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# A Gas Lift Bioreactor for Removal of Contaminants from the Vapor Phase

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**The cometabolic degradation of trichloroethylene (TCE) as a vapor by two aromatic-metabolizing pseudomonads was evaluated in an airlift reactor. These microorganisms were able to degrade 90 to 95% of TCE in air at concentrations at the reactor inlet of 300 to 4,000 μg/liter. Although exposure of the cells to high inlet concentrations of TCE (4 mg/liter) caused a decline in enzyme-specific activity and TCE removal efficiency, this loss in activity could be prevented or delayed by increasing the rate of cosubstrate addition. Under the appropriate operating conditions, the microorganisms were able to degrade even high concentrations of TCE and activity of the cells in the reactor could be maintained for periods of at least 2 weeks.**

Chlorinated compounds such as trichloroethylene (TCE) are common contaminants of groundwater and soil. At Savannah River alone, the U.S. Department of Energy estimates that approximately 4.4 million kg of TCE contaminates soils and groundwater (9). The development of in situ technologies, such as vapor extraction for soils, can be of critical value in the economical cleanup of these sites. However, such processes create air emissions that must be either trapped or destroyed to prevent atmospheric release. The object of this work was to demonstrate, on a bench scale, the successful operation of a vapor-phase biological reactor for destroying TCE in air emissions created from contaminant volatilization.

The concept of a vapor-phase bioreactor is that volatile contaminants can be carried as gases to a degradative microorganism in an aboveground reactor. This bioreactor can be used in conjunction with conventional pump-and-treat methods, as a treatment for off-gases, as a stand-alone system for destruction of harmful air emissions from point sources, and as part of integrated bioremediation systems that include in situ methods or soil vacuum extraction.

It is well documented that certain microorganisms are capable of degrading TCE and other volatile hydrocarbons under aerobic conditions, provided an appropriate cosubstrate is present (1, 3, 10, 15, 20, 23). Processes using these microorganisms are being developed to remediate soils and groundwater with both in situ and aboveground approaches (14, 16, 17).

The use of a biologically based process for the treatment of contaminants such as TCE in air has not generally been considered to be practical because of the relatively unfavorable partitioning equilibrium between air and water that is displayed by compounds such as TCE (6, 11). However, certain reactor-and-microorganism combinations have been used to remove volatile chlorinated organic compounds from vapor streams. A 66-liter trickling-bed reactor containing *Hyphomicrobium* sp. strain DM20 removes dichloromethane from air at rates of up to 4.8 g/h for a dichloromethane conversion of 80 to 95% (8). Vapor-phase TCE has been degraded in a multiple-segment trickling filter with isoprene-grown cells of *Rhodococcus erythropolis* JE77 (19).

Cometabolic TCE oxidation by certain microorganisms has been associated with enzyme inactivation and cell toxicity (5, 22), resulting in a rapid loss of degradative activity. While it may be possible to reduce the effects of toxicity or enzyme inactivation by increasing cell growth with higher cosubstrate concentrations or feed rates, competitive inhibition of phenol oxidation has been observed with TCE concentrations as low as 10 μM, and increased cosubstrate concentrations should inhibit TCE degradation (4). The identification of operating conditions for a reactor system that provided sufficient rates of cosubstrate feeding for sustained degradative activity while minimizing competitive inhibition of TCE oxidation would provide a basis for the practical application of this approach to treating TCE-contaminated airstreams. We also hypothesized that the relatively hydrophobic nature of microorganisms (enhancing uptake of TCE) and the high TCE degradation rates displayed by some cultures would cause volatile contaminants to be removed from air more efficiently than might be predicted on the basis of equilibrium partitioning between air and water.

The use of vapor-phase bioreactors to treat the off-gas from air stripping towers or soil vacuum extraction systems also separates the degradative microorganisms from the contaminated groundwater. This reactor type has several advantages, in that a nonindigenous organism is not introduced into the groundwater, the organism can be readily isolated from predators and competitive microorganisms by first filtering the air, the low specific heat of air means that the reactor can be efficiently operated at the optimum reaction temperature, and the concentration of biomass and nutrients in the reactor can easily be controlled, since they are isolated from the contaminated water. Finally, since the biomass is not washed out of the reactor, simple and efficient reactor designs such as suspended growth systems can be used to treat contaminated air.

