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Continuous Degradation of Trichloroethylene by *Xanthobacter* sp. Strain Py2 during Growth on Propene

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Propene-grown *Xanthobacter* sp. strain Py2 cells can degrade trichloroethylene (TCE), but the transformation capacity of such cells was limited and depended on both the TCE concentration and the biomass concentration. Toxic metabolites presumably accumulated extracellularly, because the fermentation of glucose by yeast cells was inhibited by TCE degradation products formed by strain Py2. The affinity of the propene monooxygenase for TCE was low, and this allowed strain Py2 to grow on propene in the presence of TCE. During batch growth with propene and TCE, the TCE was not degraded before most of the propene had been consumed. Continuous degradation of TCE in a chemostat culture of strain Py2 growing with propene was observed with TCE concentrations up to 206 μM in the growth medium without washout of the fermentor occurring. At this TCE concentration the specific degradation rate was 1.5 nmol/min/mg of biomass. The total amount of TCE that could be degraded during simultaneous growth on propene depended on the TCE concentration and ranged from 0.03 to 0.34 g of TCE per g of biomass. The biomass yield on propene was not affected by the cometabolic degradation of TCE.

A variety of aerobic bacteria containing oxygenases have been reported to degrade the pollutant trichloroethylene (TCE) cometabolically when they are grown on aliphatic hydrocarbons (4, 14, 18), aromatic hydrocarbons (7), or ammonium (20). Ensign et al. (3) showed that the propene oxidizer *Xanthobacter* sp. strain Py2 is able to degrade TCE when it is grown on propene because of the presence of propene monooxygenase.

The toxicity of TCE is a major problem encountered in the aerobic degradation of this compound, and this toxicity is probably due to the formation of highly reactive intermediates. Methane oxidizers are harmed irreversibly, and the greater the amount of TCE that the cells have transformed, the greater the effect (1). The degradation of TCE by purified methane monooxygenase results in inactivation of the enzyme (8). Other enzymes have also been shown to be affected as a result of TCE degradation (3, 17). The nature of these inactivating effects is not clear, but it has been shown that TCE oxidation products cause alkylation of macromolecules (8, 17, 23). It has been shown that in *Nitrosomonas europaea* de novo protein synthesis is required to regain oxidation activity (20).

Because of the inactivation effects caused by TCE degradation, the transformation capacity of cells is limited (1, 11, 18), and processes for continuous removal of TCE consequently should involve regeneration of inactivated biomass. Continuous aerobic degradation of TCE in a bioreactor or field situation has been observed with methane oxidizers (5, 15, 21) and with microorganisms growing with aromatic substrates (6, 11, 13).

In this study *Xanthobacter* sp. strain Py2 was chosen to study continuous degradation TCE in order to allow comparisons with data reported previously for methane- and aromatic hydrocarbon-degrading bacteria. Since the TCE transformation capacity of cells is limited, synthesis of the monooxygenase is required for continuous TCE removal. Unfortunately, propene, which induces the alkene monooxygenase, also inhibits the oxidation of chlorinated alkenes (3).

The same problem has been encountered with TCE degraders growing on methane (5), phenol (11), propane (14), and toluene (13). In all of these cases the presence of the growth substrate resulted in restoration of monooxygenase activity, but inhibited TCE degradation. To overcome the problem of inhibition of TCE degradation by the growth substrate, workers have designed two-stage bioreactors (6, 15). In these types of reactors cells are supplied with growth substrate in the first stage and then transported to the second stage, where TCE is degraded. Other workers have studied alternating the supply of growth substrate and TCE (19). Nevertheless, under controlled conditions simultaneous growth and TCE degradation in only one reactor are also possible (5, 13, 16).

We used the continuous culture technique to characterize the kinetics and physiology of simultaneous propene degradation and TCE degradation in *Xanthobacter* sp. strain Py2 cells. In this paper we first describe the transformation capacity and kinetics of TCE oxidation and then focus on TCE degradation during growth on propene in batch and continuous cultures.

MATERIALS AND METHODS

Organisms and culture conditions. *Xanthobacter* sp. strain Py2 was previously isolated with propene (22) and was grown continuously in a 1.5-liter fermentor containing mineral salts medium (9) at a dilution rate of 0.03 h^{-1}; 1.25% (vol/vol) propene in air was supplied at a rate of 100 ml/min. The impeller speed was 550 min^{-1}, the temperature was 30°C, and the pH was kept constant at 7.0 by titration with 2 M NaOH. Cells were harvested by centrifugation, washed with washing buffer (50 mM potassium phosphate buffer, pH 7.0), and resuspended in the same buffer.

