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Field-scale biofiltration of gasoline vapors extracted from beneath a leaking underground storage tank

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Abstract

Approximately 15000 L of unleaded gasoline were released into the surrounding vadose zone from a leaking underground storage tank. Initial remediation was by soil vapor extraction and combustion which soon became cost prohibitive, as added propane was required to reach the combustion limit of the extracted vapors. As a cost effective alternative, a field-scale compost based biofilter was used in conjunction with soil vapor extraction to remediate the vadose zone. The biofilter was constructed on site using 4:1 diatomaceous earth:composted horse manure. Results of a five month study showed that the biofilter removed approximately 90% of total petroleum hydrocarbons (TPH) and >90% of the BTEX compounds (benzene, toluene, ethylbenzene, xylene), achieving the stringent permit requirements set at either 90% TPH reduction or less than 1.36 kg per day of volatile organic compounds (VOC's) released to the atmosphere. The biofilter showed the capacity to readily adapt to changing environmental conditions such as increased contaminant loading, and variations in temperature and moisture. The bacterial population in the biofilter was uniformly diverse throughout the biofilter, suggesting that a consortium of bacteria was needed for efficient biodegradation. The cost of biofilter set up and operation saved 90% in the first year alone of the operating expenses incurred by soil vapor extraction and combustion.

Introduction

A recent study estimates that 300,000 soil and groundwater contamination sites across the United States exist as the result of leaking fuel storage tanks (Dowd, 1994). Such spills have resulted in contamination of the vadose zone, with the potential for leaching into the saturated zone and spreading further with groundwater flow. Among the contaminants present in significant quantities in these sites are benzene, toluene, ethyl benzene, and xylene (BTEX); all U.S. EPA priority pollutants.

The site documented in this research is typical of many found in the United States. A connecting pipe to an underground gasoline storage tank ruptured, and over a two year period released approximately 15000 L of unleaded fuel into the surrounding vadose zone. Soil vapor extraction (SVE), known to be an effective method for removing gasoline from unsaturated soils, was implemented at the site (Miller 1992). Initially, the extracted vapors were treated by incineration using an internal combustion engine. During this time levels of extracted vapors were high (approximately 110,000 $\mu g\ L^{-1}$), but after approximately 3 months of treatment, the concentrations of extracted TPH were reduced to approximately 9800 $\mu g\ L^{-1}$. As treatment progressed it became apparent that the treatment cost was becoming prohibitive. This was a result of two factors. First, as the TPH concentration decreased, it became necessary to add significant quantities of propane to achieve the vapor combustion limit. Second, it became apparent that low levels of vapors would be extracted far beyond the initial estimate of a two-year treatment for the site.

An alternative to incineration is biofiltration. Biofiltration uses a solid medium to support microbial populations. These populations can degrade the contami-

nants of a waste gas stream passed through the medium. The use of biofiltration for odor control and removal of volatile organics is well documented in Europe (Leson & Winer 1991; Atlas 1995) and to a lesser extent in the United States (Bohn 1992). In this study, biofiltration was chosen as an alternative, low cost method for treating the extracted vapors. Maricopa County, AZ issued a permit of operation for the biofilter which required that the following minimum standards be met; 90% reduction of contaminants or less than 1.36 kg per day of volatile organic compounds (VOC's) released to the atmosphere.

Successful bioremediation of petroleum hydrocarbons is known to depend on biotic as well as abiotic factors including temperature, moisture content, oxygen, and concentration of contaminant. The objectives of this research were to:

- measure the effects of the operating parameters including flow rate, contaminant concentration, temperature and moisture on TPH and BTEX removal in a field-scale biofilter;
- enumerate the bacterial population in the biofilter medium and select bacterial isolates that degrade TPH and BTEX.

Biofilter performance was evaluated over a five-month period, during which time three influent contaminant concentrations were sequentially stepped through four flow rates.

Materials and methods

Biofilter design

The biofilter was a 9.75 m (32 ft) half-cylinder (diameter, 2.44 m (8 ft)) constructed from a used underground steel storage tank. Polyvinyl chloride (PVC) piping (Payless Hardware, Phoenix, AZ) perforated at 46 cm intervals was placed along the length of the bottom of the biofilter. A 30 cm layer of washed pea gravel (Pioneer Landscape, Gilbert, AZ) was placed over the PVC piping. A 91 cm layer of biofilter medium [a 4:1 mixture of diatomaceous earth celite No. 379 (Synergistic Performance Corp., Oakland, CA) and composted horse manure with mulch (GRO-GREN Supply, Phoenix, AZ)] was placed over the pea gravel. Finally, a 15 cm layer of a 4:1 mixture of coconut based 4 x 10 mesh activated carbon and composted horse manure was placed on top (Figure 1). The biofilter material had a pH of 7.3 (determined by the saturated paste method, Page et al., 1982), a nitrogen content of 0.35%, total carbon content of 4.0% and organic carbon content of 3.2% (determined by high temperature combustion using a Carlo Erba 1500 Nitrogen, Carbon, Sulfur Analyzer, Artiola, 1990).

