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Degradation of Trichloroethylene by Methanol-Grown Cultures of *Methylosinus trichosporium* OB3b PP358

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A soluble methane monooxygenase-constitutive mutant strain of *Methylosinus trichosporium* OB3b, strain PP358, was grown with methanol as the carbon source, and the kinetics of trichloroethylene (TCE) degradation were determined. PP358 exhibited high TCE degradation rates under both oxygen- and carbon-limiting conditions. The optimal pseudo first-order rate constant for TCE was comparable to the values measured for cells grown with methane. We found that growth under oxygen-limiting conditions results in increased accumulation of polyhydroxybutyrate, which in turn correlates with higher transformation capacities for TCE. It was also shown that methanol inhibits TCE degradation only at high concentrations. Thus, methanol-grown cultures of PP358 represent an efficient system for the biodegradation of chlorinated hydrocarbons.

Methylosinus trichosporium OB3b is a methanotrophic bacterium that has received considerable attention for its ability to rapidly degrade chlorinated solvents, including trichloroethylene (TCE). The initial step in methane assimilation is catalyzed by methane monooxygenase, which oxidizes methane to methanol. Under copper starvation conditions, some methanotrophs express a non-membrane-bound, or soluble, methane monooxygenase (sMMO). This enzyme has a surprisingly broad substrate specificity (5), including the ability to degrade TCE. OB3b cells expressing sMMO exhibit very high rates of TCE degradation (15). The expression of sMMO in wild-type OB3b is repressed even in the presence of submicromolar concentrations of copper (5). *M. trichosporium* PP358 (16), which was used in this work, is a mutant of *M. trichosporium* OB3b that is defective in copper assimilation and therefore expresses sMMO constitutively (7).

In all previous studies, OB3b was grown on methane as the carbon and energy source. This organism can also grow on methanol. However, it was thought that sMMO expression is adversely affected under methanol growth conditions (3). In preliminary batch studies, we found that the rates of TCE degradation by PP358 grown on methanol are comparable to those of wild-type cells grown on methane (7). Methanol is safer to work with than methane-air mixtures and exhibits a much higher degree of aqueous solubility than methane. If high rates of TCE degradation are realized during growth with methanol then it is a much more attractive growth substrate for bioreactors performing TCE degradation. A study of degradation characteristics of *M. trichosporium* PP358 grown with methanol may help elucidate the physiological underpinnings of TCE degradation as well as characterize operational conditions for methanol-fed bioreactors.

This report focuses on continuous (chemostat) growth of PP358 on methanol and the influence of growth conditions on TCE degradation and transformation capacity. PP358 was grown in modified nitrate minimal salts medium (NMS) containing 0.1 to 0.5% (vol/vol) methanol. The composition of this medium, which was developed through optimization of batch growth of PP358, is shown in Table 1. Cells were grown in a 2.5-liter Bioflo III fermentor (New Brunswick Scientific) operated as a chemostat at 30°C, pH 6.94 ± 0.10, 400-rpm agitation, and 400- to 800-ml/min air feed rate. Steady-state, continuous-flow (chemostat) operation was determined by monitoring A_{600} and generally required four or more residence times to achieve. When a steady state was achieved in the chemostat, samples were taken and analyzed for cell dry weight, methanol concentrations, polyhydroxybutyrate (PHB) content, sMMO content and TCE degradation kinetics. Cell dry weight was determined by turbidity measurement (17). Influent and effluent methanol concentrations were determined by gas chromatography against known standards (flame ionization detector conditions: DB-624 Megabore column, 90°C, 32 ml of N_2 per min, samples centrifuged at 10,000 × *g* for 5 min to remove cells prior to assay). PHB was assayed for by the method of Braunegg et al. (4). sMMO content was measured by Western blotting (immunoblotting) (7). TCE degradation kinetics were determined in each batch by chromatography of headspace samples (16). The results of these studies are shown in Table 2.

