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# Performance of low pH biofilters treating a paint solvent mixture: Continuous and intermittent loading

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**Abstract**

Two biofilters packed with a reticulated polyurethane foam medium were inoculated with a compost-derived enrichment culture grown under acidic conditions (pH 3.0) and then operated over a period lasting 63 days. Both biofilters were supplied with a humidified gas stream containing a five-component mixture of acetone, methyl ethyl ketone, toluene, ethylbenzene, and *p*-xylene at a total VOC loading rate 80.3 g $m^{-3}$ $h^{-1}$ to simulate treatment of air emissions resulting from manufacture of reformulated paint. One biofilter was operated under continuous loading conditions and the other received intermittent loading with contaminants supplied only 8 h/day. Nutrient solution with pH 3.0 was supplied approximately once per week to provide nitrogen and other nutrients. Data are presented which demonstrate that undefined mixed cultures acclimated at low pH can successfully treat paint solvent mixtures in biofilters. The biofilter receiving continuous loading reached high overall removal efficiency (greater than 90% overall removal) 3 weeks after startup, and performance increased over time reaching overall removal in the range of 97–99% after 50 days. Performance of the intermittently loaded biofilter developed more slowly, requiring 6 weeks to stabilize at an overall removal efficiency in excess of 90%. In both biofilters, ketone components were more rapidly degraded than aromatic components, and removal of aromatic compounds was somewhat unstable even after 2 months of biofilter operation. Scanning electron microscopy (SEM) revealed that fungi dominated the microbial populations in both biofilters.


*Keywords:* Biofiltration; VOC; pH; Fungi; Paint

## 1. Introduction

Volatile organic compounds (VOCs), many of which are classified as hazardous air pollutants (HAPs), are emitted to the air on a large scale by industrial and manufacturing operations. Although process changes and material substitutions have the potential to minimize emissions and may allow compliance with environmental regulations in some applications, there is a continuing need for efficient and cost effective control technologies that can be applied to treat point and area sources. A promising air pollution control technology for many dilute waste gas streams is biofiltration.

In biofiltration contaminated air is passed through a packed-bed fixed-film bioreactor. As air moves though the bioreactor, contaminants are transferred from the air into a biofilm that grows immobilized on a solid support medium. Once in the biofilm, microorganisms biodegrade the contaminants, producing environmentally acceptable end products including carbon dioxide, water, and additional biomass. Contaminant consumption within the biofilm creates a concentration gradient that serves as a driving force for mass transfer. Because of its low cost compared to alternatives like thermal oxidation or sorption to activated carbon, biofiltration is particularly attractive for treating high flow airstreams containing low concentrations of biodegradable pollutants [1,2].

Although there have been many successful full-scale applications of biofiltration technology during the past two decades, the biological processes that occur within such systems are not yet well understood. Optimal conditions for ensuring stable operation and maximizing biodegradation rates have not been well established, and the range of conditions under which successful operation is possible have not been delineated. One particular operating parameter that can markedly impact the microbial population, and corresponding biofilter performance, is pH.

The majority of biofilters reported in the literature for treatment of VOCs have been operated at or near neutral pH (i.e.

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pH 7). Devinny et al. [1] point out that operation of biofilters at pH 7 is generally accepted as a benign condition, and designers are most familiar with it. They also point out that there is little evidence to support the notion that such operation is optimal. There are an abundance of microbial species with optimal growth rates under conditions where the pH is either acidic or alkaline [1,2]. In cases where multiple species with diverse pH requirements for growth are capable of degrading a contaminant, the pH level at which a biofilter is operated will impose a selective pressure on the microbial community. Microbial species with highest growth rates under conditions imposed will increase in relative abundance over time, and relative abundance of other species will decrease. The unique microbial population composition that results can influence biofilter performance because species differ with respect to growth rates, substrate uptake rates, and ability to remain viable under starvation conditions.

Arriaga and Revah [3] and van Groenestijn et al. [4] recently demonstrated that there can be advantages to operating biofilters at low pH. In both studies, multiple biofilters inoculated with microbial populations originating from the same source (a gasoline degrading biofilter and hydrocarbon contaminated soil, respectively) were operated at different pH levels while treating vapors contaminated by single component contaminants (hexane or toluene, respectively). Arriaga and Revah [3] observed that maximum hexane elimination capacities were higher in a pH 4 biofilter, which developed a microbial population dominated by fungi, than in a pH 7 biofilter. Likewise, van Groenestijn et al. [4] found that toluene degrading biofilters supplied with pH 4 nutrient solution exhibited markedly higher contaminant removal rates than biofilters supplied with pH 8 solution. They also found that the pH 4 biofilters were dominated by fungi while the pH 8 biofilters were dominated by bacteria. The low pH, fungal-dominated biofilters were more tolerant to unintended drying conditions within the packing medium bed [4].

