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Treatment of gaseous toluene in three biofilters inoculated with fungi/bacteria: Microbial analysis, performance and starvation response

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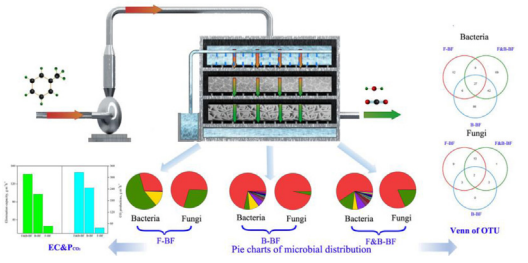
HIGHLIGHTS

- △A fungal–bacterial (F&B) biofilter showed better removal than fungal or bacterial one.
- △A maximum EC of 142 g m^{-3} h^{-1} for F&B biofilter was obtained with EBRT of 24 s.
- △F&B biofilter produced less CO_2 than bacterial one owing to the presentance of fungi.
- △Microbial community and amount was related to the inoculation and operation mode.
- △After prolonged starvation, F&B biofilter was able to convalesce in a short time.

GRAPHICAL ABSTRACT



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ABSTRACT

Bacteria and fungi are often utilized for the biodegradation of organic pollutants. This study compared fungal and/or bacterial biofiltration in treating toluene under both steady and unsteady states. Fungal biofilter (F-BF) removed less toluene than both bacterial biofilters (B-BF) and fungal & bacterial biofilters (F&B-BF) (<20% vs >60% vs >90%). The mineralization ratio was also lower in F-BF—levels were 2/3 and 1/2 of those values obtained by the other biofilters. Microbial analysis showed that richer communities were present in B-BF and F&B-BF, and that the *Hypocreales* genus which *Trichoderma viride* belongs to was much better represented in F&B-BF. The F&B-BF also supported enhanced robustness after 15-day starvation episodes; 1 day later the performance recovered to 80% of the original removal level. The combination of bacteria and fungi makes biofiltration a good option for VOC treatment including better removal and performance stability versus individual biofilters (bacteria or fungi dominated).

1. Introduction

Volatile organic compounds (VOCs) are common pollutants produced by a variety of industries, and their emissions face increasingly stringent environmental regulations. Biofilters were originally developed for odor abatement of waste gases and these filters have recently proven to be an effective and safe technology for the treatment of several VOCs at moderately high flow rates [1,2].

Water solubility is an important factor that impacts the mass transfer between gas and liquid phases. Thus, traditional biofilters commonly use bacteria as biocatalysts and are effective for treating

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hydrophilic VOCs. Several efforts have been made to improve the solubility of hydrophobic VOCs including chemical pretreatment and two-liquid phase bioreactors [1,3,4]. A number of recent studies indicated that the use of fungi presence favors the treatment of hydrophobic VOCs (with Henry's constants >1, such as hexane, pentane, etc.), which are particularly difficult to remove efficiently in bacterial biofilters due to their low transfer rates from gas to liquid phase [5].

The fungi utilized contain filamentous structures with aerial mycelia and a large surface area, which allows for easy absorption of many VOCs from the bulk gas phase [6]. Meanwhile, their resistance to low humidity favors mass transfer of hydrophobic VOCs from the gas phase to their surface [7]. In comparison to bacteria, other advantages of fungi include tolerance to low pH, starvation, etc. [8]. Various studies indicate that fungi can improve the elimination capacity (EC) of biofilters by 2-fold or greater than those dominated by conventional bacteria and can enhance the resistance to variable environments [9,10]. However, fungi have some drawbacks, as their generally lower metabolic rates, compared to aerobic bacteria, make the start-up periods much longer. In addition, their filamentous structures often lead to clogging in biofilters [5].

The first study that used fungi in treating VOCs in biofilters was done by Cox et al. [11] who reported a styrene-EC of 79 g m^{-3} h^{-1}. Several years later, the ability of fungi to use non-oxygenated aromatic compounds as the sole carbon source and completely mineralize was demonstrated [12]. Currently, studies concentrating on the removal of hydrophobic VOCs by fungi-inoculated or fungi-dominant biofilters are being conducted [13]. Toluene has been a favorite model VOC among researchers, and several new fungal isolates have been characterized for toluene degradation. The most extensively studied fungal isolates belong to the genera *Exophiala*, *Aspergillus*, *Phanerochaete*, *Cladosporium*, *Paecilomyces*, *Trichoderma*, and *Trametes*—most of which also have the ability to utilize other benzene derivatives [14–16]. Biofilters using these specific fungi as dominant degraders resulted in sustained ECs for toluene removal (55–360 g m^{-3} h^{-1}) [17–19].

