Environ. Sci. Technol. **2006**, *40,* 1022−1028

Cometabolic Degradation of TCE Vapors in a Foamed Emulsion Bioreactor

EUNSUNG KAN AND
MARC A. DESHUSSES*
Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

Effective cometabolic biodegradation of trichloroethylene (TCE) vapors in a novel gas-phase bioreactor called the foamed emulsion bioreactor (FEBR) was demonstrated. Toluene vapors were used as the primary growth substrate for *Burkholderia cepacia* G4 which cometabolically biodegraded TCE. Batch operation of the reactor with respect to the liquid feed showed a drastic decrease of TCE and toluene removal over time, consistent with a loss of metabolic activity caused by the exposure to TCE metabolites. Sustained TCE removal could be achieved when continuous feeding of mineral medium was implemented, which supported cell growth and compensated for the deactivation of cells. The FEBR exhibited its highest TCE removal efficiencies (82−96%) and elimination capacities (up to 28 g_{TCE} m^{-3} h^{-1}) when TCE and toluene vapors were fed sequentially to circumvent the competitive inhibition by toluene. The TCE elimination capacity was 2−1000 times higher than reported in other gas-phase biotreatment reports. During the experiments, 85−101% of the degraded TCE chlorine was recovered as chloride. Overall, the results suggest that the FEBR can be a very effective system to treat TCE vapors cometabolically.

Introduction

Trichloroethylene (TCE) has been extensively used as an industrial solvent and degreasing agent for several decades resulting in widespread contamination of soil, groundwater, and air (*1*, *2*). TCE is a significant environmental and health concern because of its toxicity and carcinogenic properties (*3*).

Cleanup of TCE contamination has been attempted by many different methods (*4*−*7*). Among the many treatment methods for TCE contaminated wastes, biological treatment is potentially a cost-effective and safe technique as it leads to complete degradation of TCE to harmless end products. Unfortunately, no microorganism can grow on TCE as the sole carbon and energy source under aerobic conditions. However, many microorganisms have been found to degrade TCE aerobically via cometabolism (*8*). Cometabolic degradation of TCE occurs by nonspecific action of oxygenases induced by growth substrates such as toluene, phenol, methane, and propane (*9*−*14*). However, the applicability of cometabolism for the degradation of TCE has been limited by the decrease of cell activity resulting from the toxic effect of TCE intermediates and by the competitive inhibition by growth substrates.

* Corresponding author phone: (951)827-2477; fax: (951)827-5696; e-mail: mdeshuss@engr.ucr.edu.

Several bioreactors possibly circumventing these problems have been studied including biological trickling filters, stirred tank reactors, airlift reactors, membrane bioreactors, and rotating disk biofilm reactors (*15*−*19*). Prior studies on TCE biotreatment have highlighted the significant challenges in obtaining effective TCE treatment using cometabolism. The volumetric degradation rates of TCE were often too low for the process to be practical. Usually, this was due to the low activity of the culture, to the competitive inhibition by the growth substrate, and to the toxicity of TCE degradation intermediates. Increasing cell growth rate using a high feeding rate of the growth substrate could sometimes compensate for the inactivation of cell and enzyme by TCE intermediates, but it often resulted in detrimental competitive inhibition by the growth substrate (*9*, *15*). Thus, a bioreactor system that could effectively treat TCE over extended periods of time would be desirable. On the basis of the limitations identified by previous studies, the successful bioreactor system should provide effective means to grow TCE-degrading cells, to degrade TCE and its intermediates, and to circumvent the problems of cross-inhibition.

Recently, a novel gas-phase bioreactor called the foamed emulsion bioreactor (FEBR) was developed (*20*). The FEBR consists of an emulsion of highly active pollutant-degrading microorganisms and a water-immiscible organic phase which is made into a foam with the air being treated. During the operation, the foam is continuously collapsed, and the cells with the emulsion are reused. As described elsewhere (*20*, *21*), the FEBR provided high and stable removal efficiency of toluene vapors. The high performance was primarily attributed to the high rate of gaseous pollutants mass transfer and to the high cell activity resulting from active cell growth in the system. The FEBR is expected to be suitable for TCE vapor cometabolic treatment because of the high area for gas−liquid mass transfer and the relative ease in maintaining optimum cell activity, a required condition to avoid the problem of end product toxicity on the cells. In the present paper, the cometabolic treatment of TCE vapors in FEBRs is presented and discussed. The effect of various operating strategies is examined in terms of cell growth, cell activity, and TCE removal efficiency.

Materials and Methods

Reactor Setup and Microorganism. The foamed emulsion bioreactor system consisted of a foam column as a main bioreactor (4.04-cm i.d., 40-cm high, volume of 0.51 L), a cell reservoir (0.5 L), and a defoamer (1 L) (Figure 1). The defoamer and cell reservoir volumes were not optimized at this stage of the research. *Burkholderia cepacia* G4 (gift of Professor T. K. Wood, Texas A&M University) grown on toluene as a sole carbon source was used for TCE cometabolic degradation. *B. cepacia* G4 was grown prior to each experiment by bubbling toluene-contaminated air (1−2 g m^{-3}) through a mineral medium in 4 L of bubble column reactor and was concentrated by centrifugation before each experiment. For all the experiments described herein, the FEBR was rinsed with ethanol, and the mineral medium and heat resistant reactor parts were autoclaved prior to the experiments. The toluene/TCE contaminated air was passed through two 0.22-μm sterile bacterial air vents (Gelman Sciences). This ensured that *B. cepacia* remained the dominant organism in the system. The mineral medium (*22*) contained 0.024 g L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$, 0.246 g L^{-1} $MgSO_4 \cdot 7H_2O$, 7.0 g L^{-1} Na_2PO_4, 3.0 g L^{-1} KH_2PO_4, and 2.52 g L^{-1} $(NH_4)_2HPO_4$ and trace elements as follows: 5.2 mg L^{-1} EDTA $Na_4(H_2O)_2$, 1.5 mg L^{-1} $FeCl_2$ $4H_2O$, 0.12 mg L^{-1} $CoCl_2$ $6H_2O$, 0.1 mg L^{-1} $MnCl_2$ $2H_2O$, 0.07 mg L^{-1}