For batch growth in the presence of TCE (see Fig. 3), *Xanthobacter* sp. strain Py2 pregrown with propene was inoculated into 10-ml portions of mineral salts medium in 250-ml serum flasks sealed with Teflon-lined Mininert septa (Alltech, Deerfield, Ill.). Propene was added in the gas phase to a final concentration of 1.1%. TCE was added as a saturated solution in mineral salts medium to final concentrations of 0, 36, 74, and 250 μM in the water phase. The bottles were incubated in a water bath at 30°C, and the concentrations of CO_2, propene, and TCE were monitored by gas chromatography during the experiment.

For continuous growth with propene in the presence of TCE, *Xanthobacter* sp.

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TABLE 1. Effects of cell density and TCE concentration on the amount of TCE degraded by washed propene-grown *Xanthobacter* cells[a]

Amt of biomass (mg/ml)	Amt of TCE degraded at an initial TCE concn of:					
	130 μM		260 μM		390 μM	
	TCE assay	Cl^- assay	TCE assay	Cl^- assay	TCE assay	Cl^- assay
1.8	43.6 ± 6.5	30.6 ± 1.2	55.6 ± 11.5	37.1 ± 1.3	58.2 ± 16.4	42.3 ± 1.1
3.6	43.1 ± 2.3	25.4 ± 0.3	47.3 ± 6.4	34.4 ± 0.1	46.9 ± 10.0	36.4 ± 0.2
5.5	35.0 ± 1.8	27.4 ± 0.5	39.4 ± 3.7	29.6 ± 0.3	43.3 ± 6.7	32.7 ± 0.6

[a] TCE removal was determined after 20 h of incubation. Values are expressed in milligrams of TCE oxidized per gram of biomass and were determined by the removal of TCE and by the accumulation of Cl^- ions. We assumed that three chlorine atoms represented one molecule of TCE degraded.

strain Py2 was grown in a stirred vessel as described above at a dilution rate of 0.034 ± 0.003 h^{-1}. Propene was mixed with air and a third airflow, which was saturated with TCE in a saturation column at room temperature. All gas flows were controlled by thermal mass flow controllers (Brooks Instrument B.V., Veenendaal, The Netherlands). The propene concentration was 1.25%, and the total flow rate was 106 ml/min. In- and outgoing concentrations of TCE, propene, and CO_2 were determined daily by sampling two disconnectable 0.75-liter glass containers placed in the in- and outgoing gas streams. The amounts of propene and TCE lost abiotically were less than 3%. After each change in the TCE loading rate, the reactor was operated for 5 days (corresponding to four changes of volume) before steady state was confirmed as follows: the in- and outgoing concentrations were determined and the optical density at 660 nm was constant for at least another 2 days.

Saccharomyces cerevisiae CBS 1394 was grown in 0.5-liter Erlenmeyer flasks that contained 0.3 liter of medium supplemented with 10 g of yeast extract per liter and 10 g of glucose per liter and were incubated at 30°C in a rotary shaker. Cells were harvested by centrifugation, washed with 50 mM potassium phosphate buffer, and resuspended in the same buffer. Suspensions were kept on ice and used within a few hours after they were harvested.

TCE and propene degradation assays. The transformation capacities of washed propene-grown cells were determined in 250-ml serum flasks sealed with Teflon-lined Mininert septa. TCE was added as a saturated solution (8.3 mM) in 50 mM potassium phosphate buffer at room temperature to final concentrations of 130, 260, and 390 μM. Washed *Xanthobacter* sp. strain Py2 cells were added at a final concentration of 1.8, 3.6, or 5.5 mg (dry weight) per ml, and the final volume was 10 ml. The flasks were incubated in a water bath at 30°C, and the concentration of TCE was monitored for 20 h by gas chromatography. After 20 h the concentration of accumulated Cl^- ions in the medium was determined.

Kinetic parameters of propene degradation were determined at 30°C in rubber-sealed 75-ml serum vials containing 5 ml of 50 mM potassium phosphate buffer (pH 7.0) and 0.15 to 0.37 mg of cells. To eliminate the possibility that the reaction was limited by diffusion, cultures containing two cell densities were incubated with vigorous shaking. Since the specific degradation rates were found to be the same for the two cell densities, we concluded that the degradation rate was not limited by diffusion.

The TCE degradation assays were performed with 0.25 mg of cells at 30°C in 250-ml serum flasks that were sealed with Teflon-lined Mininert septa and contained 25 ml of 50 mM potassium phosphate buffer. The initial rates of TCE degradation were determined for the first 15 min.