Similar side-by-side comparisons have not been reported, however, for treatment of complex VOC mixtures or for biofilters operated with intermittent loading during the startup phase. Treatment of multi-component VOC mixtures can be complicated by complex contaminant–contaminant interactions (e.g. inhibition, repression) [5–7], and it is not yet clear if acidophilic or acid-tolerant microbial populations able to degrade all of the components of complex VOC mixtures can be readily enriched from compost or other innocula commonly used to seed biofilters. Previous research also indicates that intermittent operation during startup can be problematic [8].

We recently reported performance of two biofilters operated at neutral pH (pH 7) for treatment of an air stream containing a five-component mixture of acetone, methyl ethyl ketone, toluene, ethylbenzene, and *p*-xylene, intended to simulate solvent emissions from manufacture and application of reformulated low-HAP paint (i.e. paints containing high concentrations of acetone in lieu of other solvents) [9]. One biofilter was operated under continuous loading conditions, while the other received contaminant loading only 8 h/day to simulate loading conditions expected at facilities where operations are discontinuous. Herein, we report performance of two biofilters inoculated with an enrichment culture grown in an acidic nutrient solution (pH 3.0) derived from the same source as the previously described neutral pH biofilters. Following inoculation, the biofilters were operated at pH 3.0 while treating a five-component waste gas stream identical to that used in pH 7.0 biofilter experiments. The primary research objectives were to establish whether microbial populations enriched from compost under acidic pH conditions could successfully degrade the VOC mixture in a low pH biofilter and to assess whether startup of such a system could be accomplished under intermittent loading.

## 2. Materials and methods

### 2.1. Experimental apparatus

Laboratory experiments employed two identical glass biofilter column reactors each configured as shown in Fig. 1. Each biofilter consisted of five packed sections, each with an inner diameter of 9.9 cm and height of 25 cm, plus a top and a bottom. The column section closest to the inlet was filled with 20 cm height packing medium, and the remaining four sections were each filled to a height of 25 cm (approximately 40 g dry weight per section), providing total bed depth of 1.2 m and total packed bed volume of 9.2 L in each column. Packing medium consisted of reticulated polyurethane foam cubes approximately 1.2 cm per side containing approximately 7 pores per cm, bulk porosity of 97%, packed bed density of 30 kg/m$^3$, and surface area (bulk) approximately 210 m$^2$/m$^3$ (Honeywell-PAI, Lakewood, CO).

Contaminant-free compressed air flowed through a pressure regulator (Arrow Pneumatics, Broadview, IL) and rotameters (Cole-Parmer) to measure and control air flow. To provide humidification, approximately 95% of the air stream supplied to each biofilter passed through an aeration stone submerged in a 20 L glass carboy filled with deionized water. The remaining 5%

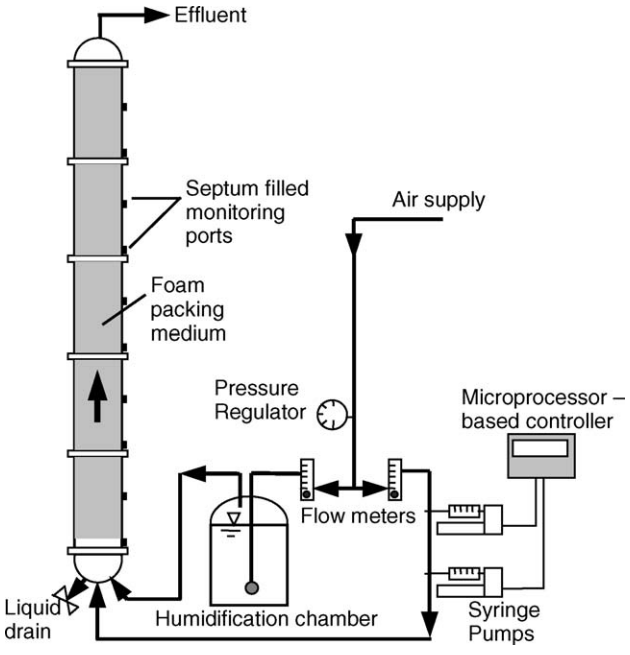


Fig. 1. Schematic diagram of laboratory-scale biofilter systems.

of the air stream flowed through two glass ports where syringe pumps (KD Scientific Inc., New Hope, PA) equipped with glass gas-tight syringes (Hamilton Scientific, Reno, NV) injected liquid VOCs (ACS reagent grade, Fisher Scientific) which evaporated. Acetone and methyl ethyl ketone were combined in one 10 mL syringe, and toluene, ethylbenzene, and *p*-xylene were combined in second 5 mL syringe. A microprocessor-based controller (Model XT, Chron-Trol Corp., San Diego, CA) turned syringe pumps on and off as needed.