10.1021/es0510055 CCC: $33.50
Published on Web 12/22/2005

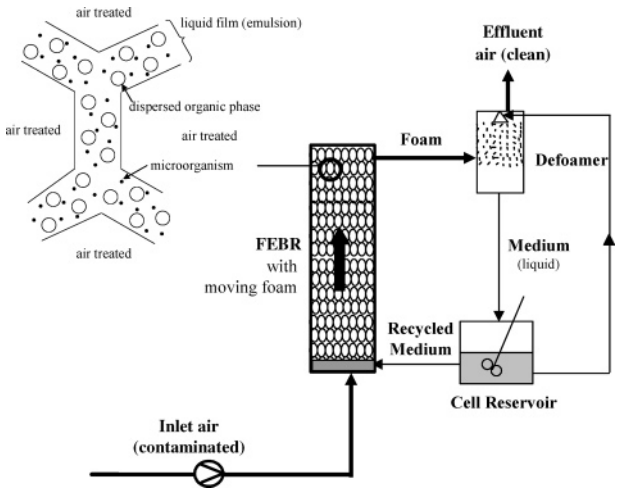

FIGURE 1. Schematic of the FEBR (Reproduced with permission from *Biotechnol. Bioeng.* 2003, *84*, 240−244. Copyright 2003 John Wiley & Sons, Inc).

$ZnCl_2$, 0.06 mg L^{-1} H_3BO_3, 0.025 mg L^{-1} $NiCl_2$ $6H_2O$, 0.025 mg L^{-1} $NaMoO_4$ $2H_2O$, and 0.015 mg L^{-1} $CuCl_2$ $2H_2O$. When chloride balances were made, trace elements were omitted from the mineral medium. Further, the liquid phase in the reactor contained 3% (v/v) oleyl alcohol as a second liquid phase and 0.2% (v/v) silicone surfactant order to obtain a stable foamed emulsion. Both oleyl alcohol and silicon oil are biocompatible and nonbiodegradable by *B. cepacia* G4.

Effect of Batch and Continuous Operations on Performance of the FEBR. To determine the effect of the operating mode on TCE removal in the FEBR, batch and continuous operations with respect to liquid feed were investigated. During batch operation, no cells or mineral medium was supplied and the cell culture was in a closed loop while toluene and TCE vapors were simultaneously fed. Water was added as needed to compensate for evaporation. The batch experiments served to determine the baseline effect of TCE on the process.

For continuous operation, 10 or 20% (vol) of the FEBR culture was replaced once per day with concentrated mineral medium (see above for composition, except that $(NH_4)_2HPO_4$ was increased to 12.6 g L^{-1} to avoid nitrogen limitation). Replacing part of the culture with the mineral medium promoted cell growth which served to compensate for cell deactivation and cell death caused by TCE biodegradation. The fill and draw mode for the liquid was selected over continuous feed and purge because of the difficulty of continuously centrifuging the reactor effluent to recover and recycle the oleyl alcohol. Two modes of toluene and TCE vapor feeding were investigated: (1) simultaneous feeding of toluene and treatment of TCE vapors and (2) alternate feeding of toluene and TCE vapors. During alternate feeding, either TCE or toluene was fed to the FEBR, switching substrate or pollutant every 12 h to avoid competitive inhibition by toluene during TCE treatment.

For the batch and continuous experiments with simultaneous feeding of TCE and toluene vapors, the operating conditions were as follows: initial biomass concentration, 7 g_{dw} L^{-1}; TCE inlet concentration, 0.06 g m^{-3}; toluene inlet concentration, 0.5 g m^{-3}; and 15 s gas retention time in the foam riser. For continuous operation with alternate feeding of TCE and toluene vapors, the operating conditions were identical except that some experiments were started with 12 g_{dw} L^{-1} of *B. cepacia* G4, and one experiment was conducted at a higher concentration of TCE (0.12 g m^{-3}) in order to explore the limits of the process. During all experiments, biomass concentrations, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (INT) reduction activity as a measurement of cell activity, toluene or TCE removal efficiency, and ammonium and chloride ion concentrations in the culture were monitored.

