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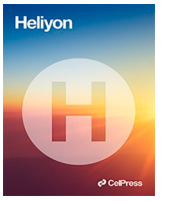

Research article

Biofiltration of toluene and ethyl acetate mixture by a fungal-bacterial biofilter: Performance and community structure analysis

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ABSTRACT

The inhibitory effect of hydrophilic volatile organic compounds (VOCs) on hydrophobic VOCs removal was found to be efficiently reduced by the fungal-bacterial biofilters (F&B-BFs) developed in the present study. Overall, the toluene and ethyl acetate mixture removal efficiencies (REs) and elimination capacities (ECs) of F&B-BFs were superior to those of bacterial biofilters (B-BFs). The REs for toluene and ethyl acetate were 32.5 ± 0.8 % and 74.6 ± 1.0 %, respectively, for F&B-BFs, in comparison to 8.0 ± 0.3 % and 60 ± 1.3 % for B-BFs. The ECs for toluene and ethyl acetate were 13.0 g m^{-3} h^{-1} and 149.2 g m^{-3} h^{-1}, respectively, for the F&B–BF, compared to 3.2 g m^{-3} h^{-1} and 119.6 g m^{-3} h^{-1} for the B-BFs. This was achieved at a constant empty bed residence time (EBRT) of 45 s. F&B-BFs exhibited a superior mineralization efficiencies (MEs) compared to B-BFs for a VOC mixture of toluene and ethyl acetate (≈36.1 % vs ~ 29.6 %). This is attributed to the direct capture of VOCs by the presence of fungi, increased the contact time between VOCs and VOCs-degrading bacteria, and even distribution of VOCs-degrading bacteria in the F&B-BFs. Moreover, compared with B-BFs, the coupling effect of genus *Pseudomonas* degradation, and *unclassified_f_Herpotrichiellaceae* and *unclassified_p_Ascomycota* adsorption of F&B–BF resulted in a reduction in the impact of the presence of hydrophilic VOCs on the removal of hydrophobic VOCs, thereby enhancing the biofiltration performance of mixtures of hydrophilic and hydrophobic VOCs.

1. Introduction

Mixtures of volatile organic compounds (VOCs) with different Henry's law constants and solubility in water are typically produced in the petrochemical, chemical, printing, and paint industries, among others. Many of these compounds are classified as hazardous air pollutants (HAPs). These pollutants are initially released into the workshops and subsequently discharged into the atmosphere, where they have been demonstrated to exert a detrimental impact on human health and the environment. In the majority of cases, the industrial off-gas streams are distinguished by high flow rates, low concentrations (<1000 ppm), volatility, and a complex composition [1–3]. In comparison to traditional control technologies, biotechnologies are distinguished by their high purification efficiency, low operational costs, practically unattended operation, and minimal emission of the secondary pollutants. This makes them particularly

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well-suited for the removal of large volumes of air streams with low concentrations of the biodegradable pollutants [4,5]. However, the application of biofiltration has been unsuccessful in controlling industrial emissions due to the poor water-solubility, biological toxicity, and the fluctuation of inlet loading rates (ILR) [6–8]. These issues have prompted widespread concern among scholars, who have sought to address them. In the existing literature on the elimination of mixed hydrophilic and hydrophobic VOCs, antagonistic type interactions have been reported. In particular, the presence of hydrophilic VOCs in contaminated streams has been found to significantly decrease the removal efficiencies (REs) of hydrophobic compounds in biofilters [9–12]. The inhibition mechanism in the biofiltration systems was attributed to the low mass transfer rates of hydrophobic VOCs from the gas phase to the biofilm. This resulted in differences in the removal performance on the macro-level [13]. It is therefore imperative to construct a highly efficient microbial degradation system with the capacity to enhance the mass transfer rates of hydrophobic VOCs while simultaneously reducing the discrepancy in removal performance between hydrophilic and hydrophobic VOCs.

The fungal-bacterial biofilter (F&B–BF) has emerged as a subject of increasing interest in the research community. In the literature surveyed, the F&B–BF was constructed by introducing pure fungi to B–BF [14,15], which employed specialized fungal degraders to capture and decompose hydrophobic VOCs directly. The aforementioned drawbacks of conventional biofilters can be overcome by this innovative technology. Its performance was superior to that of bacterial biofilters (B-BFs) and fungal biofilters (F-BFs) for the removal of mixtures of hydrophilic and hydrophobic VOCs. However, due to its construction method, it still needs to be improved before it can be used in industry. The inoculated VOC-degrading fungal species may not always flourish in F&B-BFs with extended operational periods. This is attributed to several factors, including the poorer resistance to load fluctuations [16], the lower metabolic rates [17], the narrower range of degradation [18] and the relatively slower growth rates [19]. These limitations have led to the observed instability in performance and non-universality of F&B–BF. Furthermore, for different VOCs, different fungal species that degrade VOCs should be inoculated in the reactor. This is not also conducive to the engineering applications of this technology.

A recently developed method, the two-step method for building and starting up F&B–BF, was reported by Zhai et al. [20]. In contrast to the conventional approach of inoculating pure fungi, this method is based on B–BF to construct F&B–BF. This is achieved by coupling a low pH (5.9) of mineral salt medium (MSM) control with the addition of antibiotics (20 g m^{-3} chloramphenicol) into the MSM. The aim was to achieve an optimal ratio of the 18S rRNA to 16S rRNA gene copy number (F/B) in biofilms. The presence of fungi can enhance the mass transfer rates of hydrophobic VOCs due to the aerial mycelia of fungi being able to directly contact with the gas phase and subsequently uptake hydrophobic compounds from the gas phase to the biofilm. In comparison to the previously reported F&B–BF, this particular F&B–BF exhibited high microbial diversity and robustness, as well as a strong adsorption capacity of fungi. Therefore, it can effectively overcome the antagonistic effect between hydrophilic and hydrophobic VOCs in the biofiltration process.

