impact removal by a BTF and their subsequent RE values (Liao et al., 2021). The physical/chemical processes occurring in the CWT-BTF may further compromise removal efficiency (Xu et al., 2021). The CWT1 sample had negative REs for AIHs (−20.38 %) and NAOCCs (−106.1 %). This is reasonable since the isobutane, pentane, and vinyl acetate concentrations were significantly higher in the 100 day samples compared to the samples collected at 20 days after inoculation. This result suggests that the CWT had a limited purification capacity for these VOCs, which do not react with alkali. Negative REs for dominant VOCs, including VOSCs and AIHs, were also recorded for the BTF1 and BTF2 samples, which suggests that the majority of microorganisms in the BTF had lost their capacity to remove VOCs after VOC shock loading. Therefore, the mixed culture inoculum was reintroduced into the BTF to increase TVOCs removal performance.

At 50 days after reinoculation (4th sampling event), the VOSC REs for the BTF increased from −19.1 % to 37.3 %. A comparison with the REs at 100 days after initial inoculation and 50 days after reinoculation showed that the REs for AHs improved from 5.7 % to 62.8 % and HHs from 11.5 % to 50.2 % (Figs. 3c and 3d). These results suggested that reinoculation effectively enhanced VOC REs by increasing the population densities of the degrading microorganisms. Wu et al. also reported that microbial population densities affected the operation of a BTF (Wu et al., 2018b). However, the TVOC REs dropped to 54.4 % when the inlet concentration rose to 1800 ppb (Fig. S12d). Notably, the CWT2 samples had negative REs for most VOCs (except VOSCs), which was possibly due to unforeseen factors associated with the CWT. These VOCs entered BTF2 and changed microbial behavior, which resulted in negative and low REs for AHs (−11.3 %) and HHs (13.1 %). This is because the BTF is generally more effective at removing low concentration VOCs (Liu et al., 2023).

The results for the dominant VOCs, including DMDS, DMTS, isobutane, and cyclopentane, showed that the DMDS and DMTS REs were negative prior to inoculation (Fig. S14a). However, at 20 and 100 days after initial inoculation, the DMDS REs improved to 74.3 % and 95.3 %, respectively. Similarly, the DMTS REs also increased to 89.5 % and 67.8 %, respectively. Furthermore, the BTF removed most of the cyclopentane and isobutane and had positive REs for these components (except for isobutane at 100 days after inoculation) (Figs. S14b and S14c). Notably, the BTF1 sample still had negative REs for DMDS and DMTS, whereas BTF2 had positive REs throughout the treatment process. This difference may be attributed to the higher proportion of VOSCs in BTF1 at 20 days post initial inoculation. However, at 100 days, the elevated TVOCs concentration in BTF1 may have been detrimental to microbial activity. At 50 days after reinoculation, the REs for the four dominant VOCs were positive for both BTF1 and BTF2, suggesting that the addition of more degrading microorganisms improved BTF performance (Fig. S14d).

Overall, the TVOC REs for the CWT-BTF system exceeded 54.4 % and followed the order: 20 days after initial inoculation > 100 days after initial inoculation > 50 days after reinoculation. Temperature has no

significant correlation with the REs of VOCs ($p = 0.7905$). The CWT-BTF effectively removed multi-component VOCs, including DMDS, DMTS, isobutane, and cyclopentane, at concentrations lower than 700 ppb.