Analytical Methods. Biomass concentration was monitored by protein concentration determination with BCA protein assay reagent (Pierce Chemical Co., Rockford, IL). Cells were assumed to contain 50% protein by mass. Cell activity was measured as the INT reduction activity described by Kan and Deshusses (*21*). Gaseous toluene concentrations were measured by gas chromatography (HP 5890) and an FID detector. Ammonium concentration was analyzed using an ammonia kit (CHEMetrics Inc, Calverton, VA). Chloride ions were measured by a colorimetric assay (*23*). TCE mineralization was calculated by dividing the chloride ion

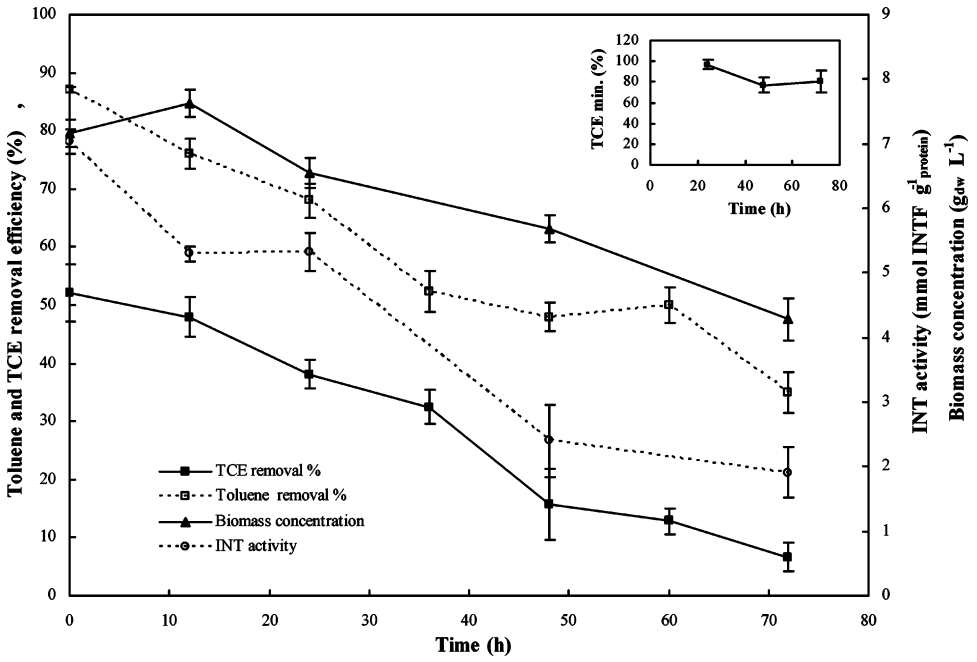

FIGURE 2. Batch operation of the FEBR treating toluene and TCE vapors (0.5 g m^{-3} and 0.06 g m^{-3}, respectively) at a gas contact time of 15 s. See Methods section for detailed conditions. INTF = INT formazan, the reduced form of INT. The inset shows TCE mineralization as % recovery of chloride ions.

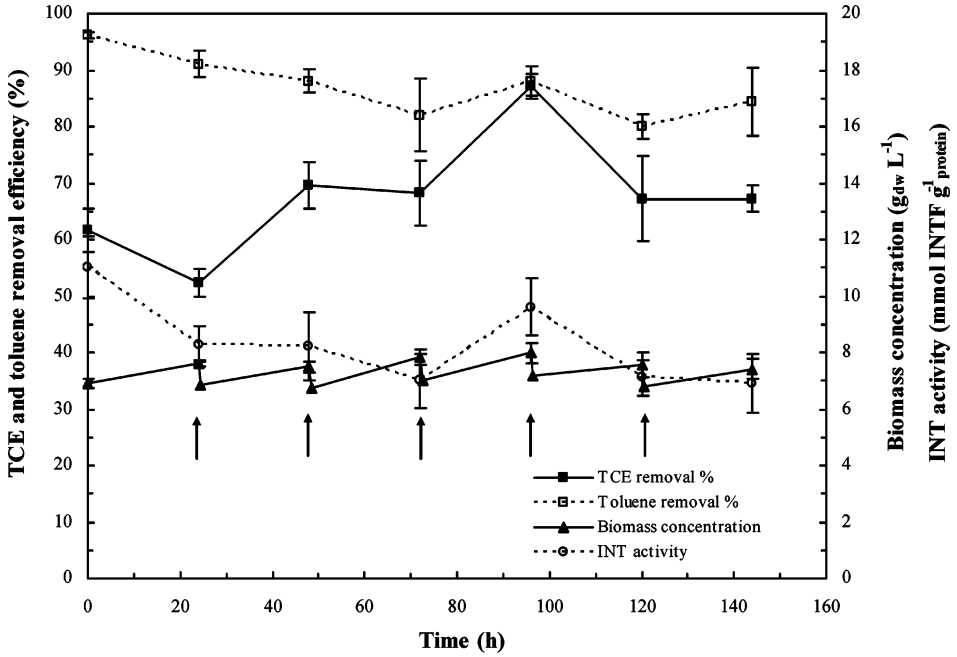

FIGURE 3. Operation of the FEBR treating TCE and toluene vapors (0.06 g m^{-3} and 0.5 g m^{-3}, respectively) at a gas contact time of 15 s and periodic replacement of 10% (v/v) of the reactor culture with mineral medium. The arrows indicate mineral medium feeding. See Methods for detailed conditions.

