

# A comparison of the performance of bacterial biofilters and fungal–bacterial coupled biofilters in BTEp-X removal

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## ABSTRACT

**Background.** Conventional biofilters, which rely on bacterial activity, face challenges in eliminating hydrophobic compounds, such as aromatic compounds. This is due to the low solubility of these compounds in water, which makes them difficult to absorb by bacterial biofilms. Furthermore, biofilter operational stability is often hampered by acidification and drying out of the filter bed.

**Methods.** Two bioreactors, a bacterial biofilter (B-BF) and a fungal–bacterial coupled biofilter (F&B-BF) were inoculated with activated sludge from the secondary sedimentation tank of the Sinopec Yangzi Petrochemical Company wastewater treatment plant located in Nanjing, China. For approximately 6 months of operation, a F&B-BF was more effective than a B-BF in eliminating a gas-phase mixture containing benzene, toluene, ethylbenzene, and *para*-xylene (BTEp-X).

**Results.** After operating for four months, the F&B-BF showed higher removal efficiencies for toluene (T), ethylbenzene (E), benzene (B), and *para*-X (*p*-Xylene), at 96.9%, 92.6%, 83.9%, and 83.8%, respectively, compared to those of the B-BF (90.1%, 78.7%, 64.8%, and 59.3%). The degradation activity order for B-BF and F&B-BF was T > E > B > *p*-X. Similarly, the rates of mineralization for BTEp-X in the F&B-BF were 74.9%, 66.5%, 55.3%, and 45.1%, respectively, which were higher than those in the B-BF (56.5%, 50.8%, 43.8%, and 30.5%). Additionally, the F&B-BF (2 days) exhibited faster recovery rates than the B-BF (5 days).

**Conclusions.** It was found that a starvation protocol was beneficial for the stable operation of both the B-BF and F&B-BF. Community structure analysis showed that the bacterial genus *Pseudomonas* and the fungal genus *Phialophora* were both important in the degradation of BTEp-X. The fungal-bacterial consortia can enhance the biofiltration removal of BTEp-X vapors.

**Subjects** Environmental Sciences, Ecotoxicology, Environmental Contamination and Remediation, Environmental Impacts

**Keywords** Bacterial biofilter, Fungal-bacterial coupled biofilter, BTEp-X, Removal performance, Microbial community structure

## INTRODUCTION

Volatile organic compounds (VOCs) and odorous compounds emitted from petrochemical industry wastewater treatment plants have become a significant source of gaseous pollutants. These emissions have been linked to poor air quality and adverse health

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effects in humans. An increasing number of government agencies are implementing stringent environmental legislation to compel polluting industries to implement effective air pollution treatment processes to control the output of VOCs and odorous gases. According to [Hu et al. \(2020\)](#), biological waste air treatment processes are not only more environmentally friendly but also less expensive than conventional technologies, such as catalytic and thermal oxidation, wet scrubbing, and activated carbon adsorption. Bacterial biofilters (B-BFs) are compost beds that allow waste gases to pass through while being absorbed and degraded by microorganisms immobilized on porous packing media ([Delhomenie et al., 2002](#)). Inorganic nutrients, such as nitrogen, phosphorous, potassium, and microelements, are regularly sprayed. B-BFs are commonly utilized for the treatment of large volumes of low-concentration VOCs or odors in air streams. Generally, B-BFs eliminate gaseous VOCs by converting them from the gas phase to the liquid phase, transferring them to biofilms, and ultimately to the microorganisms being degraded. Therefore, gas-liquid mass transfer is a crucial factor that influences VOC biodegradation by B-BFs. The rate at which pollutants transfer from air to water is proportional to Henry's law constant ([Sander, 2023](#)).

$$H_v^{cc} = C_g / C_a \quad (1)$$

where  $H_v^{cc}$  (dimensionless) represents Henry volatility (defined as  $c/c$ ), while  $C_g$  ( $\text{mol m}^{-3}$ ) and  $C_a$  ( $\text{mol m}^{-3}$ ) represent the gas-phase concentration and aqueous-phase concentration of VOC, respectively.

On the basis of Henry's law constants, VOCs were classified into three categories: hydrophilic VOCs (with a Henry's law constant at 25 °C ranging from 0.0001 to 0.099), moderately hydrophilic VOCs (with a constant of 0.1–0.99) and hydrophobic VOCs (with a constant of 1–70) ([Cheng et al., 2016a](#)). Hydrophobic VOCs are more resistant to degradation in B-BFs due to the low mass transfer from the liquid phase to the biofilm phase, compared to hydrophilic VOCs ([Zehraoui, Hassan & Sorial, 2012](#)). In contrast, the removal efficiency (RE, %) of moderately hydrophilic VOCs is determined by both mass transfer and microbial activity ([Dwivedi et al., 2004](#); [Hernández et al., 2011](#)). As shown in [Eq. \(2\)](#), a higher value of  $H_c$  results in a higher value of  $C_{go}$ , which in turn leads to a lower VOC RE.

$$C_{go} / C_{gi} = \exp[-Da_s ZRT / H_c U_0 \delta^{\varphi \tanh(\varphi)}] \quad (2)$$

$$\varphi = \delta \sqrt{k/D} \quad (3)$$

where  $C_{gi}$  and  $C_{go}$  is the inlet and outlet VOCs concentration,  $D$  is the diffusion coefficient in liquid phase ( $\text{m}^2 \text{s}^{-1}$ ), as is the the biolayer surface per volume of package,  $Z$  the depth of packed bed (m),  $R$  is the ideal gases constant ( $\text{Pa m}^3 \text{mol}^{-1} \text{K}^{-1}$ ),  $T$  is the temperature (K),  $H_c$  the Henry's law constant ( $\text{Pa m}^3 \text{mol}^{-1}$ ),  $U_0$  the superficial velocity ( $\text{m s}^{-1}$ ),  $\delta$  the biofilm thickness (m), and  $k$  is the reaction rate constant ( $\text{s}^{-1}$ ),  $h$  is the height of the filter bed, and  $\varphi$  is the Thiele number. Therefore, increasing the mass transfer of hydrophobic VOCs is beneficial for enhancing the removal performance of B-BFs. Several strategies have

been investigated to increase bioavailability, such as innovative bioreactors, biofiltration with pretreatment, the use of fungal biocatalysts, the utilization of hydrophilic compounds, and the addition of hydrophilic compounds, have been investigated ([Cheng et al., 2016a](#)). The coexistence of fungi and bacteria has clear advantages in terms of removing VOCs and maintaining performance stability. This system is particularly effective for hydrophilic and moderately hydrophobic VOCs removal, as demonstrated by [Cheng et al. \(2016b\)](#), [Lebrero et al. \(2016\)](#), and [Vergara-Fernández et al. \(2018\)](#). Fungi are especially adept at purifying hydrophobic VOCs than other organisms due to their special hyphal structure, high hydrophobins, and abundant functional groups on their cell surfaces ([Cheng et al., 2016a](#)). Furthermore, fungi enhance the availability of contaminants and nutrients for bacteria by extending their hyphae ([Kohlmeier et al., 2005](#)). Additionally, they secrete thiamine ([Deveau et al., 2010](#)), cellulase and pectinase ([Boer et al., 2005](#); [Duponnois & Garbaye, 1990](#)), and organic acids ([Duponnois & Garbaye, 1990](#); [Bharadwaj, Lundquist & Alström, 2008](#)) that provide nutrients and break down hydrophobic VOCs into intermediates with improved water solubility, thereby improving bacterial performance. The secretion of chemicals can promote fungal development, stimulate fungal enzyme activity, eliminate compounds released by fungi that hinder their growth, and enhance biofilm formation. Consequently, in the coexistence system, bacteria can work in concert to break down complex molecules ([Wang et al., 2023](#)).

This study constructed a fungal-bacterial coupled biofilter (F&B-BF) to purify a mixture of hydrophobic VOCs and compared its REs, mineralization rates (MRs), physicochemical properties of biofilm, and microbial community structure with B-BF in detail. The aim of this work was to evaluate the performance of B-BF and F&B-BF in removing mixed hydrophobic VOCs and explain the potential mechanisms responsible for the superior performance of F&B-BFs.

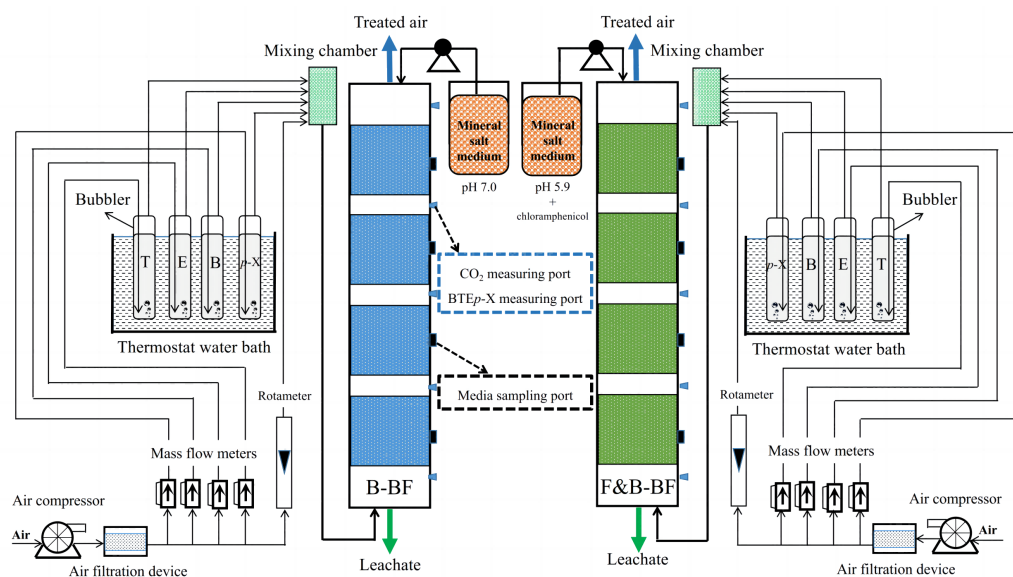
## MATERIALS & METHODS

### Mixed VOCs

This study focuses on the efflux gases from three odour treatment units (pretreatment unit, biochemical treatment unit, and sludge disposal unit) in a typical petrochemical industry wastewater treatment plant (WWTP). The detection of mixed VOCs was carried out using solid-phase microextraction (SPME)-gas chromatography (GC)-mass spectrometry (MS) for qualitative and quantitative analysis ([Tait et al., 2014](#); [Sousa et al., 2006](#)). Benzene, toluene, ethylbenzene, and *para*-xylene (BTE<sub>p</sub>-X) were the most prevalent pollutants according to the measured results. To simulate the BTE<sub>p</sub>-X gas experimentally, the method described by [Cho et al. \(2009\)](#) was used.