### 2.2. Inoculum culturing and inoculation

The microbial consortium used to inoculate the biofilters was derived from a mixture of composted wood waste (LSU Facility Services, Baton Rouge, LA) and compost derived from municipal wastewater sludge (Nation's Best, Baton Rouge, LA). A mass corresponding to 500 g dry weight of each compost was added to 2.0 L of a nutrient solution comprised of the following constituents added to deionized water: $NH_4NO_3$ 1.25 g $L^{-1}$, $KH_2PO_4$ 1.0 g $L^{-1}$, $MgSO_4{\cdot}7H_2O$ 0.5 g $L^{-1}$, $CaCl_2{\cdot}2H_2O$ 0.02 g $L^{-1}$, $CuCl_2{\cdot}2H_2O$ 0.17 mg $L^{-1}$, $CoCl_2{\cdot}6H_2O$ 0.24 mg $L^{-1}$, $ZnSO_4{\cdot}7H_2O$ 0.58 mg $L^{-1}$, $MnSO_4{\cdot}H_2O$ 1.01 mg $L^{-1}$, $Na_2MoO_4{\cdot}2H_2O$ 0.24 mg $L^{-1}$, $NiCl_2{\cdot}6H_2O$ 0.10 mg $L^{-1}$, and $FeSO_4{\cdot}7H_2O$ 1.36 mg $L^{-1}$. The resulting slurry was manually stirred, and the liquid was decanted from the slurry while retaining large solids. Decanted liquid was then diluted with additional nutrient solution. A 3.5 L volume of this initial inoculum was transferred into a 4.0 L glass kettle reactor, and the pH was adjusted to 3.0 using 1.0 M HCl.

The glass kettle reactor was then operated as a sparged-gas reactor [7,9] to enrich for microorganisms able to degrade VOCs used in subsequent biofilter experiment. The reactor, stirred by a Teflon-coated magnetic stir bar, was supplied with a VOC-contaminated air stream that entered via a gas diffuser stone. The contaminated air stream was produced by injecting liquid VOCs from a glass gas-tight syringe (by means of a syringe pump) at a rate of 0.05 mL/h per 1.5 L/min air flow. Individual VOCs were sequentially supplied to the enrichment culture as single-components for 12 h intervals (e.g., 12 h only acetone, then 12 h only toluene, etc.). The reactor was maintained at pH 3.0 by adding 1 M HCl or 5 M NaOH as necessary. Starting 7 days after inoculating the sparged-gas reactor, biomass was wasted at 1 or 2 day intervals by removing 200 mL portions of liquid and replacing with an equal volume of freshly prepared nutrient solution. This corresponds to a solids residence time (SRT) and hydraulic residence time (HRT) in the range from 17.5 to 35 days. The sparged-gas reactor was operated for a total of 100 days prior to inoculation of the biofilter.

To evaluate the bioreactor's capacity to degrade each of the VOCs, at periodic intervals, a sub-sample was removed from the reactor and oxygen uptake rate (OUR) was measured under endogenous conditions and after spiking with each contaminant using a method described previously [9]. For each VOC, the OUR measured under endogenous conditions was subtracted from the OUR measured following spiking to calculate the increase in OUR (hereafter referred to as the ΔOUR).

Before inoculation of the biofilters, the enrichment culture from the sparged-gas reactor was diluted to 10 L. Foam packing medium for each biofilter section was submerged in a separate 1.0 L volume of the liquid culture where it incubated for 15 min before being transferred into the biofilter column. Excess liquid was drained by gravity, and then the column was sealed. Time was measured in days from the start of contaminant loading following this inoculation.

### 2.3. Biofilter operation

Following inoculation, one biofilter received contaminant loading on a continuous basis (24 h/day) while the other was subjected to intermittent loading with contaminants supplied only 8 h/day. During the daily 16 h interval without contaminant loading, the intermittently loaded biofilter was supplied with uncontaminated humidified air at a flow rate identical to that during the loading condition. Both biofilters were operated at an empty bed residence time (EBRT) of 59 s, and were supplied with a VOC mixture at target concentrations and loading rates indicated in Table 1. Because the intermittently loaded biofilter received contaminant loading for only one third of the time (i.e. 8 h/24 h), contaminant mass supplied per day was one third that of the continuously loaded biofilter.