TABLE 1. Comparison of the Performance of Various Gas-Phase Cometabolic Bioreactors for the Treatment of TCE Vapors

reactor type[a]	primary substrate	organism	TCE inlet concentration (g m^{-3})	TCE removal (%)	TCE elimination capacity (g_{TCE} m^{-3} h^{-1})	reference
BTF	peptone, glucose	*B. cepacia* $PR1_{23}$	0.13−2	90−92	0.029−0.42	29
parallel BTF	phenol	*B. cepacia* G4	5−36	50−100	0.3−0.7	17
CSTR	toluene	*B. cepacia* G4	0.82	65	0.88	16
bubble column	phenol	*B. cepacia* G4	0.07−0.4	60−80	0.29−1.45	30
BF	toluene	*Pseudomonas putida* F1	0.14	30−60	1.9	18
CSTR	phenol	unidentified actinomycetes	0.18−0.46	55−75	1.6−2.1	31
CSTR	toluene	*B. cepacia* G4	0.25−11.9	7−90	0.86−3.1	12
HFMB	methanol	*M. trichosporium* OB3b PP358	0.14−0.19	54−86	1.4−3.9[c]	19
BTF	glucose	*B. cepacia* $PR1_{23}$	0.04−2.4	79−100[b]	0.36−16.3[b]	32
FEBR	toluene	*B. cepacia* G4	0.06−0.12	82−96	9−28[d]	this study

[a] CSTR, continuously stirred tank reactor; BF, biofilter; BTF, biological trickling filter; HFMB, hollow fiber membrane bioreactor; FEBR, foamed emulsion bioreactor. [b] Transient performance; at the highest rate, cell deactivation occurred in less than a day. [c] Shell volume of HFMB taken for calculation, EC is about 10 times lower if ancillary bioreactor volume is considered. [d] Divide value by 2 for operation of a hypothetical system with two FEBRs working in tandem on a 12/24 cycle (see text for details).

production by the theoretical chloride ions expected from the degraded TCE assuming that three moles of chloride were produced per one mole of TCE degraded.

Results and Discussion

It has been widely reported that TCE cometabolic biodegradation results in toxic intermediates such as TCE epoxide, which will spontaneously alkylate cellular components such as DNA, RNA, and proteins including TCE mono- and dioxygenases (see, e.g., *8*, *24*). This will ultimately lead to cell deactivation and cell death (*8*, *24*). Hence, the FEBR was initially operated as a batch with respect to the liquid so that a baseline cell deactivation rate could be obtained. Results are shown in Figure 2. During the 72-h batch operation, no external mineral nutrient solution or new cells were added to the FEBR while toluene and TCE-contaminated air was supplied continuously. TCE removal efficiency steadily dropped from 52 to 7%, while toluene removal efficiency dropped from 87 to 35% within 72 h (Figure 2). At the same time, biomass concentration and INT cell activity also dropped from 7.0 to 4.3 g_{dw} L^{-1} and from 7.1 to 1.9 mmol INTF $g^{-1}_{protein}$, respectively. This is a 39% loss in the biomass and a 73% loss of the cell activity and is consistent with the decrease in the overall performance of the FEBR. The drastic decay of the cell activity is most likely due to formation of TCE epoxide during TCE degradation which is expected to alkylate *B. cepacia* G4's toluene ortho-monooxygenase (TOM). The decrease of the biomass concentration is consistent with cell death and lysis and with the absence of significant cell growth because of nitrogen and possibly other nutrient limitation in the system. Because the system received no supply of mineral medium, only little free ammonia (7−13 mg L^{-1} after 24 h) was present in the system, indicating the existence of significant nitrogen limitation. From Figure 2, first-order cell activity and biomass decay constants of 0.0191 h^{-1} ($R^2 = 0.95$) and 0.0076 h^{-1} ($R^2 = 0.92$), respectively, were calculated. In a similar experiment, but with toluene vapors only (1 g m^{-3} compared to 0.5 g m^{-3} in Figure 2), the INT activity and the biomass decay constants were 0.009 h^{-1} and 0.0032 h^{-1}, respectively (*21*). Thus, in the presence of TCE vapors, activity and cell decay were 2.1−2.4 times faster than with toluene vapors only. This illustrates the significant