Water was delivered to the biofilter material using soaker hoses that were placed at a 20 cm depth in the biofilter and the unit was watered when necessary. Three sets of vapor collection ports (1.3 cm PVC capped at the top and placed at 15, 46 and 76 cm), tensiometers (Soilmoisture Equipment Corporation, models 2725ARL06, 2725ARL18, and 2725ARL36, Goleta, CA), and thermocouples (Thermx Southwest, S-Class 18U, San Diego, CA) were emplaced across the length (north, center, south) of the cell at 15, 46, and 76 cm depths (Figure 1). In order to maintain a mass balance within the biofilter, the unit was kept as airtight as possible. This was done by covering the biofilter unit with a heavy duty tarpaulin cover that was securely taped to the unit sides. Three 61 cm x 61 cm plywood doors were built into the biofilter cover to provide access for sampling.

Contaminated vapors were piped from the vapor extraction unit into the bottom of the biofilter using 5 cm PVC piping for upflow operation. A soil vapor extraction exhauster (Invincible Air Flow Systems, Invincible model 300, Scottsdale, AZ) was used to extract the gasoline vapors from the vadose zone. Airflow from each extraction well and ambient air flow was controlled by an inline manifold which was used to adjust the concentration of influent contaminant.

Biofilter operation

The vadose zone consisted of fine- to medium-grained sand, and the water table was approximately 65 m below the surface. Exploratory wells had been drilled to depths of 26-28 m to define the extent of the plume, and these were used as vapor extraction wells. Biofilter operation was evaluated at three different influent concentrations (500, 1200, and 2700 $\mu g\ L^{-1}$) sequentially stepped up through four different flow rates (25, 55, 75, and 95 $m^3 h^{-1}$). Although the experiment was designed for twelve stepped loading rates, only nine were actually tested, as shown in Table 1, due to breaches of the permit requirements. Each contaminant concentration cycle was started at the lowest flow rate and allowed to equilibrate for 7 days. Subsequent flow rates were equilibrated for 5 days. When the cycle was complete for one concentration, the influent concentration was increased and the cycle was started again at the lowest flow rate. For each sampling event, influent and

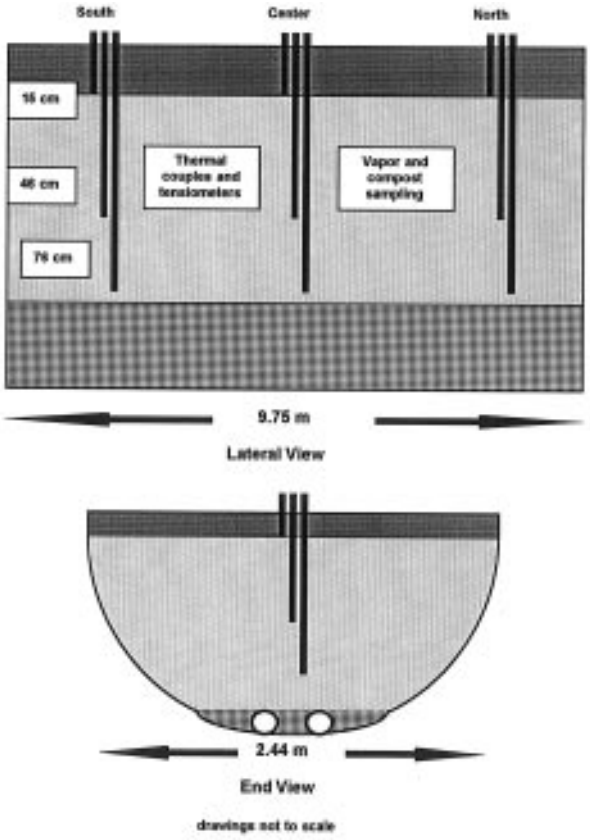

Figure 1. Schematic of the biofilter layout with lateral and end views.
15 cm 4:1 activated carbon:composted horse manure.
91 cm 4:1 diatomaceous earth:composted horse manure.
30 cm pea gravel.

effluent vapor samples were analyzed as well as vapor samples from the 15, 46, and 76 cm depths at the north, center, and south sites of the biofilter (Figure 1). In addition, temperature and moisture tension data were recorded, and biofilter medium cores were collected from each sampling site for microbiological and gravimetric moisture content analysis.

Due to heavy rains in the Phoenix area, the biofilter was shut down from day 94 to day 111 because the above ground junction point of the extraction wells became flooded with water. During this time a minimal flow of ambient air, approximately 3.5–5 m^3h^{-1}, was maintained. The experiment resumed on day 111 when the flow was set to 27.3 m^3h^{-1} and the influent vapor concentration was adjusted to 2755 μg L^{-1}. The system was allowed to equilibrate for 7 days at this setting.

Gasoline vapor analysis

Biofilter vapor samples were collected periodically from each sampling port by inserting a 0.31 cm diameter stainless steel rod into one of the collection ports. The tube was connected via tubing to a vacuum pump. The port was flushed for approximately 20 seconds and then a Tedlar™ bag (Analytical Technologies, Inc., Phoenix, AZ) was filled. Tedlar bags were stored in a dark plastic garbage bag to inhibit photodegradation of gasoline components.