For the data in Table 2, 95% confidence intervals were determined. They were as follows: for dilution rates, ±0.01 h^{-1}; for methanol, ±5% of measured value; for cell dry weight, ±10 mg/liter; and for PHB, ±0.5% (wt/wt). Although TCE kinetic assays were not performed in replicate, prior studies have indicated that these assays give values with a 95% confidence interval of approximately ±20% of the measured rate constant (7).

Cell yields. True cell yields were determined by adjusting apparent yields for endogenous metabolism (2). The rate of endogenous decay was measured in batch cultures by monitoring the decrease in A_{600}. The decay rate of anoxic PP358 was 0.003 h^{-1}, and that of aerobic PP358 was 0.004 h^{-1}. Using this batch-derived endogenous decay rate, we found the cell yield to be essentially constant over the broad range of dilution rates, with an average yield of 0.40 (standard deviation of 0.08) mg of cell (dry weight) per mg of methanol. This value is consistent with those previously described for methanotrophs (10, 19).

PHB. PHB is formed when excess acetyl coenzyme A and NADH are present in the cell (6, 20). PHB is subsequently mobilized to yield NADH under starvation conditions. PHB formation was strongly dependent on growth limitation (Table 2). In methanol-limited cultures, PHB formation was apparently independent of dilution rate. Accumulation of PHB de-

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TABLE 1. Modified nitrate minimal salts medium composition

Salt	Concn
$NaNO_3$	10 mM
KH_2PO_4	5 mM
Na_2HPO_4	5 mM
$MgSO_4$	1 mM
$FeSO_4 \cdot 7H_2O$	10 μM
$CaCl_2$	10 μM
MoO_3	10 μM
$MnCl_2$	1 μM
$CoCl_2$	1 μM
$ZnSO_4$	1 μM
H_3BO_4	1 μM
H_2SO_4[a]	10 μM
$NaOH_2$[b]	0.2 mM

[a] Added to stabilize $FeSO_4$ in concentrated solution.
[b] Added to dissolve MoO_3 in concentrated solution.

creased with increasing dilution rates for oxygen-limited cultures.

sMMO content. The amount of sMMO present in the cell is the primary determinant of the performance of the cultures in TCE degradation and was therefore of great interest. The sMMO contents of methanol-grown cells were compared by Western blotting using sMMO-specific polyclonal antisera raised in our laboratory (7) and were found to be essentially constant. Attempts to evaluate sMMO levels by enzyme-linked immunosorbent assay also showed no change in sMMO content, but this result may be partially attributed to the cross-reactivity of the polyclonal antibodies with methanol dehydrogenase, which was very strongly expressed in these strains (data not shown). These assays determine the steady-state level of the enzyme and do not distinguish between active and inactive sMMO.

TCE degradation. TCE degradation pseudo first-order rate constants (k_1) and transformation capacities (T_c) (Table 2) were measured by headspace chromatography of batch samples (17) with TCE reinjection until the rate of degradation had faded to less than 0.05 h^{-1}. Initial TCE concentrations were in the range of 500 to 3,000 μg of TCE per liter. Rate constants reported are for initial rates of TCE degradation (prior to any inhibitory effect) and were modeled by pseudo first-order kinetics (17) calculated as follows: $d(TCE)/dt = -k_1X(TCE)$, where TCE is the concentration of TCE (milligrams per liter), t is time (day), k_1 is the pseudo first-order rate constant (liters · milligram of cell [dry weight]$^{-1}$ day^{-1}), and X is the biomass concentration (milligrams of cell [dry weight] per liter). At these low TCE concentrations, the pseudo-first-order rate constant is equal to the Michaelis-Menten parameter k/K_m. To eliminate potential methanol inhibition, chemostat samples were centrifuged and resuspended in fresh modified nitrate minimal salts medium prior to TCE addition. Degradation rates were determined in the presence or absence of 20 mM formate. Formate generally increases degradation rates and capacities by supplying additional NADH, which is consumed during degradation (1, 11).

The TCE degradation rate constant of cells grown under methanol-limited conditions declined as the dilution rate increased (Table 2). In contrast, the rate constant obtained in the presence of formate was not correlated to the chemostat dilution rate. Cells grown under oxygen-limiting conditions also displayed rate constants not correlated with the dilution rate.