In this study, the F&B–BF biofilter was constructed using a novel method previously described [20]. This method involved inoculating only one type of industrial wastewater treatment plant (WWTP) activated sludge, obviating the need to inoculate target pollutant-degrading fungi. The F&B–BF biofilter is capable of enhancing the removal performance of mixed VOCs and broadening the spectrum of its application due to the strong fungal adsorption capacity and rich bacterial biodiversity. A study was conducted to overcome the antagonistic effect of the F&B–BF biofilter on the mixture of hydrophilic and hydrophobic VOCs (ethyl acetate (EA) and toluene (T)) emitted from the color printing industry. By constructing a relationship between the removal performance of binary VOC mixtures and the microbial community in the biofilter, the enhanced degradation mechanism of hydrophilic and hydrophobic VOC mixtures was revealed from the perspective of molecular biology.

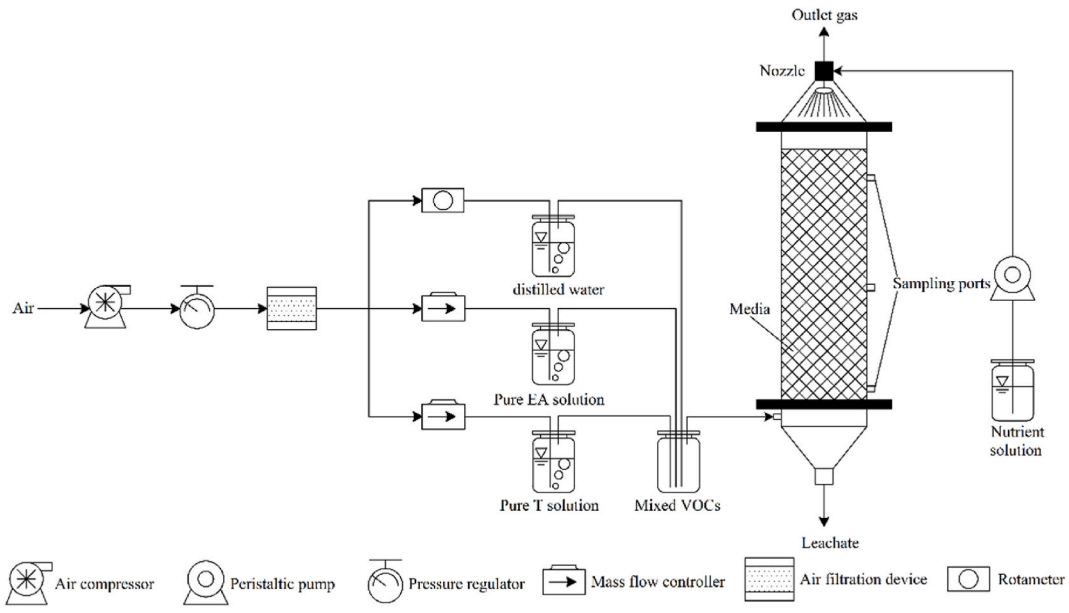

Fig. 1. Schematic diagram of the laboratory-scale biofilter.

2. Materials and methods

2.1. Mixtures of volatile organic compounds

A binary mixture stream was employed, comprising hydrophobic (toluene) and hydrophilic VOCs (ethyl acetate) (Sinopharm Chemical Reagent Co. Ltd., China, analytical reagent, >99.5 %). Their Henry's law constants (atm·m^3/mol at 25 °C) are 6.64 × 10^{-3} and 1.34 × 10^{-4}, respectively [21]. A series of concentration ratios for the mixture VOCs was fed to B–BF and F&B–BF, respectively.

2.2. Biofilter set up

A schematic diagram of the laboratory-scale biofilter was constructed in accordance with the illustration in Fig. 1. Two identical laboratory-scale biofilters (an internal diameter, 7 cm; an effective bed height, 60 cm) were made of transparent plexiglas and filled with the polyurethane foam (cube, a side length of 4–6 mm, an average pore size of 0.8 mm, a specific surface area of 400 m^2 m^{-3}, a water holding capacity of 55 g-H_2O g^{-1}, a void fraction of 95 %, a bulk density of 15 kg m^{-3}). The effective volume was 2.3 L. Sampling ports were sealed with polytetrafluoroethylene plugs and were located equidistantly along the biofilter at different heights for the collection of gas and biomass samples for analysis.

Two identical laboratory-scale biofilters were inoculated with 2500 mg volatile suspended solids (VSS) L^{-1} of aerobic activated sludge from the secondary sedimentation tank of Sinopec Yangzi Petrochemical Company WWTP (Nanjing, China). And 300 mg TOC m^{-3} benzyl alcohol as an external carbon source was added to the mineral salt medium (MSM), which favored the rapid start-up of two B-BFs. The MSM pH value of one biofilter was adjusted to 7.0 to set up the conventional biofilter (B–BF) whose bacteria were predominant. For the other biofilter based on B–BF, the pH value of MSM was decreased to 5.9, and 20 g m^{-3} of the chloramphenicol as a bacteriostat and TOC of 100 mg m^{-3} wheat bran as an external carbon source were added to MSM to rapidly construct and to start up F&B–BF by a two-step method [20]. When the ratio of 18S rRNA to 16S rRNA genes, the average absolute copy number (F/B) reached 0.38, which was higher than 0.27 [22], indicating that F&B–BF was successfully constructed and started.