Vapor samples were analyzed by gas chromatography (GC) using EPA method 8020/8015 modified volatile aromatics, including gasoline. A Hewlit Packard Co. 5890C gas chromatograph equipped with an ultra alloy-5 (5% phenylmethylsilicone) capillary column 30 m in length, inner diameter of 0.5 mm, and film thickness of 1.5 μm (Quadrex Corp., UA5-30V-1.5F, New Haven, CT) was used to analyze gas samples. The carrier gas was ultra high purity helium set at a flow rate of 30 ml min^{-1}. Hydrocarbons were detected using a flame ionization detector (FID) with ultra high purity hydrogen and breathing quality air set at flow rates of 30 ml min^{-1} and 300 ml min^{-1} respectively. Briefly, operating conditions were: initial temperature (T), 50°C; initial hold time, 8 min; ramp, 12°C min^{-1}; final T, 220°C; final hold time, 2 min; FID T, 250°C. The GC was calibrated using benzene, toluene, ethylbenzene, xylene, and gasoline standards (Alltech Assoc., Inc. Deerfield, IL). A 4-point calibration curve was analyzed for BTEX and gasoline. BTEX mass standards analyzed were 25, 50, 100, and 300 ng (EPA method 8020/8015). Gasoline mass standards analyzed were 500, 2500, 5000, and 10000 ng (EPA method 8020/8015). An internal standard, α,α,α – trifluorotoluene, was injected at a mass of 0.25 μg, and a surrogate standard, 4,4-bromofluorobenzene was injected at a mass of 1.25 μg.

Elimination rate

Rates of contaminant removal were determined from influent and effluent air phase concentrations, airflow rate, and the volume of the biofilter material using the following equation (Hodge & Devinny, 1994):

$$E_R = (C_{infl} - C_{eff}) \times \frac{Q}{Volume}$$

where: E_R = elimination rate, g m^{-3} h^{-1}; C_{infl} = influent concentration g m^{-3}; C_{eff} = effluent air phase con-

Table 1. Total petroleum hydrocarbon (TPH) removal efficiency

Day	Contaminant concentration (μg L^{-1})	Flow rate (m^3h^{-1})	TPH[1] removal at 15 cm (%)	TPH removal total effluent (%)
0	0	96.0	n.d.[2]	n.d.
28	0	96.0	n.d.	n.d.
35	0	96.0	n.d.	n.d.
55	470	26.3	79.6	89.4
60	500	56.1	88.4	90.0
65	480	70.2	89.4	89.6
70	440	96.0	88.6	88.6
77	1320	23.6	74.2	88.9
82	1040	52.0	87.7	89.9
87[3]	1440	76.0	75.2	78.5
94[4]	n.d.	<5	n.d.	n.d.
111	2755	27.4	n.d.	n.d.
118	2755	27.4	79.8	90.0
123[5]	2785	55.4	86.5	91.1

[1] The lower detection limit for TPH was 50 μg L^{-1}. Each value at 15 cm is the average of the concentration determined at the North, Center, and South sampling sites.
[2] n.d. = not determined
[3] Highest flow rate was not tested since permit conditions were not achieved.
[4] Due to heavy rains, the junction point of the extraction wells flooded stopping vapor flow. The experiment was shut down for 17 days to allow wells to clear. At this time, the lowest flow rate with the highest influent concentration was initiated and equilibrated for 7 days.
[5] Higher flow rates were not tested.

taminant concentration, g m^{-3}; Q = flow rate m^3 h^{-1}; Volume = volume of biofilter packing material, m^3.

Microbial analysis

Biofilter medium cores were taken using a 2.54 cm Oakfield® probe (Oakfield, Inc.) that was sterilized with ethanol between sample collections. Core samples were placed into Whirlpak® (Nasco) bags and stored on ice for transport to the laboratory. Serial dilutions in 0.1% peptone were made starting with 10 g samples of biofilter medium and used to determine viable plate counts. Viable counts of biofilter microorganisms were performed using two media, R2A® (Difco Laboratories, Detroit, MI) for total heterotrophic counts, and mineral salts medium (MSM) with hexadecane (MSM-C_{16}) as sole carbon and energy source for aliphatic hydrocarbon degraders. MSM contained the following (L^{-1}): Na_2HPO_4 (4.0g), KH_2PO_4 (1.0g), NH_4Cl (1.0g), $mgSO_4$ (0.2g), yeast extract (0.005g), ammonium iron(III) citrate (0.005g), and $CaCl_2$ (0.01g). Hexadecane was added at a concentration of 0.1% (v/v). Plates were incubated at 25°C for 5 days and enumerated. Colonies of interest from the spread plates of each type of media were re-streaked for isolation. Acridine orange direct counts were made to determine total bacterial numbers (Brendecke et al. 1993).

To evaluate the ability of isolated bacteria to degrade unleaded gasoline, a total of 27 and 28 isolates from the first and last sample day respectively were used to inoculate 10 ml of MSM broth amended with either 0.1% or 0.01% (v/v) of unleaded gasoline. These were incubated at 25°C with shaking and growth was indicated by turbidity. To determine degradation of BTEX, isolates were incubated at 25°C on MSM plates in an individual atmosphere of each of the BTEX compounds. Biolog® was used to identify these isolates as well as other bacteria isolated during the experiment.

Results

Gasoline vapor analysis

Table 1 shows the removal of TPH during the course of this study. Removal at the 15 cm depth (just below

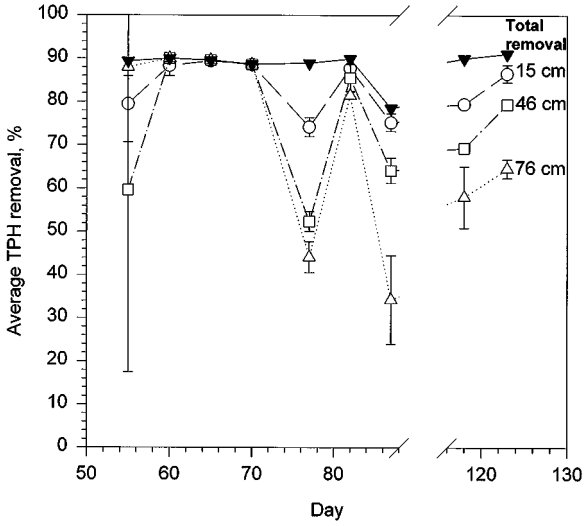

Figure 2. The percent removal of TPH from the 15, 46, and 76 cm depths as averaged from three sample sites within the biofilter, the total effluent removal, and error as indicated. The lower detection limit for TPH was 50 μg L^{-1}.