At weekly intervals, nutrients were supplied and pH was regulated by filling each of the biofilter columns with 10 L of nutrient solution and draining by gravity. The nutrient solution, adjusted to pH of 3.0, contained the same constituents as the nutrient used for enrichment culturing but at five times higher concentration to avoid nutrient limitations that may have otherwise occurred [10]. This served the dual purposes of supplying nutrients and

Table 1
Composition and loading rate of the contaminant mixture supplied to the biofilters

| Compound | Concentration ($ppm_v$) | Loading rate (g $m^{-3}$ $h^{-1}$) | Percent of total loading rate (% by mass) | Henry's law constant (dimensionless) |
|---|---|---|---|---|
| Acetone | 450 | 66 | 82 | $1.59 \times 10^{-3}$ [a] |
| Methyl ethyl ketone | 12 | 2.1 | 2.6 | $2.50 \times 10^{-3}$ [b] |
| Toluene | 29 | 6.8 | 8.5 | $2.72 \times 10^{-1}$ [a] |
| Ethylbenzene | 10 | 2.7 | 3.4 | $3.23 \times 10^{-1}$ [a] |
| *p*-Xylene | 10 | 2.7 | 3.4 | $3.14 \times 10^{-1}$ [a] |
| Total | 511 | 80.3 | 100 | – |

[a] US EPA (1996). Soil Screening Guidance: User's Guide, EPA540/R-96/018.
[b] SRC PhysProp Database (2004). http://www.syrres.com/esc/physdemo.htm.

controlling pH. All experiments were conducted at ambient laboratory temperature (22 ± 2 °C).

### 2.4. Analytical techniques

VOC concentrations in gas samples collected from the biofilters using glass gas-tight syringes were measured using a Hewlett Packard 6890 series gas chromatograph equipped a DB-624 Special Analysis Column (Capillary 60 m × 320 μm × 1.80 μm, Hewlett Packard) and flame ionization detector (FID) as described previously [6]. Headloss across the height of each biofilter packed bed was measured using a water manometer. An Orion model 290A pH meter was used to measure pH.

Immediately following biofilter inoculation, one of the biofilters was temporarily disassembled, packing medium samples were collected from each biofilter section, and biomass was dislodged from the packing medium by repeated compression and vortexing as described previously [9]. Following serial dilution, pour plates using 1.0 mL aliquots were prepared to enumerate bacteria and fungi in the inoculum. Agar plates for enumerating bacteria contained Plate Count Agar (BD, Sparks, MD) amended with 500 μg/mL cycloheximide (Sigma–Aldrich, Louis, MO) to inhibit fungal growth. Cycloheximide (dissolved in absolute ethanol) was added from a filter-sterilized stock solution after autoclaving PCA. Agar plates for enumerating fungi contained Rose Bengal Agar (BD, Sparks, MD) amended with chloramphenicol (100 μg/mL) to inhibit bacterial growth. Pour plates were incubated at 25 °C for 4 days before counting colony forming units (CFUs). Foam cubes from which biomass was removed for plate counts were rinsed with deionized water, dried at 103 °C, and weighed. Microbial concentration results are reported as CFUs per unit mass dry packing medium.

Packing medium samples were collected from various locations in the biofilters after 22 days of operation for imaging via a scanning electron microscope (SEM). Foam cubes were prepared as described previously [9] prior to imaging with a Cambridge S-260 Stereoscan SEM (Cambridge Instrument, Cambridge, UK).

### 2.5. Calculations

To evaluate treatment performance, removal efficiency (RE) for each individual VOC tested was calculated using Eq. (1):

$$\mathrm{RE}\,(\%) = \frac{C_{\mathrm{in}} - C_{\mathrm{out}}}{C_{\mathrm{in}}} \times 100 \quad (1)$$

where $C_{\mathrm{in}}$ is the VOC concentration entering the biofilter (mg/L) and $C_{\mathrm{out}}$ is the VOC concentration exiting the biofilter (mg/L). Overall removal efficiency ($\mathrm{RE_o}$) accounting for all components of the VOC mixture was calculated using Eq. (2):

$$\mathrm{RE}\,(\%) = \frac{\sum C_{\mathrm{in}} - \sum C_{\mathrm{out}}}{\sum C_{\mathrm{in}}} \times 100 \quad (2)$$

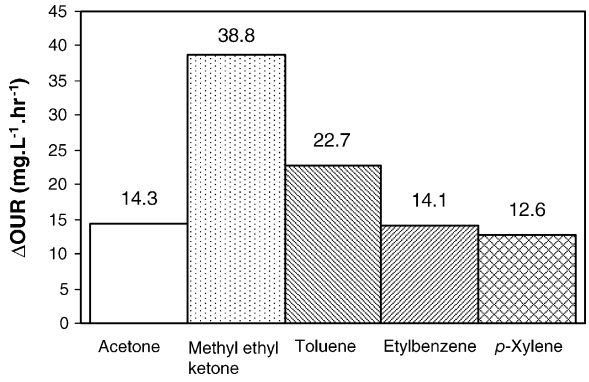


Fig. 2. Increase in oxygen uptake rate (ΔOUR) following spiking with individual contaminants in the sparged-gas reactor used to inoculate the biofilters.