Toxicity of extracellular metabolites to *Saccharomyces cerevisiae*. A 1.7-mg portion of washed *S. cerevisiae* cells was incubated aerobically in a water bath at 30°C with 10 or 20 mg of *Xanthobacter* sp. strain Py2 cells and 64 μM TCE in 250-ml serum flasks that were sealed with Teflon-lined Mininert valves and contained a total liquid volume of 10 ml. After 2.5 h the Mininert valves were exchanged for rubber seals, and the flasks were flushed with nitrogen for 30 min to remove oxygen, CO_2, and residual TCE. Then glucose was added to a concentration of 5 g/liter, and the CO_2 production was monitored.

Analytical methods. The amounts of TCE, propene, and carbon dioxide were determined by gas chromatography of 100-μl headspace samples. TCE was analyzed in triplicate with a Packard model 437 gas chromatograph equipped with a 20% Tween column (Chromosorb W AW 80-100; Chrompack, Middelburg, The Netherlands) and a flame ionization detector. The column temperature was 110°C. The concentration of TCE was expressed as the actual micromolar concentration in the liquid phase which the cells experienced. The total amount of TCE was calculated by adding the amounts present in the liquid and gas phases and using a TCE gas/water partition coefficient of 0.5 (concentration in air/concentration in water) (2).

Propene was analyzed in triplicate with a Packard model 430 gas chromatograph fitted with a Porapak R column (100-120 mesh; 110 in. [ca. 279 cm] by 0.125 in. [ca. 3.2 mm] [inside diameter]) and a flame ionization detector. The oven temperature was 180°C, and the carrier gas was N_2.

CO_2 was analyzed in duplicate with a Packard model 427 gas chromatograph fitted with a HayeSep Q column and a thermal conductivity detector at 140°C. The column temperature was maintained at 110°C, and helium was used as the carrier gas.

The standard deviations of TCE, propene, and CO_2 measurements were 3, 2, and 1%, respectively.

Dry weights were determined by centrifuging 50-ml samples, resuspending the resulting preparations in demineralized water, and drying them at 108°C.

Chloride ion concentrations were determined in triplicate by using cell-free supernatants and a microchloro counter (Marius, Utrecht, The Netherlands), which titrated chloride ions with Ag^+ released electrochemically from a silver electrode.

Chemicals. TCE (99.5% pure) was purchased from E. Merck, Darmstadt, Germany. Propene (99.995% pure) and N_2 (99.99% pure) were purchased from Hoekloos, Schiedam, The Netherlands. All other chemicals were reagent grade.

RESULTS

Transformation capacity of washed cells. Before we studied TCE degradation by a continuous culture of *Xanthobacter* sp. strain Py2, we determined the transformation capacity of washed propene-grown cells (i.e., the total amount of TCE that could be degraded by these cells in the absence of other substrates). The effects of both cell density and TCE concentration on transformation capacity were studied. Cells were incubated with TCE for 20 h, after which no residual monooxygenase activity was detected. Table 1 shows the amounts of TCE eliminated per gram of biomass as determined by gas chromatography, as well as by titration of the accumulated Cl^- ions. Most of the chlorine atoms in TCE eventually accumulated in the medium as Cl^- ions.

Our results show that the specific transformation capacity of the culture decreased with cell density, suggesting that the cells produced toxic degradation products which accumulated extracellularly.

Effect of TCE degradation products on glucose fermentation by *S. cerevisiae*. To test the hypothesis that toxic intermediates appeared extracellularly, the following experiment was performed. *S. cerevisiae* was incubated aerobically for 2.5 h in potassium phosphate buffer in the presence of *Xanthobacter* sp. strain Py2 and TCE. Then the incubation system was flushed with N_2, glucose was added, and CO_2 production by *S. cerevisiae* during anaerobic incubation was measured. In several experiments (data not shown) the fermentation rate of *S. cerevisiae* was reduced by 20 to 28% if TCE and *Xanthobacter* sp. strain Py2 had been present simultaneously during the preceding incubation. The results of one of these experiments are shown in Fig. 1. Preincubation with either TCE or strain Py2 did not reduce the fermentation rate. Anaerobically, *Xanthobacter* sp. strain Py2 did not produce any CO_2.

Kinetics of the alkene monooxygenase. Using propyne as a specific inhibitor of the monooxygenase, Ensign et al. (3) have shown that the propene monooxygenase is required for TCE degradation. Therefore, we studied the kinetics of degradation of both TCE and propene, which is the inducer of the propene monooxygenase.