3.1.4. Attenuation of non-cancer and cancer risks due to VOCs by the CWT-BTF

Certain VOCs, such as benzene, 1,1-dichloroethane, and *p*-xylene, pose significant health risks as indicated by their HRs or LCR values (Zheng et al., 2020b). The HR categories are defined as follows: $HR > 1$ indicates “high risk,” HR between 0.1 and 1 indicates “probable risk,” and $HR < 0.1$ indicates “accepted level” (Mustafa et al., 2017). The LCR classifications are defined as $LCR > 10^{-4}$, between 10^{-4} and 10^{-5} , and $< 10^{-5}$ and indicate “high risk”, “probable risk”, and “possible risk”, respectively (Li et al., 2013). The health risks results for ten typical VOCs after treatment showed that prior to CWT-BTF treatment, the HRs for most VOCs (except for methyl methacrylate at the 3rd sampling event) were greater than 1. At 20 days post initial inoculation, the 1,1-dichloroethylene, methyl methacrylate, and toluene HRs were reduced to between 0.1 and 1, which suggested that the CWT-BTF had mitigated the health risks (Fig. 5a). However, after 100 days of treatment, only the HR for toluene decreased to the “probable risk” level (0.6). Furthermore, at 50 days after reinoculation, the HRs for VOCs for all the outlets were all above 1 and the HRs for methyl methacrylate and 1,2,3-trimethylbenzene rose to 107.3 and 428.4, respectively (Figs. 5b and c). This indicated that the CWT-BTF has a very limited ability to reduce the health risk due to VOCs when there are unexpected shock loads, which suggests that future research should concentrate on developing effective techniques to further reduce the health risks associated with VOCs.

A further analysis of the LCRs for five major VOCs showed that 1,3-dichloro-1-propene (Z) and benzene LCRs decreased after the CWT-BTF treatment. However, except for dichloroethane with LCRs between 10^{-4} and 10^{-5} , the other VOC LCRs remained above 10^{-4} after CWT-BTF treatment, indicating a continued high risk. Notably, the 1,2-dichloroethane and 1,1,2-trichloroethane LCRs increased at 100 days after initial inoculation and at 50 days after reinoculation, which was probably due to the hydrophobic nature of these compounds (Fig. 5a–f). A previous study also reported that hydrophobic VOCs were difficult to remove due to the low mass transfer efficiency between the gas and liquid phases (Zehraoui et al., 2012). In general, the CWT-BTF treatment reduced the HRs and LCRs of VOCs to some extent, but due to the VOCs shock, further enhancement is needed, particularly the removal of methyl methacrylate and 1,1,2-trichloroethane. In the future, the use of surfactants and the addition of hydrophilic compounds was suggested to enhance the removal of hydrophobic VOCs in BTF (Lamprea Pineda et al., 2021). Besides, sending the high-concentration gas emitted from the pretreatment plant (such as floatation tank) to the incineration plant for incineration can effectively reduce the VOCs shock and increase the RE.

3.2. Microbial communities evolution during CWT-BTF treatment

Metagenomic sequencing was used to analyze the evolution of the microbial communities in the liquid circulating within the BTF. A total of 3.3×10^6 genes out of the 5.5×10^6 genes obtained were further analyzed after removing duplicates. The diversity analysis showed that the commercial microorganisms had significantly higher richness and diversity indices than the inoculated bacteria, and that the values increased with BTF treatment time (Table S5). This suggested that VOCs enhanced microbial diversity in a BTF.

The community structure analysis indicated that the dominant microorganisms in the BTF were significantly different from the original inoculated microbes (Fig. 6a). *Klebsiella* (18.9 %), *Klebsiella pneumoniae* (10.2 %), *Bacillus* (20.2 %), *Bacillus xiamenensis* (10.0 %), and *Burkholderia pseudomultivorans* (17.7 %) were the dominant microorganisms in the inoculum, whereas *Polynucleobacter*, *Verrucomicrobia*, and *Planctomycetota* were the dominant microorganisms in the BTF at the