The mixture of toluene and ethyl acetate air streams was generated by bubbling the compressed air stream through liquid toluene and ethyl acetate in two gas wash bottles, as described by Zhai et al. [23]. Mineral salt medium (MSM) without carbon source substrate was prepared using the following chemicals (per liter): $Na_2HPO_4{\cdot}12H_2O$ (4.5 g), KH_2PO_4 (1.0 g), $MgSO_4{\cdot}7H_2O$ (0.2 g), $CaCl_2$ (0.023 g) and using $NaNO_3$ (3.2 g) instead of $(NH_4)_2SO_4$ (2.5 g) as the nitrogen source [24], and 1 mL trace element stock solution [25].

An EBRT was kept at 45 s, the operating temperature was within the range of 20–25 °C, and the moisture content of the packing layers was maintained at 75–85 % by spraying fresh MSM from the top of B–BF and F&B–BF at a rate of 60 × 10^{-3} m^3 h^{-1} for 2 min every 2 days, respectively [26]. The mixture VOCs was fed to B–BF and F&B–BF in an up-flow mode, respectively. Periodic backwashing (1 h per week) was used to control biomass growth, as described by Han et al. [27].

2.3. Analytical methods

The concentrations of gaseous toluene and ethyl acetate were determined by gas chromatography (GC) (2014, SHIMADZU, JPN). Carbon dioxide (CO_2) was analyzed using GC fitted with a methane conversion furnace, a packed column and equipped with a thermal conductivity detector (TCD) (9890A, Shanghai Linghua Instrument Co., Ltd., CHN). The total organic carbons (TOC) were monitored using a TOC analyzer (TOC 5000A, SHIMADZU, JPN). The pH value of the MSM was detected by a pH meter (FEP20, Mettler-Toledo, CHE). The NO_3^--N concentrations in the MSM and leachate were measured according to standard methods [28]. The following parameters were calculated according to the methodology described in a previous publication [23]: RE, elimination capacity (EC), EBRT and ILR. The mineralization rate (MR) and carbon dioxide (CO_2) production rate (PCO_2) were calculated following the methods described by Sun et al. [29] and Marycz et al. [30]. Each analysis was repeated three times, and the error bars represent the mean ± S. D.

2.4. Carbon balance analysis

A carbon balance was conducted after the pseudo-steady-state phase had been achieved in the system. This can be expressed mathematically as follows:

$$m_o = m_i + mCO_2 + m_{biomass} + m_{intermediates} \quad (1)$$

where m_o and m_i represent the influent and effluent carbon rates (mg C min^{-1}) associated with a binary mixture of toluene and ethyl acetate; mCO_2 denotes the effluent carbon mass rate (mg C min^{-1}) associated with CO_2 production; mbiomass represents the biomass production rate (mg C min^{-1}) equivalent to the carbon mass utilization rate, and $m_{intermediates}$ denotes the effluent carbon rate (mg C min^{-1}) of leachate. The m_o, m_i and mCO_2 can be determined from the inlet concentration of the binary mixture of toluene and ethyl acetate, the outlet concentration, and the CO_2 outlet concentration, respectively. The dissolved CO_2 in the leachate is negligible due to its lower solubility in water (391.1 × 10^{-4} mol L^{-1}, 1atm and 20 °C) [31]. A typical composition of bacterial biofilm can be represented as $C_5H_{8.3}NO_{1.35}$ [32], while the composition of fungal-bacterial biofilm can be expressed as $C_5H_{7.94}NO_{2.24}$, as derived from the elementary analysis. Accordingly, the mbiomass was calculated based on the consumption of nitrogen sources in MSM. The $m_{intermediates}$ could be obtained from the TOC of leachate. The carbon recovery, R, was defined as the percentage ratio of the sum of m_i,

mCO_2, $m_{biomass}$ and $m_{intermediates}$ to m_o.

2.5. Bioinformatics analysis

2.5.1. Sample collection and DNA extraction

A certain amount of polyurethane foams was collected from the middle sampling port within the biofilter media on day 35 for analysis of the microbial community. The samples were centrifuged for 10 min at 12,000 rpm using a centrifuge (5417R, Eppendorf, GER). Genomic DNA was then extracted from each sample by FastDNA® Spin Kit for Soil (MP bio).

2.5.2. Illumina high-throughput sequencing and bioinformatics analysis

Primer sets 338F/806R (5′-ACTCCTACGGGAGGCAGCAG-3′/5′-GGACTACHVGGGT WTCTAAT-3′) and SSU0817F/SSU1196R (5′-TTAGCATGGAATAATRRAATAGGA-3′/5′-T CTGGACCTGGTGAGTTTCC-3′) were selected to amplify 16S rRNA and 18S rRNA, respectively. The DNA amplification, the purification of the PCR products, the high-throughput sequencing of the purified amplicons, the data processing, and the analysis were conducted in accordance with the previously reported method [23].

2.5.3. Quantitative real-time polymerase chain reaction (qRT-PCR) for bacteria and fungi

Primer sets 1369F/1492R (5′-CGGTGAATACGTTCYCGG-3′/5′-GGWTACCTTGTTAC GACTT-3′) and FungiQuant-F/FungiQuant-R (5′-GGRAAACTCACCAGGTCCAG-3′/5′-G SWCTATCCCCAKCACGA-3′) were used to perform PCR for 16S rRNA and 18S rRNA, respectively. The qRT-PCR determination was conducted in accordance with the methodology described by Cheng et al. [14]. Each sample was analyzed in triplicate, and the mean ± S.D. was employed to represent the error bar.

2.5.4. Predictions of pollutant degradation functions of biofilms

To ascertain the dissimilarities in pollutant degradation functions among the biofilms, the functional gene types and relative abundance of these biofilms were predicted using the program Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), based on 16S rRNA high-throughput sequencing data. The results were further analyzed by the Cluster of Orthologous Groups (COG) database [33].

