RESEARCH LETTER

Measurement and modeling of multiple substrate oxidation by methanotrophs at 20 °C

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Received 30 April 2008; accepted 10 July 2008.
First published online 2 September 2008.

DOI:10.1111/j.1574-6968.2008.01314.x

Editor: J. Colin Murrell

Keywords
methanotrophs; bioremediation; chlorinated ethenes.

Abstract

Earlier experiments have shown that when *Methylosinus trichosporium* OB3b was grown at 30 °C, greater growth and degradation of chlorinated ethenes was observed under particulate methane monooxygenase (pMMO)-expressing conditions than sMMO-expressing conditions. The effect of temperature on the growth and ability of methanotrophs to degrade chlorinated ethenes, however, has not been examined, particularly temperatures more representative of groundwater systems. Thus, experiments were performed at 20 °C to examine the effect of mixtures of trichloroethylene, *trans*-dichloroethylene and vinyl chloride in the presence of methane on the growth and ability of *Methylosinus trichosporium* OB3b cells to degrade these pollutants. Although the maximal rates of chlorinated ethane degradation were greater by *M. trichosporium* OB3b expressing sMMO as compared with the same cell expressing pMMO, the growth and ability of sMMO-expressing cells to degrade these cosubstrates was substantially inhibited in their presence as compared with the same cell expressing pMMO. The Δ model developed earlier was found to be useful for predicting the effect of chlorinated ethenes on the growth and ability of *M. trichosporium* OB3b to degrade these compounds at a growth temperature of 20 °C. Finally, it was also discovered that at 20 °C, cells expressing pMMO exhibited faster turnover of methane than sMMO-expressing cells, unlike that found earlier at 30 °C, suggesting that temperature may exert selective pressure on methanotrophic communities to express sMMO or pMMO.

Introduction

Chlorinated ethenes are commonly used in various industrial practices including degreasing operations, dry cleaning, dying, and textile production (Bakke *et al.*, 2007). Despite their widely perceived carcinogenicity (Bolt, 2005; Scott & Chiu, 2006), there have been significant historical releases to the environment and as a result, these compounds are often detected in substantial concentrations in subsurface soils and groundwater (Westrick *et al.*, 1984). The reductive anaerobic biodechlorination of these compounds, for example tetrachloroethylene to ethene through trichloroethylene (TCE), dichloroethylene (DCE), and vinyl chloride (VC) as intermediates, has been known for some time (Maymo-Gatell *et al.*, 1999). However, *in situ* application of anaerobic biodechlorination has been limited as this process does not result in complete dechlorination in the presence of sulfate and thus, can lead to accumulation of TCE, *cis*-dichloroethylene (*c*-DCE), *trans*-dichloroethylene (*t*-DCE), and VC (McCue *et al.*, 2003).

Aerobic biodegradation of chlorinated compounds has been widely examined as an alternative to anaerobic degradation. There have been several reports of aerobic bacterial strains that can utilize chlorinated ethenes as growth substrates (e.g. Coleman *et al.*, 2002), as well as many strains that cooxidize these compounds (Futamata *et al.*, 2001). Owing to their omnipresence in various environments, methane-oxidizing bacteria, members of the latter group, are widely used in decontamination of sites polluted with chlorinated ethenes (Hanson & Hanson, 1996). The enzyme responsible for degradation of chlorinated ethenes in methane-oxidizing bacteria is the methane monooxygenase (MMO). In the methane oxidation pathway, MMO carries out the first step, i.e. oxidation of methane to methanol

(Dalton, 1991). Two different forms of MMO have been found that do not have any significant homology in protein or DNA sequence. The soluble MMO (sMMO) resides in the cell cytoplasm, whereas the particulate MMO (pMMO) is found associated with the membrane. Most known methanotrophs express pMMO, whereas a few have also been found to express sMMO. For cells that can express both forms of the MMO, sMMO activity is only observed in copper-deficient conditions (Nielsen *et al.*, 1997).

In bioremediation studies using methanotrophs, a significant issue is the substrate specificity of the two forms of MMO. Cells expressing pMMO exhibit a greater specificity to methane whereas sMMO-expressing cells can bind a much greater range of substrates (Hanson & Hanson, 1996; van Hylckama Vlieg *et al.*, 1996; Han *et al.*, 1999). The initial rate at which methanotrophs degrade chlorinated compounds is also known to be much greater when sMMO is expressed (Han *et al.*, 1999; Lee *et al.*, 2006). Therefore, until recently, sMMO-expressing cells have been the main focus in methanotrophic degradation of chlorinated hydrocarbons.