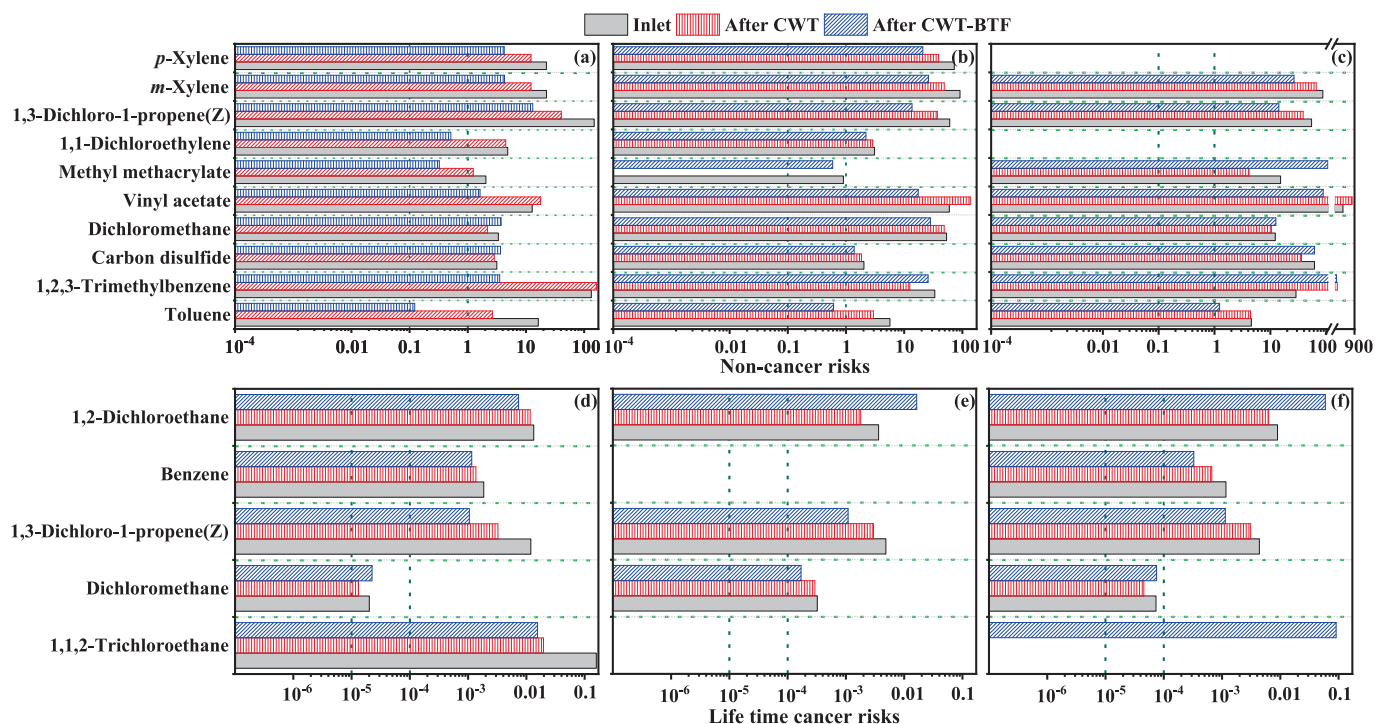


Fig. 5. Non-cancer risks and life time cancer risks of VOCs before and after CWT-BTF treatment at the 2nd (20 days after inoculation) (a, d), 3rd (100 days after inoculation) (b, e), and 4th (50 days after reinoculation) (c, f) sampling events.

2nd to 4th sampling events. This suggested that inoculated bacteria increased the dominance of indigenous microorganisms on the packing materials when stimulated by VOCs. Specifically, *Polynucleobacter* abundance increased from less than 1.0 % to 27.8 % in the BTF1 sample when the treatment time increased from 20 to 100 days. *Verrucomicrobia* and *Planctomycetota* were only detected on day 20 (2.6 % and 7.7 %) after initial inoculation (6.6 %) and at day 50 (1.4 %) after reinoculation. *Polynucleobacter* and *Planctomycetota* were detected only at 100 days after initial inoculation in the BTF2 samples, whereas *Verrucomicrobia* and *Halteria grandinella* were detected throughout the treatment process, which suggested that they may play important roles in VOCs removal. A previous study also reported that *H. grandinella*, a type of ciliate, enhanced the VOCs biodegradation potential of other microbes (Bhaskaran et al., 2008).

The correlations between the top 20 microbes and seven VOC types showed that *Fluviibacter phosphoraccumulans*, *B. xiamenensis*, and *Kiritimatiellaceae* had greater positive correlations with VOCs, except for toluene, as CWT-BTF treatment time increased. Conversely, there were negative correlations between VOCs and *Alphaproteobacteria*, *Planctomycetota*, and *Bacteroidetes* (Fig. 6b). This indicated that they may act as VOC degrading microbes in the BTF. Previous studies also demonstrated the ability of these microorganisms to degrade VOCs. For example, *Bacillus* can remove ammonia and sulfide (Su et al., 2023), while *Klebsiella* can effectively degrade AIHs, such as benzene, toluene, and xylene (Zhao et al., 2014). *Burkholderia* has been shown to degrade hydrogen sulfide, styrene, and m-xylene (Yao et al., 2022) and *Verrucomicrobia* from activated sludge is used for biological deodorization (Lebrero et al., 2013). Thus, exploring the functional roles of microbial communities improves understanding about the mechanisms associated with VOC removal in BTFs.