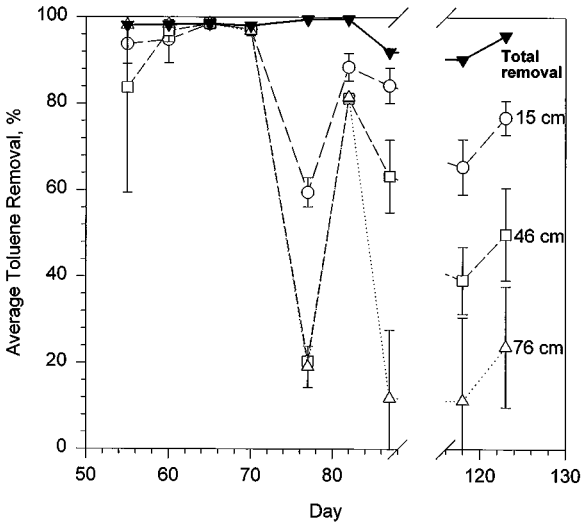

Figure 3. The percent removal of toluene from the 15, 46, and 76 cm depths as averaged from three sample sites within the biofilter, the total effluent removal, and error as indicated. The lower detection limit for toluene was 0.5 μg L^{-1}.

the activated carbon layer), which represents biological removal, ranged from 74.2 to 89.4%. However the actual effluent, which was further treated by the top 15 cm layer of activated carbon/composted horse manure mixture, reached 88.6 to 91.1% TPH removal, allowing permit requirements to be met. A removal of greater than 88% was achieved from day 55 (the start

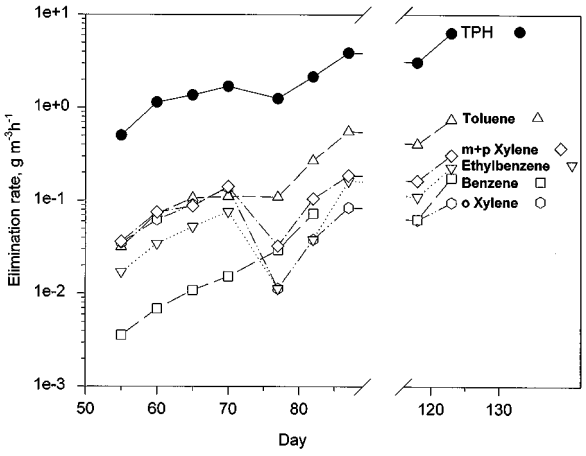

Figure 4. Total elimination rate for all contaminants; TPH and BTEX.

of effluent sampling) through day 87. At this point the contaminant concentration was 1440 μg L^{-1} and the flow rate was increased to 75.9 m^3h^{-1}. At this loading rate of 5.0 X 10^3 g m^{-3} h^{-1}, only 78.5% TPH was removed. This was considered breakthrough and the final and highest flow rate for this concentration was not tested. Similarly, for the highest contaminant concentration tested (2755–2785 μg L^{-1}) breakthrough occurred immediately for the third flow rate that was tested (74.8 m^3h^{-1}) representing a loading rate of 7.0 X 10^3 g m^{-3} h^{-1}. As a result the experiment was discontinued.

The fate of TPH and the four individual BTEX components moving through the biofilter was similar (as shown in Figures 2 and 3 which show the removal of TPH and toluene). In general, the contaminant removal increased as the contaminant traveled up through the biofilter. However, the first four sampling periods for the both total TPH and individual total BTEX compounds showed >89% removal irrespective of depth. The removal of benzene, ethylbenzene, and xylene was very similar to toluene with total removal >90% (data not shown).

Elimination rate

Elimination rates were calculated to analyze the efficiency of the biofilter in removal of TPH and BTEX over the course of this study. As shown in Figure 4, elimination rates increased over the course of the study for all components measured. The relationship between loading rate and elimination rate is shown in Figure 5. The elimination rate consistently increased

