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Cometabolic Degradation of Trichloroethylene by *Pseudomonas cepacia* G4 in a Chemostat with Toluene as the Primary Substrate

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***Pseudomonas cepacia* G4 is capable of cometabolic degradation of trichloroethylene (TCE) if the organism is grown on certain aromatic compounds. To obtain more insight into the kinetics of TCE degradation and the effect of TCE transformation products, we have investigated the simultaneous conversion of toluene and TCE in steady-state continuous culture. The organism was grown in a chemostat with toluene as the carbon and energy source at a range of volumetric TCE loading rates, up to 330 μmol/liter/h. The specific TCE degradation activity of the cells and the volumetric activity increased, but the efficiency of TCE conversion dropped when the TCE loading was elevated from 7 to 330 μmol/liter/h. At TCE loading rates of up to 145 μmol/liter/h, the specific toluene conversion rate and the molar growth yield of the cells were not affected by the presence of TCE. The response of the system to varying TCE loading rates was accurately described by a mathematical model based on Michaelis-Menten kinetics and competitive inhibition. A high load of 3,400 μmol of TCE per liter per h for 12 h caused inhibition of toluene and TCE conversion, but reduction of the TCE load to the original nontoxic level resulted in complete recovery of the system within 2 days. These results show that *P. cepacia* can stably and continuously degrade toluene and TCE simultaneously in a single-reactor system without biomass retention and that the organism is more resistant to high concentrations and shock loadings of TCE than *Methylosinus trichosporium* OB3b.**

Most monohalogenated hydrocarbons can be used as growth substrates by specific microbial cultures, while compounds with two or more halogens per molecule are generally more recalcitrant, especially when the halogens are bound to the same carbon atom (17). The latter compounds, however, are usually biodegradable via cometabolic transformation processes, provided that they have at least one carbon-hydrogen bond. Examples of such compounds are the dichloroethylenes, trichloroethylene (TCE), 1,1-dichloroethane, 1,1,1-trichloroethane, and chloroform (3, 21, 27). Cometabolic conversions of halogenated compounds rely on nonspecific enzymes, usually mono- and dioxygenases that do not specifically cleave carbon-halogen bonds but produce unstable intermediates that release halides by chemical decomposition.

The best-studied compound subject to aerobic cometabolism is TCE. A whole series of organisms have been shown to convert this compound, and attempts have been made to use this knowledge for the development of bioreactor systems for application in various branches of environmental biotechnology. The most critical factors in deciding which organism(s) to take for such bioreactor systems are the specific activity of the cells for TCE and the possible formation of toxic intermediates. On the basis of kinetic criteria, both methanotrophs and toluene oxidizers are suitable candidates (7). In methanotrophs, however, TCE conversion results in inactivation of the cells (1, 2, 5, 13, 23, 29, 30).

Pseudomonas cepacia G4 is the best-known representative of the group of toluene-oxidizing, TCE-degrading bacteria (18–20). The organism has been isolated specifically for its ability to convert TCE. The wild-type strain needs the presence of an aromatic compound such as phenol or toluene for the induction of the TCE-oxidizing enzymes. Kinetic experiments with phenol and TCE have led to the suggestion that the aromatic compound and TCE could be competitive inhibitors (10, 11). This indicates that it may be inefficient to degrade TCE in the presence of an aromatic growth substrate.

The development of a bioreactor system for the continuous degradation of TCE from air with *P. cepacia* G4 as the biocatalyst requires more quantitative data on the kinetic characteristics of the simultaneous conversion of the aromatic growth substrate and TCE and on the possible toxic effects of degradation products of TCE. The stability of the reactor system, which must degrade TCE constantly over a long period, and the ability of the system to withstand varying concentrations of TCE are also important factors. In this paper, we describe the kinetics of simultaneous TCE and toluene degradation in continuous culture. We also present a mathematical model that accurately describes the observed kinetics of TCE and toluene degradation. *P. cepacia* G4 appeared more resistant to high loadings of TCE than the methanotrophic TCE oxidizer *Methylosinus trichosporium* OB3b.