2.6. Accession number

The pyrosequencing datasets were deposited into the National Center for Biotechnology Information (NCBI) Short Reads Archive Database (accession number: SRA489557).

3. Results and discussion

3.1. Abiotic experiment

Prior to the biofiltration process, an abiotic test was conducted. The control biofilter, which did not receive any microbial inoculants, removed less than 1.53 % and 1.78 % of the influent toluene and ethyl acetate from the gaseous mixtures, respectively (data not shown). The results demonstrated that the non-biological degradation in the removal of toluene and ethyl acetate was minimal.

3.2. Comparison of the removal performance in the biofilters

During the acclimation phase, two distinct biofilters were fed with a concentration of 300 mg m^{-3} of gas-phase toluene and ethyl acetate. Following the acclimation phase, a series of experiments were conducted in accordance with the specifications outlined in Table 1.

The RE, EC, and MR indexes are crucial for evaluating the biofiltration performance of the biofilters. In this study, the REs of toluene and ethyl acetate using B–BF and F&B–BF, respectively, were compared under different concentration ratios (Fig. 2). In phase I, the concentrations of both toluene and ethyl acetate were 500 mg m^{-3}, with an EBRT of 45 s. The B–BF and F&B–BF achieved nearly stable RE values of 100 % and 100 %, respectively. When the toluene concentration was maintained at 500 mg m^{-3}, the ethyl acetate concentration increased from 1000 mg m^{-3} (phase II) to 2500 mg m^{-3} (phase V). The REs of toluene exhibited a notable decline, from 73.0 ± 0.7 % to 8.0 ± 0.3 % and 96.8 ± 0.8 % to 32.5 ± 0.8 %, while the REs of ethyl acetate exhibited a slight fluctuation, from 95 ±

Table 1
Experimental conditions for different phases.

Phase	Time (day)	Toluene concentrations (mg m^{-3})	Ethyl acetate concentrations (mg m^{-3})
I	1–10	500	500
II	11–20	500	1000
III	21–30	500	1500
IV	31–40	500	2000
V	41–50	500	2500

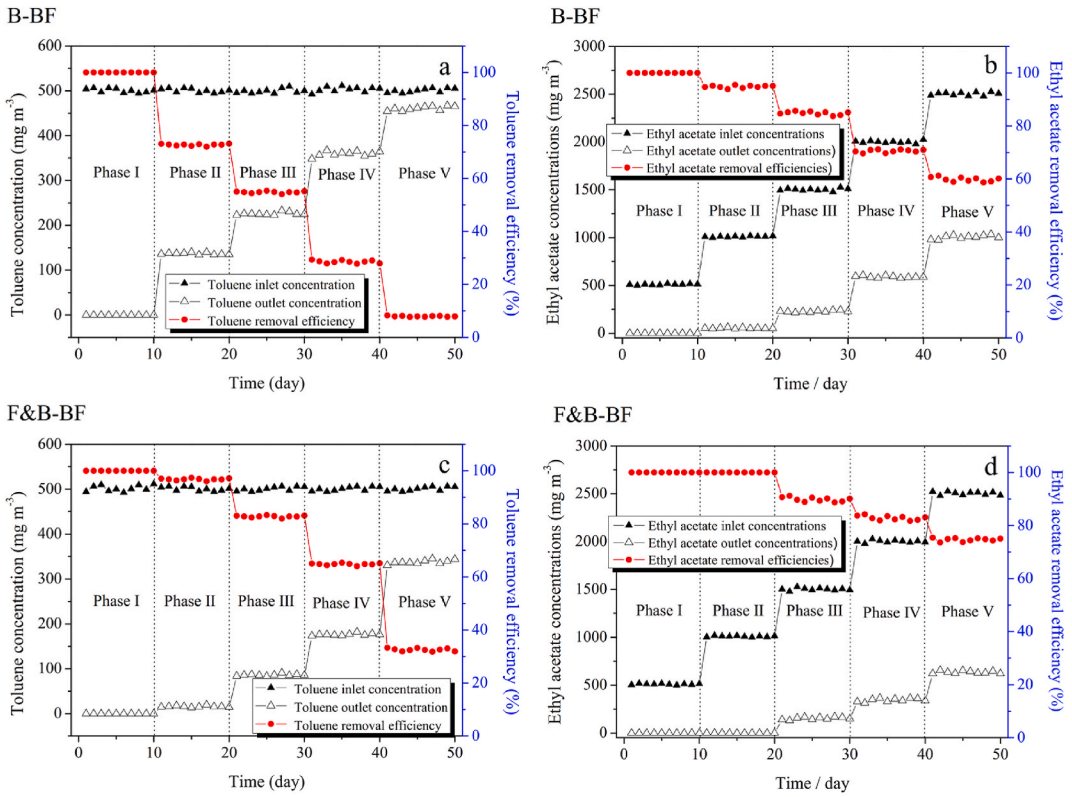

Fig. 2. Comparison of the REs of a binary mixture of toluene and ethyl acetate at different concentration ratios between B–BF and F&B–BF.

1.1 % to 60 ± 1.3 % and 100 % to 74.6 ± 1.0 % for B–BF and F&B–BF, respectively. The mixtures of gas-phase toluene and ethyl acetate ECs of B–BF and F&B–BF under different concentration ratios, respectively, were compared (Fig. 3). As ethyl acetate ILT increased from 40 g m^{-3} h^{-1} to 200 g m^{-3} h^{-1}, the ECs of toluene (3.2 g m^{-3} h^{-1} vs. 13.0 g m^{-3} h^{-1}) and ethyl acetate (119.6 g m^{-3} h^{-1} vs. 149.2 g m^{-3} h^{-1}) was compared between the B–BF and F&B–BF. The biofilters demonstrated overall higher removal performance of toluene in the presence of low concentrations of ethyl acetate. However, the impact of medium and high concentrations of ethyl acetate on the biofiltration of toluene was negative. The results were in accordance with the findings of Wang et al. [13], who had previously conducted research on the biofiltration of a mixture of methanol and n-hexane vapor. However, in comparison to B–BF, F&B–BF demonstrated a greater capacity to resist the inhibitory effects of ethyl acetate on toluene biodegradation, while also exhibiting a stronger ability to withstand the shock of high concentration loads under the same operating conditions.