3.3. Microbial community functions in a BTF

The KEGG functional profiles of the bacterial community in the BTF showed that there were six level 1 pathways and 35 level 2 pathways (Fig. S15). Energy metabolism was the most dominant level 2 pathway

followed by global and overview maps and carbohydrate metabolism. This is reasonable as VOCs serve as carbon and nitrogen sources for the microorganisms in the BTF, which would lead to the expression energy metabolism pathways for carbon and nitrogen when VOCs are removed. Previous studies also reported that microorganisms activate energy metabolism pathways to harvest metabolic energy for their growth and reproduction (Tong et al., 2021). Further analysis of the energy metabolism category identified sulfur metabolism in the samples collected after inoculation. Notably, the dimethyl sulfide degradation (ko00920) pathway was identified after searching the KEGG database. This suggested that VOSCs, which are prevalent in biowaste, are removed via sulfur metabolism. A previous study also reported that organic sulfur can be converted to inorganic sulfur by microorganisms (Gregersen et al., 2011).

The microorganisms responsible for metabolism were identified by analyzing the species that contributed the most to the top 10 most abundant energy metabolism pathways. In the inoculum, the primary microorganisms associated with energy metabolism were *Klebsiella* sp., *Burkholderia pseudomultivorans*, *Bacillus*, and *Klebsiella pneumoniae* (Fig. 7). In contrast, after inoculation, the dominant microbes related to energy metabolism at the 2nd, and 4th sampling events were *Planctomycetota bacterium* and *Verrucomicrobia bacterium*, which suggested that they had become the dominant microorganisms in the BTF. Both these microorganisms have strong VOC degradation capabilities. The replacement of initial microorganisms with other microorganisms may be attributed to the catalytic role of VOCs (Van der Heyden et al., 2019). At the 3rd sampling event, the main contributors to energy metabolism had shifted to *Polynucleobacter* sp. 32-46-5, *Polynucleobacter* sp. 32-46-207, and *Polynucleobacter* sp. 16-46-70, with *Polynucleobacter* sp. 32-46-207 exhibiting significantly higher sulfur metabolism percentages compared to other functions. However, Fig. 6a shows that *Polynucleobacter* sp. 32-46-207 (7.4 %) abundance was significantly lower than that of *Polynucleobacter* sp. es-EL-1 (27.8 %) in BTF1 at the 3rd sampling event and may have been the reason for the observed decline in VOSC removal efficiency. However, further investigation of the genes involved in the sulfur metabolic pathways is needed.