## MATERIALS AND METHODS

A reactor of 3.5-liter liquid volume based on a modified gas lift loop design (2) was used to study the biodegradation of vapor-phase TCE. The reactor was constructed of polyvinyl chloride tubing 8 cm in diameter and 30 cm long (Fig. 1). The reactor contained a polyvinyl chloride inner baffle to cause vertical circulation of the nutrient medium and cells.

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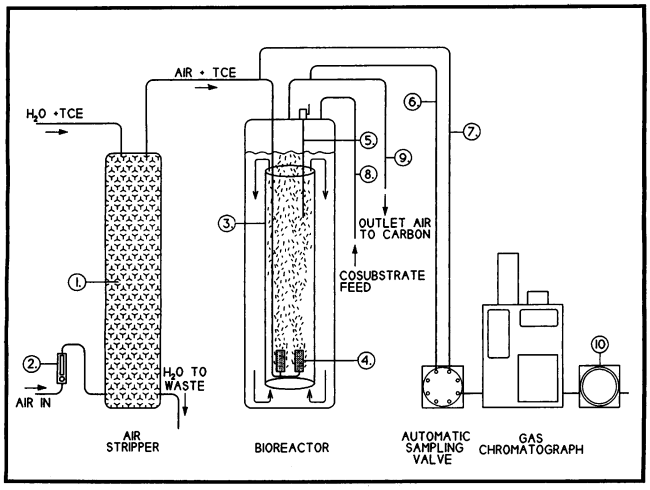


FIG. 1. Schematic of air stripper, bioreactor, and sampling system. 1, glass wool packing; 2, rotameter; 3, downcomer for air lift reactor; 4, sintered steel sparging stones; 5, sampling-and-addition tube; 6, reactor air outlet sampling line; 7, reactor air inlet sampling line; 8, cosubstrate feed tube; 9, reactor air outlet; 10, vacuum pump for sampling system.

TCE-contaminated air was fed into the bottom of the reactor through sintered stainless steel sparging stones to give a fine bubble dispersion. All fittings and connections were constructed of Teflon or aluminum tubing. Leakage of volatile contaminants such as TCE or perchloroethylene from the reactor or adsorption to reactor components was determined to be negligible by operating the reactor in the absence of biomass and analyzing the concentrations of these compounds at the inlet and outlet before, during, and after the introduction of a flow of contaminant in the airstream into the reactor.

The reactor was charged with either *Pseudomonas cepacia* G4 (15) or *Pseudomonas mendocina* KR-1 (23) grown in basal salts medium (7, 18) with phenol or toluene, respectively, as the sole carbon and energy source. The cells were suspended in the reactor at densities of $5 \times 10^8$ to $5 \times 10^9$/ml in basal salts medium, and the pH was controlled at 6.8 to 7.2 by the periodic addition of NaOH. Phenol (15%) (*P. cepacia*) or toluene (*P. mendocina*) was fed as the cosubstrate into the reactor with a syringe pump to give addition rates of 0.1 to 1.0 g/liter of reactor volume per day. The kinetics of TCE degradation were determined by introducing TCE into the reactor in an airstream at various concentrations and airflow rates and measuring the change in concentration as the air passed through the reactor.

TCE was introduced into the reactor inlet air by dissolving TCE in distilled water to a concentration of 1 g/liter. The TCE-contaminated water was placed in an aluminized Mylar bag and pumped with a peristaltic pump into an air stripping tower. The tower was constructed of 5-cm-diameter polyvinyl chloride pipe 50 cm long and was packed with glass wool. The TCE-contaminated water was dripped into the top of the tower while a metered amount of air (100 to 350 ml/min) was introduced into the bottom. The TCE-containing air was fed from the top of the stripper into the bioreactor. The TCE concentrations in the air could be adjusted by changing the flow rate of the water entering the stripping tower. This apparatus gave consistent and reliable TCE-contaminated airstreams and was able to supply a wide range of concentrations.

TCE concentrations in air entering and exiting the reactor were analyzed by sampling through T fittings attached to the reactor inlet and outlet. The sampled air was drawn through Teflon tubing to a Varian 3400 gas chromatograph (GC) fitted with an electron capture detector. Volatile compounds were analyzed on a VOCOL (Supelco) capillary column (30 m long, 0.53-mm internal diameter, 3.0-μm film thickness) run isothermally at 100°C. TCE standards in methanol were used to construct a standard curve.