Cells with low PHB contents gave increased rate constants upon addition of formate. However, degradation of TCE by cells with significant PHB contents was less affected by formate addition. Earlier, Henrysson and McCarty (13) explored the relationship between levels of PHB and sMMO activity in mixed methanotrophic cultures and showed that PHB content correlates with the rate of naphthalene oxidation, a reaction catalyzed by sMMO. They showed that cells with 10% (wt/wt) PHB or more gave a rate of degradation comparable to that obtained upon formate addition. We found that similarly low (3 to 5% [wt/wt]) PHB concentrations largely eliminated the ability of formate to increase the rate constant of TCE degradation.

Inhibition of TCE degradation. Because TCE transformation products inhibit sMMO activity (15) and deplete reducing equivalents (11), the total amount of TCE that may be de-

TABLE 2. Chemostat results[a]

Growth limitation	D[b] (h^{-1})	MeOH[c] concn (mg/liter)		Cell dry wt (mg/liter)	True yield (mg/mg of MeOH)	PHB (% [wt/wt])	Initial k_1 (liters/mg · day)		T_c (μg/mg)	
		In[d]	Out[e]				+Formate	−Formate	+Formate	−Formate
O_2	0.010	ND[f]	ND	850	ND	ND	1.4	1.0	54[g]	39[g]
	0.025	4,100	2,700	420	0.34	13	1.1	1.3	105[g]	79[g]
	0.038	3,400	2,600	380	0.49	14	1.5	1.3	110	100
	0.050	3,100	2,200	330	0.35	6	1.2	1.3	162	108
	0.070	3,200	1,900	380	0.33	7	1.0	1.0	132	98
MeOH	0.010	2,700	1	840	0.41	ND	1.4	1.0	97[g]	87[g]
	0.040	1,500	2	820	0.58	1.3	1.2	0.7	181	78
	0.050	2,300	11	810	0.37	1.2	1.1	0.7	138	57
	0.052	2,100	12	750	0.38	ND	1.2	0.5	ND	ND
	0.060	2,700	0	1,000	0.39	0.9	1.1	0.3	186	54
	0.060	1,900	6	630	0.35	3.3	ND	ND	ND	ND

[a] Dissolved oxygen (in milligrams per liter) was as follows: for methanol-limited growth, 4.3 to 7.4; for oxygen-limited growth, 0.5 to 1.9; and for batch growth, 1.2. Batch results were as follows: dilution rate, 0.000; cell weight, 690 mg (dry weight)/liter; initial k_1 with and without formate, 1.1 and 0.9 liters/mg · day, respectively; and T_c with and without formate, 21 and 26 μg/mg (calculated from change in kX). Batch results for other parameters listed in the table were not determined.
[b] D, dilution rate.
[c] MeOH, methanol.
[d] Influent methanol concentration.
[e] Effluent methanol concentration.
[f] ND, not determined.
[g] T_c calculated from change in kX.

