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A total-recycle expanded-bed bioreactor design which allows direct headspace sampling of volatile chlorinated aliphatic compounds

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Summary

A two-phase column system was developed, allowing direct headspace sampling of volatile chlorinated aliphatic compounds, including trichloroethylene, cis- and trans-1,2-dichloroethylene and vinyl chloride. Propane, methane and CO_2 were sampled in the same fashion. A comparison of two solid support matrices (crushed glass and anthracite coal) was performed. $> 97 \pm 2\%$ of the trichloroethylene added to control reactors was still present following 12 days of operation. The bioreactor consisted of an expanded-bed column containing 70 g of 60/80-mesh substratum. The liquid phase was recirculated through the expanded-bed column into a gas-recharge column fitted with a headspace sampling port which allowed direct sampling and subsequent quantification by gas-chromatography techniques.

Key words: Bioreactor; Headspace gas chromatography; Volatile chlorinated aliphatics

Introduction

The ever-increasing contamination of environmental groundwaters with halogenated compounds has generated public and scientific concern [1, 2]. Researchers have demonstrated that aerobic and anaerobic biotransformation of chlorinated ethenes can occur both in the laboratory and in the field [3–14].

Analysis of short-chain chlorinated compounds can be performed by using various gas-chromatography (GC) techniques including headspace chromatography, solvent extraction and the purge-and-trap method [4–7, 15, 16]. Difficulty arises in quantify-

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ing these compounds because chlorinated ethenes partition between the gas and liquid phases [6, 13, 15].

Typical batch-culture experiments are performed in glass vials equipped with Teflon-faced silicone septa because chlorinated aliphatics tend to adsorb to materials (e.g., butyl rubber), making quantitative analyses difficult [6, 8–10]. Liquid-phase samples have been analysed by allowing a measured amount of effluent to equilibrate in a vessel, as described above, and then performing headspace GC to quantitate the volatile components [4]. Solvent extraction of liquid-phase samples has also been utilized in quantification procedures [5]. The purge-and-trap method is another technique used to concentrate volatile compounds for quantification [7, 14]. Purge-and-trap methods are quite reliable but, like equilibration and solvent-extraction procedures, they require the removal of the liquid phase from the test system [12].

Batch cultures of bacterial consortia capable of degrading trichloroethylene (TCE) [6] have been maintained in this laboratory. The purpose of designing a total-recycle bioreactor was to determine the feasibility of utilizing the TCE-degrading capabilities of these bacterial cultures to treat environmentally contaminated waters. An important consideration in the design of the bioreactor was the need for a means of rapid sample withdrawal and subsequent quantification of chlorinated ethenes by GC techniques. We report here a bioreactor design that utilizes the direct headspace-sampling method and, thus, allows volatile component sampling without changing the volume of the liquid or gas phase of the total-recycle bioreactor system.

Materials and Methods

Design of total-recycle bioreactor system

The total-recycle bioreactor system consisted of two organic resistant borosilicate-glass chromatography columns (Pharmacia, Piscataway, New Jersey) linked in series providing a flow loop for the liquid phase (Fig. 1). The gas-recharge column provided a known headspace volume which allowed partitioning of volatile compounds (methane, propane and TCE) and afforded the use of GC techniques for quantitative analysis. The 100-cm × 2.7-cm recharge column displaced a total volume of 525 ml. The top end cap was equipped with two fittings, one of which provided a direct link from the recharge column to the expanded-bed column while the second fitting served as a port for headspace sampling. The 44-cm × 2.7-cm expanded-bed column displaced a total volume of 235 ml. This column contained 70 g of 60/80-mesh substratum (crushed glass or anthracite coal) which provided a large surface area for bacterial attachment. The substratum had a volume displacement of 45 ml. Similarly, this column had a top end cap equipped with two fittings, one of which provided a direct link to the gas-recharge column while the other was utilized as a port for sampling from or adding to the liquid phase. The end-cap O-rings were Teflon-coated to provide a gas-tight seal and all threaded fittings employed were sealed with Teflon tape. All tubing connections consisted of either Teflon tubing or organic-resistant tubing (Cole-Parmer, Chicago, Illinois). A continuous recycle-flow rate was maintained at 20 $ml \cdot min^{-1}$ with a peristaltic pump. A septum was constructed for the gas-recharge column by utilizing a 10-cm section of Size 14 organic resistant tubing that was pierced with a 26-gauge needle. Clamps were placed above and below the pierced hole to prevent headspace