MATERIALS AND METHODS

Nomenclature. The following parameters are used in this paper: a, interfacial area (square meters per cubic meter); C_g, gas phase concentration in the reactor (micromolar); $C_{g,i}$, inlet gas phase concentration (micromolar); C_l, liquid-phase concentration in the reactor (micromolar); $C_{l,i}$, inlet liquid-phase

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concentration (micromolar); $C_{l,x}$, biomass concentration in the liquid phase (milligrams per liter); C_l^*, liquid-phase concentration at gas-liquid interface (micromolar); ϕ_g, volumetric gas flow rate (liters per minute); ϕ_l, volumetric liquid flow rate (liters per minute); k_L, liquid side mass-transfer coefficient (meters per minute); $K_{i,C}$, toluene inhibition constant of TCE degradation (micromolar); $K_{i,S}$, TCE inhibition constant of toluene degradation (micromolar); K_m, Michaelis-Menten half-saturation constant (micromolar); R, volumetric conversion rate (micromoles per minute); V_l, liquid volume reactor (working volume; liters); V_{max}, maximum specific conversion rate (micromoles per minute per milligram of cells); Y_{XS}, overall yield coefficient of biomass on substrate (milligrams of cells per micromole). Subscripts denote the following parameters: X, biomass; S, growth substrate; C, cometabolized substrate (TCE).

Bacterial strain and culture conditions. *P. cepacia* G4 (19) was a gift from M. S. Shields, U.S. Environmental Protection Agency, Gulf Breeze, Fla. The organism was grown in a 1-liter fermentor with toluene as the carbon and energy source. The medium contained (per liter) 6.9 g of $Na_2HPO_4 \cdot 12H_2O$, 2.4 g of KH_2PO_4, 0.5 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4 \cdot 7H_2O$, 30 mg of yeast extract (BBL Laboratories), and 5 ml of a trace elements solution (16). Prior to sterilization, the medium was acidified with concentrated H_2SO_4 to a pH of 2 to 3. The pH in the fermentor was regulated at 7.2 with 2 N KOH. Toluene and TCE were supplied by bubbling a filtered (ACRO 50; Gelman) air-toluene-TCE mixture through the reactor. The feed of toluene was generated by passing a stream of air through a flask containing pure toluene, which was subsequently diluted into the main airflow. Addition of TCE to the continuous culture was accomplished by diluting TCE-saturated air with air containing no TCE and introducing only a fraction of the diluted TCE gas into the main airflow (21). Other conditions were as follows: working volume, 800 ml; temperature, 28°C; impeller speed, 900 rpm; airflow rate, ca. 50 ml/min; dilution rate, 0.083 h^{-1}.

Analytical methods. TCE, toluene, and oxygen were measured by gas chromatography. Concentrations in the gas phase were determined after sampling with a gas-tight syringe (Pressure-Lok, series A-2). TCE and toluene in the gas phase were analyzed with a flame ionization detector. The accuracy (standard deviation) of this method was better than 5%, with a detection limit of approximately 50 nM. Oxygen in the gas phase was analyzed on a Molsieve 5A packed column equipped with a thermal conductivity detector. With a standard deviation of less than 5%, these measurements also had a high precision. Concentrations of TCE and toluene in the liquid phase were measured with pentane-extracted samples (31). Samples (4.5 ml) were extracted with 1.5 ml of pentane containing 0.05 mM 1-bromohexane as an internal standard. Gas chromatography conditions were as described previously (24). An electron capture detector was used for the analysis of TCE, and a flame ionization detector was used for the analysis of toluene in the liquid phase. The determinations of the toluene and TCE concentrations in the liquid phase were much less reproducible than the gas phase measurements; concentration differences of up to 20% between duplicates occurred in all of the nine steady states characterized. Oxygen in the liquid phase was monitored with a probe, as described before (25).

Chloride production was determined with a colorimetric assay (15).