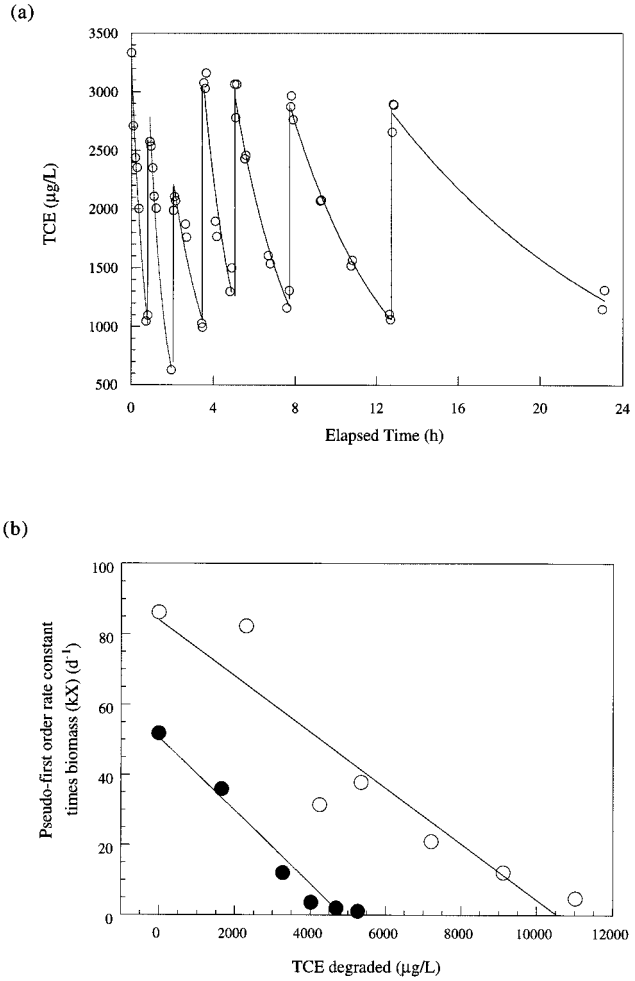

FIG. 1. TCE degradation assay and interpretation of assay to give transformation capacity. (a) Example of a multiple-dosing batch study. (b) Example of interpretation of rate data to give transformation capacity. Open circles represent data from a culture supplemented with 20 mM formate; solid circles represent data from the same culture without formate supplementation.

graded for a given amount of biomass is limited and is characterized by the transformation capacity. In this study, transformation capacities were found by either direct or indirect measurement. For direct measurement of T_c, TCE was added stepwise to cells as shown in Fig. 1a, until the rate of degradation had decreased to less than 5% of the initial rate. The total amount of TCE degraded at this ending time was divided by the total biomass of the sample to give a direct estimate of T_c.

The value of T_c also can be determined indirectly from a multiple-dosing batch study (e.g., as depicted in Fig. 1a). Alvarez-Cohen and McCarty (1) proposed that the inhibition of TCE degradation is a linear function of the amount of TCE degraded and showed a good fit of inhibition to this linear relation. We rearrange their equation as follows:

$$k_{1,n}X_n = k_{1,1}X_1 - \frac{k_{1,1}\,\Delta(TCE)_n}{T_c} \qquad (1)$$

where $k_{1,n}$ is the rate constant for the nth dosing with TCE (liters · milligram^{-1} day^{-1}), X_n is the biomass density at the nth dosing (milligrams · liter^{-1}), $\Delta(TCE)_n$ is the cumulative mass of TCE degraded at the nth dosing (micrograms · liter^{-1}), and T_c is the transformation capacity (micrograms per milligram). This definition of T_c is slightly different from that proposed by Alvarez-Cohen and McCarty (1) in that it allows the rate constant to change with time rather than assuming that all inhibition of TCE degradation is due solely to changes in the active biomass concentration. Figure 1b shows the rates derived from results in Fig. 1a plotted against the cumulative mass of TCE degraded. From equation 1, the linear fit of this data has a slope equal to $k_{1,1}/T_c$, allowing T_c to be calculated. When T_c was calculated directly (i.e., by the multiple-dosing batch study [Fig. 1a]), values were generally found to be 20% greater than those taken from plots such as those shown in Fig. 1b. The difference may be due to nonlinearity introduced by time-dependent inactivation of sMMO (see below). Although the fitting method for T_c estimation (equation 1) gives lower results than does direct measurement, it is a more convenient and time-efficient method.

Transformation capacity was found to be a complex function of dilution rate (Table 2). Cells grown with methanol limitation displayed decreasing transformation capacities with increasing dilution rates. However, the transformation capacity of oxygen-limited cells increased with dilution rate to an apparent maximum. The cause of lower transformation capacities at lower dilution rates is unclear, particularly since initial rate constants and levels of sMMO were apparently identical at the conditions tested.