First, the initial rates of degradation of both substrates by washed cells were studied as a function of the concentrations

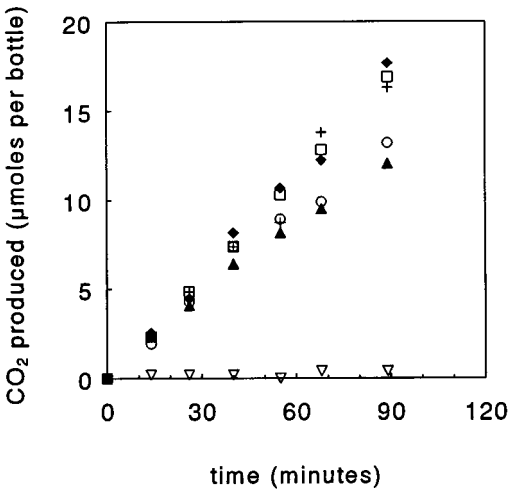

FIG. 1. Effect of preceding aerobic incubation with TCE and strain Py2 on anaerobic CO_2 production from glucose by *S. cerevisiae*. The yeast cells were pre-incubated aerobically in the presence of 64 μM TCE (◆), 10 mg of strain Py2 (+), no addition (□), 10 mg of strain Py2 and 64 μM TCE (▲), or 20 mg of strain Py2 and 64 μM TCE (○). ▽, control containing only 10 mg of strain Py2.

of the substrates. Reliable measurements of TCE degradation rates were possible only at concentrations lower than the resulting K_m (116 μM). The initial TCE degradation rates were determined during the first 15 min. Figure 2 shows that *Xanthobacter* sp. strain Py2 had a higher affinity and a higher maximal degradation rate for propene than for TCE.

Subsequently, the kinetics of simultaneous TCE degradation and propene degradation were studied. High concentrations of propene inhibited the degradation of TCE (data not shown, but this phenomenon is shown in Fig. 3B to D). To demonstrate that propene degradation was inhibited by the presence of TCE, high concentrations of TCE (concentrations greater than the K_m) were required, since the affinity of the monooxygenase for TCE is much lower than its affinity for propene. In the presence of TCE concentrations greater than the K_m for TCE the propene oxidation rate decreased rapidly during the experiment. This was probably due to irreversible loss of enzyme activity because of simultaneous TCE oxidation. Therefore, the inhibition constants (K_i) of TCE and propene could not be determined.

Batch growth on propene in the presence of TCE. Before we studied continuous degradation of TCE during growth on propene in a continuous culture, we studied batch growth of *Xanthobacter* sp. strain Py2 in the presence of TCE. Figure 3 shows the growth curves of *Xanthobacter* sp. strain Py2 when the initial concentrations of TCE in the water phase were 0, 36, 74, and 250 μM. The maximal growth rate (as determined from the CO_2 formation curve) in the presence of 0 to 74 μM TCE was 0.14 ± 0.02 h^{-1}. In the presence of 250 μM TCE the growth rate decreased to 0.08 h^{-1}. Figure 3 also shows that TCE degradation did not start before most of the propene had been consumed. This is consistent with the affinity of washed cells for the two substrates (Fig. 2), assuming that both substrates are oxidized by the same enzyme.

Continuous growth on propene in the presence of TCE. *Xanthobacter* sp. strain Py2 was grown in a chemostat culture at a dilution rate of 0.034 ± 0.003 h^{-1}, which is well below the maximal growth rate observed in the presence of 250 μM TCE (Fig. 3D). For each TCE loading rate the reactor was operated for four volume changes, and steady state was confirmed by the in- and outgoing concentrations of TCE and by the fact that