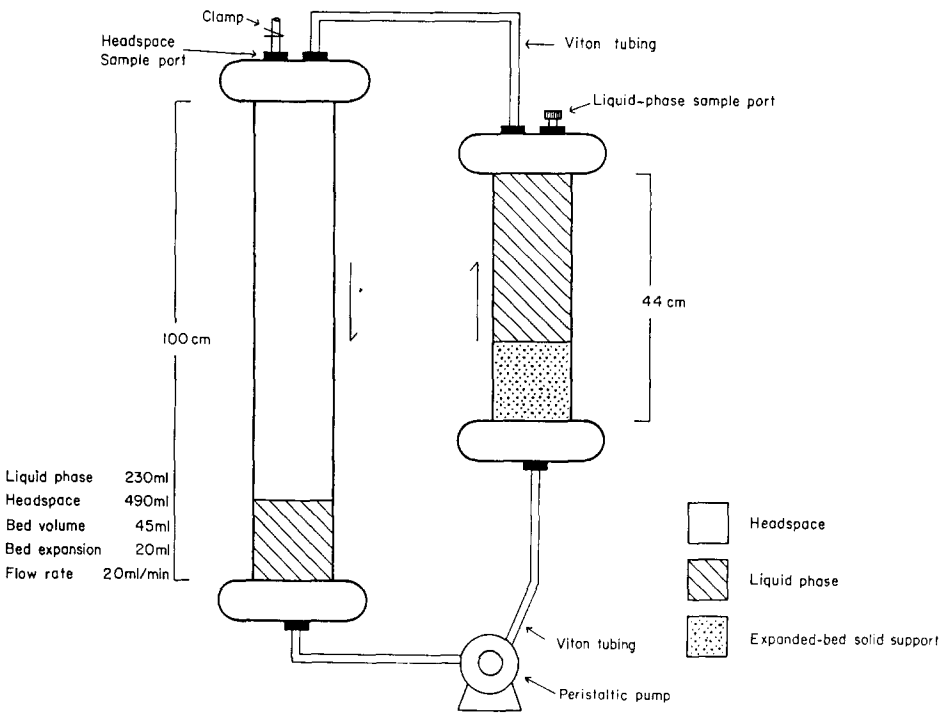

Fig. 1. Diagram showing components of expanded-bed bioreactors. Reaction vessels containing substratum were connected by Viton tubing to sparging vessels which contained appropriate gas mixtures. Continuous recirculation was accomplished by peristaltic pumping.

leakage. The columns were mounted vertically in a closed metal cabinet to prevent light exposure that could foster growth of photosynthetic microorganisms and induce photolysis of the chlorinated aliphatic compounds. All reactors were operated at room temperature (25 °C).

Medium

The liquid phase in the reactors was the same medium that was employed for maintaining TCE-degrading test cultures. The medium contained: $MgSO_4$, 0.055 g; $CaSO_4 \cdot 2H_2O$, 0.054 g; NH_4SO_3, 1.48 g; trace minerals II [17], 10 ml; 10 × vitamin solution [17], 1 ml; 10% yeast extract/trypticase (one culture only), 2 ml; 200-mM phosphate/bicarbonate buffer solution, 10 ml; nanopure-filtered water, 1000 ml. Resazurin was added (1 ml of a 0.2% solution $\cdot l^{-1}$ of medium) to monitor the aerobic status of the liquid phase. In all cases, the pH of the medium was maintained at 7.2.

Gases and chemicals

Propane and O_2 were > 99.5% pure while methane was > 98% pure. All chemicals were of reagent grade and were obtained from Mallinkrodt (Paris, Kentucky) or Sigma Chemical Co. (St Louis, Missouri). Glass-distilled solvents and reagents were purchased from J. T. Baker Chemical Co. (Phillipsburg, New Jersey).

Operation of total-recycle bioreactor system

The total liquid-phase volume of the system was 230 ml. The liquid flowed upward through the expanded-bed column containing the expanded crushed-glass substratum. It then exited the top of the expanded-bed column and entered the top of the gas-recharge column which contained 40 ml of the liquid phase. The expanded-bed column (saturated) contained 190 ml of medium. The headspace in the gas-recharge column was then 485 ml which resulted in a 2.1 : 1 volume ratio of headspace to liquid. The flow rate of the effluent was maintained at 20 $ml \cdot min^{-1}$ with a peristaltic pump creating a bed expansion of 25–30% (10–15 ml in volume).