As well as increasing degradation rates, formate can also increase transformation capacities (1). For oxygen-limited PP358, adding formate increased transformation capacities by 10 to 65% (Table 2). The transformation capacity was increased by 132 to 244% for cells grown under methanol-limiting conditions, with the exception of the dilution rate of 0.01 h^{-1}, for which only an 11% increase was observed.

Transformation capacities also correlated with PHB content. The most striking evidence of this trend is seen in Fig. 2, in which the difference in transformation capacities or rate constants with and without formate is plotted against PHB content.

When PP358 was grown under oxygen-limiting conditions, the cells possessed high TCE rate constants and transformation capacities. However, in a bioreactor these rates may be competitively inhibited by the significant methanol concentration that would remain within the reactor. Inhibition of degradation was studied in batch TCE degradation assays in the presence of different methanol and TCE concentrations. The results were fitted to the following equation:

$$\frac{d(TCE)}{dt} = \frac{\frac{k}{K_m}X(TCE)}{1 + \frac{(TCE)}{K_m} + \frac{(MeOH)}{K_I}} \qquad (2)$$

where k is the maximum specific rate (milligrams of TCE · milligram of cell [dry weight]$^{-1}$ day^{-1}), K_m is the half saturation constant (milligrams of TCE per liter), MeOH is the concentration of methanol (milligrams per liter), and K_I is the competitive inhibition constant for methanol on TCE degradation (milligrams of methanol per liter). K_I was evaluated by least-squares fitting of equation 2 to data taken from four separate sets of TCE degradation experiments. In the first set, no methanol was added, and a concentration range of 5 to 20 mg of TCE per liter was used to determine K_m. K_I was determined from the other three sets of data, where methanol was added at 0 to 7.9 g/liter and an initial TCE concentration of 3

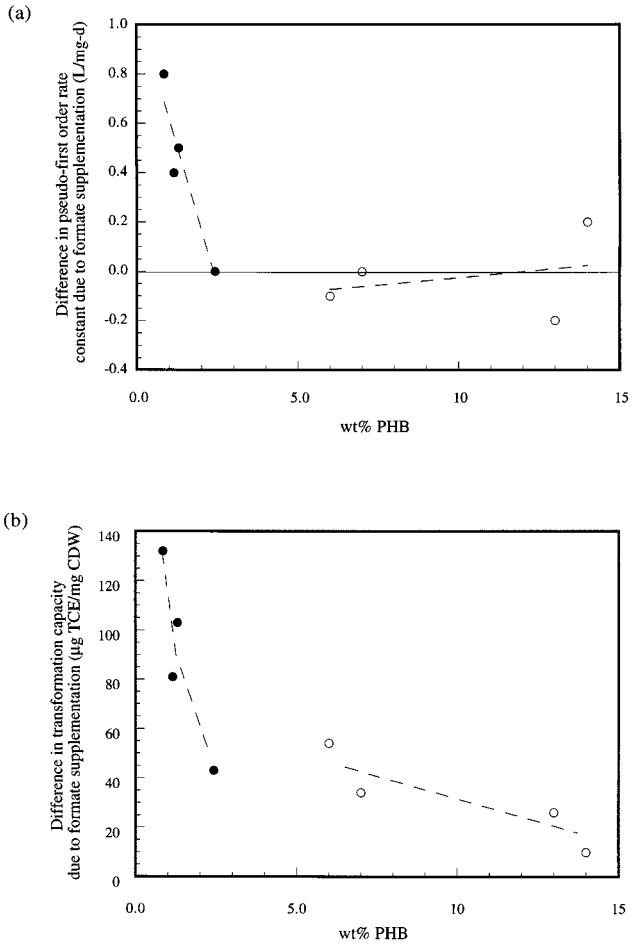

FIG. 2. Dependence on PHB content of increase in rate constant (a) and increase in transformation capacity due to formate supplementation (b). Open circles represent data from cultures grown under oxygen limited conditions; solid circles represent data from the cultures grown under methanol-limited conditions.

