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Biofiltration for the removal and 'detoxification' of a complex mixture of volatile organic compounds

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A study was performed to determine the effectiveness of using biofiltration for the removal of a complex mixture of volatile organic compounds (VOCs) air-stripped from petroleum hydrocarbons. A biofilter was constructed which contained 264 cm³ of packing material (Celite® R-635). The unit was inoculated with a mixed culture containing a hydrocarbon-degrading *Pseudomonas* sp and an *Alcaligenes* sp. Several of the major compounds in the VOC mixture were monitored individually, along with the total VOCs, using gas chromatography. The average influent concentration of the VOC mixture was 320 ppmv and the average total VOC removal rate was over 56%, with the average removal rate of the monitored individual compounds ranging from 49–90%. After 30 days of operation the average overall removal rate was 69% and the removal of the major compounds averaged 92%. The toxicity and mutagenicity of the air stream was monitored using the Microtox and Ames assays, respectively. These data show marked decreases in toxicity and mutagenicity of the air stream as a result of the biofiltration treatment. The biofiltration system, therefore, was not only effective in removing VOCs from the air stream over an extended time-period, but was also effective in greatly reducing the toxicity and mutagenicity associated with the remaining VOCs.

Keywords: biofiltration; volatile organic compounds; hydrocarbons; toxicity; mutagenicity

Introduction

Biofiltration is a biological treatment technology which is well established in many European countries [17], primarily for the removal of odorous compounds from air streams. Recently, biofiltration has been applied to the removal of volatile organic compounds (VOCs), or air toxics, including BTEX (benzene, toluene, ethyl benzene, and xylene) compounds, ethanol, gasoline vapors, methane, styrene, and various chlorinated solvents [8,10,12,13,19–23].

Over the last decade, there has been much interest in the area of biofiltration as a secondary air stream treatment technology [17]. This interest is partially due to the fact that two newer remediation technologies produce large quantities of VOCs; these systems are soil vapor extraction (SVE) and air stripping [20]. Both technologies function on the premise that the contaminants are transferred from either soil or groundwater to the air phase. Unless coupled to a secondary treatment system, the SVE and air stripping systems do not result in the destruction of the contaminants and the contaminants are released into the atmosphere for deposition and exposure elsewhere [24]. Prior to the use of biofiltration technologies, if the chemicals were not released, they must be trapped using chemical methods (eg, activated carbon or resins) or destroyed by physical methods (eg, thermal treatment). Some of these non-biological methods may produce toxic by-products and are very expensive [20]. Several researchers have therefore specifically suggested that biofiltration may be an effective method for the destruction of these secondary emissions [17,20].

The ultimate goal of biofiltration is to destroy VOCs or air toxics in the air stream [17]. However, we have been unable to locate any published reports of toxicity being directly measured during a biofiltration project. Many researchers have reported great losses of VOCs and success in the uses of biofiltration in eliminating VOCs from air streams [10,20]. However, in biological processes a reduced quantity of a compound is not always better (ie, some degradation products may be more toxic than the parent compound). There are numerous examples of biological processes leading to the formation of more toxic end-products or to the build-up of intermediate products [1,6,9]. The loss of VOCs is usually reported as percent loss of total VOCs, or as elimination capacity in $g\,m^{-3}\,h^{-1}$ [10,17,20,25]. This is most true of applications where the air stream is from a complex source containing numerous compounds (eg, SVE or air stripping systems). For these applications it is difficult to accurately quantitate exact amounts of VOCs in the influent and effluent of the biofiltration system. Often, the waste gas is poorly characterized or its make-up is indeterminable due to the complex nature of the source of these mixtures.

The development of biofiltration technology in the US is rapidly increasing; however, a more detailed understanding of the specific microbial actions on complex mixtures in air streams and how these actions affect the toxicity and mutagenicity of the air stream is needed. To this end, toxicity or mutagenicity assays may be utilized as another measure to test the efficacy of the biofiltration process. Analogous to this, a more complete understanding of complex VOC waste streams and how they are affected by biofiltration would be useful for better understanding the actions taking place in a biofiltration unit. The objectives of

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this study were to: (i) characterize and quantify individual compounds in a complex air mixture derived from air stripping of a complex source of VOCs; (ii) assess the effect biofiltration has on the toxicity and mutagenicity of the VOCs in the air stream; and (iii) assess the microbial growth and metabolic profile of the biofilter inoculum recovered from an operating biofiltration unit.

Experimental

Sources of microorganisms and chemicals

All chemicals were analytical-grade and obtained from Aldrich Chemical Co (Milwaukee, WI, USA). P-9 oil [3] was used as the source of VOCs for the air stream and was obtained from the Institute of Wood Research at Michigan Technological University (MTU). P-9 oil is commonly used in the wood preserving industry as a carrier for oil-borne wood preservatives. P-9 oil contains numerous substituted benzene components, various straight-chain alkanes, and PAHs with the single most abundant compound being 2-methylnaphthalene.