Fig. 4a showed that the mixture of toluene and ethyl acetate exhibited superior MRs for F&B–BF compared to B–BF. However, when the concentration of ethyl acetate exceeded 1500 mg m^{-3}, the discrepancy in MRs between B–BF and F&B–BF became less pronounced.

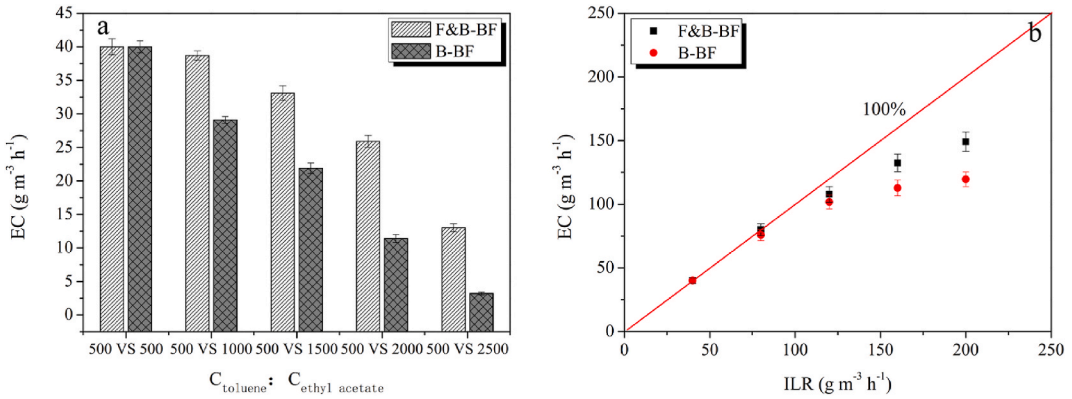

Fig. 3. Comparison of the ECs of a binary mixture of toluene and ethyl acetate at different concentration ratios between B–BF and F&B–BF (a). The red line denotes the ratio of EC to ILR reaches 100 % (b).

This indicated that the antagonistic interaction between toluene and ethyl acetate was intensified, and the growth of VOCs-degrading consortia was inhibited gradually with the increase of ethyl acetate concentration.

To investigate the fungal adsorption on the VOCs, the outlet mixed VOCs and CO_2 concentrations were continuously determined under shut-down conditions (continuous air supply). When the supply of 500 mg m^{-3} toluene and 1500 mg m^{-3} ethyl acetate was stopped, the responses of the biofilters were shown in Fig. 4b. At the outlet of B–BF and F&B–BF, 32.3 ± 2.7 and 377.8 ± 12.8 mg CO_2 m^{-3}, 0 and 29 ± 0.8 mg toluene m^{-3} and 0 and 0 mg ethyl acetate m^{-3}, respectively, were tested after 12 h of shutdown time. Twelve hours later, the concentration of toluene and CO_2 in the F&B–BF outlet exhibited a gradual decline, while the concentration of CO_2 at the B–BF outlet was below the detection limit. The presence of toluene and CO_2 detected in the F&B–BF outlet validated the efficacy of the fungal adsorption for mixed VOCs [34]. It enhanced the mass transfer rate of toluene, played a cushioning effect in the fluctuation of mixed VOCs ILR, and prolonged the reaction time between toluene and toluene-degrading consortia. In addition, low concentrations of CO_2 were detected in the B–BF outlet, likely due to the endogenous respiration of biofilms. Conversely, the ethyl acetate was barely detected at the gas outlet of the B–BF and F&B–BF, which may be attributed to its readily biodegradability.

3.3. Comparison of the carbon mass balance in the biofilters

Mixtures of toluene and ethyl acetate were employed as carbon sources for microorganisms of B–BF and F&B–BF, respectively. These mixtures were converted into four fractions, namely, CO_2, organic carbon in leachate, biomass fraction, and outlet unconverted mixed VOCs [35]. A mass balance analysis for carbon was employed to quantify the proportion of four distinct types of carbon and to gain a deeper understanding of the mixed VOCs degradation process in the B–BF and F&B–BF. The data were collected at different concentration ratios and the results of the comparison are listed in Table 2. The carbon recoveries (R) of B–BF and F&B–BF were 90.0–104.6 % and 95.0–99.3 %, respectively, which indicated the accuracy of the test results. As illustrated in Table 2, the MRs of B–BF and F&B–BF exhibited a consistent decline, from 78.7 to 29.6 % and 80.7 to 36.1 %, respectively. Concurrently, the carbon content in leachate exhibited a gradual increase, from 1.9 to 8.5 % and 1.3–6.5 %, respectively The results indicated that the growth of toluene-degrading bacteria was progressively impeded with the escalation of ethyl acetate concentrations. This phenomenon was attributed to the accumulation of acetic acid resulting from the decomposition of ethyl acetate, which led to a decline in the pH value from 5.8 ± 0.21 to 2.7 ± 0.1. In terms of the removal performance of the mixtures of hydrophilic and hydrophobic VOCs, F&B–BF demonstrated superior efficacy to B–BF in different phases due to the presence of fungi. Fungi can tolerate a low pH value, increase the mass transfer rates of toluene from the gas phase to the biofilm and extend the reaction time between toluene and toluene-degrading bacteria. Furthermore, biofilm conversion rates of F&B–BF were found to be greater than 17.0 % in this study, which is higher than 12–13 % conversion rates observed in B–BF. These findings are consistent with those reported in previous literatures [36–39]. The reason for this is that fungi are able to convert a greater quantity of carbon into biofilms than bacteria [40]. The aforementioned character enabled F&B–BF to withstand harsh environmental conditions more effectively than B–BF in biofiltration mixed VOCs.