A TCE-saturated water solution (1100 $mg \cdot l^{-1}$) [3, 5] was added to the medium; thus, the total nominal liquid-phase concentration of TCE in the reactor was 20 $mg \cdot l^{-1}$. Methane and propane were also added to the system through the headspace sample port and were maintained at 5 and 3% of the gas phase (v/v), respectively. Reactor headspaces were monitored for several weeks by GC before and after inoculation of the two test columns. The remaining column served as a control and contained 0.5% formalin and 0.2% sodium azide in order to inhibit microbial contamination.

For GC-quantitative analyses, gas-phase aliquots were sampled directly from the headspace of the recharge column. Sampling was done by inserting a GC syringe needle through the fabricated septum so that the needle tip was placed directly inside of the headspace of the recharge column. The syringe inserted into the column was flushed five times prior to sample withdrawal. After sample removal, the tubing was clamped below the point of needle entry to prevent any gas loss.

GC

TCE was analysed by using a Hewlett Packard 5890 gas chromatograph equipped with a 50-m Hewlett Packard ultra performance (Ultra 1) cross-linked methyl silicone capillary column (i.d. 0.2 mm, 0.33 μm film) and an electron-capture detector (ECD). The following conditions were used: oven temperature, 60 °C; injector temperature, 150 °C; and detector temperature, 200 °C. The split ratio was set at 10 : 1. The carrier gas was H_2 and the make-up gas was N_2. Vinyl chloride as well as cis- and trans-1, 2-dichloroethylene (DCE) were analysed with a Shimadzu GC-9A gas chromatograph equipped with a 2.44-m × 3.2-mm Poropak T packed column and a PID detector (HNU Systems, Newton, Massachusetts). The following conditions were used: oven temperature, 150 °C; injector temperature, 160 °C; and detector temperature, 230 °C. Methane and CO_2 were assayed with a Shimadzu GC-8A gas chromatograph equipped with a 2.74-m × 3.2-mm Carbosieve 8000 packed column and a TCD detector. The following conditions were used: oven temperature, 130 °C; and injector/detector temperature, 140 °C. Propane was analysed with a Shimadzu GC-9A gas chromatograph equipped with a 2.44-m × 3.2-mm Poropak N packed column and an FID detector. The following conditions were used: oven temperature, 80 °C; and detector/injector temperature, 220 °C.

Quantitative analyses were made for propane, methane and CO_2 by relating peak heights of samples to those of prepared gas standard curves. Peak areas of chlorinated volatile compounds sampled from the columns were compared with standard curves (peak areas) of the respective compounds by using a Nelson 2600 analytical chromatography system with version 4.0 (Nelson Analytical, Cupertino, California).

Through application of Henry's gas laws [6, 7] the standards were prepared in such a way that they would have the same ratio of headspace volume to liquid-phase volume.

The limits of detection for sampled compounds were as follows: TCE, 0.1 $\mu g \cdot l^{-1}$; cis-1,2-DCE, 10$\mu g \cdot l^{-1}$. trans-1,2-DCE, 10 $\mu g \cdot l^{-1}$; VC, 100 $\mu g \cdot l^{-1}$; and propane, CH_4, and CO_2, 0.05% (v/v). Detection limits for chlorinated compounds were nominal liquid-phase concentrations. Reproducibility was obtained at a signal : noise (S : N) ratio > 3.

Quantitative determination of TCE in bioreactors

When the distribution coefficient (K) or the Henry's law constant (H) for a volatile organic compound (VOC) are known, the equilibrium gas concentration (C_G) of a VOC in a closed system serves as a direct measure of its liquid concentration (C_L) under conditions of constant temperature and pressure because $C_L = C_G\ (K + V_G/V_L)$ (C_L is the initial liquid-phase concentration) [6, 7]. The terms, V_G and V_L correspond to gas-phase and liquid-phase volumes, respectively. The distribution coefficient and Henry's law constant for a given system are inversely proportional [7]. If gas chromatographic peak areas are normalized to a uniform ratio of gas- and liquid-phase volume regardless of total system volume, then the gas concentration of the VOC will be the same for either system as long as conditions of constant temperature and pressure are met and there is no significant VOC adsorption that would shift the phase equilibrium [15].