Microbial inoculum

The two bacterial isolates used in this study (GK-622 and GK-104) were recovered during previous petroleum hydrocarbon biodegradation projects at MTU [14]. GK-622 was identified as *Pseudomonas fluorescens* and GK-104 was identified as *Alcaligenes xylosoxidans* using the Biolog system (Biolog, Inc, Hayward, CA, USA) [7]. The biofilter inoculum was prepared by adding 2 ml of a stock mixed bacterial culture (GK-622 and GK-104; ~50 : 50), stored at –70°C, to a 4-L Erlenmeyer flask containing 2 L of Bushnell-Haas Broth (BHB; Difco Laboratories, Ann Arbor, MI, USA) and 20 ml of P-9 oil (0.1% v/v; ~950 mg L^{-1}). Aluminum foil was utilized to loosely cover the flasks so that oxygen limitation was not a problem. The culture was allowed to grow for 7 days and then was centrifuged (Beckman, J2-21 Centrifuge, Minneapolis, MN, USA) at 15 300 × *g* for 10 min. The resulting supernatant was then discarded and the pellet resuspended in 250 ml of BH broth. This washing procedure was repeated twice and the final resuspension was made in 200 ml of BH to yield a 10-fold concentration of cells. This inoculum was then placed in a sterile 2-L Erlenmeyer flask containing 155.7 g of the solid support (described below). This 2-L flask was then placed on a gyratory shaker at 22°C and 200 rpm for 4 h. Microbial counts on the slurry were conducted using Tryptic Soy Agar (TSA; Difco Laboratories).

Biofiltration system

The biofiltration system was composed of a borosilicate glass column with a volume of 264 cm^3 (4.0 cm × 21.0 cm). Figure 1 illustrates the set-up of the biofiltration system. The solid support in the column was the biological attachment medium Celite R-635 (Celite Corp, Lompoc, CA, USA). Celite R-635 is a Diatomite/ceramic composite which has been used with success in previous biofiltration studies [22]. The VOCs in the air stream were generated by air-stripping volatile compounds from P-9 oil. Based on preliminary studies conducted at MTU, the P-9 oil was replaced after 60 days of operation to insure that some volatiles would not be stripped completely from the mixture. In addition, at day 60, 5.0 ml of BHB was added to the column to maintain moisture and supply some additional nutrients to the microorganisms. Air was humidified by combining the air stripped P-9/VOC air stream with another air stream which was purged through distilled water. An activated carbon column was installed between the system and the air compressor to insure that no VOCs observed in the system were a result of the air stream generation machinery. VOC sampling ports were located just prior to and just after the column. A flow rate of 19 ml min^{-1} was maintained at all times and was measured at points prior to and after the column using a Hewlet Packard (HP) Bubble Flowmeter (Hewlett-Packard, Palo Alto, CA, USA). All fittings, valves, and metal parts were brass, and all tubing was teflon.

Sample collection

For each sampling time, a small ORBO-32 coconut charcoal adsorption tube (Supelco, Inc, Bellefonte, PA, USA) was attached to the influent sampling port to collect an air sample for 15 min [18]. The charcoal tube was then immediately emptied into a 4-ml borosilicate, teflon-sealed vial containing 2 ml of methylene chloride. The vial was shaken for 1 min and allowed to extract for 30 min at ambient room temperature. The methylene chloride was then removed, using a Hamilton Series 1700 gastight syringe (Hamilton, Reno, NV, USA), and placed in borosilicate glass autosampler vials at 4°C until analysis. Analyses were performed within 48 h.

Analytical methods

Extracts were analyzed by a gas chromatorgraph (GC) (HP Model 5890) with a DB-5MS 30-m macrobore (0.25 mm diameter) bonded 5% diphenyl-dimethyl polysiloxane phase column (J&W Scientific, Folson, CA, USA), and a flame ionization detector (FID). Identification of the specific components in the VOC mixture was performed with a GC (HP Model 5890 Series II; Palo Alto, CA, USA) with a mass selective detector (Hewlett Packard Model 5874; GC-MS). Temperatures for all analyses were as follows: injection port, 280°C; FID, 280°C; and oven initially 40°C. The program allowed 40°C for the initial 3 min, then oven temperature increased 15°C min^{-1} to 150°C and held at 150°C for 3 min. Helium was the carrier gas at a flow rate of 1.7 ml min^{-1} and the purge vent was turned on at 0.5 min. Analytical standards were prepared and analyzed at the start of each set of analysis. An external standard was prepared for total VOC quantification (or area sum quantification) by distilling off and collecting the most volatile fraction of the P-9 oil. The compounds present in this distilled fraction were found to be >95% the same as the VOCs present in the air stream [16]. In addition to this external calibration standard, internal standards for the five major compounds in the air stream were included in the solvent so that accurate quantification of individual compounds could be performed. Working standards were prepared from stock solutions of a 10 mg ml^{-1} P-9 distillate (for total, or area sum, VOC quantification) and 10 mg ml^{-1} each for the five model compounds. The model compounds (and corresponding internal standards) were selected based