Although methanotrophs can degrade chlorinated ethenes, it is known that these compounds compete with the growth substrate, methane, for binding to MMOs (Lee *et al.*, 2006), and the products of chlorinated ethene oxidation can inhibit microbial growth (Chang & Alvarez-Cohen, 1996; Lee *et al.*, 2006). Thus, faster degradation rates of chlorinated ethenes by sMMO-expressing methanotrophs can lead to rapid build-up of toxic products that can be detrimental to methanotrophic growth and survival as compared with pMMO-expressing cells. In previous work by Lee *et al.* (2006), the long-term effects of chlorinated ethene oxidation on the growth and degradation activity of pMMO- and sMMO-expressing cells were observed and compared at 30 °C. In these experiments, at low concentrations of mixtures of chlorinated solvents, sMMO-expressing cells were more effective at degrading these compounds, whereas at higher concentrations both methanotrophic growth and solvent degradation were significantly reduced, yielding lower total degradation than that found for pMMO-expressing cells. Although the initial rates of solvent degradation were up to an order of magnitude smaller in pMMO-expressing cells, their relatively faster growth at higher concentrations led to overall greater pollutant degradation than for the same cell expressing sMMO.

The previous study revealed a new perspective of methanotrophic degradation of chlorinated ethenes, but it did not consider that growth temperature can and will affect microbial processes and activity, for example, temperature changes are known to modulate transcription (Gadgil *et al.*, 2005), as well as the activity of expressed enzymes (Morii & Kasama, 2004). The rate of diffusion through the membrane can also be altered because membrane fluidity is known to be affected by temperature (Chu-Ky *et al.*, 2005). Therefore, the results from the previous study may not accurately reflect *in situ* biodegradation by methanotrophs as average groundwater temperature of contaminated sites in the United States typically range from 13 to 25 °C (Wiedemeier *et al.*, 1999).

Because sMMO and pMMO are known to have disparate structures and are differentially transcribed, changes in growth temperature may significantly alter the ability of cells expressing sMMO or pMMO to degrade pollutant(s). Despite its possible significance in pollutant degradation, the effect of temperature on whole-cell kinetics of methanotrophs has not yet been extensively studied, included for the primary substrate, methane. Therefore, we decided to examine mixed pollutant degradation by methanotrophs at 20 °C, to better represent *in situ* conditions and to determine what, if any effect lower growth temperatures had on the ability of methanotrophs to degrade methane and chlorinated solvents. In particular, it is our hypothesis that although lower growth temperature would reduce the growth and activity of both sMMO- and pMMO-expressing cells, pMMO-expressing cells would still be more effective than sMMO-expressing cells at degrading pollutant mixtures.

Materials and methods

Culture conditions

Methylosinus trichosporium OB3b was grown at 20 °C in 125 mL of nitrate mineral salt medium (Whittenbury *et al.*, 1970) in 2-L Erlenmeyer flasks shaken at 225 r.p.m. in a methane-to-air ratio of 1 : 2 at 1 atm of pressure. For pMMO-expressing conditions, 20 μM of copper was added as $CuCl_2$. For sMMO-expressing conditions, no copper was added. To verify which form of the MMO was expressed, the naphthalene assay was performed as described previously (Brusseau *et al.*, 1990).

Chemicals

Highest-purity methane (≥99.99%) was obtained from Airgas Company and TCE (≥99.5%) was purchased from Fisher Scientific Company (Fair Lawn, NJ). *Trans*-dichloroethylene (*t*-DCE) (≥98%) was purchased from Aldrich (Milwaukee, WI) and vinyl chloride (VC) (≥99.5%) was purchased from Fluka (Ronkonkoma, NY). Distilled deionized water was used (> 18 MΩ). Before use, all glassware was washed with detergent and then acid washed in 2 N HNO_3 for at least 24 h to remove copper and other trace metals. Nitric acid was subsequently removed by rinsing the glassware with distilled deionized water (> 18 MΩ).