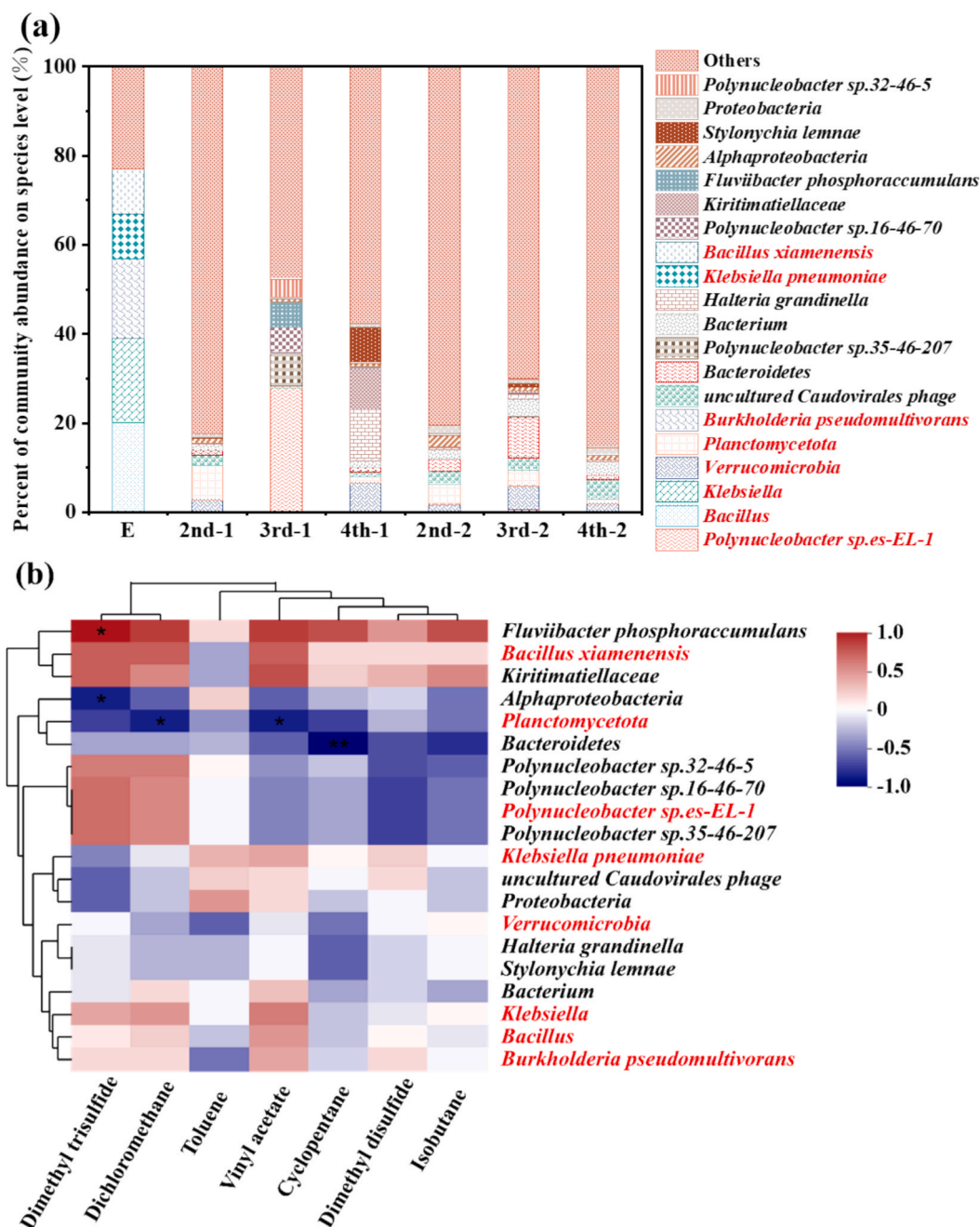


Fig. 6. Percent of microbial community abundance on the species level in the circulation water of BTF1 and BTF2 after 20 days (2nd-1 and 2nd-2) and 100 days (3rd-1 and 3rd-2) of inoculation, as well as 50 days (4th-1 and 4th-2) of reinoculation (a). Spearman correlation between microbial communities and odorous VOCs (b). The plot used asterisks as indicators for statistical significance ($p < 0.05$). No symbol was used when the $p > 0.05$ (not significant). The color transition from dark blue to dark red represents relative abundances of the community from low to high. “E” represents inoculum.

3.4. Microbial community functional genes in the BTF

To evaluate the removal mechanisms of the VOCs by the microorganisms in the BTF, we analyze the functional genes. Since VOCs and NAOCs are major components of odorous gases; the genes involved in the nitrogen and sulfur metabolic pathways were investigated (Li et al., 2019). The top 10 genes associated with sulfur and nitrogen metabolism among the 1.5×10^4 genes related to metabolism were selected to create the heatmap. The results (Fig. 8a) show that the sulfur metabolism encoding genes *cysK*, *cysJ*, and *metB* were significantly enriched in both the inoculated bacteria and the BTF, suggesting that they were crucial for sulfur metabolism and contributed to effective sulfur removal. Previous studies have reported that *cysK* and *cysJ* encoded enzymes were

involved in sulfur assimilation (Morigasaki et al., 2020), whereas *metB* encoded enzymes were involved in the *trans*-sulfuration pathway via cystathionine (Sagong and Kim, 2017). In detail, *cysJ* encoding sulfite reductase catalyzed the reduction of organic sulfur to hydrogen sulfide (Ostrowski et al., 1989). So, the high expression of these genes suggested that in the BTF, sulfite was converted to hydrogen sulfide. Based on this, we conjecture that the VOCs were gradually desulfurized by the microorganisms to dimethyl sulfide, then the dimethyl sulfide was transformed to sulfite, and finally the sulfite was reduced to hydrogen sulfide in the BTF. Essential amino acid cysteine is produced in a reaction catalyzed by *cysK* and *metB* encoding enzymes from serine by incorporation of sulfide or thiosulfate (Clausen et al., 1998; Kitabatake et al., 2000).