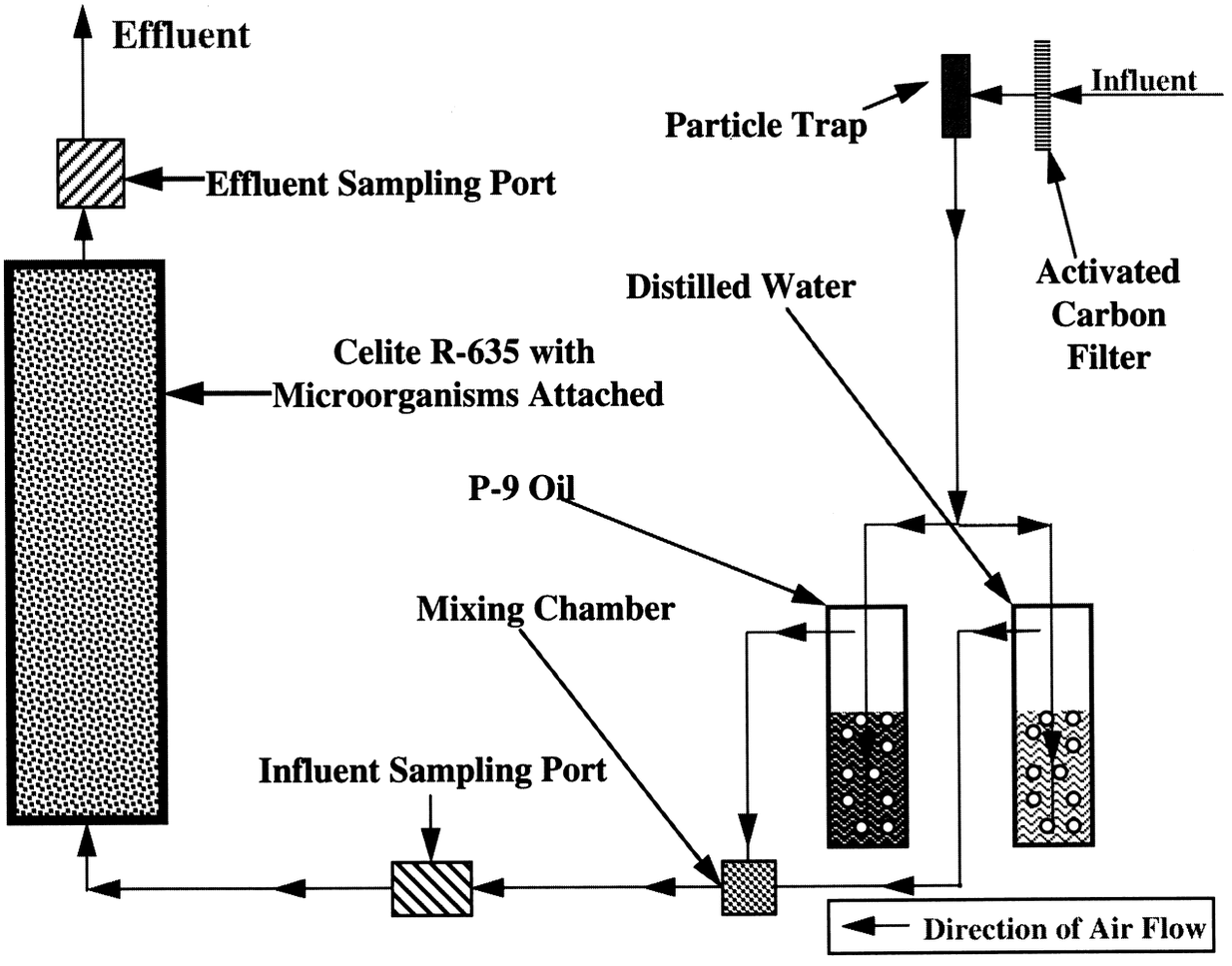

Figure 1 Schematic of biofiltration system.

upon their identification by GC-MS and the fact that they were major consitituents in the complex mixture. The two unidentified isomers were quantified using the identified isomer with most similar retention time. Separate calibrations were conducted, using internal standards, for each of the major compounds monitored in the VOC mixture. In summary, for each VOC sample analyzed both an internal standard calibration (one for each of the seven major compounds using the five internal standards) and an external calibration (for area sum quantification) were performed. All P-9 quantities are expressed as parts per million volume (ppmv). Limits of detection of this method were 0.5 ng μl^{-1} at the detector for all compounds.

Moisture content, porosity, and retention time determination

Moisture content of the Celite filter support in the column was determined by the difference in weights before and after drying samples of Celite at 60°C for 24 h at the end of the biofiltration study (after 130 days).

The actual porosity of the column was determined (after the 130-day study) using GC-FID (Buck Scientific, Stamford, CT, USA) by purging propane through the column at 91 ml min^{-1} and performing direct on-column injection of air samples at 0.5-s intervals. The oven temperature was a constant 100°C; detector temperature was 250°C; and the carrier gas (helium) was at a rate of 60 ml s^{-1}. An automated gas sampling valve (Buck Scientific) allowed 1 ml sampling of the air stream at 0.5-s intervals. Air time in the system lines was subtracted from the propane elution time in order to determine the actual retention time in the column. Porosity was calculated using the following equation:

$$P = (R_t \times F)/V_c$$

where P = porosity (%); R_t = retention time (min); F = flow rate (ml min^{-1}); and V_c = volume of column (cm^3).