For chlorinated solvents that are liquid at room temperature, i.e. TCE and *t*-DCE, saturated stock solutions

were prepared by the method developed by Chang & Alvarez-Cohen (1996). Aliquots were taken using Hamilton 1700 series gas-tight syringes (Hamilton, Reno, NV). For methane and VC, which are gaseous at room temperature, aliquots were taken using Precision Lok gas-tight syringes (Precision Samplying Corp., Baton Rouge, LA). Ten percent (v/v) VC was prepared in a 20-mL vial for low VC concentrations. Formate was prepared as 500 mM sodium formate stock solution. The amount of chlorinated solvents to be added was determined by calculation using dimensionless Henry's constants at 20 °C. Henry's constants for methane, TCE, *t*-DCE, and VC used in this study were 28.4 (Perry, 1984) 0.291 (Tse *et al.*, 1992), 0.329 (Tse *et al.*, 1992), and 0.908 (Gossett, 1987), respectively.

Measurement of Michaelis–Menten parameters at 20 °C

The whole-cell Michaelis–Menten parameters, V_{max} and K_s, for degradation of TCE, *t*-DCE, VC, and methane by *M. trichosporium* OB3b expressing either pMMO or sMMO were measured using GC as described earlier (Lontoh & Semrau, 1998). Specifically, *M. trichosporium* OB3b was grown at 20 °C as mentioned above to an $OD_{600\,nm}$ of 0.4. Cells were then diluted 1 : 1 with fresh NMS medium precooled to 20 °C. Cells were then grown to an $OD_{600\,nm}$ of *c.* 0.3 to ensure that they were in the exponential phase. The flask was then evacuated and flushed with compressed air at least 10 times to remove methane before 3-mL aliquots were transferred to 20-mL serum vials. After adding 20 mM of sodium formate, the vials were capped with Teflon-coated rubber butyl stoppers (National Scientific Co., Duluth, GA) and crimp sealed with aluminum caps. The corresponding protein concentration was determined using the Bio-Rad protein assay kit (Hercules, CA).

For degradation assays by *M. trichoporium* OB3b expressing sMMO, varying concentration ranges were used, specifically 22–220, 8–950, and 18–500 μM for TCE, *t*-DCE, and VC, respectively. Initial samples were taken after the vials were vigorously shaken for 1 min. For TCE and *t*-DCE, 100 μL of headspace was injected into an HP5890 Series II gas chromatograph equipped with a 75 m DB-624 column with 0.53 mm internal diameter (J&W Scientific Co.) using a Precision Lok gas-tight syringe. An electron capture detector was used for both TCE and *t*-DCE, with injector, oven, and detector temperatures set to 160, 120, and 250 °C, respectively. Subsequent injections were made every 2 min for TCE over 14 and 3 min for *t*-DCE over 16 min. For VC, an HP5890II gas chromatograph was used with a flame ionization detector, with injector, oven, and detector temperatures set to 140, 120 and 250 °C, respectively. Injections were made every 1.5 min over 6 min. For degradation assays of *M. trichosporium* OB3b expressing pMMO, significantly longer time intervals were used due to the slower degradation rate. Injections were made every 30 min for TCE over 2–3 h, 18 min for *t*-DCE over 72 min, and 5 min for VC over 30 min. The concentration ranges used for pMMO degradation assays were 5–90, 14-300, and 20–510 μM for TCE, *t*-DCE, and VC, respectively.

For methane consumption assays, concentration ranges of 0.5–260 and 8–430 μM were used for *M. trichosporium* OB3b expressing pMMO and sMMO, respectively. Serum vials were shaken at 200 r.p.m. at 20 °C. For sMMO-expressing cells, samples were taken every 60 min over 8 h. For pMMO-expressing cells, samples were taken every 20 min over 80 min for low concentrations (< 35 μM), and every 90 min over 6 h for higher concentrations. For methane measurements, an HP6890 with a flame ionization detector and a GS-Molesieve column with 0.53 m inner diameter and 30 m length was used, with injector, oven, and detector temperatures set to 185, 75, and 250 °C, respectively. The normalized degradation rates were fit to the Michaelis–Menten equation using KALEIDAGRAPH v.4.0 (Synergy Software).