Modeling. A mathematical model was used to describe the simultaneous conversion of TCE and toluene by *P. cepacia* G4 during steady-state growth on toluene. The model was based on the following assumptions: (i) the rate of degradation of TCE and toluene by *P. cepacia* G4 can be described by Michaelis-Menten-type kinetics adapted to include competitive inhibition; (ii) the gas phase and the liquid phase in the chemostat are ideally mixed; (iii) the overall growth yield of the cells on toluene is not affected by the conversion of TCE; and (iv) mass transfer from the gas phase via the aqueous phase to the cells can be described by the film model (32). Mass-transfer resistance is supposed to be located solely in the liquid phase.

The model is based on five equations. For the degradation kinetics, Michaelis-Menten-type equations are used, assuming a competitive inhibition between the substrate (toluene) and contaminant (TCE):

$$R_s = -V_{max,s} \frac{C_{l,s}}{C_{l,s} + K_{m,s}\left(1 + \frac{C_{l,c}}{K_{i,s}}\right)} C_{l,x} \quad (1)$$

$$R_c = -V_{max,c} \frac{C_{l,c}}{C_{l,c} + K_{m,c}\left(1 + \frac{C_{l,s}}{K_{i,c}}\right)} C_{l,x} \quad (2)$$

The symbols used are explained above in the nomenclature section. The constant overall growth yield on the substrate is given by

$$Y_{XS} = -\frac{R_X}{R_S} \quad (3)$$

Finally, mass balances for both the gas and liquid phases are formulated as follows:

$$\phi_g(C_{g,i} - C_g) - k_L a\,(C_l^* - C_l)\,V_l = 0 \quad (4)$$

$$\phi_l(C_{l,i} - C_l) + k_L a\,(C_l^* - C_l)\,V_l + RV_l = 0 \quad (5)$$

Determination of kinetic parameters. The $k_L a$ values for toluene (1.67 min^{-1}) and TCE (1.75 min^{-1}) were calculated from the $k_L a$ value for oxygen, using the equation described by Westerterp et al. (32). The $k_L a$ value for oxygen (2.61 min^{-1}) was determined by the steady-state oxygen balance method (26).

The kinetic constants $V_{max,S}$ (0.07 μmol/min/mg of cells), $V_{max,C}$ (5.0×10^{-3} μmol/min/mg of cells), $K_{m,C}$ (6 μM), and $K_{m,S}$ (25 μM) were estimated from the work of Paul de Graaf in our laboratory (6), who determined these values with *P. cepacia* G4 growing on toluene in a chemostat at dilution rates of 0.07 and 0.09 h^{-1}. These measurements were done by determining substrate depletion rates in batch incubations (30-ml flasks with 10 ml of medium) containing mineral medium, substrate, and cells freshly collected from the chemostat cultures. The flasks were vigorously shaken in order to constantly maintain a distribution close to equilibrium between the liquid and gas phases. Control experiments with the addition of substrate to the liquid phase only indicated that rapid equilibration indeed occurred (mass-transfer coefficient, $k_L a$, ≥6 min^{-1}). The rate of depletion of toluene and TCE was monitored over a 15-min period by gas chromatographic analysis of headspace samples taken with a syringe through viton septa. Degradation rates in the liquid phase were calculated by using the partition coefficients of toluene and TCE (6, 12). The validity of the applied method was also checked by comparing separate gas and liquid phase measurements.

The inhibition constants (K_i values) for TCE and toluene

TABLE 1. Effects of TCE loading rate on TCE and toluene conversion characteristics at nine different steady states