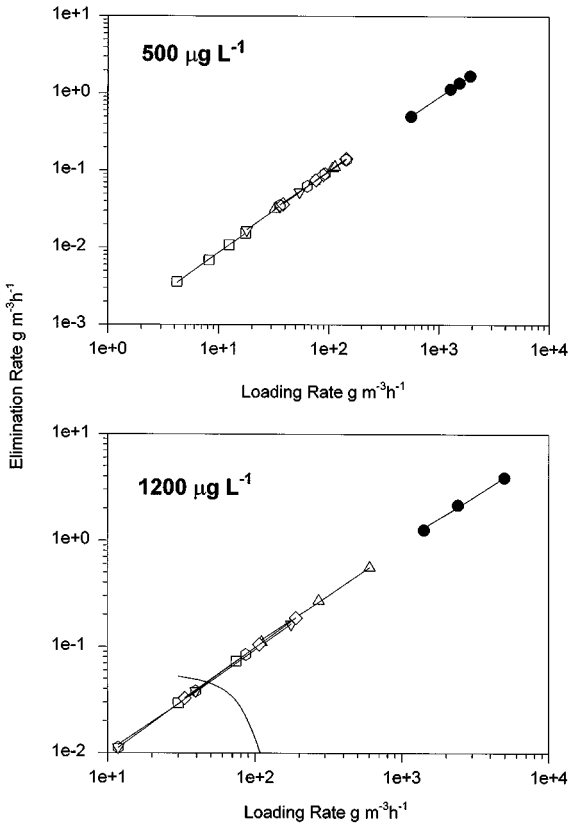

Figure 5. Elimination rate versus loading rate for the low, 500 μg L^{-1}, and medium, 1200 μg L^{-1}, influent contaminant concentrations. Experimental sample values are shown with symbols: ● TPH, □ benzene, △ toluene, ▽ ethylbenzene, ◇ m&p-xylene, and o-xylene; regression as the line. The poor fit for benzene at the medium concentration, 1200 μg L^{-1}, resulted from the total effluent removal showing zero removal at one point. Regressions were not determined for the highest concentration because there were only two data points taken before breakthrough occurred. The linear regression equations used for all contaminants were:
$y = (9.5e_4 \pm 4.9e_5)x + (1.8e_3 \pm 4.3e_3)$ *at 500*μgL^1
$y = (8.3e_4 \pm 1.9e_4)x + (6.5e_2 \pm 1.2e_1)$ *at 1200*μgL^1

as the loading rate increased, indicating a correlation (Figure 5).

Temperature and moisture analysis

Temperature measurement showed that although the temperature was uniform throughout the biofilter, the overall temperature increased steadily over the course of the 5 month experiment ranging from a low at day 55 of 11.0 ± 0.3°C to 29.1 ± 2.2°C on the final sampling day. This was not surprising since the experiment ran from November until April. In general, elimination rate increased with temperature and regression of these data

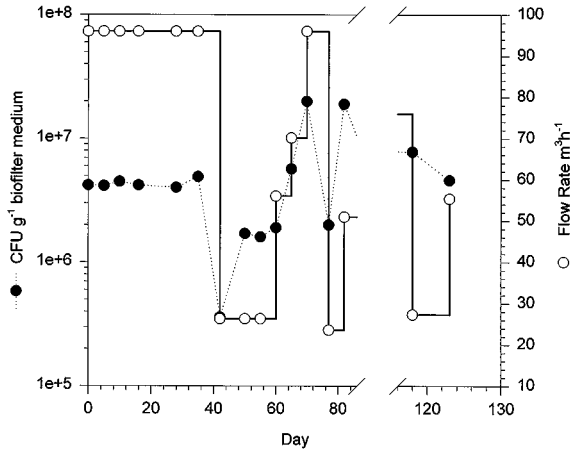

Figure 6. Viable plate counts of bacteria isolated on MSM-C_{16} medium from samples collected from the north 46 cm depth compared to the flow rate over the course of the experiment.

gave an r^2 = 0.7. Benzene, ethylbenzene, and xylene had even less correlation yielding r^2 values <0.7 (data not shown). Soil moisture varied within the biofilter as determined by either tensiometer readings or gravimetric analysis. The tensiometer readings were calibrated using water retention curves determined from a sample of the compost (Danielson and Sutherland, 1995). Gravimetric moisture determinations ranged between 50–90% for all samples and fluctuated by as much as 30% at some sample points over the course of the experiment (data not shown).

Microbial analysis

Following construction, the biofilter was flushed with ambient air for 35 days at 55 m^3h^{-1} to establish and equilibrate baseline microbial populations. Viable counts performed on Days 0, 5, 10, 16, 28, and 35 were similar and averaged 1.3 X 10^7 ± 2.3 X 10^6 CFU per gram of dry compost on R2A medium. Viable counts on MSM-C_{16} medium were approximately 1/2 log lower averaging 5.7 X 10^6 ± 8.3 X 10^5 CFU per gram of dry compost. Total direct microscopic counts were approximately 2 logs higher than viable counts, averaging 9.7 X 10^8 ± 1.8 X 10^8 cells per gram of dry compost. On day 35, the flow rate was lowered to 26.3 m^3h^{-1} and contaminant vapors were introduced at the lowest concentration. Results of periodic microbial plate counts are shown in Figure 7 for MSM-C_{16}. R2A counts showed a similar pattern but were 1/2 log higher (data not shown). No significant difference in either viable plate counts or in direct counts was observed