3.4. Comparison of microbial communities in the biofilters

A genus-level analysis enables the comprehension of the interrelationship between the composition of the microbial community composition and the biofiltration performance. It facilitates the investigation of the VOCs degradation mechanisms of diverse biofilters. The comparison of microbial communities at the genus level between B–BF and F&B–BF is illustrated in Fig. 5. As illustrated in Fig. 5a, *Pseudomonas* was the most prevalent bacterial genus in B–BF and F&B–BF. A number of previous studies have indicated that the genus *Pseudomonas*, which is suitable for growth in neutral conditions, with a pH range of 7.0–8.0 [41–43]. Furthermore, the degradation of toluene [44–46] and ethyl acetate [47–49] has been demonstrated. The relative abundance of *Pseudomonas* decreased

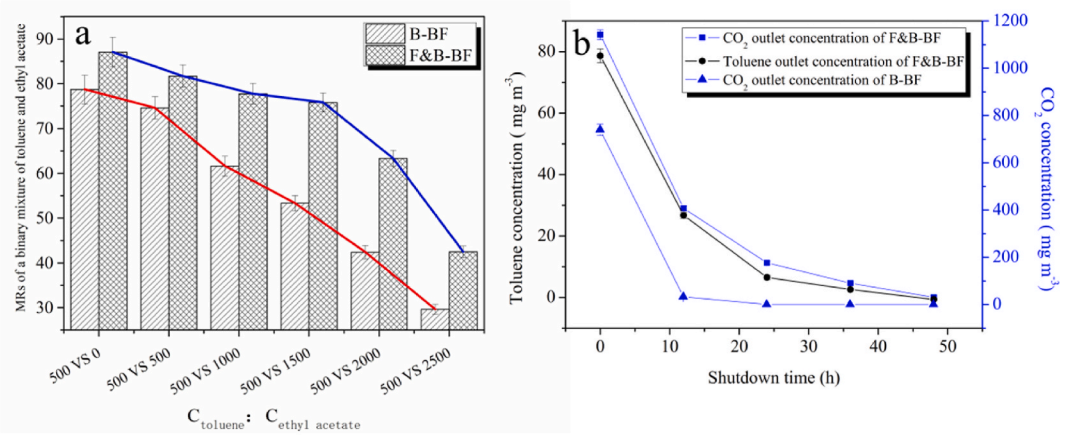

Fig. 4. Comparison of the MRs of a binary mixture of toluene and ethyl acetate at different concentration ratios (a) and the response of shut-down (b) between B–BF and F&B–BF.

Table 2
Carbon balance analysis of each phase.

Phase	m_o	B–BF								
		m_i	%	mCO_2	%	$m_{biomass}$	%	$m_{intermediates}$	%	R
500 TL	36.5	0.0	0.0	28.7	78.7	4.5	12.3	0.7	1.9	92.9
500 TL+500EA	58.3	0.0	0.0	43.5	74.6	7.4	12.6	1.6	2.8	90
500 TL+1000EA	80.2	12.2	15.3	49.4	61.6	10.0	12.5	2.9	3.6	93
500 TL+1500EA	102.0	26.5	26.0	54.4	53.3	13.0	12.7	5.8	5.7	97.7
500 TL+2000EA	123.8	51.8	41.9	52.5	42.4	16.2	13.1	8.9	7.2	104.6
500 TL+2500EA	145.6	77.5	53.2	43.1	29.6	18.6	12.8	12.4	8.5	104.1
Phase	**m_o**	**F&B–BF**								
		m_i	**%**	**mCO_2**	**%**	**$m_{biomass}$**	**%**	**$m_{intermediates}$**	**%**	**R**
500 TL	36.5	0.0	0.0	29.5	80.7	6.3	17.3	0.5	1.3	99.3
500 TL+500EA	58.3	0.0	0.0	43.9	75.3	10.9	18.6	1.5	2.6	96.5
500 TL+1000EA	80.2	1.2	1.5	57.4	71.6	14.7	18.4	2.8	3.5	95
500 TL+1500EA	102.0	13.0	12.7	64.6	63.3	18.7	18.3	4.8	4.7	99
500 TL+2000EA	123.8	27.9	22.5	62.4	50.4	23.3	18.8	6.7	5.4	97.1
500 TL+2500EA	145.6	52.4	36.0	52.6	36.1	26.9	18.5	9.5	6.5	97.1

Unit:g-C/(m^3 h).