Steady state no.	Load (μmol/h)		Toluene conversion			TCE conversion			Growth yield (g of cells/mol)
	TCE	Toluene	μmol/h	%	nmol/min/mg of cells	μmol/h	%	nmol/min/mg of cells	
1	0	520	510	99	28.0				48.8
2	5.6	480	470	99	26.1	5.0	90	0.28	51.0
3	7.6	510	500	97	34.0	5.3	70	0.36	41.7
4	12.6	580	560	97	26.5	8.5	67	0.40	49.9
5	20.2	590	570	97	33.9	12.5	62	0.74	41.3
6	30.1	560	550	97	31.4	17.9	60	1.02	44.7
7	59.6	600	570	95	32.8	19.6	33	1.12	42.7
8	116	560	510	91	29.5	25.7	22	1.49	45.3
9	263	600	420	71	37.5	18.0	7	1.61	36.9

with *P. cepacia* were also determined by substrate depletion experiments. The initial rates of depletion in the gas phase of toluene, with different amounts of TCE present, were determined. For the other K_i value, the initial rates of depletion in the gas phase of TCE, with different amounts of toluene present, were determined. The results were as follows: $K_{i,S}$ = 30 μM and $K_{i,C}$ = 5 μM.

RESULTS

P. cepacia G4 grew well in a chemostat with toluene as the carbon and energy source. At a toluene addition rate of 520 μmol/h to a 1-liter fermentor with a working volume of 800 ml, operated at a dilution rate of 0.08 h^{-1}, a steady-state cell density of 0.37 g/liter was reached. This corresponds to a growth yield of 49 g of cells per mol of toluene converted. The specific activity of the cells for toluene under those conditions was 28 nmol/min/mg of cells.

Simultaneous degradation of toluene and TCE. In order to evaluate the possibilities for simultaneous degradation of TCE and toluene, the chemostat was operated at the same constant dilution rate and toluene flux as described above and tested with a series of TCE addition rates, ranging from 5.6 to 263 μmol/h. The total gas flow through the system was kept constant, so the increases in the TCE loading rates resulted in increases in the TCE concentrations in the incoming airstream (Table 1). After each increase in the TCE loading rate, the reactor conditions were kept fixed for at least 3 days (which is five times the average residence time) to ensure that the measurements were made under steady-state conditions. Measurements made after another 24 h confirmed that the system was indeed in steady state. At the lowest TCE flux applied, 5.5 μmol/h, 90% of the TCE was removed from the incoming gas stream. The TCE conversion rate, i.e., the TCE loading rate minus the TCE effluent rate per milligram of bacterial cells present in the chemostat, was 0.28 nmol/min/mg of cells at that moment. Higher TCE fluxes resulted in higher concentrations of TCE in the liquid phase, higher specific TCE removal activities of up to 1.6 nmol/min/mg of cells, and lower TCE conversion efficiencies (Fig. 1). TCE loading rates of up to 116 μmol/h lowered the toluene conversion efficiency but did not significantly affect the growth yield of the cells on toluene (Table 1). A further increase in the TCE loading rate to 263 μmol/h resulted in a growth yield of 36.9 g of cells per mol of toluene converted, a distinct reduction compared with the growth yield of 45 ± 4 g/mol observed at TCE loading rates at or below 116 μmol/h.

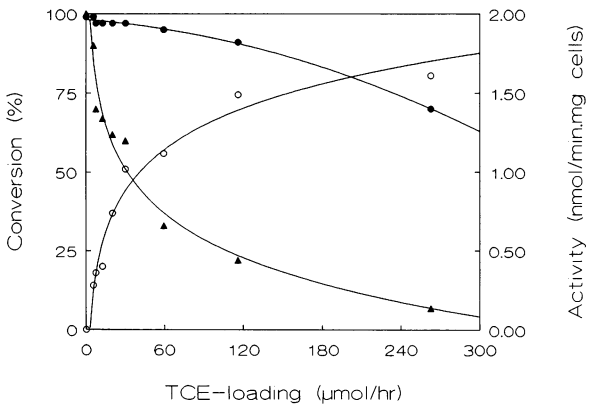

FIG. 1. Simultaneous degradation of toluene and TCE by *P. cepacia* G4 growing on toluene in a chemostat at a dilution rate of 0.08 h^{-1}. ▲, conversion of TCE; ●, conversion of toluene; ○, TCE degradation activity in the chemostat.

Chloride balances (Table 2) indicated that all three chlorine atoms from TCE were liberated as chloride.