Research into toluene-polluted gases biofiltration has fundamentally been focused on the performance at steady or un-steady states, seeking conditions that resulted in optimal or suboptimal operation, maximum ECs, etc. Extensive reviews have also been reported on bacterial analysis based on the DGGE (denatured gradient gel electrophoresis) method. However, studies on the simultaneous analysis of fungal and bacterial structure are relatively scarce [20]. In order to accurately assess the differences between fungal and bacterial biofiltration, systematic comparative studies of different microbial dominators and their roles/responses during the biofilter's performance under steady or un-steady states are necessary.

In this study, three lava rock biofilters – one inoculated with fungus (F-BF), one inoculated with bacteria (B-BF), and another with both fungi and bacteria (F&B-BF) – were created for toluene removal. The bacterial and fungal communities in the biofilters were investigated using real-time polymerase chain reaction (RT-PCR) and pyrosequencing to probe for new insights into the relationship between microbial community and operational performance in biofitration. In addition, the biofilters were also operated under adverse conditions (e.g., instantaneous impact load and starvation) to study the influences of environmental factors on toluene removal and microbial structures.

2. Materials and methods

2.1. Nutrient medium and inoculum

The mineral salt medium used for continuous biofilter experiments had the following chemical composition (per liter distilled water): 0.5 g K_2HPO_4, 0.1 g $MgSO_4{\cdot}7H_2O$, 4.5 g KH_2PO_4, 2 g NH_4Cl, and 2 mL of vitamins and trace minerals. In accordance with previously determined optimum pH values for bacterial and fungal growth (bacteria and fungi were neutral, 5.0–7.0, and weakly acidic <6.0) [21], the original pH for B-BF, F-BF and F&B-BF were adjusted to 7.0, 5.9, and 5.9, respectively.

We used a pure fungus named LW-1 with the ability to biodegrade toluene, which was isolated and identified as *Trichoderma viride* (CCTCC no. M2014176). Strain preservation, culture conditions, and spore production have been described in an early patent application [21]. Similarly, an activated sludge was collected from the aerobic tank of a sewage treatment plant in Hangzhou (Zhejiang, China) and used for bacterial inoculation. Initially, this activated sludge was continuously exposed to toluene for three weeks; at which point, the acclimated sludge appeared capable of toluene biodegradation.

Different inoculants were utilized in starting the biofilters: acclimated sludge was inoculated in B-BF, while *T. viride* and the acclimated sludge was inoculated in F-BF and F&B-BF. Most studies used activated sludge and special degraders to shorten the initial stages of biofiltrations [22]. Activated sludge not only provide a better micro-environment for microbial growth, but also possess a variety of species that are helpful in the formation of biofilms (e.g., species that produce extracellular polymeric substances that encourage cell aggregation) [23]. The only difference between F-BF and the other biofilters was the addition of the antibiotic streptomycin sulfate, which was added to the circulating mineral salt medium to inhibit bacterial growth [6].

2.2. Biofilter setup

Three identical laboratory-scale biofilters were set up as shown in Appendix A Fig. S1. Each biofilter had an internal diameter of 7.7 cm, a height of 98 cm, and were packed with lava rock (5–8 mm in diameter) up to a total bed volume of 4.5 L. A perforated plate at the bottom provided support for the packing material while another plate at the top acted as a distributor for the mineral salt medium addition. Gas sampling ports sealed with rubber septa were available at equal intervals along the biofilter height.

To generate toluene vapors, compressed air was split into two portions. The majority of the dry air was maintained at the desired humidity using a humidification unit placed in a water bath. The minor air stream was bubbled through liquid toluene in a stainless steel tank to generate the contaminated air stream. The two streams were then combined in a mixing flask and fed into the reactor in an upflow mode. The operating temperature was room temperature (25–30 °C), and the relative humidity of the inlet toluene vapor was ~45%. To maintain adequate nutrients and moisture content, the mineral salt medium described before was added once every 3 days. 1000 mL nutrient solution was sprayed into the biofilters and circulated continuously for 15–20 min.

2.3. Biofilter inoculation and operation

T. viride LW-1 was maintained on potato dextrose agar at 4 °C for preservation. To prepare cell suspensions, the fungus was grown in several 500 mL air-tight glass vials (working volume of 250 mL) on toluene. Each vial contained an initial toluene concentration of 50–100 mg L^{-1}. The media were maintained under shaking conditions at 160 rpm and at a constant temperature of 30 °C. After 96–120 h of growth, an aliquot with an optical density (OD_{600nm}) of 1.0 was harvested by centrifugation at 5000 rpm for 20 min and re-suspended in fresh nutrient medium.

The initial amount biomass for both biofilters was identical in all cases (characterized as protein amount). Streptomycin sulfate was added to prevent bacterial growth in F-BF. It is worth mentioning