### Experimental setup

The experimental system is illustrated in [Fig. 1](#). Two bioreactors, B-BF and F&B-BF, were established to eliminate BTE<sub>p</sub>-X from an upflow waste air feed. The lab-scale biofilter was made of transparent Plexiglas and had an internal diameter of 8 cm, an effective bed height of 100 cm, and a working packed bed volume of 5 L. A perforated Teflon mesh was placed at the bottom to act as a support for the packing material and distribute gas-phase



**Figure 1** Schematic diagram of the B-BF and F&B-BF for BTEp-X waste gas treatment.

Full-size [DOI: 10.7717/peerj.17452/fig-1](https://doi.org/10.7717/peerj.17452/fig-1)

pollutants, while another perforated mesh at the top was used to distribute the mineral salt medium (MSM). Gas sampling ports sealed with rubber septa were available at equal intervals (10 cm) along the biofilter height and were used to collect gas samples by gas-tight syringes (250  $\mu$ L, Hamilton, Switzerland) to determine the concentrations of toluene (T), ethylbenzene (E), benzene (B), and *para*-X (*p*-Xylene), and CO<sub>2</sub> at different heights of the outlet. And the media sampling port was located in the middle of each bed layer vertically to collect packing samples using tweezers for bioinformation analysis (Fig. 1). Each sample was analyzed in triplicate. The system was supplied with compressed air *via* an air pump and filtered before being divided into five channels. Four of these channels were precisely controlled by four mass flow meters (CS200, Beijing Sevenstar Flow Co., LTD., China) and passed through vessels containing B, T, E, and *p*-X, respectively. And the fifth channel was just clean air. After mixing in a tank (inner diameter = 5 cm, effective bed height = 15 cm), these five streams were fed into the B-BF in an up-flow mode. Another mixture of BTEp-X was created following the same procedure to be introduced into the F&B-BF. The thermostat water bath (DK-S24, Shanghai Jing Hong Laboratory Instrument Co., Ltd., China) maintained a constant temperature of 25 °C for liquids B, T, E, and *p*-X, ensuring their consistent volatility throughout the experiment. Polyurethane foam (1 cm<sup>3</sup>) was used as the packing material due to its large specific surface area, high porosity, resistance to compaction, and low cost, as reported by *Dorado et al. (2009)*.

### Method for construction and start-up

The B-BF start-up was initiated by introducing activated sludge from a WWTP in the petrochemical sector. An inducer-target graded acclimation strategy and a gas-liquid phase joint inoculation method were used (*Devinny, Deshusses & Webster, 2017*). The activated

sludge was mixed with a nutrient solution and continuously dripped onto the filter bed packings from the top. Simultaneously,  $100 \text{ mg m}^{-3}$  of T exhaust gas was supplied to the filter bed to allow the biofilm to adhere to the packings. The degradation efficiency was low during the early stages of membrane hanging when the biofilm had not yet matured. After purification, T was reintroduced to the filter bed using an air pump. After the biofilm matured, T was replaced with a mixture of B, T, E, and *p*-X to simulate exhaust gas for domestication purposes. The start-up of B-BF was considered complete when the removal rate exceeded 80%. The F&B-BF was built and launched based on the B-BF, utilizing the low pH of the MSM and antibiotics (Zhai, Jiang & Long, 2020). The start-up of F&B-BF was considered to be complete when the ratio of the 18S rRNA to 16S rRNA gene copy number (F/B) was greater than 0.27 (Veiga *et al.*, 1999). The B-BF and F&B-BF were operated at room temperature with an empty bed residence time (EBRT) of 120 s, 90 s and 60 s, respectively, by modifying the inflow of air. The MSM utilized in the B-BF was described by van Groenestijn & Liu (2002). It contained the following per liter of demineralized water: 4.5 g  $\text{KH}_2\text{PO}_4$ , 0.5 g  $\text{K}_2\text{HPO}_4$ , 2.0 g  $\text{NH}_4\text{Cl}$ , 1.0 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 2 mL of a solution of minerals and 2 mL of a vitamin solution. For the F&B-BF, the components of MSM were adjusted as follows: pH 5.9,  $\text{NO}_3^-$  substituting for  $\text{NH}_4^+$  as the nitrogen source, and the addition of  $20 \text{ g m}^{-3}$  chloramphenicol as antibiotics (Zhai, Jiang & Long, 2020). To maintain adequate nutrient and moisture contents within the filter bed, a liquid medium was periodically sprinkled at a rate of  $20 \text{ mL min}^{-1}$  (10 min), once every 3 d, from the top of the biofilter. The pH of the recirculated nutrient medium was maintained constant, at 7.0 for B-BF and 5.9 for F&B-BF using a  $0.1 \text{ mol L}^{-1}$  solution of sodium hydroxide or hydrochloric acid, depending on the experimental program. The pH was measured by means of a pH electrode (EASYFERM 120, Hamilton) attached to the nutrient collection tank and an on-line pH controller coupled to an electro valve (DO 9765T, Dual  $3\frac{1}{2}$  Digit pH redox indicator and regulator, Italy). Biofilter efficiency is influenced by the biomass attached to the packing inside the biofilters (Moussavi *et al.*, 2009). However, a high concentration of biomass does not guarantee superior removal efficiencies. Instead, it can lead to the rapid accumulation of excess biomass, an increase in pressure drop ( $\Delta P$ ), and ultimately clogging and poor removal performance (Kennes & Veiga, 2002; Ryu, Cho & Chung, 2010). To control the excessive biomass accumulation, weekly backwashing was performed with 1 L of  $20^\circ\text{C}$  water from the top of B-BF and F&B-BF for 1 h, respectively (Han, Wang & Liu, 2018).

### Analytical procedures

Gas samples collected from the inlet and outlet of the B-BF and F&B-BF were measured for individual gaseous BTE*p*-X concentrations using a gas chromatograph (GC) (2014, SHIMADZU, Japan) equipped with a Rtx-5 capillary column (30 m, 0.32 mm ID,  $0.5 \mu\text{m}$ ) and a flame ionization detector (FID), as previously described (Lee *et al.*, 2002). The injector, oven, and detector were set to temperatures of 210, 100, and  $250^\circ\text{C}$ , respectively. The concentration of carbon dioxide ( $\text{CO}_2$ ) was analyzed using a GC (9890A, Shanghai Linghua Instrument Co., Ltd., China), fitted with a methane conversion furnace, a TDX-01 packed column (0.7 m, 2 mm) and equipped with a FID. The injection and oven

temperatures were 350 °C and 90 °C, respectively, with the FID set at 250 °C (Zhai, Jiang & Long, 2020). The  $\Delta P$  across the height of the biofilter was periodically observed using a glass U-tube water manometer with an operating range of 0–40 cm. The removal performance of BTEp-X was compared between B-BF and F&B-BF using the following parameters: EBRTs, REs, and MRs. Each error bar represents the mean  $\pm$  SD.

$$\text{Empty bed residence time (s)} : EBRT = V/Q \times 3600 \quad (4)$$

$$\text{Removal efficiency(\%)} : RE = (C_{in} - C_{out})/C_{in} \times 100\% \quad (5)$$

$$\text{B mineralization rate(\%)} : MR_B = (C_{CO_2,out,B} - C_{airborne CO_2,in})/3.38C_{in,B} \times 100\% \quad (6)$$

$$\text{T mineralization rate(\%)} : MR_T = (C_{CO_2,out,T} - C_{airborne CO_2,in})/3.35C_{in,T} \times 100\% \quad (7)$$

$$\text{E mineralization rate(\%)} : MR_E = (C_{CO_2,out,E} - C_{airborne CO_2,in})/3.32C_{in,E} \times 100\% \quad (8)$$

$$\text{p-X mineralization rate(\%)} : MR_{p-X} = (C_{CO_2,out,p-X} - C_{airborne CO_2,in})/3.32C_{in,p-X} \times 100\% \quad (9)$$

where  $Q$  is the gas flow rate ( $\text{m}^3 \text{h}^{-1}$ ), and  $V$  is the effective volume of the filter bed ( $\text{m}^3$ ).  $C_{in}$  and  $C_{out}$  represent the inlet and outlet individual BTEp-X concentrations ( $\text{mg m}^{-3}$ ), respectively.  $C_{airborne CO_2,in}$  represents the inlet airborne  $\text{CO}_2$  concentrations ( $\text{mg m}^{-3}$ ), while  $C_{CO_2,out,B,T,E,p-X}$  represents the outlet  $\text{CO}_2$  concentrations ( $\text{mg m}^{-3}$ ) of individual BTEp-X, respectively. These concentrations were measured using a carbon dioxide isotope analyzer (CCIA-36-EP, LGR, USA).