Microbial enumeration

Five grams of the Celite solid support were removed from separate sections of the column at the end of the 130-day study. Celite was removed from 15 sections of the column (five in the upper third, five in the middle third, and five in the bottom third of the column). Five grams of the celite were placed in 125-ml Erlenmeyer flasks containing 25 ml of BH broth. These flasks were then placed on a gyratory shaker at 250 rpm for 30 min at ambient temperatures

(~22°C) to remove attached bacteria. The BHB from the flasks was then plated on TSA for bacterial enumeration and incubated at 26°C for 24 h.

Toxicity and mutagenicity studies

Air samples were collected as described above, with small ORBO-32 tubes. Toxicity and mutagenicity samples were collected according to previously described methods [2,15].

Toxicity analysis was performed per standard Microtox procedures for the Organic Solvent Solubilization method [4]. All Microtox data were reduced by on-line software and results are presented as the Effective Concentration (EC_{50}) in percent of the extract (ie, percent of the extract which causes a 50% light reduction from the organisms in the test) per 285 cm^3 of air sampled. Both 5-min and 15-min toxicity tests were performed. Mutagenicity analysis was performed using a modification of the microsuspension version of the *Salmonella*/microsome (or Ames) assay [5]. Tester strain TA98 with and without S9 metabolic activation was utilized. Ten microliters of sample were incorporated per plate. The results are reported as revertants per 285 cm^3 of air. Spontaneous revertant levels in DMSO were 42 (38 for test with S-9) revertants per plate for a 10-μl sample. The positive controls yielded 867 revertants for 2-nitrofluorene at a concentration of 400 ng $plate^{-1}$ in the test without S9. The positive control, 2-aminoanthracene, used in the test with S9 incorporation, yielded 191 revertants at a concentration of 97.5 ng $plate^{-1}$. Spontaneous, revertant levels were subtracted from all plate counts prior to graphing to provide net revertant levels.

Results and discussion

Characterization of the VOC stream

The GC-MS characterization of the VOCs within the air stream revealed that more than 100 different compounds were present in air-stripped P-9 oil VOC mixture [16]. However, the HP Chemstation database of compounds was only able to positively identify five major compounds in the mixture, ie, toluene, *m*-xylene, 2-ethyltoluene, 1,2,4-trimethylbenzene, and 1,3,5-trimethylbenzene. An unidentified xylene isomer and an unidentified trimethylbenzene isomer were also identified; however their specific structure was not determined as part of this study. All other peaks in the chromatograms were in too low abundance to be accurately identified. Because of the abundance of these seven compounds in the VOC mixture, they were chosen for individual monitoring for degradation.

Total VOC removal

The distillate fraction, which was used for quantification of the total VOCs, was found to contain greater than 90% of the same compounds which were found in the VOC samples taken from the air stream [16]. Thus, the responses of the distillate standard and the VOC samples were assumed to be equivalent in the GC-FID system [16]. Overall removal of VOCs in the biofilter is illustrated in Figure 2 as Elimination Capacity (g m^{-3} h^{-1}) *vs* Load (g m^{-3} h^{-1}). A second evaluation of the same biofiltration unit, set-up in an identical manner (Figure 2) yielded similar results. The total degradation, or VOC percent removal, of the system

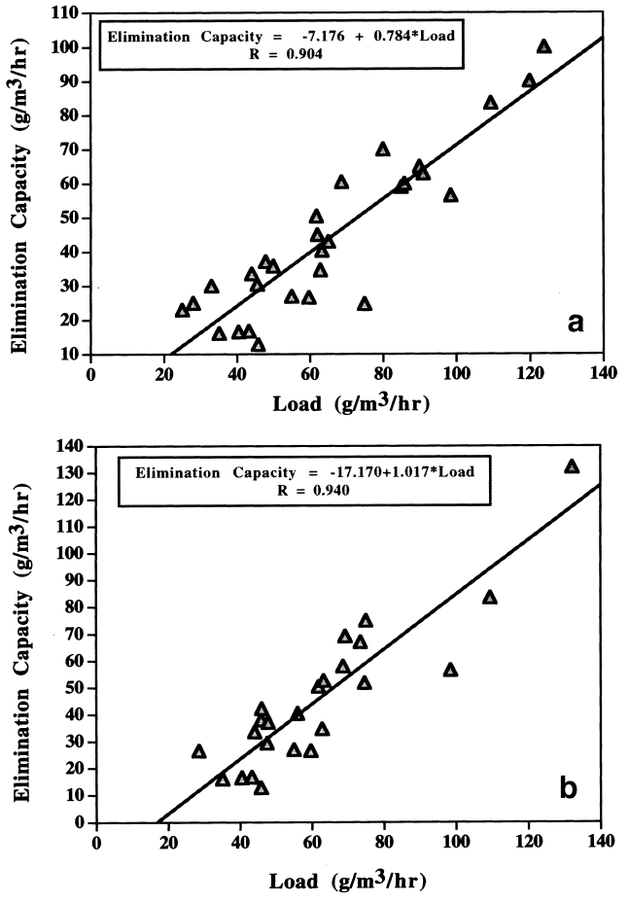

Figure 2 Overall VOC removal (a) Trial 1 and (b) Trial 2.

varied and was found to be as high as 100% and as low as approximately 5% (Figure 3). The overall average percent removal, over the 130-day study, was found to be 57% (s.d. ± 30.5). After 30 days of operation, the average removal increased to 69% (s.d. ± 23.9). The overall average ppmv of the influent was 320 (s.d. ± 93.7). Thus for the majority of its operation, the biofiltration unit was removing an average of 221 ppmv of VOCs per 264 cm^3 of total biofilter column volume.