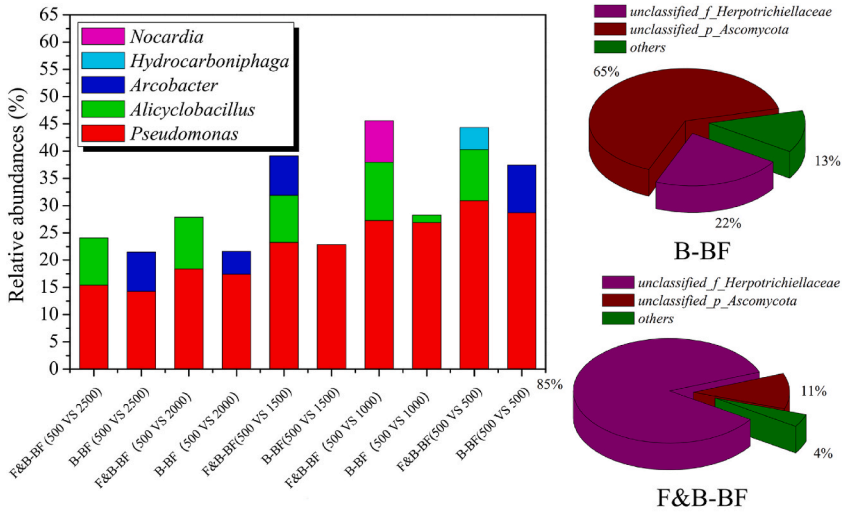

Fig. 5. The microbial community comparison at the genus level between B–BF and F&B–BF as a function of $C_{toluene}$: $C_{ethyl\ acetate}$.

from 26.9 to 14.3 % for B–BF and 27.3 to 15.4 % for F&B–BF, respectively, as the ethyl acetate concentrations increased from 1000 to 2500 mg m^{-3}. This indicates that the growth of the genus *Pseudomonas* was retarded with increasing ethyl acetate concentration due to the decrease in pH value. In addition, the REs dropped from 73.0 ± 0.7 to 8.0 ± 0.3 % (B–BF) vs. 96.8 ± 0.8 to 32.5 ± 0.8 % (F&B–BF) for toluene and from 95.0 ± 1.1 to 60.0 ± 1.3 % (B–BF) vs 100.0 to 74.6 ± 1.0 % (F&B–BF) for ethyl acetate, and that the ECs of toluene were induced from 40.0 to 3.2 and 13.0 g m^{-3} h^{-1} for B–BF and F&B–BF, respectively. The results indicated a positive correlation between the removal performance of B–BF and F&B–BF and the relative abundance of the genus *Pseudomonas*. From Fig. 6, the average copy number of 16S rRNA genes (bacteria) in the biofilm was determined to be 8.69×10^3 copies ng^{-1} of F&B–BF, which was lower than 1.96×10^4 copies ng^{-1} for B–BF. Although the relative abundances of the genus *Pseudomonas* were similar, the absolute content of the genus *Pseudomonas* was decreased from B–BF to F&B–BF. This is in contradiction with the removal performance of F&B–BF being superior to B–BF.

The fungal community structure at the genus level in B–BF and F&B–BF was qualitatively and quantitatively analyzed. As shown in Fig. 5b, the most abundant fungal genus in B–BF was *unclassified_p_Ascomycota* (65.0 %), followed by *unclassified_f_Herpotrichiellaceae* (22.0 %) and others (13.0 %), in that order. In F&B–BF, *unclassified_f_Herpotrichiellaceae* was the dominant fungal genus, comprising 85 % of the total, followed by *unclassified_p_Ascomycota* (11.0 %), and others (4.0 %). If *unclassified_f_Herpotrichiellaceae* and *unclassified_p_Ascomycota* were regarded as a single entity, their relative abundance was 87.0 % for B–BF and 96.0 % for F&B–BF, respectively. The results indicated that there was no significant difference in the fungal communities between B–BF and F&B–BF, suggesting that the structure of the fungal community was stable. As illustrated in Fig. 6, the average copy number of the 18S rRNA genes (fungi) increased

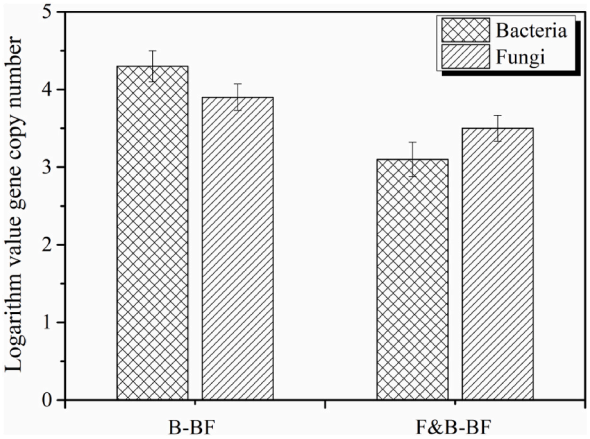

Fig. 6. Logarithm of absolute copy number of bacteria and fungi of B–BF and F&B–BF.

from 1.27×10^3 copies ng^{-1} in B–BF to 3.31×10^3 copies ng^{-1} in F&B–BF. As a result, more VOCs were captured, the contact time between toluene and the genus *Pseudomonas* was prolonged, the genus *Pseudomonas* was evenly distributed [50], and the influence of a low pH value was weakened. Consequently, although the absolute copy number of *Pseudomonas* was decreased by 2.25 times, F&B–BF successfully mitigated the inhibition of hydrophilic VOCs on hydrophobic VOCs removal. This was attributed to the fungal adsorption coupled with bacterial degradation. This was the fundamental reason why the biofiltration performance of F&B–BF was superior to B–BF.

3.5. Toluene-degradation pathway using F&B–BF

The degradation pathway of toluene in biofiltration of mixtures of gas-phase toluene and ethyl acetate by F&B–BF was predicted using PICRUSt. The results of the main pollutant degradation functions of the four biofilms from the F&B–BF under different concentration ratios of toluene to ethyl acetate are presented in Fig. 7. The relative abundance of benzoate degradation genes in biofilms was considerably higher than that of the majority of the other predicted functional categories. As the concentration of ethyl acetate in the VOC mixture decreased, the abundance of the benzoate degradation function in the biofilm increased. This indicated that the benzoate degradation function was gradually inhibited as the concentration of ethyl acetate concentration increased.