Samples of reactor inlet and outlet air were drawn through an automatic sampling system on the GC with a vacuum pump, captured in a 50-μl sampling loop, and injected into the GC automatically once every 20 min to 1 h. The automatic sampling system was composed of an A410 UWT 10-port air-activated valve (Valco Instruments Co., Houston, Tex.). The valve was controlled by using the autosampler software in the data processor on the Varian GC. Abiotic losses of TCE in the reactor and sampling system were monitored by operating the reactor without cells or with cells that had been killed by the addition of 100 ml of 10 M NaOH. Under these conditions, TCE concentrations at the reactor inlet and outlet reached equilibrium within 2 h. Perchloroethylene was used as a nondegradable tracer in some experiments with live cells. Abiotic losses were determined to be less than 10%.

Changes in reactor effluent TCE concentrations were analyzed at a variety of inlet TCE concentrations, airflow rates, and cosubstrate feed rates for initial cell densities ranging from $5 \times 10^8$ to $5 \times 10^9$ cells/ml. TCE degradation at inlet TCE concentrations of 300 and 600 μg and of 1 and 4 mg per liter was analyzed. Airflow rates were varied between 100 and 350 ml/min. Phenol (15%) was fed into the reactors at feed rates of 0.1 to 1.0 g/liter of reactor volume per day. Residual concentrations of cosubstrate or TCE in the reactor were analyzed by withdrawing and extracting 5 ml of reactor contents with pentane and analyzing the extract. TCE concentrations were determined by injecting a 10-μl sample of the pentane extract into the GC. Phenol concentrations in the pentane extract were analyzed by GC.

For each separate set of conditions, the reactor was permitted to come to equilibrium, as indicated by stable inlet and outlet TCE concentrations for at least 5 h. Samples were taken every 20 min to 1 h until at least 50 inlet and outlet samples had been analyzed. The operating parameters of the reactor were then changed to reflect a new set of conditions, and the reactor was again allowed to come to equilibrium before the next set of samplings and analysis took place. The inlet and outlet TCE concentration data were averaged to give the percent TCE removal.

Phenol hydroxylase specific activity was determined by assaying whole cells in a phenol hydroxylase assay (4, 12). Phenol was added at a final concentration of 100 μM to a 5-ml suspension of cells at a density of $5 \times 10^8$/ml. At 1-min intervals, 1 ml of the cell suspension was transferred and residual phenol was detected by the addition of 4-aminoantipyrine and $K_3Fe(CN)_6$. The cells were removed by centrifugation before the $A_{500}$ of the supernatant was measured. Units of phenol hydroxylase activity are expressed as nanomoles of phenol oxidized per minute per milligram of whole cell protein. The density of the cells in the reactor was monitored by periodically removing a sample of the reactor contents and measuring the $A_{550}$ with a Spectronic 20 spectrophotometer. The optical density was correlated with cells per milliliter by calibrating the spectrophotometer by using serial dilution plate count methods with *P. cepacia*.

TABLE 1. TCE degradation by *P. cepacia* G4 in a 3.5-liter reactor

| Input TCE concn (μg/liter of air) | Output TCE concn (μg/liter of air) | Cell density (per ml) | Phenol feed (g/liter/day)[a] | Airflow (ml/min) | % TCE removal |
|---|---|---|---|---|---|
| 4,000 | 200 | $1 \times 10^9$ | 0.5 | 100 | 94 |
| 1,000 | 120 | $1 \times 10^9$ | 0.5 | 100 | 88 |
| 600 | 54 | $1 \times 10^9$ | 0.5 | 100 | 91 |
| 300 | 12 | $1 \times 10^9$ | 0.5 | 100 | 96 |
| 300 | 15 | $5 \times 10^9$ | 0.5 | 100 | 95 |
| 300 | 15 | $1 \times 10^9$ | 0.5 | 100 | 95 |
| 300 | 45 | $5 \times 10^8$ | 0.5 | 100 | 85 |
| 300 | 9 | $1 \times 10^9$ | 1.0 | 100 | 97 |
| 300 | 15 | $1 \times 10^9$ | 0.75 | 100 | 95 |
| 300 | 21 | $1 \times 10^9$ | 0.5 | 100 | 93 |
| 300 | 24 | $1 \times 10^9$ | 0.2 | 100 | 92 |
| 300 | 30 | $1 \times 10^9$ | 0.1 | 100 | 90 |
| 300 | 120 | $1 \times 10^9$ | 0.5 | 350 | 60 |
| 300 | 33 | $1 \times 10^9$ | 0.5 | 175 | 89 |

[a] Grams of phenol fed per liter of reactor volume per day.

Viable cells were also quantitated by serial dilution plate count methods.