to 5 mg/liter was used. The values of TCE concentration as a function of time were fitted by a nonlinear parameter estimation technique in a Microsoft Excel 4.0 spreadsheet. The sum of squared residuals, normalized for the number of time points in each batch degradation, was minimized by varying k and K_m or k and K_I and using a generalized reduced gradient search method for optimization. To determine K_I, all three sets of data were simultaneously used. The parameters that best fit the data were K_m = 36 mg of TCE per liter, K_I = 1.2 g of methanol per liter and k = 12 mg of TCE · mg of cells^{-1} day^{-1}. These values compare well with other published results. Specifically, for methane-grown OB3b previous estimates of K_m were 4.6 mg/liter (9) and 19 mg/liter (15), and that of k was 55 mg of TCE · mg of cells^{-1} day^{-1} (15). In another study, the TCE degradation by a mixed culture of methanotrophs was reduced by 50% upon addition of 0.4 g of methanol per liter (8).

Toxicity of TCE degradation. sMMO catalyzes a reaction in which TCE is oxidized in the presence of oxygen and NADH to yield NAD^+ and TCE epoxide, which then spontaneously breaks down in water (9). During TCE degradation, the rate of reaction decreases as TCE is transformed. The decrease in degradation rate has been called toxicity, although the viability of the cells may not be compromised by TCE degradation. We therefore prefer to call this phenomenon degradation inhibition. Two sources of degradation inhibition may be identified: reduction in catalytic rate due to reversible or irreversible product inhibition and loss of degradative capacity due to physiological changes in the cell.

To distinguish between the effect of product inhibition and inhibition due to physiological changes occurring in resting cells which are not degrading TCE, TCE was added to one of duplicate samples at time 0 and at later times to the other sample. TCE was added to the second sample 4 to 9 h later, and the TCE degradation rate constant was determined to show the effect of physiological changes occurring in the resting state. Incubation of methanotrophs without growth substrate has been shown to cause an exponential decay in the degradation rate (11), with the decay accelerated by aeration (1). Incubation of methanol-grown PP358 without growth substrate caused a similar decrease in the rate constant (Table 3). However, the rate constant exhibited by these resting cultures was higher than the rate constant of samples that degraded TCE for the same period. Thus, the decrease in rate constant is greater for cells degrading TCE than for resting cells. For example, cells grown under oxygen-limiting conditions at a dilution rate of 0.025 h^{-1} had a TCE degradation rate constant of 0.7 liter · mg^{-1} day^{-1} after 6.8 h of incubation without TCE. This was substantially greater than the rate constant of 0.2 liter · mg^{-1} day^{-1} for cells that degraded TCE for the same

TABLE 3. Time-dependent inhibition of TCE degradation

Growth limitation	Dilution rate (h^{-1})	Initial rate constant[a] (liters/mg · day)		Incubation time (h)	Rate constant (liters/mg · day) of cells incubated:			
					+TCE[d]		−TCE[e]	
		+Ft[b]	−Ft[c]		+Ft	−Ft	+Ft	−Ft
O_2	0.000	1.1	0.9	4.9	0.4	0.4	0.6	0.6
	0.025	1.1	1.3	6.8	0.7	0.8	1.3	1.0
	0.038	1.2	1.2	9.0	0.2	0.2	0.7	0.7
	0.070	1.0	1.0	8.5	0.1	0.1	0.5	0.7
	0.050	1.2	1.2	5.0	0.3	0.2	0.7	0.2
MeOH	0.060	1.1	0.3	8.3	0.2	0.1	1.0	0.2
	0.050	1.1	0.7	5.7	0.5	0.1	0.9	0.3

[a] Rate constant of cells when TCE was added at time 0.
[b] Incubation with 20 mM formate supplementation.
[c] Incubation without formate.
[d] Rate constant of cells that had been degrading TCE during the incubation time, evaluated at the specified time.
[e] Rate constant of cells that had been resting during the incubation time, evaluated at the specified time.