Figure 3 illustrates the percent removal of the VOCs over

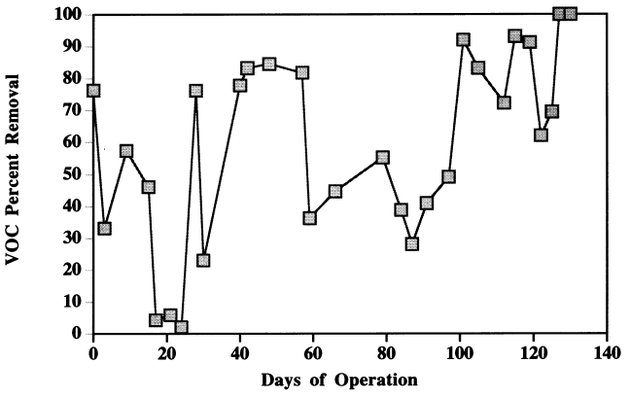

Figure 3 Total VOC percent removal (-▫-).

time showing the overall increase in percent removal as the column operated. The increases and decreases of the influent VOC concentrations over time were a result of inconsistent room temperatures, which fluctuated as much as 8°C. The flow to the biofilter was controlled on a flow basis and not a VOC mass basis. The P-9 oil in the system was changed 60 days into the study to prevent the complete stripping of highly volatile compounds from the mixture. This change did not affect the influent concentration, but a small decrease in removal efficiency occurred at day 60 and may have been a result of the P-9 oil change. Five milliliters of BHB were also added to supply the microbial population with some additional nutrients and did not appear to greatly affect biofilter performance.

Upon completion of the study (both trials), GC-MS analysis of extracts from the Celite resulted in a lack of identifiable petroleum compounds. In addition, when the consortium of organisms was grown on vapor phase petroleum hydrocarbons (PHCs) as the sole-source of carbon and energy [14], an extract of the biomass, analyzed by GC-MS, revealed no identifiable compounds. Thus, we conclude that the material entering the biofilter is being biodegraded and not adsorbed or bioaccumulated by either the microbes or the biofilter solid support.

While the overall degradation in the column was found to be 57% (69% after the first 30 days of operation) and this is less than the desirable 100% degradation, it is substantial given the column volume is only 264 cm^3. The average influent concentration was approximately 320 ppmv, meaning giving an average of 181 ppmv degraded (221 ppmv after 30 days) in the column at any given time. This is important since the smallest column we could find used in the literature contained a volume 100 times greater than the column discussed in this paper, and its removal efficiency was below 50% hydrocarbon reduction at influent concentrations ranging from 200–1000 ppmv [20]. This illustrates the overall effectiveness of the microbes and the solid support of this unit.

Specific compound degradation

The degradation of the major compounds within the complex mixture varied from 49% to 90%. The seven compounds monitored were (in elution order on the GC-FID): toluene, *m*-xylene, an unidentified xylene isomer, and unidentified trimethylbenzene isomer, 1,2,4-trimethylbenzene, 2-ethyltoluene, and 1,3,5-trimethylbenzene. Toluene and 1,3,5-trimethylbenzene showed the most complete degradation with average percent removals of 90% (Table 1). After the first 30 days of biofilter operation, toluene and 1,3,5-trimethylbenzene demonstrated removal rates of 100% and 96%, respectively.

The average percent removals of *m*-xylene, an unidentified xylene isomer, an unidentified trimethylbenzene isomer, and 1,2,4-trimethylbenzene are also listed in Table 1. With the exception of the unidentified xylene isomer, all compounds showed average percent removals greater than that of the total VOC percent removal. Again, after 30 days of biofilter operation these compounds demonstrated higher removal rates and less variability in the removals (Table 1). Levels of 2-ethyltoluene were monitored, but the data are not included in any calculations because its very low concentration caused a lack of accurately quantifiable data points. The levels of this compound were so low that only five out of a total of 31 samples showed enough of the compound to accurately quantify. The average removal of these five samples was 69% and the average analyte level in the five samples was 1.8 ppmv. After the initial 30 days of operation the average removal of the five monitored compounds (toluene, *m*-xylene, unidentified trimethylbenzene isomer, 1,2,4-trimethylbenzene, and 1,3,5-trimethylbenzene) was 92%; a 20% increase in removal efficiency.