## RESULTS

Under the operational conditions described, the microorganism and reactor system were relatively insensitive to the inlet TCE concentration, cell density, or phenol feed rate (Table 1). Concentrations of TCE up to 4,000 μg/liter of inlet air were degraded by at least 90%. TCE degradation of 85 to 95% was observed when the cell density was varied from $5 \times 10^8$ to $5 \times 10^9$ cells per ml and the phenol feed was varied from 0.1 to 1 g/liter of reactor volume per day. The parameter that had the greatest impact on the efficiency of TCE removal by this reactor system was the rate of inflowing air. At higher airflow rates, with a fixed TCE concentration, the percent TCE removal was as low as 60% when the airflow rate equaled 350 ml (10% of the liquid volume of the reactor) per min. These results indicate that the efficiency of mass transfer in this reactor does limit the percent TCE degradation at higher airflow rates. Although higher flow rates cause the introduction of greater amounts of TCE into the reactor, mass transfer is limiting removal efficiency because the reactor will degrade only 60% of TCE at 100 μg per min when TCE is introduced with an airflow rate of 350 ml/min but will degrade 94% of TCE at 400 μg per min when TCE is introduced into the reactor in an airstream flowing at 100 ml/min.

*P. cepacia* G4 and *P. mendocina* KR-1 both degrade TCE. We compared the performances of each organism for the ability to degrade TCE, in the extent of TCE removal, and in longevity of oxidation activity in the gas lift reactor. Both bacteria performed equally well in removing greater than 90% of the TCE over a period of 3 days. Phenol is the cosubstrate for *P. cepacia*, but toluene must be used as the cosubstrate for *P. mendocina* (unpublished data). Toluene is a less desirable cosubstrate because of its flammability and greater toxicity; therefore, the majority of experiments were conducted by using *P. cepacia* G4 with phenol as the cosubstrate.

After baseline kinetics were determined and the two microorganisms were compared, operational parameters affecting reactor performance were evaluated. The goal of this evaluation was to identify operating conditions that would achieve optimum TCE removal with minimum cosubstrate feeding while maintaining reliability of the degradative reaction. TCE was fed into the reactor inlet at different concentrations and airflow rates, and percent removal was measured over time. Optimum TCE removal efficiency at minimum cosubstrate feed for *P. cepacia* was established to be 300 μg/liter of TCE inlet feed at an airflow rate of 100 ml/min with a phenol feed rate of 0.1 g/liter/day. Figure 2 shows that greater than 90% TCE removal efficiency was maintained for a period of 5 days under these operating conditions. However, the activity of the microorganisms against TCE declined rapidly between days 5 and 8. This coincided with a large decline in phenol hydroxylase specific activity over the course of the experiment. A reactor operated under identical conditions of phenol feeding and airflow with no TCE in the inlet air displayed no loss of enzyme activity over the same period (data not shown).

The inactivation of phenol hydroxylase in the reactor could be accelerated by increasing the concentration of TCE in the inlet airstream while keeping the phenol feed rate and cell density constant. TCE degradation efficiency was measured over 42 h with a TCE inlet concentration of between 3,000 and 4,000 μg/liter of inlet air flowing at 100 ml/min. The bacterium could degrade even this high level of TCE in the air with removal efficiencies of between 90 and 95% for 24 h. After this time, the TCE removal efficiency declined rapidly within a few hours. A corresponding loss of phenol hydroxylase activity was also observed. This illustrates the necessity for balancing the cell density and cosubstrate feed rate with the inlet concentrations of TCE. At lower TCE concentrations and the appropriate cosubstrate feed rate, the reactor could be operated for extended periods.

Once optimum removal efficiency had been established, the longevity of the degradative activity over time was evaluated. TCE degradation by *P. cepacia* could be maintained for 14 days with an inlet TCE concentration of 450 to 500 μg/liter of air at a 100-ml/min flow. Greater than 95% removal of TCE in the inlet air was observed while the reactor was operated with a phenol feed rate of 0.2 g/liter day. The microorganism-reactor combination degraded 800 to 900 mg of TCE over the course of the 14-day experiment, while consuming 2.8 g of phenol. In another set of experiments performed under similar conditions, the activity of the microorganism could be maintained for periods in excess of 30 days (data not shown).