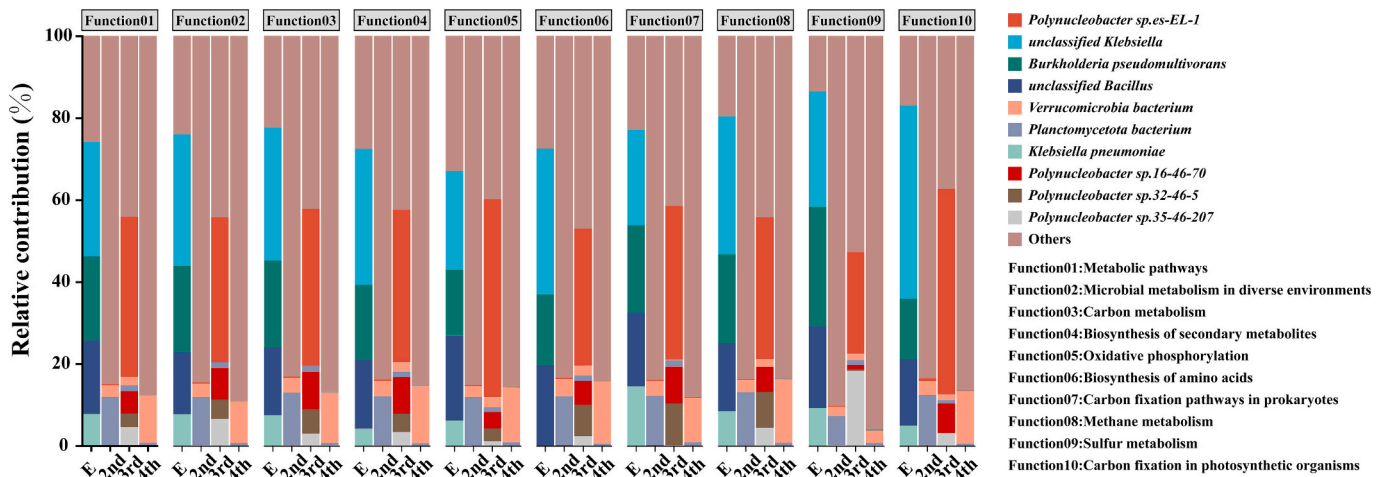


Fig. 7. Species contribution to the energy metabolism pathways at the 2nd (20 days after inoculation), 3rd (100 days after inoculation), and 4th (50 days after reinoculation) sampling events.