In another experiment, trichloroethanol was detected as an intermediate of TCE degradation at a concentration of 0.5 μM in the liquid phase, at a dilution rate (D) of 0.04 h^{-1} and a TCE loading rate of 24 μmol/liter/h (see below).

Effect of dilution rate on TCE elimination capacity. In order to evaluate the effect of the growth rate on the TCE removal efficiency of *P. cepacia* in continuous culture, we did an experiment in which we lowered the dilution rate in the fermentor from 0.08 to 0.04 h^{-1} while maintaining the volumetric loading rate for toluene at 700 μmol/liter/h. The experiment was performed in a 3-liter fermentor with a working volume of 1,275 ml. The TCE loading rate was set at 24 μmol/liter/h, at a TCE concentration of the incoming gas of 6.3 μM. These conditions were comparable to those in one of the experiments done at D = 0.08 h^{-1}. As expected, the biomass concentration in the fermentor operated at D = 0.04 h^{-1} was two times the biomass concentration at D = 0.08 h^{-1}.

TABLE 2. Chloride balances

TCE loading rate (μmol/h)	TCE conversion rate (μmol/h)	Chloride production rate (μmol/h)	
		Expected	Measured
7.6	5.3	15.9	18.8
20.2	12.5	37.5	38.1
59.6	19.6	58.8	63.9
116	25.7	77.1	79.7

TABLE 3. Effect of dilution rate on TCE and toluene conversion characteristics of *P. cepacia* G4

Dilution rate (h^{-1})	Toluene loading rate (μmol/liter/h)	Biomass (g/liter)	TCE loading rate (μmol/liter/h)	$C_{g,C,i}$ (μM)	$C_{g,C}$ (μM)	TCE conversion		Sp act for TCE (nmol/min/mg of cells)
						μmol/liter/h	%	
0.04	700	0.66	23.7	6.3	1.2	19.0	80	0.48
0.08	740	0.35	25.3	6.5	2.4	15.6	62	0.74

A comparison of the results of these two experiments shows that lowering the dilution rate resulted in a higher volumetric activity of the system (Table 3).

Resilience of the system towards high doses of TCE. Knowing that TCE conversion does not result in inactivation of the cells, it is of interest to know whether the organism can withstand a temporary exposure to high concentrations of TCE. Therefore, we exposed the cells to a high load of TCE. The baseline conditions were chosen in such a way that one of the steady states described above was repeated: $D = 0.08\ h^{-1}$; toluene addition rate, 600 μmol/h; TCE addition rate, 12.5 μmol/h; $C_{g,TCE,i} = 5$ μM. Once steady state was achieved, the TCE load was instantaneously increased more than 200-fold to 2,750 μmol/h, $C_{g,TCE,i} = 1{,}100$ μM. This load was applied for 12 h, i.e., for about one average residence time in the reactor. The high TCE load resulted in TCE concentrations of up to 1.1 mM in the liquid phase; toluene degradation was inhibited completely, growth stopped, and washout of the cells occurred (Fig. 2). When the TCE load was reduced to the original nontoxic level, the system recovered, toluene degradation resumed, cells grew again, and the cell density came back to the original value. TCE degradation resumed also, but later, when the toluene level in the liquid phase had come down to a concentration of about 1.2 μM.

These results show that *P. cepacia* G4 can withstand exposure to high concentrations of TCE for a considerable period of time.

Modeling the cometabolic degradation of TCE and toluene. In order to understand the interplay between toluene and TCE degradation by *P. cepacia* G4, we developed and tested a simple mathematical model, which was based on the assumption that degradation of TCE and toluene can be described by two Michaelis-Menten-type equations adapted to take competitive inhibition into account. The input variables used to test