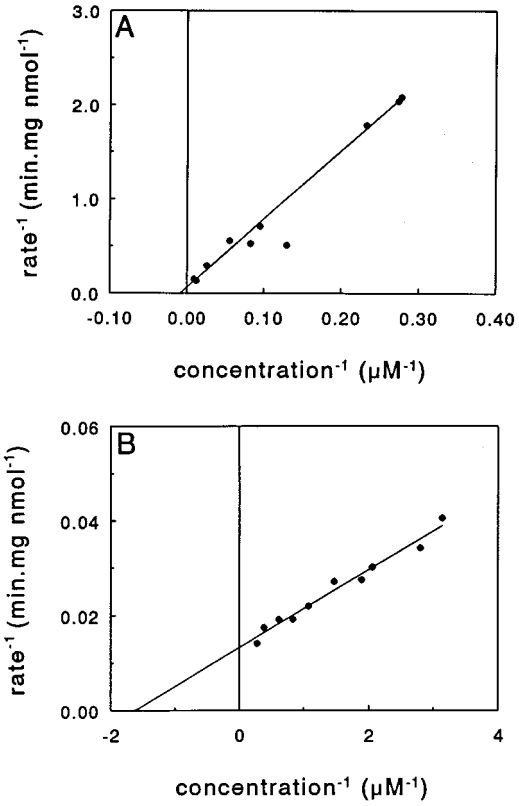

FIG. 2. Lineweaver-Burk plots of TCE degradation (A) and propene degradation (B) by *Xanthobacter* sp. strain Py2. Concentrations are expressed in micromoles per liter of liquid phase. The parameters resulting from the linear least-square fit (the outlying data point in panel A was omitted from the fit) are as follows: for TCE, V_{max} = 16 nmol min^{-1} mg^{-1} and K_m = 116 μM; and for propene, V_{max} = 75 nmol min^{-1} mg^{-1} and K_m = 0.62 μM.

the optical density at 660 nm was constant for at least another 2 days. Figure 4 shows that *Xanthobacter* sp. strain Py2 grew propene limited in the presence of TCE. The amount of TCE degraded increased as the TCE concentration increased, although the level of TCE conversion decreased (Fig. 4A). The highest TCE load tested (461 μmol $liter^{-1}$ h^{-1}) resulted in a steady-state concentration in the liquid phase of 206 μM and a volumetric TCE removal rate of 54 μmol $liter^{-1}$ h^{-1}. Figure 5 shows the amount of TCE removed per gram of biomass as a function of the TCE concentration in the liquid phase.

The rate of carbon recovery was between 85 and 112% (Fig. 4B), indicating that the overall variability of the data was limited. The yields of biomass and CO_2 on propene were constant (Fig. 4B) irrespective of the TCE concentration in the liquid. The constant biomass yield suggests that TCE degradation did not cause a significant increase in the maintenance requirements due to cell damage or loss of reducing equivalents. An interruption in the propene supply for several hours at the highest TCE concentration tested caused a washout of the fermentor.

DISCUSSION

Xanthobacter sp. strain Py2 cells were able to degrade a limited amount of TCE, and this amount varied with the cell density (Table 1). In the presence of the highest biomass concentration (5.5 mg of biomass per ml) and lowest TCE concentration (130 μM) tested, 87% of the TCE was degraded.