## 3. Results

The increase in oxygen uptake rate (ΔOUR) observed in the sparged-gas reactor used to inoculate the biofilters following spiking with each of the VOCs is shown in Fig. 2. The increase in OUR following spiking with individual VOCs was highest for methyl ethyl ketone (38.8 mg $\mathrm{L^{-1}\,h^{-1}}$) followed by toluene (22.7 mg $\mathrm{L^{-1}\,h^{-1}}$), acetone (14.3 mg $\mathrm{L^{-1}\,h^{-1}}$), ethylbenzene (14.1 mg $\mathrm{L^{-1}\,h^{-1}}$), and *p*-xylene (12.6 mg $\mathrm{L^{-1}\,h^{-1}}$). For all five compounds tested, there was an increase in OUR following contaminant spiking, demonstrating that at the time of biofilter inoculation, the enrichment culture was able to degrade all of the contaminants used in subsequent biofilter experiments.

Results from plate counts conducted immediately after inoculation of the biofilters revealed fungal CFU concentrations of $5.7 \times 10^4$ CFUs per g dry packing medium and bacterial CFU concentrations of $5.9 \times 10^3$ CFUs per g dry packing medium (ratio of fungal to bacterial CFUs = 9.1).

Overall VOC removal efficiency ($\mathrm{RE_o}$) in each of the biofilters (calculated using Eq. (2)) following inoculation is shown in Fig. 3. After the first 24 h of operation (when the first data were collected), overall VOC removal efficiency in the continuously loaded biofilter was 21% (Fig. 3, top). Separately conducted abiotic sorption experiments conducted in an identical column packed with polyurethane foam but without microbial inoculation indicate that complete contaminant breakthrough would have occurred within 2 h in the absence of biological activity (data not shown). Thus, it is clear that the microbial population was able to degrade contaminants almost immediately following inoculation. Aside from a sudden unexplained decrease in removal efficiency observed immediately after a nutrient addition on day 22, performance of the continuously loaded biofilter gradually increased and reached a level in excess of 90% after day 23. After day 57, overall VOC removal efficiency was greater than 99%.

Data points shown in Fig. 3 (bottom) for the intermittently loaded biofilter correspond to measurements at the mid-point of the daily 8 h loading interval. On day 1 (24 h after start of operation), overall VOC removal efficiency was 16%. Overall removal efficiency was somewhat unstable, ranging from 24 to

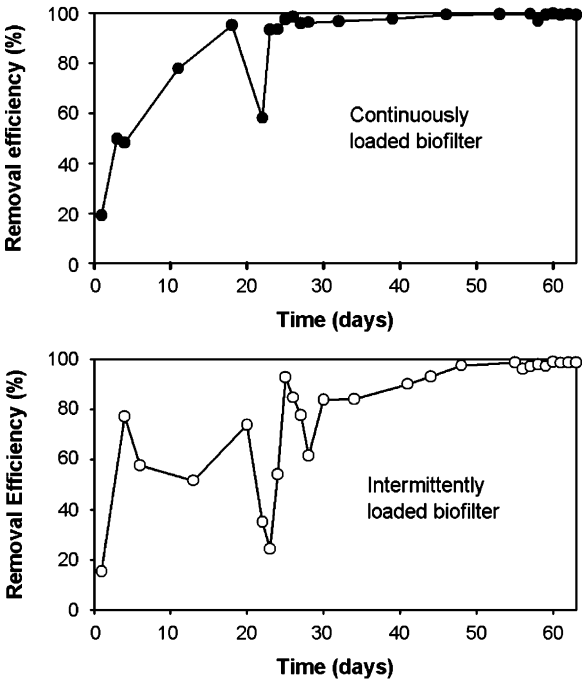


Fig. 3. Overall VOC removal efficiency in the continuously loaded biofilter (top) and intermittently loaded biofilter (bottom).