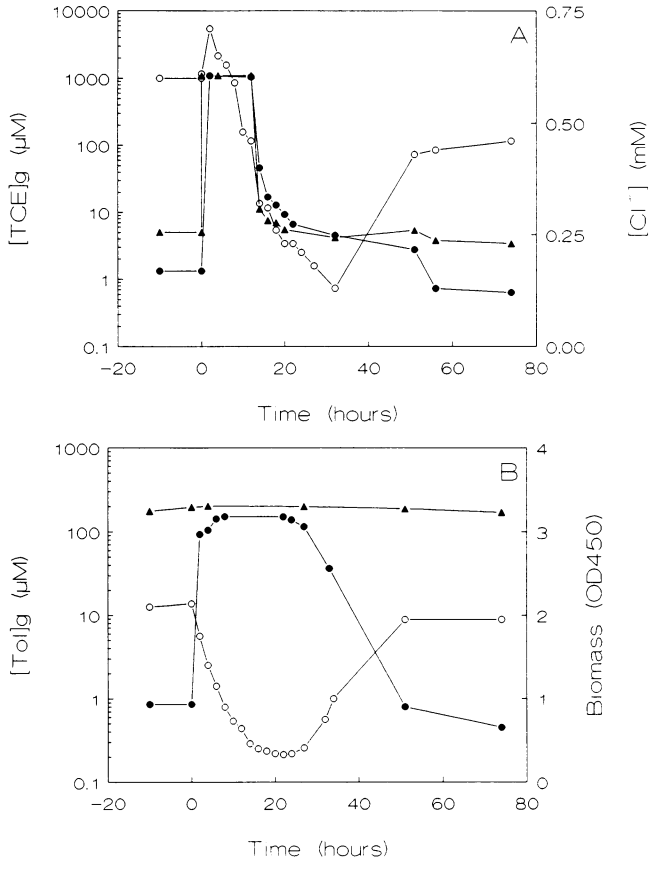

FIG. 2. Resilience of *P. cepacia* G4 towards a stepwise increased load of TCE from 12.5 to 2,750 μmol/h, maintained over the next 12 h and then reduced again to the original level. (A) ▲, $C_{g,C,i}$; ●, $C_{g,C}$; ○, $[Cl^-]$. (B) ▲, $C_{g,S,i}$; ●, $C_{g,S}$; ○, optical density at 450 nm (OD_{450}).

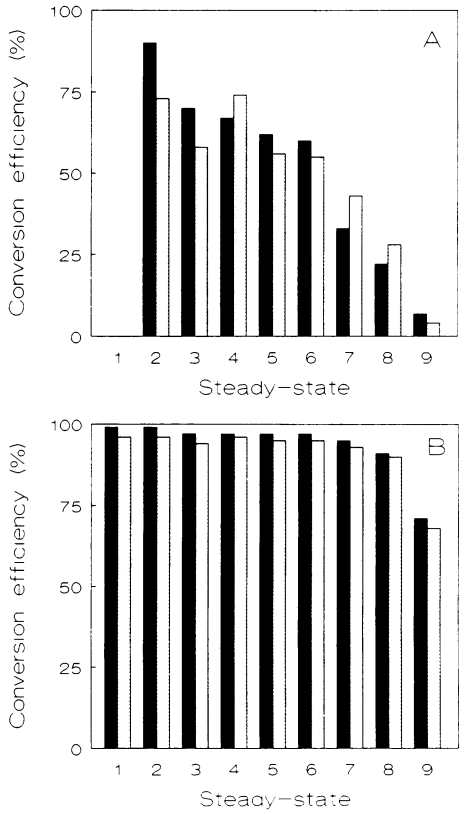

FIG. 3. Comparison of predicted (▨) and experimentally determined (■) conversion efficiencies of toluene and TCE at nine different steady states in the chemostat. (A) TCE; (B) toluene.