Table 1 Degradation of each of the seven monitored compounds and total VOCs

Compound	Average percent removal (%)[a]	% Removal after 30 days[a]	Avg. concentration (influent ppmv)[a]
Toluene	90 (±28.3)	100 (±20.3)	8.3 (±3.5)
m-Xylene	85 (±45.5)	94 (±17.7)	18.8 (±4.1)
Xylene isomer	49 (±45.5)	57 (±42.6)	7.6 (±1.4)
TMB isomer[b]	71 (±47.4)	86 (±29.5)	4.6 (±1.3)
1,2,4-TMB[c]	63 (±69.7)	85 (±39.1)	3.3 (±2.5)
2-Ethyltoluene	69 (±77.3)[b]	100 (±0.0)[b]	1.8 (±0.6)[b]
1,3,5-TMB	90 (±26.8)	96 (±15.3)	5.4 (±1.5)
Total VOCs	57 (±30.5)	69 (±23.9)	320 (±60.5)

[a]Mean ± s.d. of 30 samples (30 influent and 30 effluent).
[b]2-Ethyltoluene calculation only included five samples due to other samples being below acceptable detection and/or quantification limits.
[c]TMB = Trimethylbenzene.

As illustrated by Figure 4, after 20 days of biofilter operation, with influent concentrations ranging from 4–16 ppmv, no toluene was detected in the effluent. Similarly, after approximately 20 days of operation, *m*-xylene (Figure 5) showed only 2 days of measurable analyte in the effluent stream. In fact, after day 40 of the operation, *m*-xylene was detected in the effluent on only 2 out of 21 days. The unidentified trimethylbenzene isomer, 1,2,4-trimethylbenzene, and 1,3,5-trimethylbenzene (Table 1) also showed much more consistent and complete removal after 30 days of operation. The unidentified xylene isomer was the only exception, having inconsistent removal and having the lowest overall percent removal at 49%.

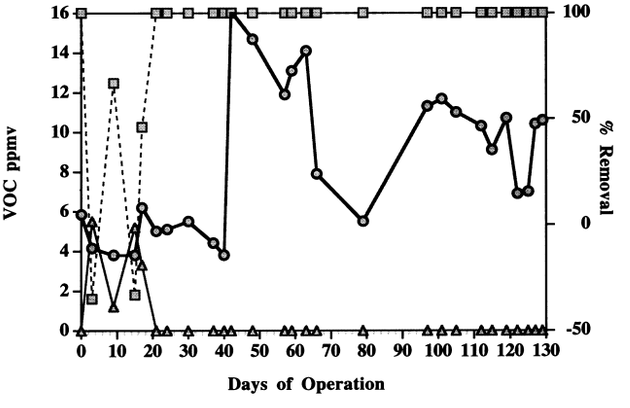

Figure 4 Toluene removal from the complex VOC mixture. (–●–) Influent ppmv; (–△–) effluent ppmv; (---□---) % removal.

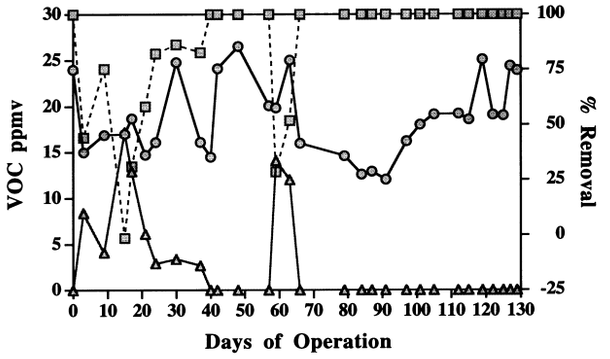

Figure 5 *m*-Xylene removal from the complex VOC mixture. (–●–) Influent ppmv; (–△–) effluent ppmv; (--□--) % removal.

Three of four most abundant compounds (ie, ppmv concentration) in the influent VOC mixture were degraded with the most efficiency, again with the unidentified xylene isomer being the exception. Table 2 shows the rank order of each compound as it relates to overall percent removal, average ppmv concentration in the influent air stream, and volatility. The three compounds with the highest concentration were also the three most degraded in the system. All three of these compounds showed 84+% removal, with two of the compounds over 90% (Table 1). Furthermore, with the exception of 1,3,5-trimethylbenzene, percent removal mirrored the order of volatility.

The specific compounds which were monitored in the complex mixture showed highly varying degradation rates. This was to be expected due to the differences in solubility and the microorganisms' metabolic abilities to degrade these compounds. The solubility of the compounds in the water phase is a leading factor in their ability to be degraded within biofiltration systems [17]. Interestingly, the solubility did not appear to be the major factor in removal efficiency of the compounds. The unidentified xylene isomer is more soluble (no matter what isomer) than any of the trimethylbenzenes, yet the xylene isomer had only about one-half the average percent removal of the 1,3,5-trimethylbenzene compound. This suggests that in this situation the metabolic capabilities of the microorganisms present may have been the limiting factor in the degradation of these compounds, and not the solubility. This is a finding other researchers have also noted [11].