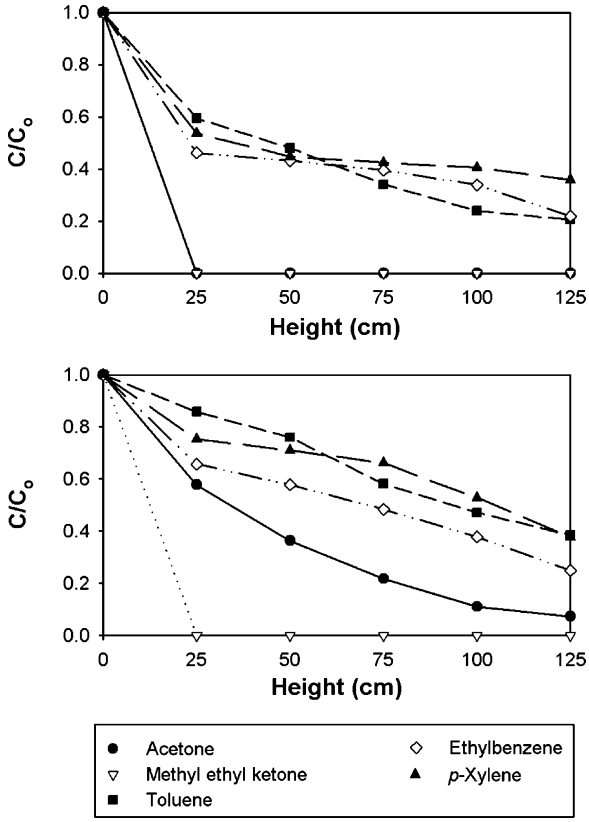


Fig. 4. VOC concentration profiles measured on day 39 in the continuously loaded biofilter (top) and intermittently loaded biofilter (bottom).

93%, but generally increased during the next several weeks. Overall removal efficiency exceeded 90% from day 43 onward, generally fluctuating between 95 and 98% during the remainder of operation.

VOC removal profiles within the biofilters on day 39 are shown in Fig. 4. In the continuously loaded biofilter, acetone and methyl ethyl ketone were completely removed in the first 25 cm bed depth. Toluene, ethylbenzene, and *p*-xylene were removed more slowly and penetrated the entire column depth. Contaminant removal profiles in the intermittently loaded biofilter followed the same general trends. Methyl ethyl ketone was completely removed by 25 cm of bed depth. Acetone, however, penetrated much further into the bed with only 43% removed by the end of the first section (25 cm) and only 93% removed by the end of the column (125 cm). Aromatic compounds were gradually removed along the bed height and were not completely removed.

Removal efficiency for each compound present in the VOC mixture is shown in Fig. 5 (calculated using Eq. (1)). In the continuously loaded biofilter, methyl ethyl ketone was completely removed throughout the entire period of biofilter operation. Removal of acetone (which comprised 82% of the contaminant mass loading, see Table 1) was 16% on day 1, quickly increased to more than 50% by day 4, and exceeded 99% after day 23. The biofilter's removal capacity for the three aromatic components of the contaminant mixture developed more slowly and exhibited more variability than removal capacity for the two ketones. Removal efficiencies for the three aromatic compounds approached 90% by day 47; however, there was still variability and excursions below 70% occurred between days 47 and 63 (at which time performance monitoring stopped).

Removal efficiency for individual compounds in the intermittently loaded biofilter followed the same general trends as observed in the continuously loaded biofilter but with somewhat slower increase in performance, particularly in the case of acetone. Methyl ethyl ketone was completely removed starting shortly after startup. Acetone removal gradually improved over time, reaching essentially complete removal by day 43. Removal of aromatic compounds was more sporadic but generally improved over time.

Fig. 6 shows an SEM image of the surface of foam packing removed from the inlet section of the continuously loaded biofilter on day 22. As shown, filamentous fungi dominated the microbial community covering the packing medium surface. SEM images of packing media collected from the intermittently loaded biofilter also revealed the presence of large numbers of filamentous fungi, though with less complete coverage of the packing media surface (data not shown).

At the end of operation (day 63), headloss across the packed bed depth of the continuously loaded biofilter and intermittently loaded biofilter was 0.8 and 0.4 cm $H_2O$, respectively. The high porosity (approximately 0.97) of the polyurethane foam packing medium was apparently able to accommodate biomass accumulation without a dramatic increase in pressure drop over the time course of the experiment. Similarly low headloss has been reported in other studies using high porosity packing media in fungal-dominated biofilters [6,11].