*P. cepacia* also appeared to be relatively robust in its resistance to transient high concentrations of TCE in the

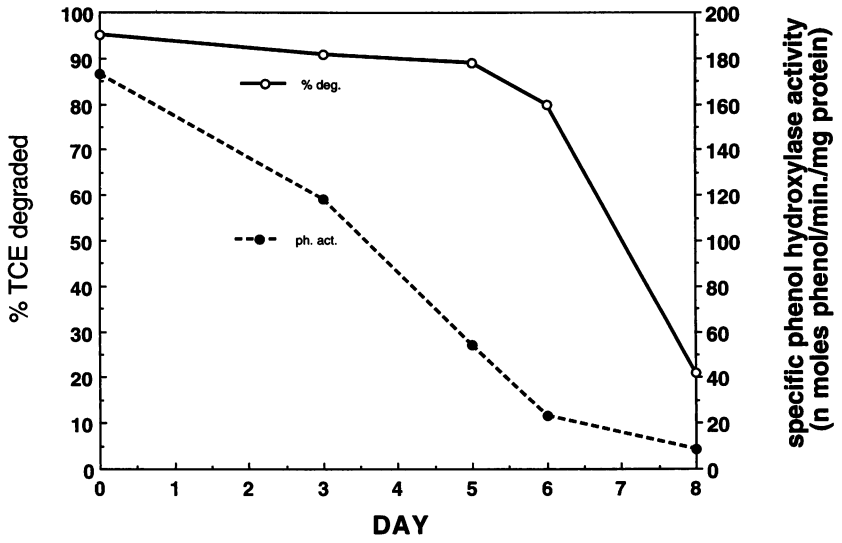


FIG. 2. TCE degradation with minimum cosubstrate feed. A bioreactor containing *P. cepacia* was fed 0.1 g of phenol per liter of volume per day. Airflow was 100 ml/min, initial biomass density was $1 \times 10^9$ cells per ml, and inlet TCE concentration was 300 to 350 μg/liter of air. Reactor performance was monitored for 8 days. ○, percent TCE removal (% deg.); ●, phenol hydroxylase specific activity (ph. act.).

form of inlet spikes deliberately introduced into the reactor by increasing the rate of TCE-contaminated water fed into the air stripper (Fig. 3). The steady-state concentration of TCE at the inlet of the reactor was maintained at approximately 350 μg/liter of air. On five occasions over a 7-day period, the inlet TCE concentration was increased to as much as 10 mg of TCE per liter of air for 1 to 6 h. The inlet TCE concentration was then returned to the steady-state value. The microorganism maintained TCE removal efficiencies in the range of 90 to 95% for the 7-day interval. The high-concentration TCE spikes were degraded by at least 95%. After 7 days of operation with five high-concentration episodes, the reactor performance declined to approximately 50% TCE removal, with efficiency gradually improving until