Table 2 Rank order of each compound in percent removal, concentration in the influent air stream, and volatility

Compound	Volatility[a] (1–5)	Concentration[c] (1–5)	Removal rank (%)[c]
Toluene	1	2	1
1,3,5-Trimethylbenzene	5	3	2
m-Xylene	2	1	3
Trimethylbenzene isomer[b]	3[d] 4	4 5	4 5
1,2,4-Trimethylbenzene			

[a]As determined by GC retention time in the DB-5MS column.
[b]Unidentified trimethylbenzene isomer.
[c]2-Ethyltoluene was excluded due to an insufficient number of quantifiable data points; the unidentified xylene isomer was not included as it did not show greater removal than the total VOC percent removal.
[d]Assumed to be 1,2,3-trimethylbenzene.

It is difficult to compare removal efficiency of these compounds with those found in other studies. If researchers wanted to monitor toluene, for example, in a biofiltration system they would simply use only toluene in the influent air stream [8]. In at least two other cases of evaluating a complex mixture of air-stripped compounds, the investigators chose to monitor total VOCs and not individual compounds [10,20]. Thus, comparing the removal efficiency of toluene, for example, in this type of complex mixture to a system that was designed for only toluene removal would be difficult and possibly erroneous. To our knowledge this is the first biofiltration laboratory study incorporating the monitoring and quantification of specific compounds within a complex VOC mixture.

Moisture content, porosity, and retention time determination

The actual moisture content of the column was determined to be 40.5%. This is well within the recommended range for effective biofiltration [17]. The actual porosity of the column as determined by the propane test (described previously), was 40.0%. The actual retention time in the column was determined to be 3.0 min.

Microbial growth

The initial microbial inoculum contained 3.79×10^8 bacteria (CFU ml^{-1}), with approximately equal amounts of each isolate. A check of the Celite 8 h after inoculation revealed that there were 7.22×10^3 bacteria (CFU ml^{-1}) which could be recovered from 5 g of Celite (or 1.44×10^3 CFU g^{-1} of Celite). These microbial counts increased in all three regions of the column tested at the end of the 130-day study. As was expected, the recovery of bacteria from the bottom (influent), middle and top (effluent) regions of the column yielded very different microbial counts. The top region (effluent region) contained 1.88×10^7 CFU g^{-1}, 2.70×10^7 CFU g^{-1} in the middle region, and 5.27×10^8 in the bottom region (influent region). While no enumeration was conducted, there was a significant amount of biomass found immediately surrounding the inlet gas area. This concentration of biomass did not result in any pressure drops during operation of the system, but was the largest visible concentration of microbial growth within the column. There were only two colony types recovered on the enumeration TSA plates. The two colony types visually appeared to be the same two isolates (GK-622 and GK-104) which were originally inoculated in the column and were in approximately the same proportion (ie 50 : 50) for all samples examined.

The number of microorganisms was more than an order of magnitude higher at the influent region of the column than at the upper regions of the column, suggesting that this is where most of the biodegradation was taking place within the column. This was to be expected as other researchers have also noted that biomass accumulation at the influent regions of biofilters may cause pressure drops in systems [17]. The solid support chosen for use in this study, Celite R-635, appeared to provide enough intraparticle porosity to allow for sufficient microbial growth, while

still allowing an adequate amount of interparticle porosity to allow for contact of the analytes in the air stream with the liquid phase in the column. Another study [22] pointed to the advantages of Celite over some other types of solid supports. While we did not compare Celite R-635 to other solid supports in this study, we found that it did serve as an excellent solid support. From the start-up of the biofiltration unit to shut-down, microbial growth was visibly present in all areas of the column, there was no compaction of the packing material, and no pressure drops were observed due to changes in interparticle porosity.

Toxicity and mutagenicity results

EC_{50}s were calculated for each of the influent samples (Figure 6); however, at no time was the software able to calculate an EC_{50} for any of the effluent samples. All samples were standardized for 285 cm^3 of air sampled. Preliminary work at MTU revealed that this level would allow for accurate detection of influent toxicity/mutagenicity. Larger or smaller amounts of air would not produce reliable results in the Microtox assay. Figure 6 shows the EC_{50}s in the influent for the 5-min test ranged from 0.57 to 0.81 (15-min test data are not presented here). The toxicity of the influent samples, while remaining somewhat constant, decreased slightly from an initial EC_{50} of 0.60 to 0.76 after 35 days of operation. The toxicity of the influent then showed a slight increase in toxicity to an EC_{50} of 0.56 on day 68 after the P-9 oil was changed in the system. Overall, toxicity results demonstrated a good relationship with the VOC removal data, indicating that the degradation in the biofiltration system is effective in reducing the toxicity of the air stream.

The mutagenicity data further substantiated the trends observed in the toxicity testing and VOC degradation evaluation. Figure 7 shows the mutagenic response reported as net revertants (tester strain TA98 without S9) per 285 cm^3 of air sampled. The influent mutagenic activity was always higher than in the effluent, with the highest mutagenic activity in the effluent observed at days 7 and 14 of the operation. This corroborated the VOC degradation data (Figure 2), in that during this period of operation the system had not stabilized and was showing inconsistent VOC degradation. At no time after 20 days of operation did net revertant levels of the effluent exceed 15 revertants per plate. Similar to the toxicity results, influent (and effluent to a small amount) revertants increased slightly at day 68 of operation, or immediately after the incorporation of new P-9 oil into the system.