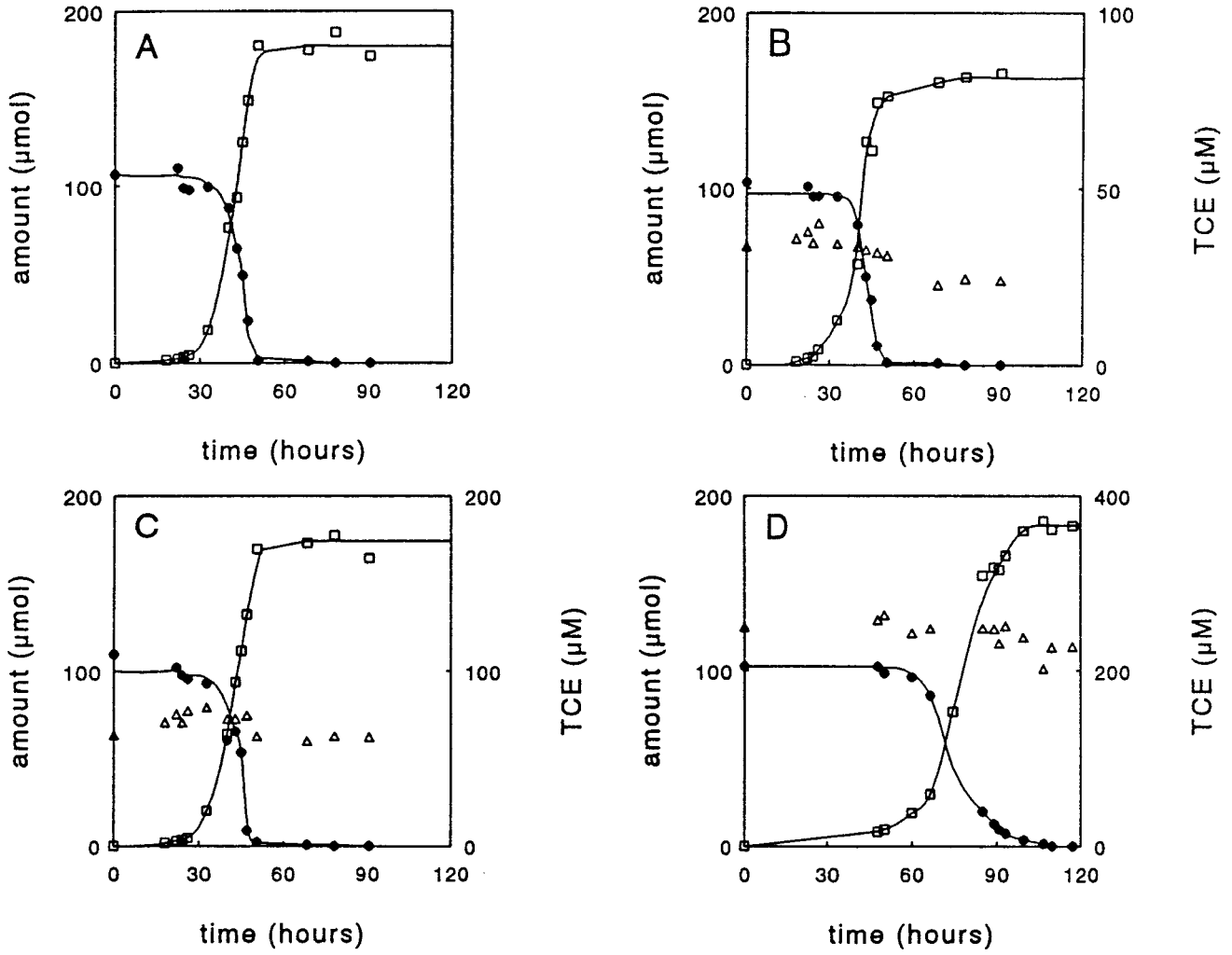

FIG. 3. Effect of TCE on the growth of strain Py2 on propene. The initial TCE concentrations were 0 μM (A), 34 μM (B) 76 μM (C), and 250 μM (D). The *y* axes on the left indicate the propene (●) and CO_2 (□) concentrations (in micromoles per flask); the *y* axes on the right indicate the TCE concentrations (△) (in micromoles per liter of liquid).

The lower initial TCE concentrations used in this experiment are in the range of the K_m value for TCE (116 μM). Therefore, the lower specific transformation capacity at a high cell density could have been due to substrate limitation. At the highest TCE concentration tested (390 μM), however, a maximum of 30% of the compound was converted. Therefore, in this case the decrease in transformation capacity observed with increasing cell density could not have been due to kinetic effects. We speculate that toxic metabolites (e.g., carbon monoxide [10], hydrolysis products of the TCE epoxide or chloral [8]) accumulate in the medium to a greater extent at higher cell densities, resulting in stronger inhibition. Alvarez-Cohen and McCarty (1), however, observed the opposite effect of cell density on transformation capacity. In mixed methanotrophic cultures the transformation capacity was slightly higher at higher cell density. The values reported by these authors (0.025 to 0.033 g of TCE per g of biomass) (1) are similar to the values which we found for strain Py2. With phenol-oxidizing microorganisms Hopkins et al. (11) observed a much higher transformation capacity (0.24 g of TCE per g of cells).

Our hypothesis that toxic metabolites appear extracellularly was supported by the observed inhibition of yeast cells (Fig. 1) incubated with *Xanthobacter* sp. strain Py2 cells degrading TCE. Incubation with either TCE or strain Py2 had no effect on the fermentation rate of the yeast, clearly demonstrating that inhibitors were formed during incubation with both TCE and strain Py2. The nature of the inhibitory products formed from TCE by strain Py2 is not known. Because of its volatile nature carbon monoxide is unlikely to play a role, since the bottles were flushed with N_2 before glucose was added. Diffusible hydrolysis products of the TCE epoxide or chloral, however, might be responsible for the observed inhibition. Such hydrolysis products have been found to inactivate the purified methane monooxygenase by covalent modification (8). On the other hand, compounds produced by the *Xanthobacter* strain itself as a stress response to TCE could also be responsible for the inhibition of the yeast. Irrespective of the nature of these inhibitors, degradation of TCE presumably results in the extracellular appearance of toxic metabolites. This observation has great impact on the TCE removal process and implies that biomass and liquid from a reactor in which TCE is degraded should be handled with care.

The Michaelis-Menten half-saturation constant for TCE was found to be 116 μM, which is considerably higher than the