### Qualitative analysis of biofilm

The relative biofilm hydrophobicity was determined according to the method described by Rosenberg, Gutnick & Rosenberg (1980), Xie et al. (2019) and Mu et al. (2020). In brief, one mL of bacteria ( $OD_{400} = 0.6$ ) was placed into glass tubes and 250  $\mu\text{L}$  of *n*-hexadecane (Macklin, H810865) was added. The  $OD_{400}$  of the aqueous phase was measured before and after extraction with *n*-hexadecane. The relative biofilm hydrophobicity can be calculated using Eq. (10):

$$\text{The relative biofilm hydrophobicity(\%)} : H = (OD_0 - OD)/OD_0 \times 100. \quad (10)$$

Where  $OD_0$  and  $OD$  are the  $OD_{400}$  before and after extraction with *n*-hexadecane, respectively. The experiments were conducted with three independent cultures per condition. Biofilm extracellular polymeric substances (EPS) were extracted accurately and analyzed promptly using heat extraction as follows: heating for 10 min at 80 °C (1 bar), two ultracentrifugations 4 °C (20000G, 20 min and 10000G, 15 min), purification with a 3500D dialysis membrane, 4 °C, 24 h, as described by Comte, Guibaud & Baudu (2006). The EPS samples were freeze-dried immediately after extraction and stored at  $-18$  °C until use. The zeta potential was roughly calculated using the Helmholtz-Smoluchowski method based on electrophoretic mobility measurements obtained from a Doppler electrophoretic light scattering analyzer (Zetamaster, Malvern Instruments, Malvern, UK) (Hiementz, 1986).

## Microbial analysis

Biofilms were sampled from the middle layer of the B-BF and F&B-BF at designed intervals on days 112, 140 and 168. As detailed in a prior study, sample DNA extraction, quantitative real-time polymerase chain reaction (qRT-PCR) for bacteria and fungi, Illumina high-throughput sequencing, and bioinformatics analysis were carried out using the method described by *Zhai et al. (2017)*.

## RESULTS AND DISCUSSION

### VOC determination

Based on the *in situ* measurements, B, T, E, *p*-X were selected as model VOCs for moderately hydrophobic chemical emissions commonly found in a typical petrochemical industry WWTP. The main VOC components and concentrations are shown in [Table 1](#).

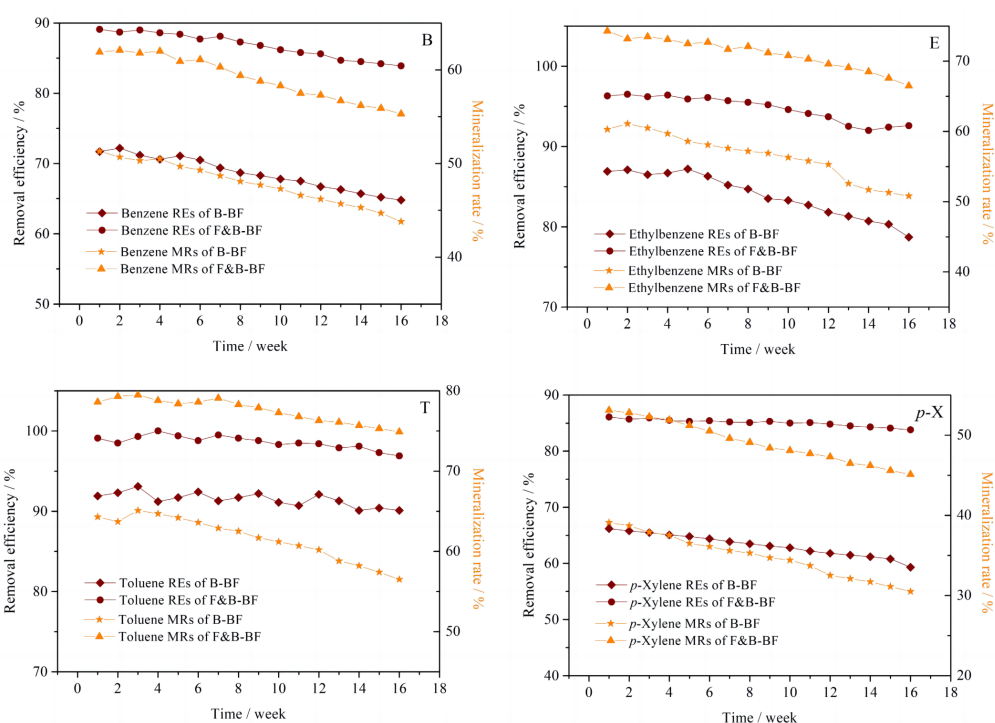
### Removal performance comparison

After the successful start-up, the BTE*p*-X REs and MRs of the B-BF and F&B-BF were compared over the following 112 days at an EBRT of 90 s. During the 112-day operation period, the influent concentrations of BTE*p*-X were as follows:  $B = 35.8 \pm 2.3 \text{ mg m}^{-3}$ ,  $T = 22.7 \pm 1.5 \text{ mg m}^{-3}$ ,  $E = 9.5 \pm 0.7 \text{ mg m}^{-3}$ , and  $p\text{-X} = 14.4 \pm 1.2 \text{ mg m}^{-3}$  for the B-BF and F&B-BF, respectively. According to *Kim & Deshusses (2005)*, the REs decrease as the Henry's law constants increase. The Henry's law constants for B, T, *p*-X, and E at 25 °C are 0.557, 0.673, 0.699 and 0.798, respectively, which were obtained from the SRC Phys Prop Database (<http://esc.syrrees.com>) (*Sieg, Fries & Puttmann, 2008*). Therefore, it can be assumed that the REs for B are the highest, followed by T, with *p*-X and E having the lowest. However, the data presented in [Fig. 2](#) shows that the B-BF removed individual VOCs with the following efficiency over the tested concentration range: T, 90.1–93.1%; E, 78.7–87.2%; B, 64.8–72.2%; and *p*-X, 59.3–66.2%. It is important to note that B-BFs are only effective for removing VOCs with low and medium Henry's law constants due to mass transfer limitations (*Deshusses & Johnson, 2000; Zhu et al., 2004*). Additionally, previous studies have shown that B and *p*-X are more resistant to removal than T and E (*Hassan & Sorial, 2009; Garcíá-Pea et al., 2008*). The F&B-BF showed RE<sub>T,E</sub> values of over 95% and 90%, respectively, compared to the REs of B and *p*-X, which were between 80% and 90% ([Fig. 2](#)). Its RE<sub>T,E,B,p-X</sub> was superior to that of the B-BF. The production of CO<sub>2</sub> is a crucial characteristic for assessing the biodegradation rates of pollutants, as it can reveal critical details about the extent of VOC mineralization. Similarly, for the F&B-BF, the MRs of individual BTE*p*-X outperformed those of the B-BF. These results were obviously higher than those previously reported in the literature (*Sun et al., 2020; Ghasemi et al., 2020*), indicating improved performance. Although compounds with higher Henry's law constants generally have lower removal rates, the BTE*p*-X were efficiently degraded and mineralized by the F&B-BF under the experimental operating conditions. The presence of fungi in the F&B-BF allows for direct capture of BTE*p*-X from the gas phase to biofilms, resulting in higher partitioning of pollutants to biofilms than the B-BF.

Cell surface hydrophobicity (CSH) is a crucial factor that affects microbial adsorption and degradation (*Yuan et al., 2018*). Strains with high hydrophobicity can enhance the

**Table 1** Main components and concentration of VOCs ( $\text{mg m}^{-3}$ ).

Wastewater treatment unit	Benzene (B)	Toluene (T)	Ethylbenzene (E)	<i>para</i> -Xylene ( <i>p</i> -X)
Pretreatment unit (Grill well-Catchment tank-Regulating tank-Oil-water separator-Cavitation air flotation-Dissolved air flotation)	564.2	259.4	30.1	142.2
Biochemical treatment unit (Anaerobic-aerobic process)	323.3	63.2	4.8	36.7
Sludge disposal unit (Surplus sludge dewatering and drying process)	60.2	5.7	19.8	82.3
Negative pressure gas collector	35.8	22.7	9.5	14.4

**Figure 2** Comparison of the performance in BTE*p*-X removal between the B-BF and F&B-BF.Full-size DOI: [10.7717/peerj.17452/fig-2](https://doi.org/10.7717/peerj.17452/fig-2)

degradation of hydrophobic VOCs (Zhang *et al.*, 2010). Fungi generally have higher CSH than bacteria (Zhang *et al.*, 2019), which is why the fungal biofilter (F-BF) outperformed the B-BF in removing hydrophobic VOCs removal (Cheng *et al.*, 2016b). Analysis revealed that after 4 months of stable operation, the relative hydrophobicities of the biofilms in the B-BF and F&B-BF were 21–35% and 76–89%, respectively. This difference in hydrophobicity was one of the reasons why the F&B-BF outperformed the B-BF in BTE*p*-X removal.