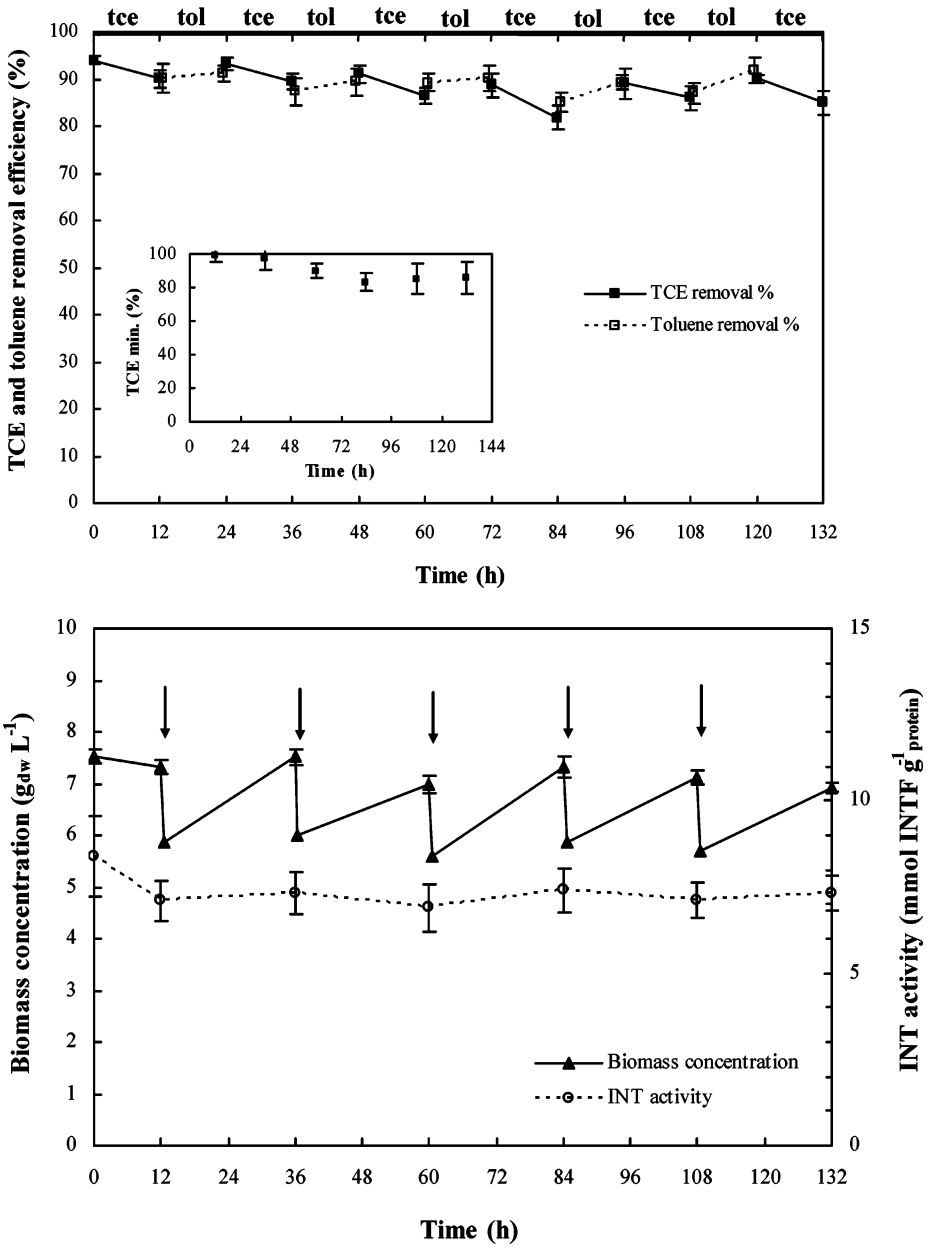

FIGURE 4. Operation of the FEBR when periodically alternating TCE and toluene feeding. Conditions: toluene inlet, 1 g m^{-3}; TCE inlet, 0.06 g m^{-3}; empty bed retention time (EBRT), 15 s. See Methods for details. The arrows indicate mineral medium feeding. The inset shows TCE conversion to chloride ions.

impact of TCE and its degradation intermediates on the overall cell metabolism. Chloride ion measurements provided evidence that TCE was degraded rather than being removed by absorption into the oleyl alcohol. The percentage of TCE mineralization during the experiment ranged from 96% after 24 h to 80% after 72 h. TCE mineralization to chloride ions decreased as the cell activity and biomass concentration decreased over time, suggesting that only partial degradation of TCE occurred. Although they could not be measured, the most likely chlorinated intermediates are highly reactive TCE epoxides and dichloroacetate (*25*−*28*). The latter compound may accumulate if the activity of the dichloroacetate halidohydrolase is such that this enzyme causes a bottleneck in the degradation pathway (*25*, *26*).

On the basis of the results obtained during this experiment, it was identified that the greatest concern was to keep a high cell activity and high biomass concentration to sustain a high removal efficiency of TCE. Thus, cell growth should be implemented to compensate for cell death and cell deactivation. This was achieved by periodically purging a fraction of the culture and feeding mineral medium and toluene. Figure 3 presents the results of the FEBR operation when 10% (vol) of the FEBR culture was replaced every 24 h by the same volume of the concentrated mineral medium. The 10% vol fraction replacement was determined from the first-order deactivation rate (0.019 h^{-1}) plotted in Figure 2, and model simulations (not shown) which revealed that a dilution rate of 0.1 day^{-1} would result in a high and constant biomass concentration. Examination of Figure 3 shows that during each cycle, biomass concentration increased as a result of growth, and a relatively stable cell activity was maintained for over 132 h of operation. The specific biomass growth rates during each FEBR cycle was 0.10 ± 0.03 day^{-1}, that is, almost equal to the dilution rate of 0.1 day^{-1}, indicating that the FEBR was operated at a steady state during the experiment. TCE removal efficiency ranged from 52 to 70% and peaked at 87% at 96 h. This corresponds to TCE elimination capacities (EC = flow × (inlet−outlet concentration)/foam