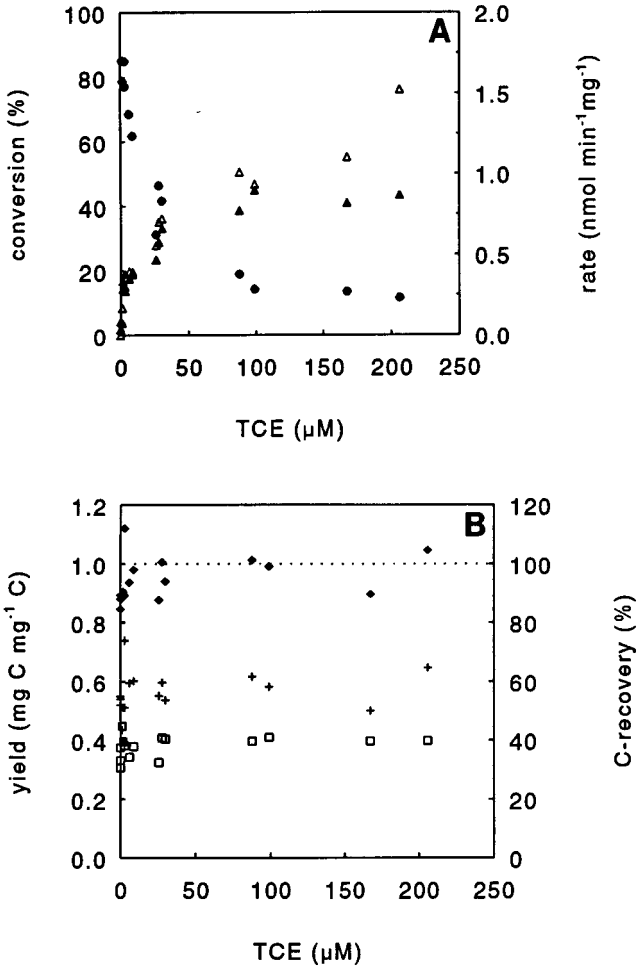

FIG. 4. TCE degradation by strain Py2 in continuous cultures during growth on propene. The TCE load was varied between 15 and 461 μmol liter^{-1} h^{-1} and resulted in steady-state concentrations in the liquid phase (indicated on the *x* axes). (A) Symbols: ●, level of TCE conversion; △, TCE removal rate as determined by gas chromatography; ▲, TCE removal rate as determined by the accumulation of Cl^-. (B) Yields (in milligrams of C/milligrams of C) of biomass (□) and CO_2 produced (+) and rates of recovery (◆). For biomass a C content of 0.45 g of carbon per g of biomass was assumed.

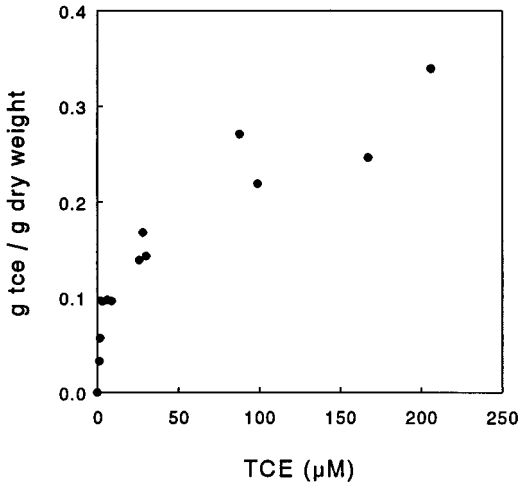

FIG. 5. Amount of TCE removed per gram of biomass during continuous growth of strain Py2 on propene. Data were obtained from Fig. 4A.

values reported for *Pseudomonas cepacia* G4, 3 μM (7) and 6 μM (13). Our half-saturation constant value was, however, in the same range as the values found for the methane oxidizers *Methylosinus trichosporium* OB3b (145 μM [17] and 126 μM [12]) and *Methylomonas methanica* 68-1 (225 μM [12]).

Propene and TCE, which are probably substrates for the same enzyme (3), each influence the rate of degradation of the other. The propene oxidation rates decreased rapidly in the presence of excess TCE. On the other hand, a high concentration of propene prevented TCE degradation (Fig. 3B to D). In this context it should be noted that the specificity constant (maximum rate of metabolism $[V_{max}]/K_m$) for propene is approximately 900 times greater than the specificity constant for TCE. The low specificity constant of the enzyme for TCE thus allows batch growth on propene in the presence of 250 μM TCE (Fig. 3D). During batch growth TCE is not degraded until most of the propene has been consumed. A similar pattern of subsequent substrate degradation has been found for the isoprene-utilizing organism *Rhodococcus erythropolis* JE77 (4).

The mutual influence of growth substrate utilization and TCE degradation has also been observed with various other organisms (7, 11–14, 16). Folsom et al. (7) suspected that the influence of phenol on TCE oxidation and vice versa in *P. cepacia* G4 can be explained by a competitive mechanism. Landa et al. (13) have actually reported K_i values for competitive inhibition by toluene during TCE oxidation and vice versa with the same organism; the reported values (K_i for toluene, 30 μM; K_i for TCE, 5 μM) are very close to the independently determined K_m values (25 and 6 μM, respectively), suggesting that competitive inhibition occurs, but no Lineweaver-Burk plots are given by these authors. For *Xanthobacter* sp. strain Py2 the type of inhibition could not be established because of the quick and irreversible loss of enzyme activity in the presence of high concentrations of TCE.