Chen *et al.* (2017) discovered that a higher negative zeta potential of EPS is more beneficial for generating migration potential and increasing the probability of collision between the pollutant molecules and degrading strains. The analysis results show that



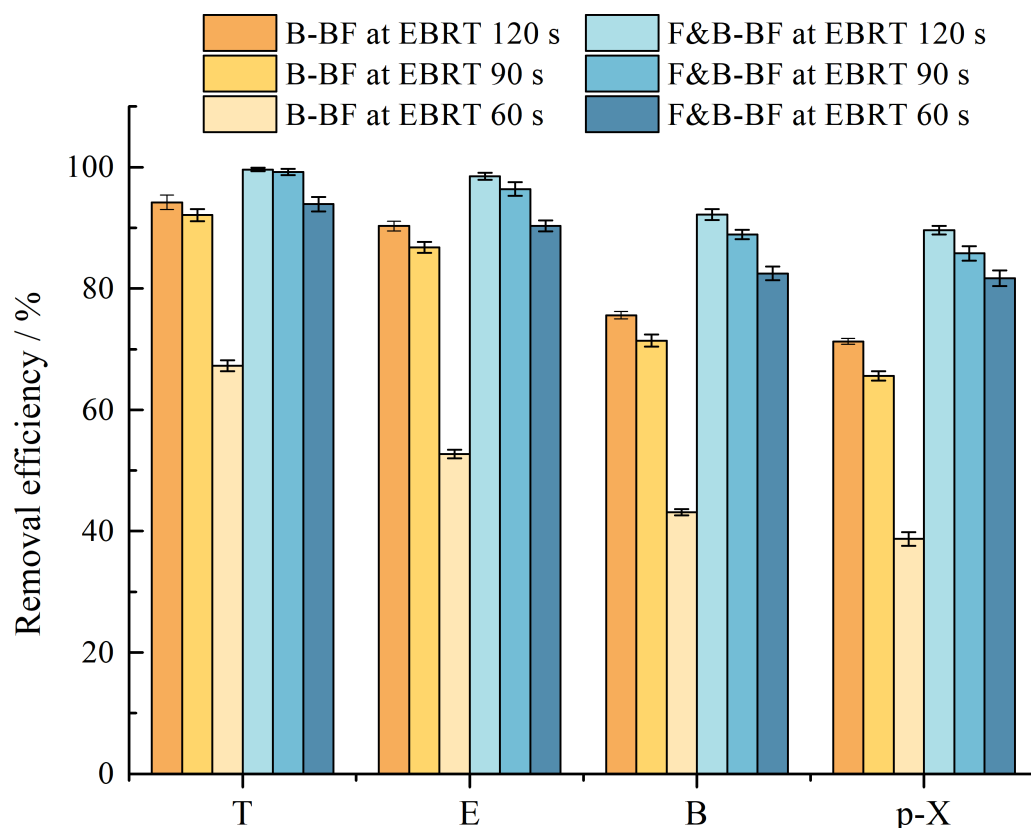
the zeta potential of the biofilm EPS in the B-BF changed from  $-13.7$  mV to  $-9.5$  mV. However, the zeta potential of the biofilm EPS in the F&B-BF showed a more significant slow-growth trend ( $-18.3$  mV to  $-16.1$  mV) following a four-month load operation test. Based on the experimental results, it is evident that the negative zeta potential of the biofilm EPS in the F&B-BF was higher than in the B-BF. The results indicate that the BTE $p$ -X are more likely to come into contact with degrading strains in the F&B-BF than in the B-BF. This is why the BTE $p$ -X removal performance of the F&B-BF was superior to that of the B-BF. These results further elucidated the excellent VOC purification performance of the fungal/bacterial coexistence system from the point of view of physicochemical cell surface properties.

### EBRTs comparison

The removal performances of BTE $p$ -X were compared between the B-BF and the F&B-BF at three different EBRTs, *i.e.*, 120 s, 90 s and 60 s, respectively, with stable operation for 3 weeks. [Figure 3](#) shows a reduction in the REs and MRs of the B-BF and the F&B-BF after the EBRT changed from 120 s to 60 s. For the B-BF, a sudden decrease in T, E, B, and  $p$ -X REs from approximately 94.2% to 67.3%, 90.3% to 52.7%, 75.6% to 43.1%, and 71.3% to 38.7%, respectively, was observed. Insufficient contact time between the gas-phase pollutants and the biofilm was the main reason for the low RE<sub>T,E,B</sub> and  $p$ -X values. Substrate toxicity, competitive inhibition, and the formation of toxic intermediates by nonspecific enzymes may have also been responsible for the poor results ([Bielefeldt & Stensel, 1999](#)). Comparatively, a gradual decrease in the RE<sub>T,E,B</sub> and  $p$ -X values of the F&B-BF was observed when the EBRT was reduced from 120 s to 60 s. Specifically, the values decreased from 99.6% to 93.9%, 98.5% to 90.3%, 92.2% to 82.5%, and 89.6% to 81.7%, respectively. Fungal-bacterial consortia were found to exhibit synergistic activity due to the large hyphal surfaces and greater number of mesopores in the fungal strains, which enhance the hydrophobic VOCs capture capacity of the F&B-BF ([Cheng et al., 2017](#); [Zhu et al., 2017](#)). It is probable that the adsorption of BTE $p$ -X by fungal mycelium increased the contact time between BTE $p$ -X and their degrading strains to some extent. This helped to maintain a higher removal performance of the F&B-BF compared to the B-BF at a relatively short EBRT. In addition, the BTE $p$ -X degrading bacteria might be uniformly distributed within the biofilms because of “Fungal highways”. This reduced the distance between the bacteria and their substrates, resulting in an increased the contact frequency between the biocatalysts and their hydrophobic substrates ([Kohlmeier et al., 2005](#)). Furthermore, fungi might rapidly consume intermediate metabolites that accumulate and inhibit bacterial growth. This resulted in higher REs and MRs for BTE $p$ -X in a shorter EBRT ([Cheng et al., 2017](#)). In summary, the optimal EBRT for engineering applications was determined to be 90 s.

### Long-term stability comparison

The long-term stability is very important for practical application of bioreactors. [Figure 4](#) displays the removal performance of the B-BF and F&B-BF during the period from Day 113 to 168. The F&B-BF proved to be more stable than the B-BF when run at the same inlet



**Figure 3** Comparison of the effect of different EBRTs in BTE<sub>p-X</sub> removal between the B-BF and F&B-BF.

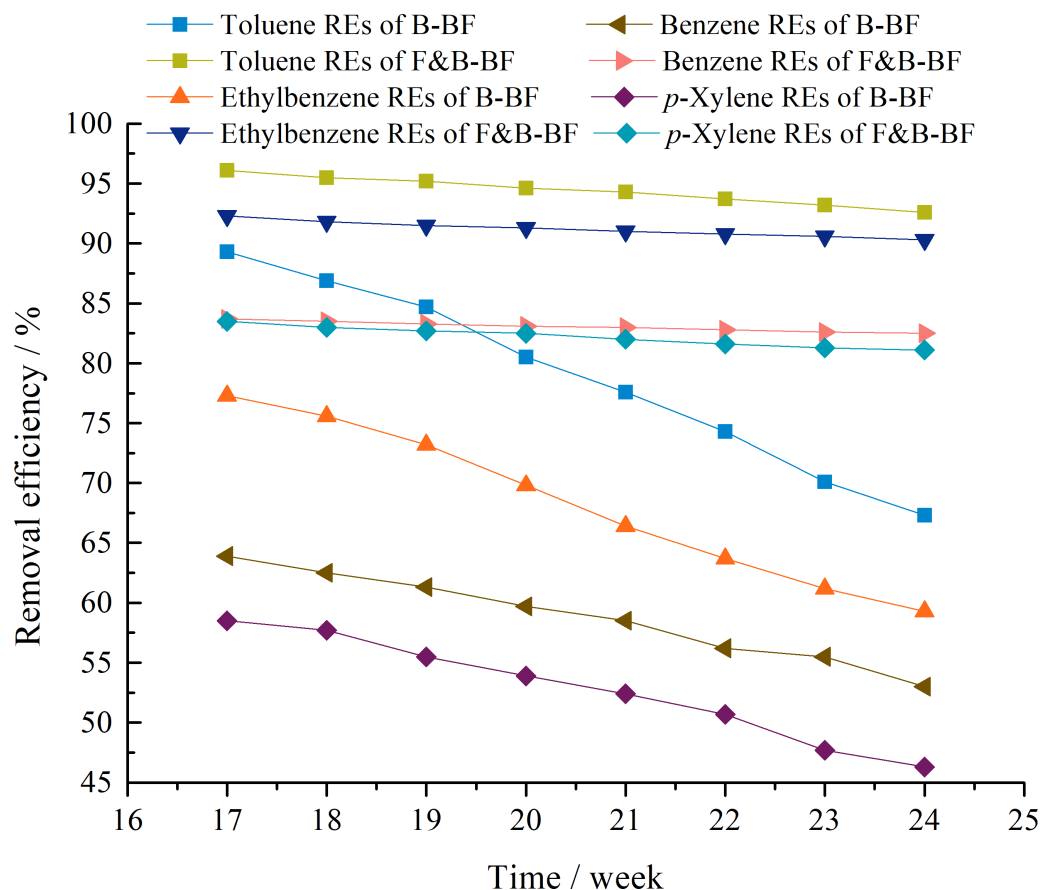
Full-size DOI: [10.7717/peerj.17452/fig-3](https://doi.org/10.7717/peerj.17452/fig-3)

BTE<sub>p-X</sub> concentrations with an EBRT of 90 s. During Days 113–168 of the F&B-BF, the RE<sub>T,E</sub> values exceeded 90%, and the RE<sub>B,p-X</sub> values were above 80%, whereas they were approximately 59–67% and 46–53% for the B-BF. These results suggest that the long-term operation of F&B-BF in BTE<sub>p-X</sub> removal was more stable than that of the B-BF.

In general, the pressure drop is an important parameter of long-term stability that can be used to indirectly characterise biomass formation, packing cracking and gas flow channelling in biofiltration systems. As shown in Fig. 5, the pressure drop of the F&B-BF increased slowly from 50 Pa to 69 Pa, while for the B-BF,  $\Delta p$  changed more significantly from 83 Pa to 118 Pa. This indicates that less carbon was converted into biofilm in the F&B-BF and more carbon was oxidized to CO<sub>2</sub> in BTE<sub>p-X</sub> removal than in the B-BF. Another reason for this is the lower growth rate of fungi (Cheng et al., 2017). Therefore, it can be inferred that the F&B-BF runs more stably and longer in BTE<sub>p-X</sub> removal than the B-BF.