Results

TCE partitioning and quantification

Two support materials were evaluated for their possible utility in the bioreactors. Anthracite coal and crushed glass, both at 60–80 mesh, were satisfactory for bacterial attachment and TCE degradation through the use of bacterial test consortia (data not shown). 70 g of the respective substrata were added to the saturated fluidized-bed column. Table 1 shows the results of the comparison of the two support materials in the bioreactors after the addition of TCE at a total nominal liquid-phase concentration

TABLE 1

COMPARISON OF SUPPORT MATERIALS AND TCE PARTITIONING IN BIOREACTORS THROUGH USE OF HEADSPACE SAMPLING TECHNIQUE

Substratum	TCE concentration		
	5 min	45 min	24 h
Anthracite coal (60/80 mesh)	19.2 ± 0.81	13.0 ± 0.6	5.0 ± 0.3
Crushed glass (60/80 mesh)	20.0 ± 0.8	20.0 ± 0.8	19.9 ± 0.7

Results are mean of duplicate experiments. TCE-saturated water (1100 $mg \cdot l^{-1}$) was added to make initial total nominal liquid concentration of 20 $mg \cdot l^{-1}$. Liquid phase was medium; pH = 7.2. 1 SD.

of 20 $mg \cdot l^{-1}$. Partitioning of TCE into the coal was extensive, resulting in decreased headspace concentrations in comparison with those of standards prepared at the same phase ratios without coal. The crushed glass showed no indication of significant TCE adsorption over the 24-h period (Table 1).

The insignificant adsorption of the crushed-glass substrata ($P > 0.01$) led to further investigation of its possible utility as a solid support matrix for bacterial studies. The liquid-phase TCE concentration in the bioreactors was evaluated in relation to external TCE standards. The ECD peak area responses at nominal TCE concentrations were virtually identical, indicating that the TCE concentration in the bioreactors was comparable to that seen in the standard solutions. A mass distribution calculation for TCE ($K=2.74$) [2] revealed that $\approx$ 45% of the total nominal amount of TCE in the liquid phase migrated to the gas phase of the reactors.

Similar results were obtained for the other chlorinated compounds with the crushed-glass substratum (data not shown). The results indicated that one can use GC-headspace analysis to monitor the subsequent change of TCE concentration in the bioreactors when studying the degradative capabilities of bacterial consortia after their introduction into the system.

Bioreactor-gas dynamics: before inoculation

To determine if reactors behaved in the same fashion, medium, along with TCE, propane and methane, was added to reactors prior to inoculation with test cultures. Table 2 shows the results of headspace analysis over a 5-day period. No appreciable loss of any of the analytes was observed and all the concentrations measured in the reactors were identical within analytical uncertainty. There was no CO_2 formation in any of the reactors; this indicated that there was no significant biological activity.

Bioreactor gas dynamics: after inoculation

Two reactors were inoculated with test cultures by recirculating 1l ($\approx$ 240 $mg \cdot l^{-1}$ dry wt biomass) of each of the respective cultures through the reactor substrata for

TABLE 2

CONCENTRATION OF VOLATILE COMPONENTS IN BIOREACTORS BEFORE INOCULATION

Sample	TCE ($mg \cdot l^{-1}$)	Propane ($mmol \cdot l^{-1}$)	Methane ($mmol \cdot l^{-1}$)	CO_2 ($mmol \cdot l^{-1}$)
Day 0				
Reactor 1	20.0 ± 0.81	0.93 ± 0.06	2.25 ± 0.10	ND
Reactor 2	19.8 ± 0.9	0.93 ± 0.06	2.25 ± 0.10	ND
Reactor 3	19.9 ± 0.9	-	2.24 ± 0.10	ND
Day 5				
Reactor 1	19.9 ± 0.7	0.93 ± 0.04	2.25 ± 0.12	ND
Reactor 2	19.7 ± 0.8	0.91 ± 0.06	2.25 ± 0.10	ND
Reactor 3	19.8 ± 0.8	-	2.23 ± 0.14	ND

Liquid phase was medium; pH = 7.2; mean is based on two sample injections; ND = not detected. ± = 1 SD.

5 h. Reactors were drained after inoculation. At this time, medium (500 ml) was recirculated through each of the reactors for 1 h to flush each system. The reactors were then drained and 230 ml of fresh medium was added to each reactor. Column 1 served as a control and contained 0.5% formalin and 0.2% sodium azide. The headspace of each recharge column was then purged with pure O_2 for 1 h. TCE was then added to each reactor at a total nominal liquid-phase concentration of 20 $mg \cdot l^{-1}$, followed by methane and propane which were 5 and 3% (v/v) of the headspace, respectively. Table 3 shows the results of headspace analysis over a 9-day period. Methane consumption, propane consumption and CO_2 formation were observed in the test columns. The control reactor showed no loss of any of the analytes and no CO_2 production. Also, no loss of TCE occurred during the 4-day period. At Day 9, Reactors 2 and 3 indicated TCE losses of 70 and 50%, respectively, of the initial contaminant in comparison with the inhibited control.