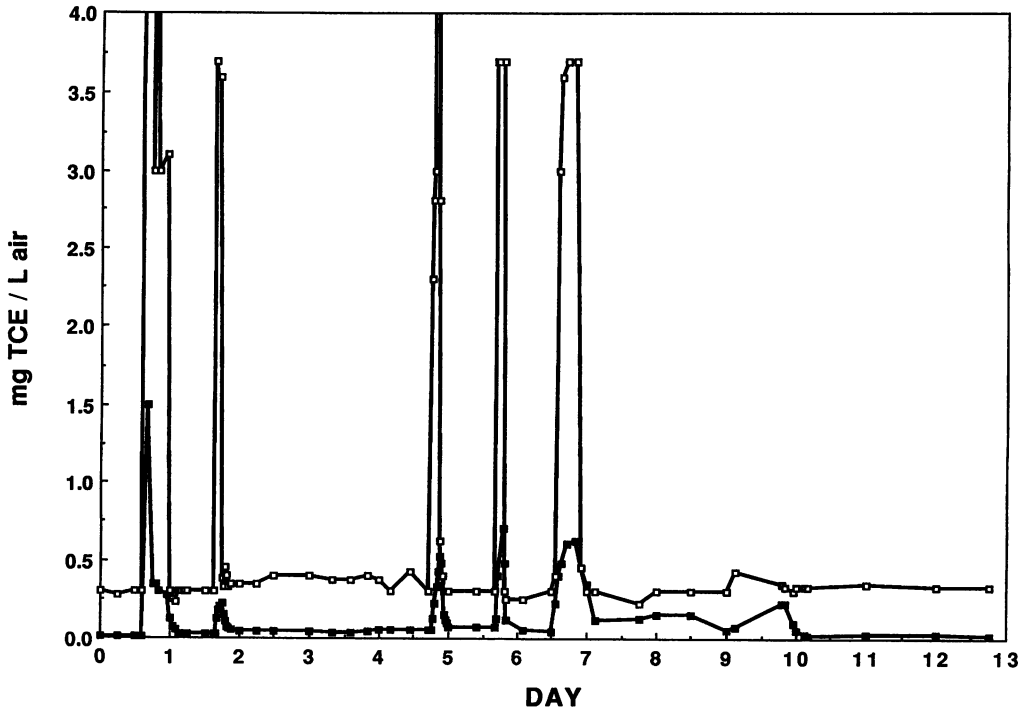


FIG. 3. Effects of TCE shocks on degradation. A reactor containing *P. cepacia* operated under conditions described in the legend for Fig. 2, with a phenol feed rate of 0.2 g/liter/day, was exposed to transient high TCE concentrations in the inlet air. Performance was monitored for 13 days. □, inlet TCE concentrations; ■, outlet TCE concentrations.

90 to 95% degradation was again measured by day 10. At the end of this experiment, the microorganisms were killed by the addition of 100 ml of 10 M NaOH and the outlet concentration of TCE increased to the same concentration as the inlet concentration (data not shown), demonstrating that biological activity is necessary for the observed reductions in TCE concentration.

## DISCUSSION

Both *P. cepacia* and *P. mendocina* display characteristics that make them useful as a means to degrade TCE in a vapor-phase reactor system. These cultures could remove 90 to 95% of the TCE introduced under most conditions of airflow rates and TCE concentrations tested. Cosubstrate feed did not appear to cause competitive inhibition of TCE degradation. These reactors did not completely eliminate TCE from the airstream: 5 to 10% of the TCE escaped from the reactor outlet.

Under certain conditions of reactor operation, loss of TCE removal and phenol hydroxylase specific activity was observed within 1 to 5 days. The deleterious effects on cell viability or enzyme specific activity caused by TCE oxidation have been observed with both *Pseudomonas putida* F1 (21, 22) and *Methylosinus trichosporium* (5). It has been hypothesized that toxic or reactive intermediates produced during the degradation of TCE cause inactivation of the catalytic enzyme(s). Apparently, phenol hydroxylase activity in *P. cepacia* G4 decreases until, below a threshold value, the extent of TCE removal is also affected. We have found that increasing the cosubstrate feed rate prevents this loss in enzyme specific activity. Increased cosubstrate feeding may cause an increase in the synthesis of phenol hydroxylase.

The TCE removal efficiency of this reactor decreased to about 60% when the airflow rate was increased to 350 ml (10% of the total liquid reactor volume) per min. This may be due to inefficiencies of mass transfer and not the biodegradative capacity of the cells. Since this reactor design uses rising bubbles to generate agitation and no attempts were made to increase bubble retention time or reduce bubble coalescence, this reactor is not a highly efficient mass transfer device. Further reactor improvements, such as the addition of an agitation system to increase shear in the reactor and the retention time of the bubbles, may improve mass transfer characteristics and allow the same volume of cells to treat a much larger volume of air.

The concentrations of residual TCE, phenol, and potential TCE metabolites in the reactor fluid were analyzed periodically during each run. At no time were any detectable levels of TCE or phenol found in the reactor contents unless the cells had lost degradative activity. No TCE degradation intermediates could be detected by GC analysis under the conditions used.

The oxidation of TCE by organisms such as *P. cepacia* or *P. mendocina* results in the liberation of 3 mol of chloride per mol of TCE degraded (23). This was manifested during the reactor runs by a constant decrease in pH that was adjusted when necessary with sodium hydroxide, causing an accumulation of NaCl in the reactor over time. We have preliminary data concerning the operation of these reactors for periods in excess of 30 days without any deleterious effects on the cells caused by the accumulation of sodium chloride or unidentified TCE metabolites in the reactor.

The results presented here are promising for commercial applications of a biological treatment system for TCE in air. Microorganisms were used for the degradation of a non-growth substrate with cosubstrate feeding that did not measurably interfere with degradation and maintained biological activity. Relatively high concentrations of the contaminant were rapidly degraded, and problems of enzyme inactivation could be overcome by adjusting the cosubstrate feed rate. Although only TCE was tested in this reactor, along with perchloroethylene, which was not degraded, the activity of aromatic-degrading microorganisms against other chlorinated compounds such as dichloroethylene and vinyl chloride has been documented (13). We suspect that this reactor-and-microorganism combination can be adapted to treat air streams containing a mixture of chlorinated alkenes, as is often found at contaminated sites.

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