time period. Thus, 0.5 liter · mg^{-1} day^{-1} of the degradation inhibition in cells degrading TCE was apparently due to degradation and not to time without growth substrate. However, this comparison also shows that a substantial fraction of the decline in rate constant during TCE degradation is time dependent and independent of TCE degradation. This is illustrated for the same case, for which the TCE degradation rate constant decreased from 1.2 liters · mg^{-1} day^{-1} prior to incubation of cells to 0.7 liter · mg^{-1} day^{-1} after 6.8 h without TCE. In this case, 0.5 liter · mg^{-1} day^{-1} of degradation inhibition was due to time spent without growth substrate. These time-dependent losses accounted for 17 to 75% of the decrease in rate constants observed in TCE-degrading cultures. For the methanol-limited cells, the addition of formate substantially decreased the reduction in rate constant upon incubation. In cells grown under oxygen limitation, formate had no effect on degradation inhibition due to incubation.

The loss of TCE-degrading capacity in cells held in a resting state indicates that the relationship between transformation capacity and decrease in activity is complex. This time effect should be taken into account when modeling transformation capacity. For experiments performed over short periods of time, the linear model of transformation capacity (1) may be appropriate, but longer experiments require the development of a time-dependent model. However, our results indicate that the relationship between the time of incubation and the loss of degradation activity is complex.

Approximately half of the observed decrease in degradation rate constant was dependent on TCE addition. This decrease is likely the result of competitive inhibition, such as the CO inhibition observed by Henry and Grbić-Galić (12), and enzyme inactivation due to covalent modification by TCE epoxide (15).

The results described above imply that transformation capacities may be increased by avoiding the conditions leading to carbon starvation of cells and by removing competitive inhibitors. The continuous-flow system employed by Taylor et al. (18) in which batch-grown OB3b is attached to sand grains is one such design. Also, it may be possible to regenerate sMMO in viable cells. These considerations indicate that the ideal design for high transformation capacities is one in which degradation products are removed from cells and cells are regenerated with growth substrate periodically.

Conclusions. PP358 exhibited excellent TCE degradation ability under all growth conditions. Compared with that under oxygen-limiting conditions, PP358 grown under methanol-limiting conditions had somewhat lower degradation rates, but these lower rates could be relieved by the addition of formate. Even without formate, degradation rates were still substantial. Consequently, the design of reactors to bioremediate TCE with methanol-grown PP358 is impeded neither by choice of oxygen or methanol limitation nor by dilution rates used.

Under the oxygen-limiting growth conditions reported here, cells contained significant levels of PHB, while methanol-limited cells contained relatively little PHB. Since PHB is formed when NADH is present at high levels (6, 20), it is likely that oxygen limitation results in high levels of reducing equivalents in these cultures. Conversely, cells with lower levels of reducing equivalents would tend to have a lower PHB content. This hypothesis is supported by the effect of formate on TCE degradation. Formate is converted by methanotrophs into carbon dioxide, yielding NADH (14). Therefore, formate addition should increase the reducing equivalent pool in PP358. Indeed, when formate was added to methanol-limited cells, degradation rates and transformation capacities were significantly enhanced. On the other hand, in oxygen-limited cells degradation rates and transformation capacities were largely unaffected by formate addition. These findings correlate with the hypothesis that oxygen-limited cells are at a higher energy state than methanol-limited cells and that this putative energy state strongly affects the TCE degradation characteristics of PP358.