Discussion

> 96% of the initial TCE added to the bioreactors at initial nominal liquid-phase concentrations of 20 $mg \cdot l^{-1}$ was still present following 12 days of operation. Discrepancies in the total TCE applied to inhibited soil columns were observed by researchers studying continuous-flow systems and subsequent mass-balance determinations [14]. Anaerobic continuous-flow column studies involving biotransformation of chlorinated ethenes did not include results for comparable inhibited controls [12]. The lack of comparable controls in previous studies gives rise to analytical questions and data interpretation.

The mass distribution of TCE in the bioreactors revealed that ≈ 45% of the total nominal amount of TCE in the liquid phase migrates to the gas phase [2]. Intuition

TABLE 3

CONCENTRATION OF VOLATILE COMPONENTS IN BIOREACTORS AFTER INOCULATION

Sample	TCE ($mg \cdot l^{-1}$)	Propane ($mmol \cdot l^{-1}$)	Methane ($mmol \cdot l^{-1}$)	CO_2 ($mmol \cdot l^{-1}$)
Day 0				
Reactor 1	20.0 ± 0.91	0.93 ± 0.06	2.25 ± 0.12	ND
Reactor 2	19.7 ± 0.8	0.91 ± 0.04	2.25 ± 0.12	ND
Reactor 3	19.8 ± 0.8	-	2.25 ± 0.14	ND
Day 4				
Reactor 1	19.9 ± 0.9	0.91 ± 0.06	2.25 ± 0.12	ND
Reactor 2	19.7 ± 0.7	0.23 ± 0.02	1.13 ± 0.06	0.85 ± 0.04
Reactor 3	19.7 ± 0.7	-	0.57 ± 0.04	0.76 ± 0.04
Day 9				
Reactor 1	19.0 ± 0.9	0.93 ± 0.04	2.25 ± 0.14	ND
Reactor 2	5.5 ± 0.3	ND	0.66 ± 0.02	0.91 ± 0.04
Reactor 3	9.0 ± 0.5	ND	0.23 ± 0.02	0.89 ± 0.04

Liquid phase was medium; pH = 7.2; mean is based on two sample injections; ND = not detected. Reactor 1 was an uninoculated control containing 0.5% formalin and 0.2% sodium azide. Reactors 2 and 3 were inoculated with test consortia. ± = 1 SD.

suggests that the precision of the subsequent GC-headspace analysis performed on reactor samples should be acceptable because roughly half of the added mass of TCE is in each phase.

Partitioning of TCE into the solid support (Table 1) demonstrates that care is necessary in the choice of substratum. ≈ 75% of the TCE added to reactors containing anthracite coal at initial liquid-phase concentrations of 20 $mg \cdot l^{-1}$ was absorbed to the solid support. > 85% of this TCE could be retrieved after thermal desorption (data not shown). Similar results were obtained in TCE-adsorption studies involving soils [15].

In choosing a solid support for a bioreactor of this type, researchers should consider three things: bacterial attachment to the support, the ability of the attached bacteria to actively degrade the compound of interest and the inert characteristics of the support. Previous studies with glass beads indicated that the beads were inert to chlorinated ethenes; however, the smooth glass beads did not promote bacterial attachment to create the necessary biomass. The crushed-glass substratum showed no indication of TCE adsorption and displayed positive characteristics for microbial attachment and biomass formation. This substratum was, therefore, chosen for further studies in which microbial consortia capable of biologically degrading TCE were introduced into the bioreactor system.

It is shown here that removal of the liquid-phase sample from the reactor is not necessary to determine the concentration of volatile compounds such as TCE. Avoidance of removal of liquid-phase samples has multiple advantages. First, the ratio of headspace to liquid-phase volume remains undisturbed as a result of sampling only a small volume of the reactor gas phase; thus, mass-balance calculations and interpretation of bioreactor operation are simplified. Second, the ease of direct headspace-GC sampling allows for rapid analysis, in contrast with time-consuming solvent extraction procedures or purge-and-trap techniques used by other investigators [5, 7, 12].

A total-recycle system similar to the one described here should be beneficial for quantitative analysis of other volatile organic compounds that display liquid phase : headspace partitioning similar to that of TCE. This technique could prove useful in investigating microbial degradation of volatile contaminants in efforts to design pilot-scale processes. Various operational parameters could be studied simultaneously, including test cultures, substrata, solid supports and waste composition.

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