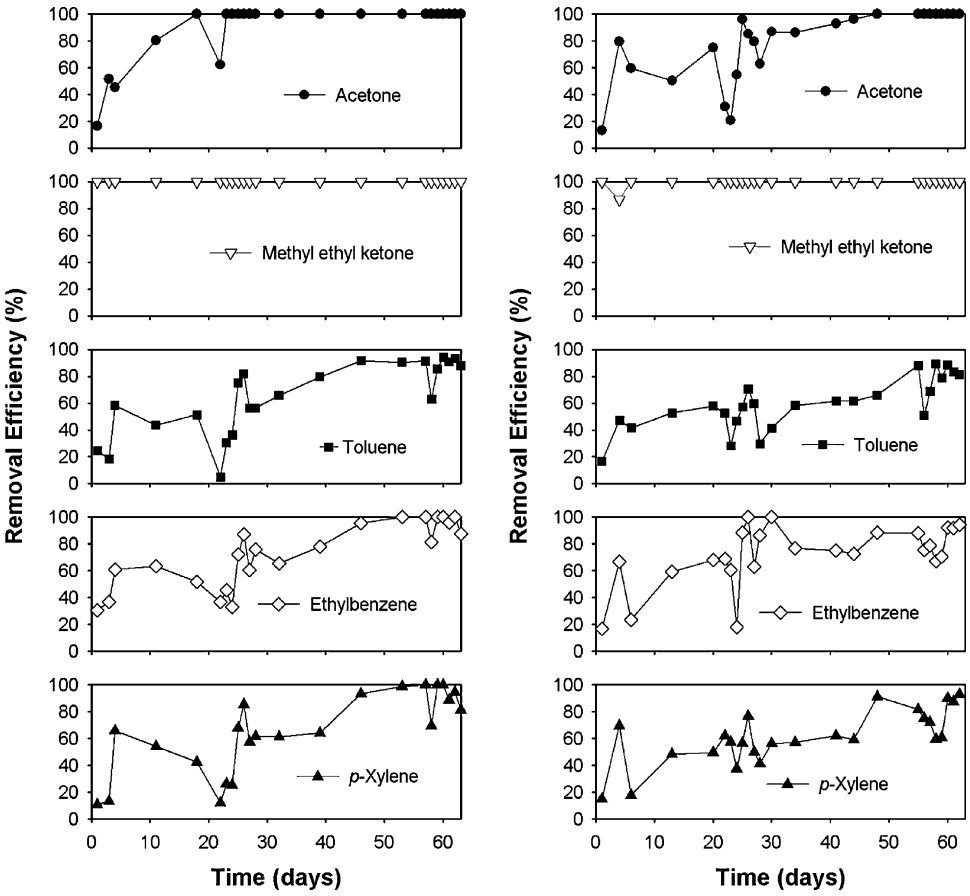


Fig. 5. Removal efficiency for each component of the VOC mixture in the continuously loaded biofilter (left) and the intermittently loaded biofilter (right).

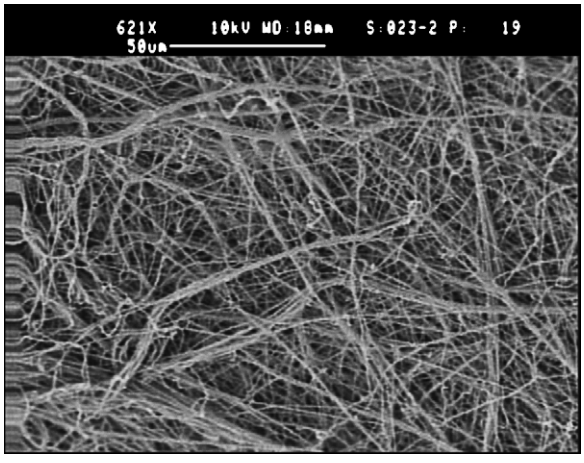


Fig. 6. SEM image of microbial colonization in the continuously loaded biofilter after 22 days of operation.

## 4. Discussion

Although previous research has demonstrated that sudden uncontrolled pH declines can adversely affect biofilter performance [1,12], data presented herein demonstrate that when microbial populations are acclimated and maintained under acidic conditions, they can readily degrade mixtures of VOCs representative of those found in paint manufacturing and paint application facilities. Plate count data from the period immediately following biofilter inoculation suggest that the low pH conditions in the sparged-gas reactor imposed a sufficiently high selective pressure to enrich for fungi over bacteria. The ratio of fungal to bacterial CFUs in the acidic (pH 3.0) inoculum used for experiments described herein, 9.1, was substantially higher than the inoculum produced in a neutral pH (7.0) sparged-gas reactor started on the same day with the same compost and operated under identical VOC loading conditions (which had a fungal CFU to bacterial CFU ratio of 0.033) [9]. SEM photos of the packing media removed from the biofilters after three weeks of operation confirmed that the microbial population that developed over time in both of these low pH biofilters was dominated by fungi.

Fungi can degrade a variety of VOCs [3,4,11–23], and fungal species generally exhibit maximum growth rates at pH levels lower than for bacteria [4]. Consequently, low pH conditions likely serve as a selective pressure that increases relative abundance of fungi [3,4]. Enrichment of fungi may be advantageous because some fungi can apparently exhibit contaminant degradation rates equal to or higher than those of bacteria-dominated biofilters [3,4,6,21,22]. Additionally, it has been hypothesized that aerial mycelia of fungi in direct contact with the gas phase can offer faster contaminant mass transfer rates than flat aqueous bacterial biofilm surfaces [3,4,22]. Because of lower requirements for water, fungi may also be more tolerant to

unintended drying conditions within the packing medium bed [4].