### Recovery period comparison

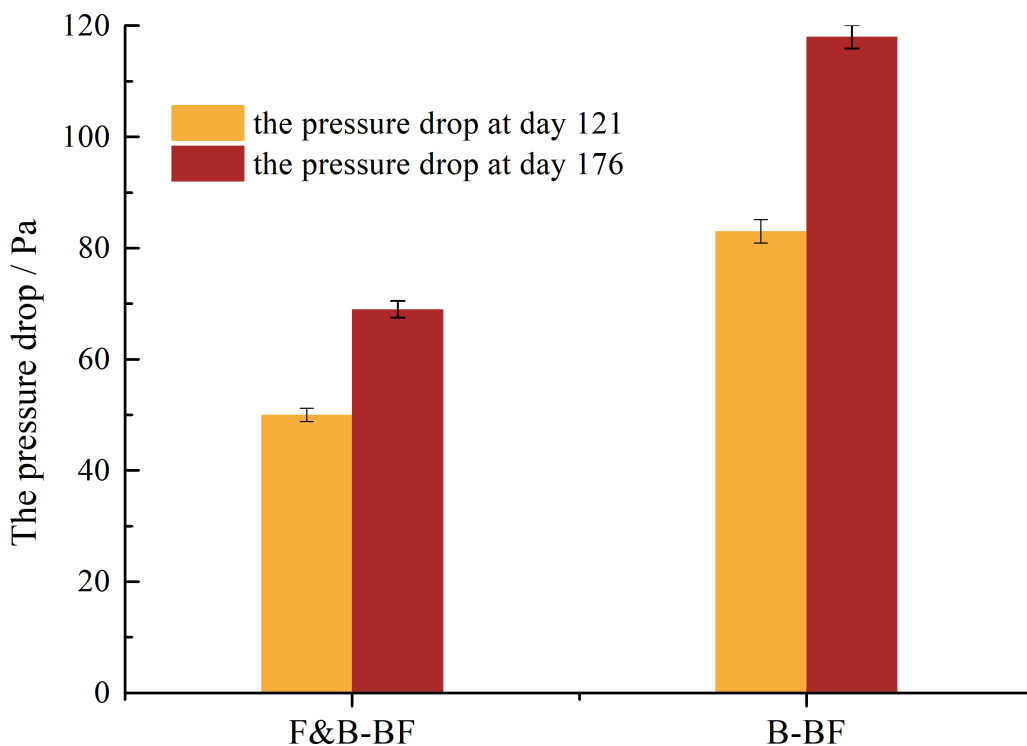
In real-world scenarios, biofiltration systems often experience periods of starvation during holidays, festivals, unexpected accidents, and maintenance closures. The ability of biofiltration systems to recover quickly is crucial for their employment in industrial



**Figure 4** Comparison of the stability of long-term operation in BTE $p$ -X removal between the B-BF and F&B-BF.

Full-size DOI: [10.7717/peerj.17452/fig-4](https://doi.org/10.7717/peerj.17452/fig-4)

settings. To compare the robustness of the systems, the effect of pollutant starvation was investigated on Day 169. After 3 days of starvation, the inlet BTE $p$ -X concentrations were restored to their previous values. For the F&B-BF, the REs were 98.5% for T, 95.7% for E, 88.1% for B and 85.4% for  $p$ -X after 2 days, which then recovered to normal levels. However, the B-BF took 5 days to reach the values of RE $_T$ -93.3%, RE $_E$ -83.7%, RE $_B$ -70.8% and RE $_{p-X}$ -64.9%, respectively (Fig. 6). The results indicate that the F&B-BF can restore the removal performance more quickly than the B-BF after a period of interruption of gaseous BTE $p$ -X supply. This is attributed to the fact that the fungi release the adsorbed BTE $p$ -X and continuously supply carbon resources for their degrading strains in the F&B-BF (Zhai, Jiang & Long, 2020). During a supply interruption of gaseous BTE $p$ -X, the F&B-BF can maintain a certain number and activity of the BTE $p$ -X degrading strains. However, when the supply of BTE $p$ -X was interrupted for the B-BF, the microorganisms had to undergo endogenous respiration due to insufficient carbon sources. The prolonged interruption time resulted in a significant decrease in the absolute number of the BTE $p$ -X degrading bacteria, from  $3.81 \times 10^7$  copies  $\mu\text{L}^{-1}$  to  $6.29 \times 10^5$  copies  $\mu\text{L}^{-1}$ . As a result, the removal performance of the B-BF in BTE $p$ -X removal recovered more slowly than the F&B-BF.



**Figure 5** Comparison of the change of  $\Delta p$  in long-term operation between the B-BF and F&B-BF.

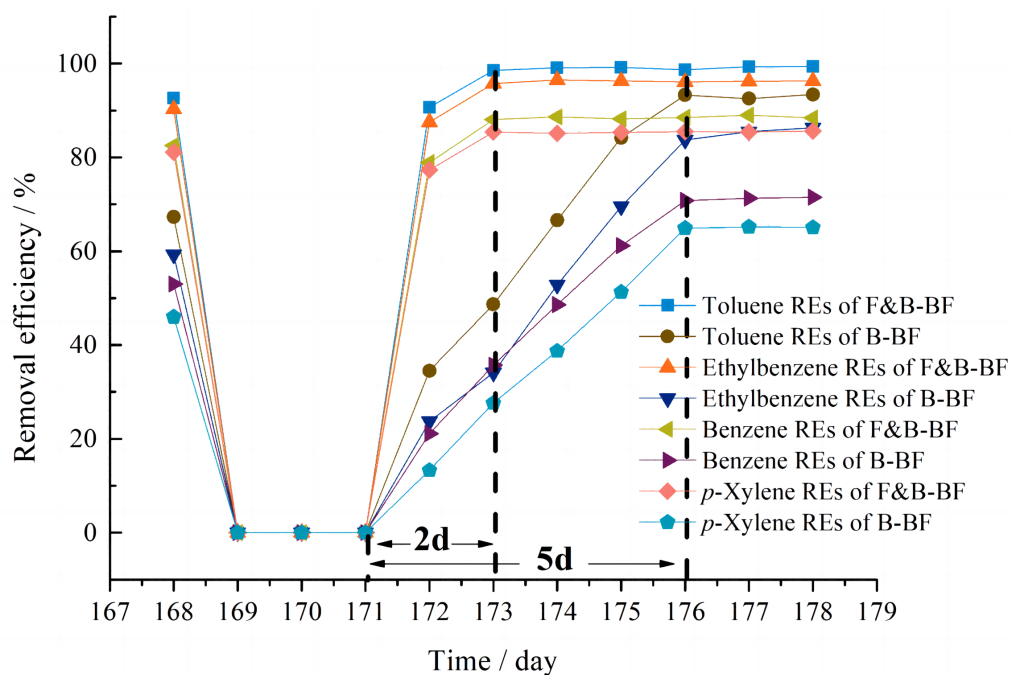
Full-size [DOI: 10.7717/peerj.17452/fig-5](https://doi.org/10.7717/peerj.17452/fig-5)

Additionally, the 3-day interruption resulted in a slight decrease in the pressure drop of the B-BF and the F&B-BF. This suggests that the starvation protocol could be an alternative measure for controlling the pressure drop and maintaining long-term stability.

### Microbial community diversity comparison

Biofilms, consisting of bacteria, fungi, actinomycetes, and algae, play a crucial role in breaking down pollutants in biofiltration systems. Typically, a B-BF contains  $10^6$ – $10^{10}$  cfu of bacteria and actinomycetes, and  $10^3$ – $10^6$  cfu of fungi per gram of bed (Ottengraf, 1987). Degrading microbes usually account for 1–15% of the total microbial population in a B-BF (Pedersen et al., 1997; Delhomenie et al., 2001). The total bacterial and fungal counts in the samples were analyzed using qRT-PCR. The logarithmic value of the gene copy number of the samples collected at different steady-state stages (Days 112, 140 and 168) from the B-BF and F&B-BF was compared. The average copy number of 16S rRNA genes (bacteria) was  $4.16 \times 10^7$  copies  $\mu\text{L}^{-1}$  vs.  $3.87 \times 10^6$  copies  $\mu\text{L}^{-1}$ , and the 18S rRNA genes (fungi) was  $3.72 \times 10^5$  copies  $\mu\text{L}^{-1}$  vs.  $7.13 \times 10^6$  copies  $\mu\text{L}^{-1}$  for the B-BF and F&B-BF, respectively. Thus, F/B ranged from 0.0089 to 1.84. These data suggest that the BTEp-X removal performance increased with the F/B in the biofilms.

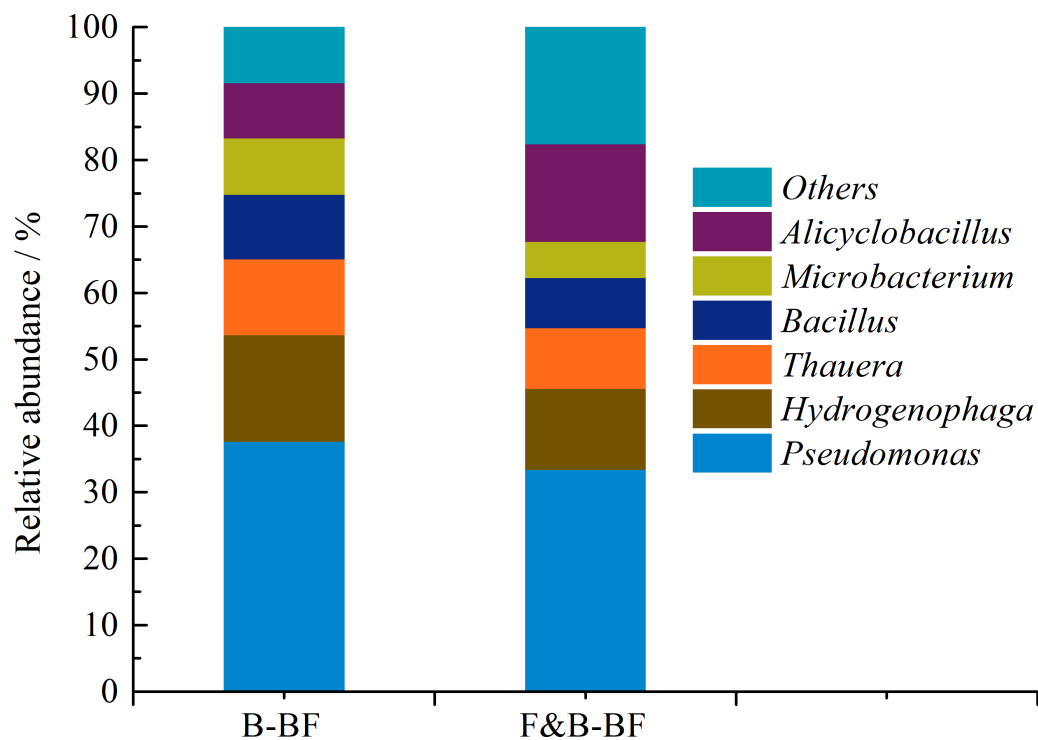
The structure of the microbial community, as well as the number of microbes, can significantly affect the operational performance of biofiltration systems (Guo et al., 2015). The characterisation of microbial diversity can provide insights into how the coexistence of fungi and bacteria enhances BTEp-X removal, aiding in the optimization of the design