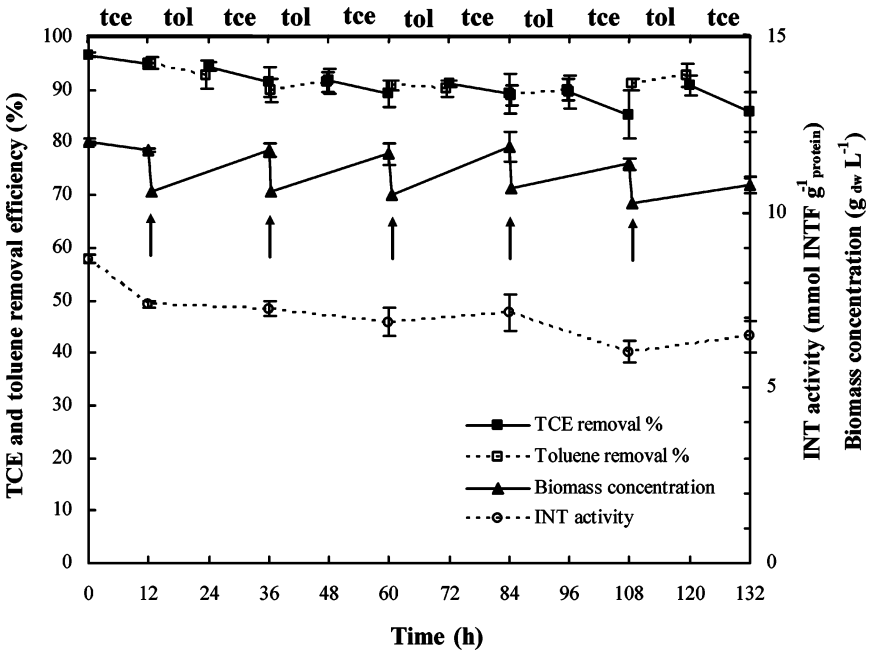

FIGURE 5. Operation of the FEBR when periodically alternating TCE and toluene feeding. Conditions: toluene inlet, 1 g m^{-3}; TCE inlet, 0.12 g m^{-3}; EBRT, 15 s. See Methods for details. The arrows indicate mineral medium feeding.

bed volume) ranging from 9 to 11 g m^{-3} h^{-1}. Toluene removal efficiency ranged from 80 to 96% at elimination capacities ranging from 96 to 115 g m^{-3} h^{-1}. The toluene removal obtained in the presence of TCE compares well with the removal efficiency of toluene (89−94%) when treated alone for 360 h of operation using the same mineral medium feeding strategy (*21*). Thus, TCE had virtually no effect on toluene removal. Most of the TCE was degraded to chloride by the microorganisms, as 87−101% of the degraded chlorine in TCE was recovered as chloride. No nitrogen limitation was observed in the culture for the duration of the experiment as a result of the continuous feeding of the concentrated ammonium phosphate medium. As mentioned, the TCE elimination capacity obtained in these experiments reached 11 g_{TCE} m^{-3} h^{-1}. This is significantly higher than the performance reported by others (Table 1) and is remarkable as the high performance was obtained at much lower TCE concentrations and at much shorter gas residence times. The lower concentrations and shorter gas residence time are more relevant to application in the field. The high performance of the FEBR is likely to be due to both the high cell density and activity that was maintained during the experiments and the high rate of TCE mass transfer because of the high surface area for gas−liquid mass transfer and the presence of oleyl alcohol as a second liquid phase. Other experiments (not shown) conducted without oleyl alcohol resulted in poor removal of TCE vapors.

However, the highest TCE removal obtained during the experiments shown in Figures 2 and 3 was only 80% probably because of the competitive inhibition of the toluene ortho-monooxygenase by toluene, the growth substrate (*9*). Therefore, to avoid competitive inhibition, sequential feeding of TCE and toluene vapors was implemented in the FEBR. Either toluene or TCE was fed in 12-h increments. In the field, this would require two bioreactors operating in tandem, with one bioreactor treating TCE while the culture in the other bioreactor would be regenerated with toluene. The 12-h half-cycle time was determined on the basis of the results of the batch operation of Figure 2 identifying that the TCE removal efficiency would not decrease significantly over that time. Also, others had found that TCE removal and toluene oxygenase activity dropped within 24 h of exposure to TCE alone, although temporarily higher TCE removal efficiencies could be obtained in the absence of a growth substrate (*18*). During the alternate feeding operation, 20% of the culture was replaced by the concentrated mineral medium every 24 h to stimulate a more vigorous cell growth during feeding with toluene. Another possibility to achieve continuous treatment of TCE and to avoid competition by the growth substrate would be to have a separate reactor to grow the cells on toluene (or any other substrate that induces toluene monooxygenase, e.g., phenol) and to either periodically or continuously add active cells to the FEBR treating TCE. Obviously, the selection of the system to regenerate the cells will have a significant impact on reactor design and on treatment cost. A detailed evaluation of these aspects is outside the scope of this paper. Herein, we focused on the sequential treatment−regeneration mode conducted on a 24-h total cycle time.

Results of the alternate feeding operation indicated that TCE removal efficiency rapidly reached 94% and was maintained between 82 and 94% (89% ± 3.5%), while toluene removal efficiency ranged from 85 to 92% for the 134 h of operation (Figure 4). Such high TCE removal efficiency was clearly due to the absence of competitive inhibition during treatment and to the effective regeneration of the cells during the growth periods. During the growth periods, the specific growth rate was on average 0.22 d^{-1}, and no nitrogen limitation was experienced. A high cell activity was maintained throughout (6.9−7.4 mmol INTF $g^{-1}_{protein}$ min^{-1}). As shown in the inset of Figure 4, the operation provided a high TCE mineralization of 85−99% except for the 83% value associated with the relatively poor removal (82%) of TCE at 84 h. The TCE elimination capacity during the 12-h feeding of TCE reached 13−14 g_{TCE} m^{-3} h^{-1}, which is higher than the EC of 9−11 g_{TCE} m^{-3} h^{-1} obtained during the simultaneous feeding of toluene and TCE. This was expected because when TCE is degraded alone, there is no competitive inhibition by toluene. These results suggest that continuous TCE treatment could be obtained with two identical FEBRs operated in tandem or with one FEBR fed with the appropriate amount of active TCE-degrading cells grown in an ancillary bioreactor. Such configuration and mode of operation may

prove useful when higher removal percentages of TCE are required.