As illustrated in Fig. 8, benzaldehyde and benzoate were the intermediate metabolites of toluene. As evidenced by Table 3, benzaldehyde dehydrogenase and catechol 1,2-dioxygenase were pivotal enzymes in the toluene metabolism process. This indicates that the side-chain methyl group of toluene was first oxidized and subsequently generated benzyl alcohol, benzaldehyde, benzoic acid and catechol in the biofiltration process using a F&B–BF. This suggests that the toluene-degradation pathway by a mixture of fungi and bacteria is similar to that of bacteria [51]. The results indicated fungi did not play a primary role in the degradation of gaseous VOC mixtures, but rather in the adsorption of a mixture of VOCs.

4. Conclusions

The F&B–BF developed in the present study successfully reduced the influence of hydrophobic VOCs degradation in the presence of hydrophilic VOCs. This was achieved by the presence of fungi, which attributed to its adsorption capacity, well solved the mass transfer of hydrophobic VOCs, increased the contact time between VOCs and VOCs-degrading bacteria and successfully alleviated the inhibition of low pH value on the enzymes activity of toluene-degrading bacteria. Consequently, the removal performance of the mixtures of toluene and ethyl acetate in F&B–BF was superior to that of B–BF. High-throughput sequencing analysis indicated that the relative abundance of *Pseudomonas* was positively correlated with the removal performance for B–BF and F&B–BF. The functional genes prediction using PICRUSt and the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic pathway analysis for toluene indicated that the toluene-degradation pathway using F&B–BF was similar to that of B–BF.

Data availability statement

Data included in article/supp.

Ethics statement

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

Our funding statement has been amended as follows:
1.The natural science foundation of Gansu province of China (22JR5RE1041).

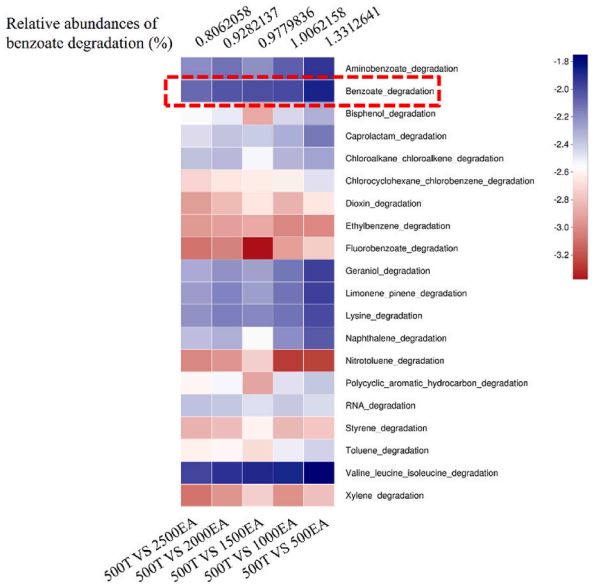

Fig. 7. Heatmap of functional genes relating to pollutant degradation predicted using PICRUSt as a function of different $C_{toluene}$: $C_{ethyl\ acetate}$.

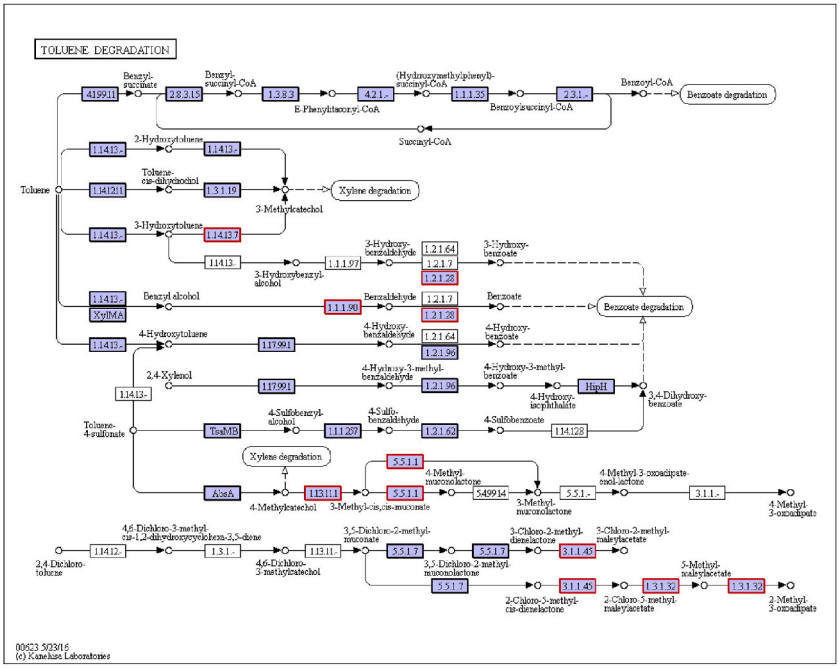

Fig. 8. The KEGG metabolic pathway of toluene. The numbers in the boxes represent enzyme commission numbers for the enzymes, defined in Table 3.

Table 3
Enzymes related to metabolic pathway of toluene in the fungal-bacterial biofilter.

Enzyme number	KEGG orthologous number and definition
1.14.13.7	K03380 Phenol 2-monooxygenase
1.1.1.90	K00055 Aryl-alcohol dehydrogenase
1.2.1.28	K00141 Benzaldehyde dehydrogenase
1.13.11.1	K03381 Catechol 1,2-dioxygenase (C12O)
5.5.1.1	K01856 Muconate cycloisomerase

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CRediT authorship contribution statement

Jian Zhai: Writing – review & editing, Supervision, Project administration, Funding acquisition. **Chunhua Jiang:** Writing – original draft, Software, Formal analysis. **Xiaojuan Xue:** Methodology, Formal analysis, Data curation. **Hai Wang:** Writing – original draft, Supervision, Resources, Project administration, Conceptualization.

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

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