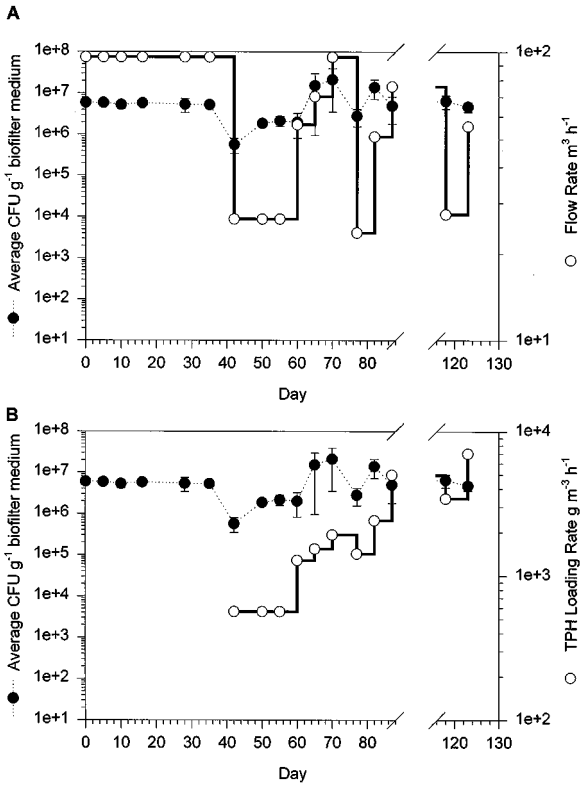

Figure 7. Average viable plate counts of bacteria isolated on MSM-C_{16} medium from samples collected from the north biofilter sampling site.*(A)* Compares the plate counts with flow rates used. *(B)* Compares plate counts with loading rates used. Depths as indicated and error bars are shown.

between the beginning and end of this experiment with direct counts averaging $3.3 \times 10^9 \pm 9.6 \times 10^8$ for the last day. However, there were significant fluctuations in viable plate counts during the experiment, for example MSM-C_{16} counts dropped by 1 log from day 28 to day 42 and then steadily increased again until Day 70, followed by a second decrease. These fluctuations appear to be related to changes in flow rate, and therefore loading rate, which showed similar patterns over the time course of the experiment (Figure 6). For example, the correlation between viable counts and flow rate for the first contaminant concentration tested had an $r^2 = 0.8$.

There were approximately 13 colony types with distinct differences in color and morphology, isolated on R2A media during each sampling. Differences in morphology and coloration were more limited on the MSM-C_{16} media; resulting in 9-10 distinct colony types each sampling period. Re-streaking isolates from MSM-C_{16} onto R2A resulted in some colonies expressing coloration after transfer. Gram stains were made from all isolated colonies and showed that Gram negative and positive organisms were present in equal numbers. The Gram stain information was used to screen the isolates further for Biolog identification. Successful Biolog identification was limited. Only six positive identifications were made from the eighteen isolates from the last sample day; those isolates that were able to use the unleaded gasoline as the sole carbon source. Horse manure inhibits growth of fungi, so no fungal inhibitors were added to either media. There were some fungal colonies on the plates, but these were not included in the viable counts. Actinomycetes were included in the viable counts, and were more prevalent on the MSM-C_{16} medium, although they were not the dominant population present.

Sole carbon source utilization studies of the bacterial isolates resulted in three groupings:

1) Isolates that could use unleaded gasoline, as well as each BTEX compound, as sole carbon source (see the top third of Table 2);
2) Isolates that could use one or more of the compounds (see the middle of Table 2); and
3) Isolates that were unable to use any of the compounds as sole carbon source for growth (see the bottom third of Table 2).

In the first group, there was a total of eleven isolates from day 0 and six from day 123. Of the eleven isolates from day 0 there were four pairs that exhibited the same morphology. One isolate had the same morphology, on the first sample day as on the last sample day. In the second grouping, there was a total of 26 isolates from day 0 and day 123. There were five pairs of isolates that had similar colony morphology from the first sample day and the last sample day. There was one pair from day 0 and a set of five from day 123 that appeared to be the same. The last group had eight isolates from day 0 and eight isolates from day 123, with two pairs that were similar between samplings.

Discussion

Effect of influent concentration

A total of nine loading rates were tested starting with the lowest concentration and flow rate. At the lowest concentration, 440–500 μg L^{-1}, 88% TPH removal occurred immediately, at the 76 cm depth (see days 55–70, Table 1 and Figure 2). This was likely due to high initial rates of sorption that occurred until

Table 2. Carbon source utilization of bacterial isolates taken from Day 0 and Day 123

Media and isolate # Day 0	Day 123	Unleaded	Benzene	Toluene	Ethyl-benzene	Xylene
MSM 4 & 6		+	+	+	+	+
MSM 5 & 7 & R2A 12		+	+	+	+	+
MSM 9 & R2A 13		+	+	+	+	+
MSM 10[1]	MSM 5[1]	+	+	+	+	+
R2A 5&7		+	+	+	+	+
R2A 14		+	+	+	+	+
	MSM 3	+	+	+	+	+
	MSM 7	+	+	+	+	+
	R2A 5	+	+	+	+	+
	R2A 6	+	+	+	+	+
	R2A 15	+	+	+	+	+
MSM 1	R2A 4	+	–	+	–	+
MSM 3	R2A 3	+	–	–	+	+
R2A 2		+	–	–	–	–
R2A 4&6	MSM 1	+	–	–	–	–
R2A 10		+	–	+	+	+
R2A 9	MSM 11	+	–	–	–	–
R2A 17	R2A 17	+	–	–	–	–
	MSM 2, 4, 6, 9 & R2A 13	+	–	–	–	–
	R2A 10	+	–	–	–	–
	R2A 11	+	–	+	–	+
	R2A 12	+	–	–	–	–
	R2A 16	+	–	–	–	–
MSM 2 & R2A 8		–	–	–	–	–
MSM 8		–	–	–	–	–
R2A 1		–	–	–	–	–
R2A 3		–	–	–	–	–
R2A 11	R2A 9	–	–	–	–	–
R2A 15		–	–	–	–	–
R2A 16	MSM 8	–	–	–	–	–
	R2A 1	–	–	–	–	–
	R2A 2	–	–	–	–	–
	R2A 7	–	–	–	–	–
	R2A 8	–	–	–	–	–
	MSM 10 & R2A 14	–	–	–	–	–

[1] Isolates listed in the same line had similar colony morphology and sole carbon source utilization patterns.

sorption sites were saturated. At the two successive influent concentrations tested, 1040- 1440 and 2755-2785 μg L^{-1}, removal of contaminant occurred at all depths. Removal ranged between 74.2 and 89.4% at the 15 cm depth, and was attributed to microbial activity. Additional contaminant removal from 0.2–10.2% was achieved after flow through the top activated carbon layer. Figure 5 shows that for the loading rates used during this experiment the biofilter removed TPH and BTEX with equal efficiency.