The alternate feeding operation with periodic feeding of mineral nutrients was applied to a higher TCE inlet concentration to determine whether this operating strategy could work on a different condition and to demonstrate the flexibility of the process (Figure 5). In this experiment, the TCE inlet concentration was increased from 0.06 g_{TCE} m^{-3} to 0.12 g_{TCE} m^{-3}, that is, a doubling of the TCE loading to the reactor. Therefore, the feeding rate of the mineral medium was reduced to 10% of the culture volume per day to increase cell retention time in the system and possibly to obtain a greater cometabolic capacity. The initial biomass concentration was increased from 7 g_{dw} L^{-1} to 12 g_{dw} L^{-1} to start the experiment closer to the expected steady-state biomass concentration value. During the 132-h experiment, TCE and toluene removal efficiency ranged from 85 to 96% and from 89−95%, respectively. The good reactor behavior was attributed to the high cell activity and high biomass concentration and the absence of competitive inhibition. The periodic replacement of 10% of the culture supported active cell growth (11−12 g_{dw} L^{-1}, average specific growth rate of 0.11 day^{-1}), high cell activity (6−7 mmol INTF $g^{-1}_{protein}$), and high TCE mineralization (86−99%). As a result, the FEBR exhibited a maximum TCE elimination capacity of 28 g m^{-3} h^{-1} with a removal efficiency of 96%. As discussed above (Table 1), this is markedly higher than previous reports of TCE gas-phase biotreatment. Interestingly, the overall TCE biodegradation rate obtained from the experiments reported in Figures 3−5 was in good agreement with the rate predicted using a previously published kinetic model of TCE biodegradation by cometabolism (*12*, see Supporting Information). During the experiment of Figure 5, the transformation yield (g TCE degraded/g toluene supplied) was on average 0.12 $g_{TCE}/g_{toluene}$. This falls between the value of 0.057 $g_{TCE}/g_{toluene}$ obtained with *B. cepacia* G4 in chemostats operated such that active biomass growth occurred and the value of 0.39 $g_{TCE}/g_{toluene}$ obtained with the same culture maintained in a nongrowth status in a fed batch reactor (*12*, *16*). Thus, the FEBR systems appear to allow a better use of the primary growth substrate than a conventional chemostat.

Overall, the results presented and discussed herein clearly indicate that the FEBR is a promising system for cometabolic removal of TCE vapors. Possible enhancement of the process could come from optimizing key operating parameters such as cycle time, growth substrate concentration, and concentration of the auxiliary phase.

Acknowledgments

This work was supported by a grant from the National Science Foundation (grant no. BES 0086860, New Technologies for the Environment).

Supporting Information Available

An existing biokinetic model (*12*) of TCE biodegradation by cometabolism was used to estimate the expected TCE elimination capacity of our FEBR. The kinetic relationships and numerical results of the comparison are presented in the Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Westrick, J. J.; Mello, J. W.; Thomas, R. F. The groundwater supply survey. *J. Am. Water Works Assoc.* **1984**, *5*, 52−59.

(2) Wackett, L. P.; Gibson, D. T. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. *Appl. Environ. Microbiol.* **1988**, *54*, 1703−1708.

(3) Eder, E. Toxicology of C1−C3 chlorinated hydrocarbons. *Chemosphere* **1991**, *23*, 1783−1801.

(4) Cheng, S. F.; Wu, S. C. The enhancement methods for the degradation of TCE by zero-valent metals. *Chemosphere* **2000**, *41*, 1263−1270.

(5) Park, J.; Choi, E.; Cho, I. H.; Kim, Y. G. Solar light induced degradation of trichloroethylene (TCE) using TiO2: Effects of solar light intensity and seasonal variations. *J. Environ. Sci. Health, Part A* **2003**, *38*, 1915−1926.

(6) Nelson, M. J.; Kinsella, J. V.; Montoya, T. In situ biodegradation of TCE contaminated groundwater. *Environ. Prog.* **1990**, *9*, 190−196.

(7) Kelly, C. J.; Bienkowski, P. R.; Sayler, G. S. Kinetic analysis of a tod-lux bacterial reporter for toluene degradation and trichloroethylene cometabolism. *Biotechnol. Bioeng.* **2000**, *69*, 256−265.

(8) Newman, L. M.; Wackett, L. P. Trichloroethylene oxidation by purified toluene 2-monooxygenase: Products, kinetics, and turnover-dependent inactivation. *J. Bacteriol.* **1997**, *179*, 90−96.

(9) Folsom, B. R.; Chapman, P. J.; Pritchard, P. H. Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4; kinetics and interactions between substrates. *Appl. Environ. Microbiol.* **1990**, *56*, 1279−1285.

(10) Jean, P. A.; Arvin, E. Modeling of the cometabolic biodegradation of trichloroethylene by toluene-oxidizing bacteria in a biofilm system. *Environ. Sci. Technol.* **1997**, *31*, 3044−3052.

(11) Oldenhuis, R.; Vink, R. L. J. M.; Janssen, D. B.; Witholt, B. Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. *Appl. Environ. Microbiol.* **1989**, *55*, 2819−2826.

(12) Landa, A. S.; Sipkema, E. M.; Weijma, J.; Beenackers, A. A. C. M.; Dolfing, J.; Janssen, D. B. Cometabolic degradation of trichloroethylene by *Pseudomonas cepacia* G4 in chemostat with toluene with as the primary substrate. *Appl. Environ. Microbiol.* **1994**, *60*, 3368−3374.