The specific rates of TCE degradation by cells during growth on propene in the fermentor (Fig. 4) were significantly lower than the initial rates (e.g., at 100 μM 7.4 nmol min^{-1} mg^{-1}) determined in the absence of propene. The lower rates in the fermentor were probably due to competition between propene and TCE for the monooxygenase and possibly also to lower levels of active monooxygenase in cells growing in the presence of TCE.

Although the specific rates of TCE degradation in the chemostat were relatively low, the transformation capacity of the cells (0.34 g/g) was six- to seven-fold higher than the amount of TCE degraded by washed cells in the absence of propene (Table 1) at a similar concentration of TCE. The higher transformation capacity in the presence of propene may have been due to replenishment of reducing equivalents by propene oxidation. In phenol-oxidizing microorganisms the TCE degradation capacity was improved by 102% in the presence of an external source of reducing equivalents (1). With a methanotrophic culture the energy-generating substrate formate was found to increase the transformation capacity by 58%, indicating that depletion of stored energy reserves by TCE oxidation was a factor which determined the transformation rate and capacity (11). However, apart from the replenishment of reducing equivalents by propene oxidation, de novo synthesis of the propene monooxygenase in the presence of the inducer propene is probably the main reason for the increased transformation capacity.

Other workers (Table 2) have reported similar values for the transformation capacity of toluene and phenol oxidizers (11,

TABLE 2. Amounts of growth substrate required per gram of TCE degraded and transformation capacities of biomasses during continuous degradation

Prepn	Growth substrate(s)	Amt of substrate required (g of substrate consumed per g of TCE degraded)	Transformation capacity (g of TCE per g of biomass)	Reference
Mixed culture	Methane	23–1,200		5
Mixed culture	Methane	77[a]	0.036[a]	1
Mixed culture	Methane + propane	11–30	0.015–0.08[b]	19
Methylosinus trichosporium OB3b	Methane	320–1,200	1.7×10^{-4}–6.8×10^{-3}	16
Mixed culture	Phenol	9[a]	0.24[a]	11
P. cepacia G4	Toluene	14–71	0.027–0.152	13
Xanthobacter sp. strain Py2	Propene	4–23	0.03–0.34	

[a] Degradation capacity was determined in the absence of an energy-generating substrate.
[b] Per day.

13), whereas lower values have been reported for methane oxidizers (16). Table 2 also shows the amounts of growth substrates required for degradation of TCE. The values in Table 2 are very difficult to compare since they depended not only on the TCE concentration but also on whether the growth substrate and TCE were supplied alternately. In general, substantial amounts of a volatile growth substrate(s) are required for cometabolic degradation of TCE, which results in high costs and contamination of air or water with the residual growth substrate(s).

Although a continuous culture of strain Py2 can grow in the presence of a wide range of TCE concentrations, it is susceptible to interruptions in the propene supply at a high concentration of TCE, which causes a washout of the fermentor. The cells are apparently not able to recover from exposure to TCE in the absence of propene. Thus, although simultaneous oxidation of propene during TCE degradation results in lower rates of TCE degradation, it also prevents total inactivation of the biocatalyst.

The biomass yield (Fig. 5) of strain Py2 on propene was not affected by the simultaneous TCE degradation, even when the concentration of TCE in the liquid was 206 μM. With the toluene-oxidizing organism *P. cepacia* G4 Landa et al. (13) also observed a constant biomass yield over a wide range of TCE concentrations up to 14 μM, but at 80 μM TCE the yield was lower. On the basis of the energy required for the initial step in the oxidation of TCE (1 NADH), a detectable decrease in the yield can hardly be expected when the propene flux (at least 15 nmol min^{-1} mg^{-1}) is compared with the TCE flux (1.5 nmol min^{-1} mg^{-1} at most). However, we anticipated that an increase in the monooxygenase turnover rate due to inactivation by TCE would increase the apparent maintenance requirements and hence decrease the yield on propene. However, it is possible that the inactivation of the cells by TCE degradation was compensated for by energy gained from the oxidation of TCE degradation products.

In conclusion, our results clearly demonstrate that *Xanthobacter* sp. strain Py2 cells growing on propene can be used to continuously degrade TCE over a wide range of concentrations. However, a disadvantage of this organism is its low affinity for TCE, which results in rather low specific degradation rates at low TCE concentrations.

ACKNOWLEDGMENTS

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