**Figure 6** Comparison of the performance recovery cycle in BTEp-X removal between the B-BF and F&B-BF.

Full-size DOI: [10.7717/peerj.17452/fig-6](https://doi.org/10.7717/peerj.17452/fig-6)

and operation of biofiltration techniques. To identify and compare the bacteria and fungi present in the biofilm on the surface of packing materials, Illumina high-throughput sequencing was utilized to investigate the microbial community diversity. As shown in Fig. 7, the major bacterial genus in the B-BF was *Pseudomonas* (37.6%), followed by *Hydrogenophaga* (16.1%), *Thauera* (11.4%), *Bacillus* (9.7%), *Microbacterium* (8.5%), and *Alicyclobacillus* (8.3%). Similarly, the bacterial community in the F&B-BF was mainly composed of *Pseudomonas* (33.4%), followed by *Hydrogenophaga* (12.2%), *Thauera* (9.1%), *Bacillus* (7.6%), *Microbacterium* (5.4%), and *Alicyclobacillus* (14.7%). There are differences in the relative abundance of bacterial genera between the B-BF and F&B-BF. The ability of the genus *Pseudomonas* to degrade BTEX has been reported (Attaway & Schmidt, 2002; Haque, De Visscher & Sen, 2012). Fahy et al. (2008) reported that strains of *Hydrogenophaga* and *Pseudomonas* can metabolize B, T, and p-X. Lin, Van Verseveld & Röling (2002) reported that members of the *Thauera* cluster can degrade BTEX in a denitrifying environment. *Bacillus sphaericus* can degrade BTEX at a faster rate and can be used to treat highly polluted air streams in biofilters (Kumar & Chandrajit, 2011). In addition, the bacterium *Microbacterium* has been reported to be nonpathogenic, rhizosphere-based, and capable of degrading BTEX and naphthalene, with a high level of tolerance for several hydrocarbons (Wongbunmak et al., 2017). Furthermore, the genus *Alicyclobacillus* has the ability to degrade phenol (Aston et al., 2016), which is a significant degradation byproduct of T, B and E (Cheng et al., 2016b; Olsen, Kukor & Kaphammer, 1994). The relative abundance of the genus *Alicyclobacillus* increased from 8.3% in the

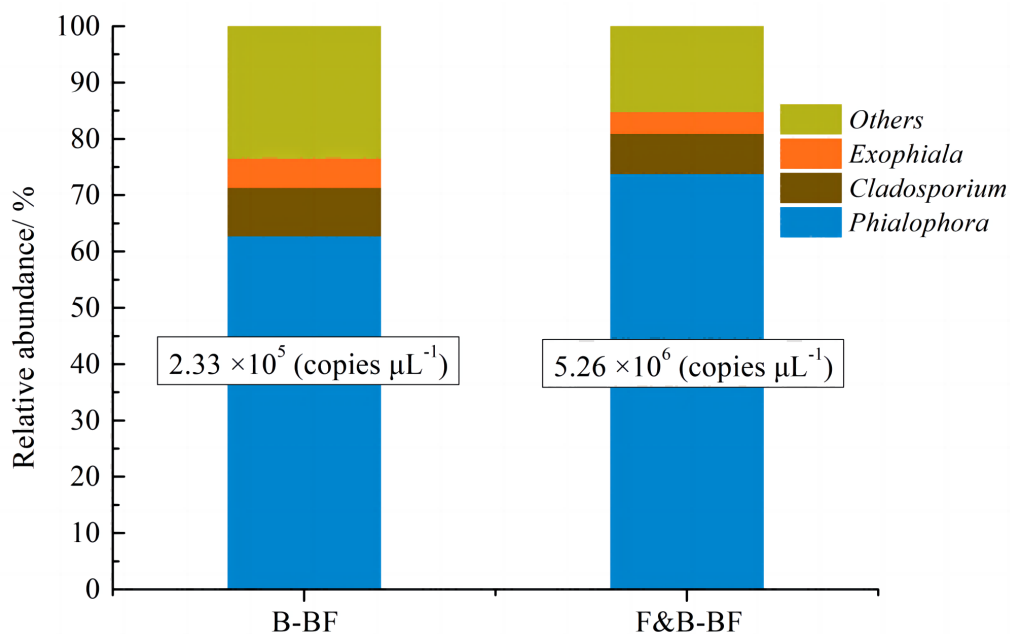


**Figure 7** Comparison of the bacterial community structure and relative abundance at the genus level between the B-BF and F&B-BF.

Full-size DOI: [10.7717/peerj.17452/fig-7](https://doi.org/10.7717/peerj.17452/fig-7)

B-BF to 14.7% in the F&B-BF. This suggests that the intermediates of BTEp-X were more easily degraded furtherly by the F&B-BF than by the B-BF, which may explain why the BTEp-X MRs of the F&B-BF were significantly higher than those of the B-BF.

The results of high-throughput sequencing indicate that the fungal community is primarily composed of *Phialophora*, *Cladosporium* and *Exophiala*. Among these, *Phialophora* is the dominant fungus in the B-BF and F&B-BFs. As shown in Fig. 8, the relative abundance of *Phialophora* increased from 62.7% in the B-BF to 73.8% in the F&B-BF, and the absolute amount increased significantly from  $2.33 \times 10^5$  copies  $\mu\text{L}^{-1}$  (B-BF) to  $5.26 \times 10^6$  copies  $\mu\text{L}^{-1}$  (F&B-BF). The genus *Phialophora* belongs to the phylum *Ascomycota*, which is one of the few groups of fungi capable of transforming a wide range of organic pollutants (Harms, Schlosser & Wick, 2011). It is suggested that the genus *Phialophora* possesses a high capacity to directly transform BTEp-X from the gas phase to the biofilm. Additionally, it has been reported that this genus is capable of degrading T and other volatile aromatic compounds (Isola et al., 2013). It has a combined ability to adsorb and degrade BTEp-X. *Cladosporium* and *Exophiala* can also readily utilize B, E, T, and styrene as sole carbon and energy sources (Qi, Moe & Kinney, 2002). The study found a positive correlation between the removal performance of BTEp-X and the fungal quantity of biofilms in biofilters.



**Figure 8** Comparison of the fungal community structure and relative abundance at the genus level between the B-BF and F&B-BF.

Full-size DOI: [10.7717/peerj.17452/fig-8](https://doi.org/10.7717/peerj.17452/fig-8)

## CONCLUSION

The F&B-BF outperformed the B-BF in terms of BTE $p$ -X removal. The study also yielded the following specific observations in addition to the aforementioned general conclusion.

1. Under experimental conditions, the F&B-BF achieved RE $T,E,B$  and  $p$ -X values of 98.7%, 96.3%, 87.6% and 85.3%, respectively, at an EBRT of 90 s.
2. In the F&B-BF and the B-BF, T is the most biodegradable pollutant, followed by E, B and then  $p$ -X.
3. By comparison with the B-BF, the F&B-BF performed more efficiently in BTE $p$ -X removal due to the fungal adsorption, the higher hydrophobicity and negative potential of the biofilms
4. The F&B-BF recovered faster, lasted longer, and operated with greater reliability than the B-BF in BTE $p$ -X removal.
5. The fungal genus *Phialophora* contributed significantly to the mass transfer from the gas phase to the biofilm and the degradation of the BTE $p$ -X. Meanwhile, the bacterial genus *Pseudomonas* and *Alicyclobacillus* were primarily responsible for the degradation of BTE $p$ -X and its intermediate metabolites, such as phenol.

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### Competing Interests

The authors declare there are no competing interests.

### Author Contributions

- Hai Wang performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Xiaojuan Xue analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Xujun Nan analyzed the data, prepared figures and/or tables, and approved the final draft.
- Jian Zhai conceived and designed the experiments, authored or reviewed drafts of the article, and approved the final draft.

### Data Availability

The following information was supplied regarding data availability:

The raw data is available in the [Supplementary Files](#).

### Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.17452#supplemental-information>.

## REFERENCES

- Aston JE, Apel WA, Lee BD, Thompson DN, Lacey JA, Newby DT, Reed DW, Thompson VS. 2016. Degradation of phenolic compounds by the lignocellulose deconstructing thermoacidophilic bacterium *Alicyclobacillus Acidocaldarius*. *Journal of Industrial Microbiology and Biotechnology* **43**:13–23 DOI [10.1007/s10295-015-1700-z](https://doi.org/10.1007/s10295-015-1700-z).
- Attaway HH, Schmidt MG. 2002. Tandem biodegradation of BTEX components by two *Pseudomonas* sp. *Current Microbiology* **45**(1):30–36 DOI [10.1007/s00284-001-0053-1](https://doi.org/10.1007/s00284-001-0053-1).