Data reported herein demonstrate low pH can impart a sufficiently large selective pressure to result in fungal-dominated biofilter microbial populations treating VOC mixtures as suggested previously for single component waste gas streams [4]. It should be noted, however, that fungi were also present in substantial quantities (though at lower relative abundance) in the neutral pH biofilters enriched at pH 7 from the same compost as the initial inoculum for experiments described herein [9]. Thus, the selective pressures that will lead to fungal predominance are not yet entirely clear.

Contaminant removal profiles along the heights of biofilter beds observed in the study described herein are consistent with previously reported biofilters treating similar solvent mixtures. Laboratory and pilot-scale biofilters treating mixtures of ketones and aromatic hydrocarbons have revealed that removal of ketones (acetone and methyl ethyl ketone) generally precedes that of aromatic compounds (toluene, ethylbenzene, and *p*-xylene) [5–7,9,24]. Low pH conditions in the biofilters described herein did not result in a trend different from previously reported biofilters. It is not clear from the data collected why this consistently observed trend occurred. Faster removal of ketones may result because of higher aqueous partitioning coefficients (i.e., lower Henry's law constants, see Table 1) leading to faster mass transfer. It may also result from inherent differences in rates of enzyme-catalyzed reactions. Experiments demonstrating that toluene can be rapidly removed when other constituents (e.g. ethyl acetate or ketones) are absent [5,25] suggests that substrate inhibition or catabolic repression likely occurs in such systems.

Regardless of the exact cause, removal of aromatic components of the VOC mixture was somewhat unstable even after 2 months of biofilter operation. This may have important implications in cases where treatment objectives require removal of specific contaminants rather than considering contaminant removal as an aggregate parameter. For example, in terms of overall removal efficiency, both biofilters systems described herein consistently removed greater than 95% of the influent contaminant mass after day 48. High overall removal efficiency (>95%) was possible even when removal efficiency for aromatic compounds was lower (e.g. 70%) because the aromatic compounds comprised only 12.2% of influent contaminant mass flow (total for all three aromatic constituents, see Table 1). Maintaining high removal efficiency for aromatic compounds present at relatively low levels in VOC mixtures, a potential treatment goal in some air pollution control applications, may be difficult to achieve in practice.

In spite of the fact that many industrial operations utilize processes that generate VOC contaminated waste gas streams on an intermittent basis, relatively few studies investigated biofilter performance under intermittent loading conditions [9,26–28]. Results presented herein indicate that low pH biofilters can be successfully operated with intermittent VOC loading; however, removal efficiency following startup increased relatively slowly in comparison of the biofilter operated with continuous loading. Slower performance increase during startup has also been observed for intermittently loaded biofilters operated at neutral pH [9]. Use of an activated carbon load equalization system such as that described by Li and Moe [29] may have allowed more complete removal of aromatic compounds in the intermittently loaded biofilter.

Although the low pH fungal-dominated biofilters described herein successfully treated the paint VOC mixture, performance increase during the period immediately following startup was slower than the previously reported neutral pH biofilters inoculated with an enrichment culture derived from the same starting materials and treating a waste gas stream identical in composition [9]. The continuously loaded low pH biofilter took approximately 3 weeks longer to reach 90% overall removal than did the continuously loaded neutral pH biofilter. Likewise, the intermittently loaded low pH biofilter took approximately 1.5 weeks longer to reach 90% overall removal than did the intermittently loaded neutral pH biofilter. Overall VOC removal after 48 days of operation, however, was quite similar in both the low pH biofilters described herein and the neutral pH biofilters described previously. The slower startup in the low pH biofilters may have resulted from the fact that fungi generally have slower growth rates than bacteria [3,30]. It was recently reported that addition of the surfactant Tween 20 allowed more rapid attainment of high removal rates in a biofilter inoculated with a pure culture of the fungus *Exophila lecanii-corni* [30]. Further research is necessary to determine whether surfactant addition could also speed startup in biofilters containing undefined mixed cultures as employed here. Further research is also necessary to evaluate whether low pH biofilters have advantages over conventionally operated neutral pH systems for treatment of complex VOC mixtures.

## 5. Conclusions

Data presented herein demonstrates that when microbial populations are acclimated and maintained under acidic conditions, they can readily degrade mixtures of VOCs representative of those found in paint manufacturing and paint application facilities. The low pH, fungal-dominated biofilters successfully treated the paint VOC mixture, though with slower development of performance during the startup period than a neutral pH biofilter reported previously. Among the VOCs tested, the two ketones (acetone and methyl ethyl ketone) were more rapidly degraded than the aromatic compounds (toluene, ethylbenzene, and *p*-xylene). Removal of aromatic components of the VOC mixture was somewhat unstable even after 2 months of biofilter operation. Although data demonstrate that low pH biofilters can be started up successfully under intermittent loading condition, such operation can lengthen the time interval needed to achieve high removal.

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