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REFERENCES

1. **Alvarez-Cohen, L., and P. L. McCarty.** 1991. Effects of toxicity, aeration, and reductant supply on trichloroethylene transformation by a mixed methanotrophic culture. Appl. Environ. Microbiol. **57:**228–235.
2. **Bailey, J. E., and D. F. Ollis.** 1986. Biochemical engineering fundamentals. McGraw-Hill, New York.
3. **Best, D. J., and I. J. Higgins.** 1981. Methane-oxidizing activity and membrane morphology in a methanol-grown obligate methanotroph, *Methylosinus trichosporium* OB3b. J. Gen. Microbiol. **125:**73–84.
4. **Braunegg, G., B. Sonnleitner, and R. M. Lafferty.** 1978. A rapid gas chromatographic method for the determination of poly-β-hydroxybutyric acid in microbial biomass. J. Appl. Microb. Biotechnol. **6:**29–37.
5. **Burrows, K. J., A. Cornish, D. Scott, and I. J. Higgins.** 1984. Substrate specificities of the soluble and particulate methane monooxygenases of *Methylosinus trichosporium* OB3b. J. Gen. Microbiol. **130:**3327–3333.
6. **Dawes, E. A., and P. J. Senior.** 1973. The role of energy reserve polymers in micro-organisms. Adv. Microb. Physiology **10:**136–266.
7. **Fitch, M. W., D. W. Graham, R. G. Arnold, G. E. Speitel, Jr., S. K. Agarwal, P. Phelps, and G. Georgiou.** 1993. Phenotypic characterization of copper-resistant mutants of *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. **59:**2771–2776.
8. **Fogel, M. M., A. R. Taddeo, and S. Fogel.** 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. **51:**720–724.
9. **Fox, B. G., J. G. Borneman, L. P. Wackett, and J. D. Lipscomb.** 1990. Haloalkene oxidation by the soluble methane monooxygenase from *Methylosinus trichosporium* OB3b: mechanistic and environmental implications. Biochemistry **29:**6419–6427.
10. **Goldberg, I., J. S. Rock, A. Ben-Bassat, and R. I. Mateles.** 1976. Bacterial yields on methanol, methylamine, formaldehyde and formate. Biotechnol. Bioeng. **18:**1657–1668.
11. **Henry, S. M., and D. Grbić-Galić.** 1991. Influence of endogenous and exogenous electron donors and trichloroethylene oxidation toxicity on trichloroethylene oxidation by methanotrophic cultures from a groundwater aquifer. Appl. Environ. Microbiol. **57:**236–244.
12. **Henry, S. M., and D. Grbić-Galić.** 1991. Inhibition of trichloroethylene oxidation by the transformation intermediate carbon monoxide. Appl. Environ. Microbiol. **57:**1770–1776.
13. **Henrysson, T., and P. L. McCarty.** 1993. Influence of the endogenous storage lipid poly-β-hydroxybutyrate on the reducing power availability during cometabolism of trichloroethylene and naphthalene by resting methanotrophic cultures. Appl. Environ. Microbiol. **59:**1602–1606.
14. **Jollie, D. R., and J. D. Lipscomb.** 1990. Formate dehydrogenase from *Methylosinus trichosporium* OB3b. Methods Enzymol. **188:**331–334.
15. **Oldenhuis, R., J. Y. Oedzes, J. J. Van der Waarde, and D. B. Janssen.** 1991. Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. Appl. Environ. Microbiol. **57:**7–14.
16. **Phelps, P., S. Agarwal, G. E. Speitel, Jr., and G. Georgiou.** 1992. *Methylosinus trichosporium* OB3b mutants having constitutive expression of soluble methane monooxygenase in the presence of high levels of copper. Appl. Environ. Microbiol. **58:**3701–3708.
17. **Speitel, G. E., Jr., , R. C. Thompson, and D. Weissman.** 1993. Biodegradation kinetics of *Methylosinus trichosporium* OB3b at low concentrations of chloroform in the presence and absence of enzyme competition by methane. Water Res. **27:**15–24.
18. **Taylor, R. T., M. L. Hanna, N. N. Shah, D. R. Shonnard, A. G. Duba, W. B. Durham, K. J. Jackson, R. B. Knapp, A. M. Wijesinghe, J. P. Knezovich, and M. C. Jovanovich.** 1993. In situ bioremediation of trichloroethylene-contaminated water by a resting-cell methanotrophic microbial filter. Hydrol. Sci. J. **38:**323–342.
19. **Whittenbury, R., K. C. Phillips, and J. F. Wilkinson.** 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. **61:**205–218.
20. **Williams, A. M.** 1988. The biochemistry and physiology of poly-beta-hydroxybutyrate metabolism in *Methylosinus trichosporium* OB3b. Ph.D. thesis. Cranfield Institute of Technology, Cranfield, United Kingdom.