During the experiment, the two lowest measured total TPH removals occurred at day 70 (88.6%) and day 87 (78.5%, considered breakthrough). These samples occurred at the highest flow rates tested for each respective contaminant concentration, e.g., 96 m^3h^{-1} for the lowest contaminant concentration tested, and 76 m^3h^{-1} for the intermediate contaminant concentration (Table 1). Breakthrough was also observed at $>$55.4 m^3h^{-1} for the highest concentration tested. Despite the fact that there was higher absolute removal

Table 3. Cost comparison of SVE with combustion and SVE with biofiltration[1]

	Combustion	Biofiltration
Set up cost		$1000 PVC piping
		$1500 diatomaceous earth
		$4000 activated carbon
		$500 composted horse manure
		$250 tank[2] and cutting
Monthly maintenance	$200 monitoring[3]	$200 monitoring
	$17 electricity	$17 electricity
	$5000 internal combustion engine[4]	
	$2000 propane	
Year 1 of operation	$86,604	$9854
Monthly maintenance	$200 monitoring	$200 monitoring
	$17 electricity	$17 electricity
	$5000 internal combustion	
	$2000 propane	
Subsequent years of operation	$86,604	$2604

[1]Does not include setup costs for extraction wells and assumes that these would be equal for each type of remediation.
[2]The tank for this experiment was previously used.
[3]Cost of monitoring 2 samples per month.
[4]Assumes that an engine would be rented. A VR Systems V-3 internal combustion engine retails for approximately $75,000.

of TPH (Figure 5), the biofilter was not able to meet performance requirements (90% TPH removal) at these combinations of flow rates and contaminant concentrations. These results indicate that biofilter performance was not adequate at high flow rates.

Low flow rates also seemed to affect biofilter performance. For example, days 55, 77 and 118 correspond to the lowest flow rate tested for each contaminant concentration. On each of these days, biological removal (TPH removal at 15 cm) was lower than at higher flow rates (Table 1). There are several possible explanations for this behavior. The bacterial populations may have required a longer acclimation period to accomodate the decreased flow rate and simultaneous increase in contaminant concentration (Zilli et al. 1993). In addition, Ottengraf (1986) has described the phenomena of reduced contaminant removal, termed diffusion limitation, in his description of a biophysical model of biofilter biofilms. At decreased contaminant levels, the equilibrium concentration gradient formed around the medium used for biofiltration is driven by the diffusion rate of the contaminant into the biolayer. At low concentrations bioremediation is limited by this diffusion rate of the contaminants into the biolayer (Ottengraf 1986; Leson and Winer 1991; Zilli et al. 1996). Thus, at low contaminant concentration and high flow rate, residence times are too short for diffusion. In summary, there is an intricate interplay among the concentration, the flow rate and the bacteria that developed during the five months of bioremediation that precluded our being able to fully evaluate this complex field experiement.

Interestingly, the period of inoperation, from day 87 to day 111, did not seem to diminish biofilter efficiency, which immediately returned to $>$90% when contaminant vapors were reintroduced. This finding concurs with other published studies (Tang et al. 1995; Martin & Loehr 1996). Overall, flow rates that were either too low ($<$50 m^3h^{-1}) or too high ($<$70 m^3h^{-1}) resulted in suboptimal performance. Low flow rates resulted in decreased bacterial populations except for the final phase of the study and high flow rates resulted in residence times that were too short for the biodegradation of contaminant vapors and thus, breakthrough occurred. Since the only oxygen available to the biofilter for maintaining aerobic conditions was through the air manifold of the influent line, a calculation was made to determine whether the amount of available O_2 in the vapor phase was sufficient for complete biodegradation. It is known that TPH and BTEX are degraded by bacteria under aerobic conditions. Assumptions for this calculation included: 1) 21% of vapor influent con-

tained oxygen for all loading rates; 2) 50% of the TPH carbon was mineralized to CO_2; and 3) octane (C_8H_{18}) was representative of TPH. Using these assumptions and the relationship:

$$\underset{(TPH)}{C_8H_{18} + O_2 + NH_3} \rightarrow \underset{(cell\ mass)}{C_5H_7NO_2 + CO_2 + H_2O}$$

a mass balance was calculated for the amount of O_2 needed for complete oxidation of the compounds of interest. As an example, for each mole of TPH, 7.5 moles of O_2 would be needed for oxidation. Based on this, there was an excess of O_2 available in the vapor phase for mineralization at all contaminant concentrations and flow rates used. It should be noted that the diffusion of oxygen into the liquid phase of the biofilm could also have been limiting resulting in the development of anaerobic microsites within the compost material.