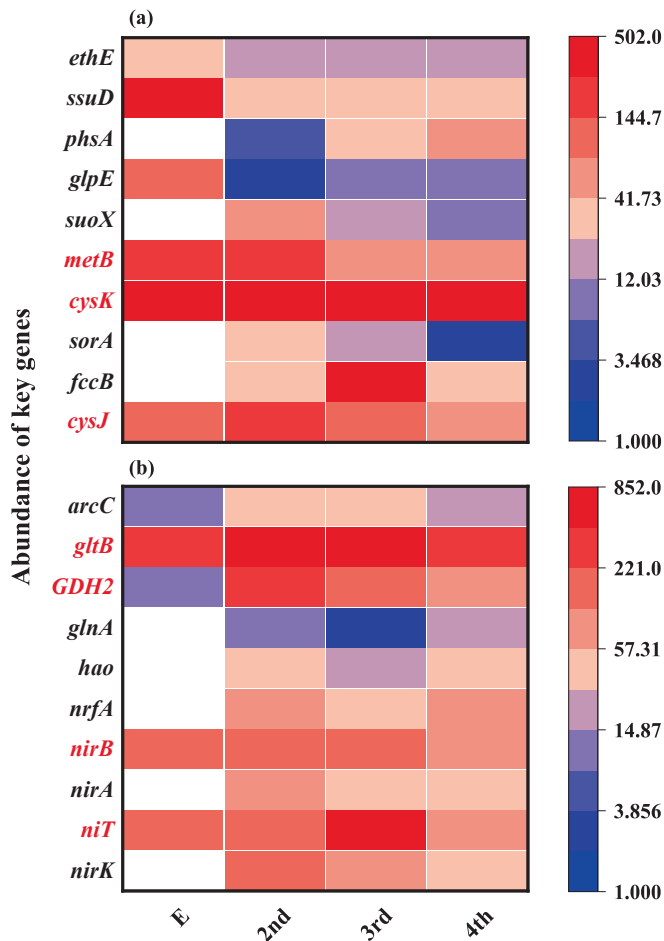


Fig. 8. A heatmap showing the abundance of key genes related to sulfur (a) and nitrogen metabolism (b) at 2nd (20 days after inoculation), 3rd (100 days after inoculation), and 4th (50 days after reinoculation) sampling events. “E” represents inoculum, and white block represents the genes were not detected.

The *gltB*, *GDH2*, *nirB*, and *niT* nitrogen metabolism genes were enriched in all the samples (Fig. 8b). Specifically, *GDH2* and *niT* abundances were lower in the inoculum compared to the BTF. However, *fccB*, *nirA*, and the nitrite reductase encoding gene *nirK* were highly expressed in the BTF, suggesting that the inoculation microorganisms activated

functional genes associated with the nitrogen metabolic pathway. Furthermore, *gltB* and *GDH2* encode glutamate synthase (Lee et al., 2013) and an α -subunit of mitochondrial glutamate dehydrogenase (Tarasenko et al., 2009), respectively, which suggests that they might have roles in the assimilation of nitrogen-containing odorous gases. Particularly, nitrate/nitrite conversion into ammonia was catalyzed by nitrate/nitrite reductase encoded by genes such as *nirB* and *niT* (Exley et al., 1993; Harborne et al., 1992). The high abundance of these genes suggested that nitrogen-containing organic compounds were first degraded to nitrite and then to ammonia in the BTF. The ammonia was used for synthesizing amino acid by *GDH2* and *gltB* encoding enzymes (Belitsky and Sonenshein, 1998; Vanoni and Curti, 1999).

4. Conclusions

A pilot-scale integrated CWT-BTF system was used to remove VOCs from a biowaste treatment plant and attenuate the health risks. The results showed that the waste unloading hall had a higher concentration of TVOCs than the pretreatment plant and that VOCs were the predominant VOCs. Prior to inoculation, the TVOC RE for the CWT-BTF were -40.4% . At 20 days after initial inoculation, the TVOC RE increased to 84.0% and a biofilm had formed. However, at 100 days, RE decreased to 76.4% probably due to shock loading with VOCs. Reinoculation increased the REs for VOCs, AHs, and HHs, but the TVOC RE dropped to 54.4% when the inlet concentration rose to 1800 ppb.

The CWT-BTF significantly decreased the HRs and LCRs. The inoculum bacteria, including *Bacillus*, *Klebsiella*, and *Burkholderia pseudomultivorans*, enabled the indigenous microorganisms, such as *Polynucleobacter*, *Verrucomicrobia*, and *Planctomycetota*, which are the main contributors to energy metabolism pathways, to become dominant in the BTF under VOCs stimulation. *Polynucleobacter* sp. 32-46-207, which is involved in sulfur metabolism, had a potential role in VOCs removal. *cysJ*, *cysK*, and *metB* were the key genes related to sulfur metabolism, whereas *gltB*, *GDH2*, *nirB*, and *niT* were related to nitrogen metabolism. This research provides insights into the response and adaptation mechanisms shown by microorganisms in a BTF and offers guidance for constructing more versatile degrading bacteria when there is shock loading with VOCs.

CRedit authorship contribution statement

Qihao Feng: Writing – original draft, Investigation, Formal analysis. **Zhishu Liang**: Writing – review & editing, Supervision. **Wen Liao**: Methodology, Data curation. **Guiying Li**: Writing – review & editing, Conceptualization. **Weiping Zhang**: Writing – review & editing.

Taicheng An: Supervision.

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.wasman.2025.115124>.

Data availability

The data that has been used is confidential.

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