TABLE 4. Values of variables calculated with the mathematical model and compared with those determined experimentally in nine different steady states

Steady state no.	$C_{g,C}$ (μM)		$C_{g,S}$ (μM)		$C_{l,X}$ (mg/liter)		$C_{l,C}$ (μM)		$C_{l,S}$ (μM)	
	Exptl	Model	Exptl	Model	Exptl	Model	Exptl	Model	Exptl	Model
1	0.0	0.0	1.6	7.2	383	371	0.0	0.0	1.4	16
2	0.2	0.5	1.5	6.6	375	370	0.0	1.1	0.2	15
3	0.8	1.1	4.9	9.6	313	307	0.4	2.3	6.4	24
4	1.4	1.1	5.3	7.4	450	442	0.5	2.4	4.5	16
5	2.4	2.8	5.5	11	350	342	1.0	6.2	4.5	28
6	3.9	4.3	4.8	11	365	353	2.0	9.4	5.2	26
7	13	11	9.4	15	365	359	5.6	23	10	38
8	27	24	14	18	363	356	14	52	9.1	50
9	74	73	52	61	233	218	80	164	126	187

the model were the same as those used in the actual experiments. With this input, it was possible to closely predict the conversion efficiency of both toluene and TCE (Fig. 3). For TCE loading rates of up to 120 μmol/h, the output of the model showed good agreement also with experimentally determined concentrations of toluene and TCE in the gas phase and with the cell density (Table 4). Some fluctuation in the actual amount of biomass can be explained from the measured fluctuations in the toluene feed rate (Table 1). The experimental concentrations of toluene and TCE in the liquid phase did not coincide with the values predicted by the model (Table 4). However, the conversion activities of the cells for TCE and toluene in the fermentor (Table 1) were in accordance with the predicted rates using calculated concentrations in the liquid phase (Table 4).

DISCUSSION

The results indicate that it is possible to cometabolically degrade TCE continuously and stably in a completely mixed system with growing cells. The chemostat was run for more than 6 weeks at various TCE loading rates without toxic effects of TCE or TCE conversion products. The growth yield and the specific activity of the cells for toluene were not affected by TCE loading rates of up to 145 μmol/liter/h. Previous experiments in our laboratory with *M. trichosporium* OB3b in a similar experimental setup (21, 22) showed that the growth yield of the cells on methane decreased with increasing TCE conversion until the system collapsed completely at a TCE loading rate of 13 μmol/liter/h. The highest volumetric activity of the reactor and the highest observed specific activity of the cells were at least 20- and 75-fold higher for *P. cepacia* G4 than for strain OB3b (Table 5). The hypothesis is that the *M. trichosporium* cells get poisoned by conversion products of TCE. Apparently, *P. cepacia* can convert TCE without inactivation of the cells.

TABLE 5. Comparison of TCE transformation characteristics of *P. cepacia* G4 and *M. trichosporium* OB3b in continuous culture

Characteristic	*P. cepacia*	*M. trichosporium*[a]
Growth substrate	Toluene	Methane
Biomass concn (g/liter)	0.4	2.5
Toxicity of TCE transformation	Not observed	Yes
Conversion ratios (μmol of TCE/mmol of growth substrate)	10–50	0.008–0.4
TCE loading rates (μmol/liter/h)		
Stable	7–330	0.08–2.3
Not stable[b]	Not observed	13
$C_{g,C,i}$ (μM)		
Stable	2–80	0.02–0.6
Not stable[b]	Not observed	3.1
Highest sp act for TCE (nmol/min/mg of cells)	1.6	0.024
Highest volumetric activity (nmol/liter/min)	530	25

[a] Data from the work of Oldenhuis (21) and Oldenhuis and Janssen (22).
[b] Highest degradation rate observed with washout occurring.

P. cepacia G4 also had the capacity to survive temporary high concentrations of TCE. The same was recently found for strain G4 growing in an airlift reactor in the presence of phenol (8). This is important for its potential use in the cleanup of contaminated waste streams in which TCE concentrations can vary considerably.

It is not clear why *M. trichosporium* OB3b is more sensitive to TCE conversion than *P. cepacia* G4. TCE epoxide, the first intermediate in the TCE degradation route of *M. trichosporium* OB3b, can covalently bind to various nucleophilic sites on biological macromolecules (14, 23). Alternatively, the epoxide may decompose to acyl halides, which are extremely reactive and toxic (14). Conceivably, *P. cepacia* is more resistant to TCE, either because less reactive products are formed in this organism or because the organism is less sensitive to the damage caused by TCE degradation products.