Hodge and Devinny (1994) showed that biofiltration using activated carbon as the filter medium had greater elimination rates than a compost that was tested, but a lower degradation rate and buffering capacity which could limit its long term effectiveness. Activated carbon that has been used for the adsorption of contaminants also creates the need to dispose of contaminated material. Column studies have shown that BTEX compounds were reduced by at least 75% in mixed contaminant waste streams and that to improve removal a series of biofilters may be required (Togna & Singh 1994). In this study, activated carbon was placed over the biological component of the system to sorb any undegraded contaminants. Composted horse manure was added to the activated carbon with the intent to stimulate biodegradation of sorbed compounds, but no evaluation of this was made during this study.

Effects of temperature and moisture on Bioremediation

Zhou and Crawford (1995) have shown that increasing the temperature increases the rate of bioremediation due to the increased metabolism of microorganisms. In this study, temperatures ranged from 11°C at the beginning to 29°C during the 85 day experiment. The increased temperature was somewhat correlated to increased elimination rates for TPH and toluene (r^2 = 0.7), but was not well correlated with elimination rates for the other contaminants (data not shown). However, there are other factors that could have influenced this correlation that were not considered. Changes in temperature affects not only the microorganisms present, but also the contaminants. As temperature increases, the amount of contaminant transferred to the air phase also increases, but simultaneous increases in the flow rate can decrease the transfer of contaminants resulting in diminished performance of the biofilter (Zilli et al. 1993). While the biofilter adapted well to increasing temperatures, preliminary studies in this system, showed that when the internal temperature of the compost exceeded 45°C, the biofilter began to perform poorly (data not shown). So the biofilter was shut down for July, August, and September when daily temperatures ranged from 38 – 45°C. Such high temperatures may cause increased toxicity of hydrocarbons to degrading organisms (Atlas, 1994).

Though adequate moisture is needed for microorganisms, saturated conditions during gasoline degradation should be avoided, as degradation proceeds more readily in aerobic conditions. The moisture content within the biofilter fluctuated over a wide range. After an initial wetting with the soaker hoses, the extracted soil vapor was sufficiently humid to maintain adequate moisture in the system.

Bacterial isolates

Based on qualitative visual observation, the bacteria that were isolated over the course of this experiment did not appear to vary significantly. Some of these isolates were identified using Biolog, but one problem with the use of the Biolog system is that it requires that organisms be grown on specific types of media prior to analysis. Many of the isolates did not grow adequately on the recommended media to allow Biolog identification. For example, of 19 isolates from sample day 123 that were able to grow with gasoline as a sole carbon source, only 6 could be identified with Biolog. A second example was an unusual sample dominated by yellow gram negative colonies that was collected from the 46 cm depth on day 65. No conclusive identification of this isolate was made.

Some isolates that were identified by Biolog included *Rhizobium meliloti* which was isolated prior to hydrocarbon input, as well as *Rhizobium loti* isolated after contaminant was introduced. We found this interesting since composted horse manure, which presumably contained digested alfalfa was used as the inoculum. Rhizobia have been shown to degrade hydrocarbon compounds (Shimp et al., 1993). Other potential hydrocarbon degrading isolates include *Bacillus insolitus*, *B. licheniformis*, and *B. pasterii* which were present at various sample depths within the biofilter.

Pseudomonas stutzerii was also prevalent throughout the biofilter and persisted during the sampling period indicating that these organisms were distributed within the biofilter.

The bacteria isolated and tested for their sole carbon source use showed that there were a total of eight bacteria that appeared to be the same from the background sample as the acclimated sample. Of those isolates that could degrade all of the contaminants only one was the same from days 0 and 123, the rest of the isolates from this group changed over the course of the experiment. The numbers of bacteria isolated were similar from day 0 and 123. There were several bacteria isolated on the same day on R2A and MSM that were the same. Further experiments are being carried out using various molecular methods and systematics to determine if these isolates are genetically the same.

Cost comparison

Table 3 shows a cost comparison of the biofiltration of gasoline vapors compared to combustion of the said vapors at the Phoenix site studied. The cost incurred for defining the extent of contamination and installation of extraction wells was assumed to be the same for each type of remediation and would vary depending on the site and contractor. As shown in this Table, even with all of the capital costs for the biofilter imposed on the first year of operation, this technique saved 90% of the cost of vapor combustion. Therefore expenses in subsequent years of operation provide even higher savings. Activated carbon was the most expensive material cost for the biofilter, but was able to reduce emissions to within the permit requirements when biological treatment alone was not sufficient.

Conclusions

Biofiltration of VOC's is a practical and cost effective method of remediation. This field-scale biofiltration system had an overall contaminant removal of 90–98% for TPH and BTEX compounds while operating over a wide array of conditions: flow rate, contaminant concentration, temperature, and moisture. Efficient operation of the biofilter required adequate flow rates, 50 to 70 m^3h^{-1}, allowing optimal residence times for the contaminant compounds. Contaminant loading rate was also a factor in efficient operation of the biofilter system. Temperature had a limited effect overall, though increased temperature did correlate to an increase in elimination rate for TPH and toluene. Most importantly, the bacterial population was uniform throughout the biofilter and was active over a wide array of operating conditions. Few of the organisms isolated were able to degrade all of the BTEX components suggesting that optimal biofilter performance required a consortium of bacteria. The compost based system with a final activated carbon layer successfully operated within permit requirements with significant savings in operating costs.

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