Both the toxicity and mutagenicity data corroborated the GC quantification of VOC removal. As far as we are aware this is the first attempt to incorporate direct toxicity testing into a biofiltration study. While there was an obvious difference in toxicity levels between the influent and effluent samples, the limits of this test system may have been reached. The manufacturer's method for organic solubilization allows for the use of a maximum of 1% organic solvent in the test. In order to detect some toxicity level in the effluent samples we attempted using a higher concentration of solvent (and thus analyte), but were unable to obtain any results due to toxicity problems with the solvent in the test. The Microtox test is an aqueous-based toxicity test and the application of highly volatile air-borne samples may be exceeding its capabilities. However, given the consistent results on the toxicity of the influent samples it is assumed that it performed well enough to provide a screen for reduced toxicity as a result of the biofiltration process. Based upon other work completed at MTU [15], we are confident the samples incorporated into the Microtox test accurately reflected what was actually present in the air stream. Future work will be undertaking to further develop these procedures (and novel ones) and apply this method to other types of VOC samples.

Similarly, the mutagenicity results were in agreement with the toxicity and VOC degradation data. While the biofilter was stabilizing (ie, after the first 20 days of operation) there was some mutagenic activity observable in the effluent samples, albeit much lower than the influent samples. After the initial 20 days of operation the effluent samples possessed revertant levels similar to spontaneous revertant levels. Mutagenicity levels of the effluent samples rose slightly after day 60, when the P-9 oil was changed, and then dropped after day 71. This very slight increase was presumably due to introduction of a fresh source of P-9 oil. Overall, the toxicity, mutagenicity, and VOC removal corroborated with one another in determining the

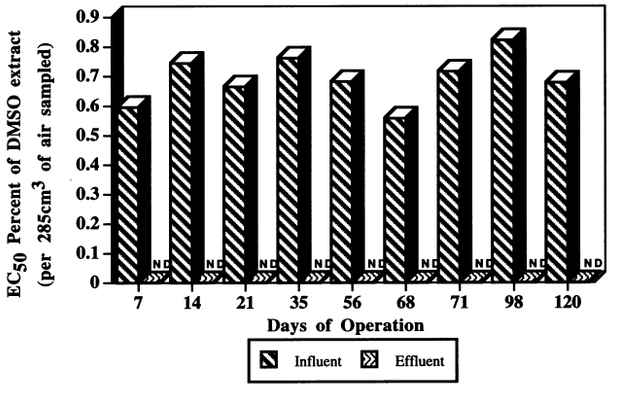

Figure 6 Toxicity of biofilter influent and effluent samples. No effluent samples had detectable toxicity. ND = Not determinable under these test conditions and at this sample concentration.

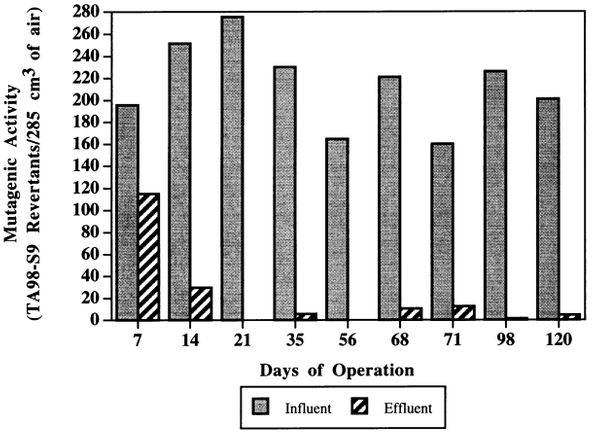

Figure 7 Mutagenic activity of influent and effluent biofilter VOC samples.

biofilter was successful in removing VOCs and reducing the ecotoxicity of the air stream.

This biofiltration study is unlike any other previously reported in terms of the types of information collected. This study incorporated several factors which were novel to biofiltration system monitoring. Analytically we were able to identify, monitor, and quantify individual compounds within a complex VOC mixture, as well as quantify the total VOCs. The data on specific compound degradation aided in determining the various rates of degradation for each compound and not just an overall rate for the complex mixture. We were able to speculate on limiting factors of degradation (eg, solubility issues, microbial metabolism, etc). In addition, we were able to directly measure decreases in toxicity and mutagenicity of the VOCs in the air stream as a result of the biofiltration process. The toxicity and mutagenicity data appeared to support the efficacy of the biofiltration process. However, the toxicity sample collection methods utilized in this study are currently being improved, and greatly revised, so that better and more quantitative data may be obtained in the future with more complex systems. These new approaches to monitoring biofiltration systems will allow future researchers to rapidly monitor more parameters in the operating biofiltration units and consequently obtain more data on the degradative processes. Finally, given the success of the column of the size used in this project, it may prove possible to design a small biofiltration unit, or several small units in series, which could be taken and utilized at any site where biofiltration is needed. Size has always been, and is currently, one of the major factors precluding the use of biofilters at many sites where VOC treatment is required.

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