The specific TCE degradation rate of *P. cepacia* in the chemostat increased with increasing TCE concentrations according to Michaelis-Menten kinetics, even though the increased TCE concentrations were accompanied by increased toluene concentrations in the liquid phase. The maximally observed specific TCE conversion activity of the cells in the chemostat was 1.6 nmol/min/mg of cells, almost twofold lower than the activity of cells grown on an aromatic substrate and tested for TCE conversion in the absence of this substrate (6, 11). The maximal TCE conversion activities in the reactor were reached at toluene concentrations in the liquid phase of 40 to 150 μM, although at these toluene concentrations inhibition of TCE conversion already occurred.

Our mathematical model for the cometabolic conversion of TCE in the presence of toluene as the growth substrate describes the conversion efficiency of both compounds at the different steady states quite well. The strength of the model is that it is based on some generally accepted principles and, more importantly, needs as input variables only the ingoing flows and concentrations of toluene and TCE.

Usually it is assumed that $K_{i,S}$ (the inhibition constant of TCE on toluene conversion) is equal to $K_{m,C}$ (the Michaelis-Menten half-saturation constant for TCE conversion), while $K_{i,C}$ is equal to $K_{m,S}$ (4). Our data indicate that this is not the case with simultaneous toluene and TCE conversion. This may

be caused by the fact that the measurements were done with whole cells and not with purified enzyme. Factors other than competition for the active site of the enzyme may influence the effect of toluene on TCE conversion and vice versa, e.g., reductant supply or substrate transport to the enzyme. With the same model, but using the K_m of toluene as the K_i on TCE conversion and vice versa, a washout of the system at TCE loading rates higher than 150 μmol/h was predicted (data not shown), indicating the necessity of using the measured values of the different constants in the model.

The model is based on the assumption that the growth yield of the cells on toluene is constant. This implies that TCE conversion does not affect the yield. However, it can be expected that this assumption no longer holds at high TCE loading rates, because TCE can become toxic to the cells and because TCE oxidation by toluene monooxygenase in *P. cepacia* G4 requires reducing equivalents. Nevertheless, the experimental results show that the assumption is applicable over a wide range of TCE loading rates.

A comparison of the measured and the predicted liquid phase concentrations shows that the latter are consequently higher. This can partly be explained by the sampling procedure. In the period needed to obtain a sample of the liquid phase from the chemostat, the conversion of both compounds certainly will proceed, which causes a significant reduction of the actual concentration. For an evaluation of the performance of the reactor system, however, these considerations are only of minor importance since the amounts of volatile compounds that left the system via the liquid were never more than 2 and 5% of the total load of toluene and TCE, respectively.

The potential volumetric activity of the chemostat is determined by the amount of biomass present in the system. In a cometabolic system, the latter is usually determined by the flux of the primary substrate through the reactor. Theoretically, a higher TCE conversion capacity can also be obtained by lowering the dilution rate of the fermentor, because this results in an increase in the amount of biomass. This was indeed observed after lowering the dilution rate from 0.08 to 0.04 h^{-1}. An additional advantage of such an approach is that a lower dilution rate results in a lower concentration of the growth-limiting substrate. Since toluene as the growth-limiting substrate inhibits the degradation of TCE, this suggests that the growth rate should be as low as possible. How low it may become is determined by the amount of chloride that is produced from TCE, since an increased residence time will result in accumulation of chloride in the chemostat.

The results obtained with *P. cepacia* G4 offer a good starting point for the development of an efficient bioreactor system for the microbial degradation of gaseous waste streams. The volumetric activities reported here are in the order of 0.8 to 4 g of TCE per m^3 per h and compare favorably with values of 0.06 to 0.6 g/m^3/h reported in the literature (9, 22, 28). In evaluating and comparing different systems, one should be aware that whether TCE is already dissolved in the aqueous phase or still has to be transferred from the gas phase may make a difference. The concentration range at which the system has to be effective also strongly affects the specific conversion rate. Recent legislation in western Europe requires TCE concentrations in off-gases to be below 100 mg/m^3.

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