- Bharadwaj DP, Lundquist PO, Alström S. 2008.** Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens. *Soil Biology & Biochemistry* **40**(10):2494–2501  
DOI [10.1016/j.soilbio.2008.06.012](https://doi.org/10.1016/j.soilbio.2008.06.012).
- Bielefeldt AR, Stensel HD. 1999.** Modeling competitive inhibition effects during biodegradation of BTEX mixtures. *Water Research* **33**:707–714  
DOI [10.1016/S0043-1354\(98\)00256-5](https://doi.org/10.1016/S0043-1354(98)00256-5).
- Boer WD, Folman LB, Summerbell RC, Boddy L. 2005.** Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**:795–811 DOI [10.1016/j.femsre.2004.11.005](https://doi.org/10.1016/j.femsre.2004.11.005).
- Chen DZ, Jiang NX, Ye JX, Cheng ZW, Zhang SH, Chen JM. 2017.** Comparative investigation on a hexane-degrading strain with different cell surface hydrophobicities mediated by starch and chitosan. *Applied Microbiology and Biotechnology* **101**(9):3829–3837 DOI [10.1007/s00253-017-8100-4](https://doi.org/10.1007/s00253-017-8100-4).
- Cheng Y, He HJ, Yang CP, Zeng GM, Li X, Chen H, Yu GL. 2016a.** Challenges and solutions for biofiltration of hydrophobic volatile organic compounds. *Biotechnology Advances* **34**(6):1091–1102 DOI [10.1016/j.biotechadv.2016.06.007](https://doi.org/10.1016/j.biotechadv.2016.06.007).
- Cheng Z, Li C, Kennes C, Ye J, Chen D, Zhang S, Chen J, Yu J. 2017.** Improved biodegradation potential of chlorobenzene by a mixed fungal-bacterial consortium. *International Biodeterioration & Biodegradation* **123**:276–285 DOI [10.1016/j.ibiod.2017.07.008](https://doi.org/10.1016/j.ibiod.2017.07.008).
- Cheng ZW, Lu LC, Kennes C, Yu JM, Chen JM. 2016b.** Treatment of gaseous toluene in three biofilters inoculated with fungi/bacteria: microbial analysis, performance and starvation response. *Journal of Hazardous Materials* **303**:83–93  
DOI [10.1016/j.jhazmat.2015.10.017](https://doi.org/10.1016/j.jhazmat.2015.10.017).
- Cho E, Galera MM, Lorenzana A, Chung WJ. 2009.** Ethylbenzene, o-xylene, and BTEX removal by *Sphingomonas* sp. D3K1 in rock wool-compost biofilters. *Environmental Engineering Science* **26**(1):45–52 DOI [10.1089/ees.2007.0144](https://doi.org/10.1089/ees.2007.0144).
- Comte S, Guibaud G, Baudu M. 2006.** Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties. Part I. Comparison of the efficiency of eight EPS extraction methods. *Enzyme and Microbial Technology* **38**(1-2):237–245 DOI [10.1016/j.enzmictec.2005.06.023](https://doi.org/10.1016/j.enzmictec.2005.06.023).
- Delhomenie MC, Bibeau L, Bredin N, Roy S, Broussau S, Brzezinski R, Kugelmass JL, Heitz M. 2002.** Biofiltration of air contaminated with toluene on a compost-based bed. *Advances in Environmental Research* **6**(3):239–254  
DOI [10.1016/S1093-0191\(01\)00055-7](https://doi.org/10.1016/S1093-0191(01)00055-7).
- Delhomenie MC, Bibeau L, Roy S, Brzezinski R, Heitz M. 2001.** Influence of nitrogen on the degradation of toluene in a compost-based biofilter. *Journal of Chemical Technology & Biotechnology* **76**(9):997–1006 DOI [10.1002/jctb.472](https://doi.org/10.1002/jctb.472).
- Deshusses MA, Johnson CT. 2000.** Development and validation of a simple protocol to rapidly determine the performance of biofilters for VOC treatment. *Environmental Science and Technology* **34**(3):461–467 DOI [10.1021/es9909172](https://doi.org/10.1021/es9909172).
- Deveau A, Brulé C, Palin B, Champmartin D, Rubini P, Garbaye J, Sarniguet A, Frey-Klett P. 2010.** Role of fungal trehalose and bacterial thiamine in the improved

- survival and growth of the ectomycorrhizal fungus *Laccaria bicolor* S238N and the helper bacterium *Pseudomonas fluorescens* BBc6R8. *Environmental Microbiology Reports* 2(4):560–568 DOI 10.1111/j.1758-2229.2010.00145.x.
- Devanny JS, Deshusses MA, Webster TS. 2017.** *Biofiltration for air pollution control*. Boca Raton: CRC Press DOI 10.1201/9781315138275.
- Dorado AD, Rodriguez G, Ribera G, Bonsfills A, Gabriel D, Lafuente J, Gamisans X. 2009.** Evaluation of mass transfer coefficients in biotrickling filters: experimental determination and comparison to correlations. *Chemical Engineering & Technology* 32(12):1941–1950 DOI 10.1002/ceat.200900275.
- Duponnois R, Garbaye J. 1990.** Some mechanisms involved in growth stimulation of ectomycorrhizal fungi by bacteria. *Canadian Journal of Botany* 68(10):2148–2152 DOI 10.1139/b90-280.
- Dwivedi P, Gaur V, Sharma A, Verma N. 2004.** Comparative study of removal of volatile organic compounds by cryogenic condensation and adsorption by activated carbon fiber. *Separation and Purification Technology* 39(1-2):23–37 DOI 10.1016/j.seppur.2003.12.016.
- Fahy A, Ball AS, Lethbridge G, Timmis KN, Mcgenity TJ. 2008.** Isolation of alkali-tolerant benzene-degrading bacteria from a contaminated aquifer. *Letters In Applied Microbiology* 47(1):60–66 DOI 10.1111/j.1472-765X.2008.02386.x.
- García-Pea I, Ortiz I, Hernández S, Revah S. 2008.** Biofiltration of BTEX by the fungus *Paecilomyces variotii*. *International Biodeterioration & Biodegradation* 62(4):442–447 DOI 10.1016/j.ibiod.2008.03.012.
- Ghasemi R, Golbabaee F, Rezaei S, Pourmand MR, Masoorian E. 2020.** A comparison of biofiltration performance based on bacteria and fungi for treating toluene vapors from airflow. *AMB Express* 10(1):8 DOI 10.1186/s13568-019-0941-z.
- Guo XC, Miao Y, Wu B, Ye L, Yu HY, Liu S, Zhang XX. 2015.** Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics. *Bioresource Technology* 197:99–105 DOI 10.1016/j.biortech.2015.08.049.
- Han MF, Wang C, Liu H. 2018.** Comparison of physical technologies for biomass control in biofilters treating gaseous toluene. *Journal of the Air & Waste Management Association* 68(10):1118–1125 DOI 10.1080/10962247.2018.1469556.
- Haque F, De Visscher A, Sen A. 2012.** Biofiltration for BTEX removal. *Critical Reviews in Environmental Science and Technology* 42(24):2648–2692 DOI 10.1080/10643389.2011.592764.
- Harms H, Schlosser D, Wick LY. 2011.** Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews Microbiology* 9(3):177–192 DOI 10.1038/nrmicro2519.
- Hassan AA, Sorial G. 2009.** Biological treatment of benzene in a controlled trickle bed air biofilter. *Chemosphere* 75(10):1315–1321 DOI 10.1016/j.chemosphere.2009.03.008.
- Hernández M, Quijano G, Muñoz R, Bordel S. 2011.** Modeling of VOC mass transfer in two-liquid phase stirred tank, biotrickling filter and airlift reactors. *Chemical Engineering Journal* 172(2–3):961–969 DOI 10.1016/j.ccej.2011.07.008.

- Hiementz PC. 1986.** *Principles of colloid and surface chemistry*. New York: Marcel Dekker Inc.
- Hu XR, Han MF, Wang C, Yang NY, Deng JG. 2020.** A short review of bioaerosol emissions from gas bioreactors: health threats, influencing factors and control technologies. *Chemosphere* **253**:126737 DOI [10.1016/j.chemosphere.2020.126737](https://doi.org/10.1016/j.chemosphere.2020.126737).
- Isola D, Selbmann L, Hoog GSD, Fenice M, Onofri S, Prenafeta-Boldú FX, Zucconi L. 2013.** Isolation and screening of black fungi as degraders of volatile aromatic hydrocarbons. *Mycopathologia* **175**(5–6):369–379 DOI [10.1007/s11046-013-9635-2](https://doi.org/10.1007/s11046-013-9635-2).
- Kennes C, Veiga MC. 2002.** Inert filter media for the biofiltration of waste gases—characteristics and biomass control. *Reviews in Environmental Science and Biotechnology* **1**(3):201–214 DOI [10.1023/A:1021240500817](https://doi.org/10.1023/A:1021240500817).
- Kim S, Deshusses MA. 2005.** Understanding the limits of H<sub>2</sub>S degrading biotrickling filters using a differential biotrickling filter. *Chemical Engineering Journal* **113**(2–3):119–126 DOI [10.1016/j.cej.2005.05.001](https://doi.org/10.1016/j.cej.2005.05.001).
- Kohlmeier S, Smits THM, Ford R, Keel C, Harms H, Wick LY. 2005.** Taking the fungal highway: mobilization of pollutant degrading bacteria by fungi. *Environmental Science & Technology* **39**(12):4640–4646 DOI [10.1021/es047979z](https://doi.org/10.1021/es047979z).
- Kumar RMA, Chandrajit B. 2011.** Biodegradation of waste gas containing mixture of BTEX by *B. Sphaericus*. *Research Journal of Chemical Sciences* **1**(5):52–60.
- Lebrero R, Lopez JC, Lehtinen I, Perez R, Quijano G, Munoz R. 2016.** Exploring the potential of fungi for methane abatement: performance evaluation of a fungal-bacterial biofilter. *Chemosphere* **144**:97–106 DOI [10.1016/J.chemosphere.2015.08.017](https://doi.org/10.1016/J.chemosphere.2015.08.017).
- Lee EY, Jun YS, Cho K-S, Ryu HW. 2002.** Degradation characteristics of toluene, benzene, ethylbenzene, and xylene by *Stenotrophomonas maltophilia* T3-c. *Journal of the Air & Waste Management Association* **52**(4):400–406 DOI [10.1080/10473289.2002.10470796](https://doi.org/10.1080/10473289.2002.10470796).
- Lin B, Van Verseveld HW, Röling WFM. 2002.** Microbial aspects of anaerobic BTEX degradation. *Biomedical and Environmental Sciences* **15**(2):130–144.
- Moussavi G, Bahadori MB, Farzadkia M, Yazdanbakhsh A, Mohseni M. 2009.** Performance evaluation of a thermophilic biofilter for the removal of MTBE from waste air stream: effects of inlet concentration and EBRT. *Biochemical Engineering Journal* **45**:152–156 DOI [10.1016/j.bej.2009.03.008](https://doi.org/10.1016/j.bej.2009.03.008).
- Mu YQ, Xie TT, Zeng H, Chen W, Wan CX, Zhang LL. 2020.** *Streptomyces*-derived actinomycin D inhibits biofilm formation via downregulating *ica* locus and decreasing production of PIA in *Staphylococcus epidermidis*. *Journal of Applied Microbiology* **128**:1201–1207 DOI [10.1111/jam.14543](https://doi.org/10.1111/jam.14543).
- Olsen RH, Kukor JJ, Kaphammer BA. 1994.** A novel toluene-3-monooxygenase pathway cloned from *Pseudomonas pickettii* PKO1. *Journal of Bacteriology* **176**(12):3749–3756 DOI [10.1128/jb.176.12.3749-3756.1994](https://doi.org/10.1128/jb.176.12.3749-3756.1994).
- Ottengraf SPP. 1987.** Biological-systems for waste-gas elimination. *Trends in Biotechnology* **5**(5):132–136 DOI [10.1016/0167-7799\(87\)90007-2](https://doi.org/10.1016/0167-7799(87)90007-2).
- Pedersen AR, Moller S, Molin S, Arvin E. 1997.** Activity of toluene-degrading *pseudomonas putida* in the early growth phase of a biofilm for waste gas treatment.