(13) Wackett, L. P.; Brusseau, G. A.; Householder, S. R.; Hanson, R. S. Survey of microbial oxygenases: Trichloroethylene degradation of propane-oxidizing bacteria. *Appl. Environ. Microbiol.* **1989**, *55*, 2960−2964.

(14) Rasche, M. E.; Hyman, M. R.; Arp, D. J. Factors limiting aliphatic chlorocarbon degradation by *Nitrosomonas europaea*: cometabolic inactivation of ammonia monooxygenase and substrate specificity. *Appl. Environ. Microbiol.* **1991**, *57*, 2986−2994.

(15) Oldenhuis, R.; Oedzes, J. Y.; van der Waarde, J. J.; Janssen, D. B. Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. *Appl. Environ. Microbiol.* **1991**, *57*, 7−14.

(16) Mars, A. E.; Houwing, J.; Dolfing, J.; Janssen, D. B. Degradation of toluene and trichloroethylene by *Burkholderia cepacia* G4 in growth-limited fed-batch culture. *Appl. Environ. Microbiol.* **1996**, *62*, 886−891.

(17) Lee, E. Y.; Ye, B. D.; Park, S. H. Development and operation of a trickling biofilter system for continuous treatment of gas-phase trichloroethylene. *Biotechnol. Lett.* **2003**, *25*, 1757−1761.

(18) Cox, C. D.; Woo, H.; Robinson, K. G. Cometabolic biodegradation of trichloroethylene in the gas phase. *Water Sci. Technol.* **1998**, *37*, 97−104.

(19) Pressman, J. G.; Georgiou, G.; Speitel, G. E. A hollow-fiber membrane bioreactor for the removal of trichloroethylene from the vapor phase. *Biotechnol. Bioeng.* **2000**, *68*, 548−556.

(20) Kan, E.; Deshusses, M. A. Development of foamed emulsion bioreactor for air pollution control. *Biotechnol. Bioeng.* **2003**, *84*, 240−244.

(21) Kan, E.; Deshusses, M. A. Continuous operation of foamed emulsion bioreactors treating toluene vapors. *Biotechnol. Bioeng.* **2005**, *92*, 364−371.

(22) Luu, P. P.; Yung, C. W.; Sun, A. K.; Wood, T. K. Monitoring trichloroethylene mineralization by *Pseudomonas cepacia* G4 PR1. *Appl. Microbiol. Biotechnol.***1995**, *44*, 259−264.

(23) Bergmann, J. G.; Sanik, Determination of trace amounts of chlorine in naphtha. *J. Anal. Chem.* **1957**, *29*, 241−243.

(24) Yeager, C. M.; Bottomley, P. J.; Arp, D. J. Cytotoxicity associated with trichloroethylene oxidation in *Burkhoderia cepacia* G4. *Appl. Environ. Microbiol.* **2001**, *67*, 2107−2115.

(25) Nelson, M. J. K.; Montgomery, S. O.; O'Neill, E. J.; Pritchard, P. H. Aerobic metabolism of trichloroethylene by a bacterial isolate. *Appl. Environ. Microbiol.* **1986**, *52*, 383−384.

(26) Newman, L. M.; Wackett, L. P. Trichloroethylene oxidation by purified toluene 2-monooxygenase: products, kinetics and

turnover-dependent inactivation. *J. Bacteriol.* **1997**, *179*, 90−96.
(27) Timkovich, R.; Thrasher, J. S. Carbon monoxide oxygenase activity of cytochrome cd1. *Biochemistry* **1988**, *27*, 5383−5388.
(28) Lamzin, V. S.; Aleshin, A. E.; Strokopytov, B. V.; Yukhnevich, M. G.; Popov, V. O.; Harutyunyan, E. H.; Wilson, K. S. Crystal structure of NAD-dependent formate dehydrogenase. *Eur. J. Biochem*. **1992**, *206*, 441−452.
(29) Shields, M. S.; Reagin, M. J.; Gerger, R. R.; Somerville, C.; Schaubhut, R.; Campbell, R.; Hu-Primmer, J. Constitutive degradation of trichloroethylene by an altered bacterium in a gas-phase bioreactor. In *Hinchee RE*; Leeson, A., Semprini, L., Ong, S. K., Eds.; Bioremediation of chlorinated and polycyclic aromatic hydrocarbon compounds; Lewis Publishers: Boca Raton, FL, 1994; pp 50−65.
(30) Hecht, V.; Brebbermann, D.; Bremer, P.; Deckwer, W. D. Co-metabolic degradation of trichloroethylene in a bubble column bioscrubber. *Biotechnol. Bioeng.* **1995**, *47*, 461−469.
(31) Lee, S.; Strand, S.; Stensel, H. D. Sustained degradation of trichloroethylene in a suspended growth gas treatment reactor by an actinomycetes enrichment. *Environ. Sci. Technol.* **2000**, *34*, 3261−3268.
(32) Sun, A. K.; Wood, T. K. Trichloroethylene mineralization in a fixed-film bioreactor using a pure culture expressing constitutively toluene ortho-monooxygenase. *Biotechnol. Bioeng.* **1997**, *55*, 674−685.

Received for review May 27, 2005. Revised manuscript received November 7, 2005. Accepted November 15, 2005.

ES0510055