- Biotechnology and Bioengineering* **54**(2):131–141  
DOI [10.1002/\(SICI\)1097-0290\(19970420\)54:2<131::AID-BIT5>3.0.CO;2-M](https://doi.org/10.1002/(SICI)1097-0290(19970420)54:2<131::AID-BIT5>3.0.CO;2-M).
- Qi B, Moe WM, Kinney KA. 2002.** Biodegradation of volatile organic compounds by five fungal species. *Applied Microbiology and Biotechnology* **58**(5):684–689  
DOI [10.1007/s00253-002-0938-3](https://doi.org/10.1007/s00253-002-0938-3).
- Rosenberg M, Gutnick D, Rosenberg E. 1980.** Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. *FEMS Microbiology Letters* **9**(1):29–33 DOI [10.1111/j.1574-6968.1980.tb05599.x](https://doi.org/10.1111/j.1574-6968.1980.tb05599.x).
- Ryu HW, Cho KS, Chung DJ. 2010.** Relationships between biomass, pressure drop, and performance in a polyurethane biofilter. *Bioresource Technology* **101**(6):1745–1751  
DOI [10.1016/j.biortech.2009.10.018](https://doi.org/10.1016/j.biortech.2009.10.018).
- Sander R. 2023.** Compilation of Henry's law constants (version 5.0.0) for water as solvent. *Atmospheric Chemistry and Physics* **23**:10901–12440  
DOI [10.5194/acp-23-10901-2023](https://doi.org/10.5194/acp-23-10901-2023).
- Sieg K, Fries E, Puttmann W. 2008.** Analysis of benzene, toluene, ethylbenzene, xylenes and *n*-aldehydes in melted snow water via solid-phase dynamic extraction combined with gas chromatography/mass spectrometry. *Journal of Chromatography A* **1178**(1–2):178–186 DOI [10.1016/j.chroma.2007.11.025](https://doi.org/10.1016/j.chroma.2007.11.025).
- Sousa ET, Rodrigues FD, Martins CC, de Oliveira FS, Pereira PAD, de Andrade JB. 2006.** Multivariate optimization and HS-SPME/GC-MS analysis of VOCs in red, yellow and purple varieties of *Capsicum chinense* sp. peppers. *Microchemical Journal* **82**(2):142–149 DOI [10.1016/j.microc.2006.01.017](https://doi.org/10.1016/j.microc.2006.01.017).
- Sun ZQ, Ding C, Xi JY, Lu LC, Yang BR. 2020.** Enhancing biofilm formation in biofilters for benzene, toluene, ethylbenzene, and xylene removal by modifying the packing material surface. *Bioresource Technology* **296**:122335  
DOI [10.1016/j.biortech.2019.122335](https://doi.org/10.1016/j.biortech.2019.122335).
- Tait E, Perry JD, Stanforth SP, Dean JR. 2014.** Identification of volatile organic compounds produced by bacteria using HS-SPME-GC-MS. *Journal of Chromatographic Science* **52**(4):363–373 DOI [10.1093/chromsci/bmt042](https://doi.org/10.1093/chromsci/bmt042).
- van Groenestijn JW, Liu JX. 2002.** Removal of alpha-pinene from gases using biofilters containing fungi. *Atmospheric Environment* **36**(35):5501–5508  
DOI [10.1016/S1352-2310\(02\)00665-9](https://doi.org/10.1016/S1352-2310(02)00665-9).
- Veiga MC, Fraga M, Amor L, Kennes C. 1999.** Biofilter performance and characterization of a biocatalyst degrading alkylbenzene gases. *Biodegradation* **10**(3):169–176  
DOI [10.1023/A:1008301415192](https://doi.org/10.1023/A:1008301415192).
- Vergara-Fernández A, Yáñez D, Morales P, Scott F, Aroca G, Diaz-Robles L, Moreno-Casas P. 2018.** Biofiltration of benzo[ $\alpha$ ]pyrene, toluene and formaldehyde in air by a consortium of *Rhodococcus erythropolis* and *Fusarium solani*: effect of inlet loads, gas flow and temperature. *Chemical Engineering Journal* **332**:702–710  
DOI [10.1016/j.cej.2017.09.095](https://doi.org/10.1016/j.cej.2017.09.095).
- Wang CJ, Wang ZW, Cheng ZW, Wang JJ, Zhang SH, Chen JM. 2023.** Research progress on the purification of volatile organic compounds by fungi/bacteria and

- their coexistence systems. *Chinese Journal of Applied and Environmental Biology* **29**(2):474–483 DOI [10.19675/j.cnki.1006-687x.2021.11056](https://doi.org/10.19675/j.cnki.1006-687x.2021.11056).
- Wongbunmak A, Khiawjan S, Suphantharika M, Pongtharangkul T. 2017.** BTEX and naphthalene-degrading bacterium *Microbacterium esteraromaticum* strain SBS1-7 isolated from estuarine sediment. *Journal of Hazardous Materials* **339**:82–90 DOI [10.1016/j.jhazmat.2017.06.016](https://doi.org/10.1016/j.jhazmat.2017.06.016).
- Xie TT, Zeng H, Ren XP, Wang N, Chen ZJ, Zhang Y, Chen W. 2019.** Antibiofilm activity of three *Actinomyces* strains against *Staphylococcus epidermidis*. *Letters in Applied Microbiology* **68**:73–80 DOI [10.1111/lam.13087](https://doi.org/10.1111/lam.13087).
- Yuan X, Zhang X, Chen X, Kong D, Liu X, Shen S. 2018.** Synergistic degradation of crude oil by indigenous bacterial consortium and exogenous fungus *Scedosporium boydii*. *Bioresource Technology* **264**:190–197 DOI [10.1016/j.biortech.2018.05.072](https://doi.org/10.1016/j.biortech.2018.05.072).
- Zehraoui A, Hassan AA, Sorial GA. 2012.** Effect of methanol on the biofiltration of *n*-hexane. *Journal of Hazardous Materials* **219**:176–182 DOI [10.1016/j.jhazmat.2012.03.075](https://doi.org/10.1016/j.jhazmat.2012.03.075).
- Zhai J, Jiang CH, Long C. 2020.** Two-step method for the construction and start-up of a fungal-bacterial biofilter for treating gaseous toluene. *Environmental Progress & Sustainable Energy* **39**(5):e13404 DOI [10.1002/ep.13404](https://doi.org/10.1002/ep.13404).
- Zhai J, Shi P, Wang Z, Jiang HT, Long C. 2017.** A comparative study of bacterial and fungal-bacterial steady-state stages of a biofilter in gaseous toluene removal: performance and microbial community. *Journal of Chemical Technology and Biotechnology* **92**(11):2853–2861 DOI [10.1002/jctb.5302](https://doi.org/10.1002/jctb.5302).
- Zhang C, Jia L, Wang SH, Qu J, Li K, Xu LL, Shi YH, Yan YC. 2010.** Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. *Bioresource Technology* **101**(10):3423–3429 DOI [10.1016/j.biortech.2009.12.083](https://doi.org/10.1016/j.biortech.2009.12.083).
- Zhang XM, Cheng ZW, Yu JM, Chen JM, Zhu QQ. 2019.** Absorption of different VOCs by fungus and bacterium and analysis of cell surface. *China Environmental Science* **39**(3):1268–1277 DOI [10.19674/j.cnki.issn1000-6923.2019.0153](https://doi.org/10.19674/j.cnki.issn1000-6923.2019.0153).
- Zhu M, Zhou K, Sun X, Zhao Z, Tong Z, Zhao Z. 2017.** Hydrophobic N-doped porous biocarbon from dopamine for high selective adsorption of *p*-Xylene under humid conditions. *Chemical Engineering Journal* **317**:660–672 DOI [10.1016/j.cej.2017.02.114](https://doi.org/10.1016/j.cej.2017.02.114).
- Zhu XQ, Suidan MT, Pruden A, Yang CP, Alonso C. 2004.** Effect of substrate Henry's constant on biofilter performance. *Journal of the Air & Waste Management Association* **54**(4):409–418 DOI [10.1080/10473289.2004.10470918](https://doi.org/10.1080/10473289.2004.10470918).