Effect of mixed chlorinated solvents on cell growth at 20 °C

The growth rates of *M. trichosporium* OB3b cells in the presence of different concentrations of mixed pollutants were measured using the procedure described previously by Lee *et al.* (2006). Specifically, *M. trichosporium* OB3b was grown to the mid exponential phase ($OD_{600\,nm}$ *c.* 0.4) and then diluted to an $OD_{600\,nm}$ of *c.* 0.04. After the cells were regrown to an $OD_{600\,nm}$ of *c.* 0.06 at 20 °C, the headspace was removed by applying a vacuum and replacing with air 10 times. Five milliliters of aliquots were then aseptically transferred into specially constructed serum vials. Sodium formate was added at a concentration of 20 mM and the serum vials were sealed with Teflon-coated butyl-rubber stoppers (National Scientific Co., Duluth, GA). Using a Precision Lok syringe, 5 mL of headspace in the serum vials were replaced with 5 mL of methane to yield a concentration of 270 μM. VC, *t*-DCE, and TCE were then added to yield initial equimolar concentrations ranging from 10 to 100 μM. The initial and final concentrations of the chlorinated solvents were measured using the procedure developed earlier (Lee *et al.*, 2006). To measure any abiotic loss, the same procedure was repeated with *M. trichosporium* OB3b cells of the same density inactivated with the addition of sodium hydroxide as described earlier (Lontoh & Semrau, 1998).

Calculation of the competition parameter, Δ

The Δ model developed by Lee *et al.* (2006) was used to evaluate the effects of chlorinated ethenes on the oxidation

of methane by methanotrophs. The dimensionless parameter Δ was calculated using the following equation to yield values ranging from 1 (i.e. no chlorinated ethenes present) to negative numbers (i.e. the combined rates of chlorinated ether turnover are greater than the rate of methane turnover).

$$\Delta = \left(\frac{V_{\max}^{G} \times S^{G}}{K_{s}^{G} + S^{G}} - \sum_{j=1}^{n} \frac{V_{\max}^{P_i} \times P_i}{K_{s}^{P_i} + P_i} \right) / \frac{V_{\max}^{G} \times S^{G}}{K_{s}^{G} \times S^{G}} \quad (1)$$

In this equation, S^G and P_i are concentrations of the growth substrate and the *i*th pollutant, respectively. $V_{\max}^{G}$ and $V_{\max}^{P_i}$ are the maximum rates of growth substrate and pollutant transformation, respectively and K_S^G and $K_S^{P_i}$ are the half-saturation constants for the binding the of the growth substrate and pollutant.

Results and discussion

For *M. trichosporium* OB3b grown at 20 °C and expressing pMMO, both the maximal uptake rate ($V_{\max}$) and pseudo first-order uptake rate ($V_{\max}/K_s$) for methane were significantly greater than those for VC, *t*-DCE, and TCE degradation (Table 1). For the same cell expressing sMMO, however, the maximal uptake rate of methane was considerably smaller than that measured for chlorinated ethenes. Furthermore, for *M. trichosporium* OB3b expressing sMMO, the pseudo first-order rate for methane uptake was of the same order of magnitude as that measured for VC, *t*-DCE, and TCE degradation. The pseudo-first-order rate constant for methane uptake by *M. trichosporium* expressing pMMO, however, was one to two orders of magnitude greater than that measured for degradation of the chlorinated ethenes. These results are similar to that found for the same cell grown at 30 °C, i.e. both the maximal uptake and pseudo-first order rates of methane by pMMO-expressing cells were greater than that measured for different chlorinated ethenes, but sMMO-expressing cells degraded chlorinated solvents at rates comparable to that found for methane (Oldenhuis *et al*., 1991; Lee *et al*., 2006).

It is interesting to note that at 20 °C, methane oxidation by pMMO-expressing cells was greater than that measured under sMMO-expressing conditions. This finding was unexpected as at 30 °C, methane oxidation by sMMO-expressing cells was greater than that by pMMO-expressing cells (Oldenhuis *et al*., 1991; Lee *et al*., 2006).

The growth of *M. trichosporium* OB3b expressing either sMMO or pMMO in the presence of varying equimolar concentrations of VC, *t*-DCE, and TCE was examined at 20 °C as shown in Table 2. The relative growth rate, μ/μ_0, i.e. the ratio of the specific growth rate in the presence of chlorinated ethenes to that in absence of these cometabolites, was used to determine the effect of these chlorinated ethenes on methanotrophic growth. It is evident from Table 2 that an increase in equimolar concentration of the chlorinated ethenes was coupled with a decrease in the relative growth of both pMMO- and sMMO-expressing cells. The growth of *M. trichosporium* OB3b expressing sMMO, however, decreased much more significantly in the presence of chlorinated ethenes than the same cell expressing pMMO. Notably, there was no growth observed for sMMO-expressing cells when the equimolar concentrations of chlorinated ethenes were greater than 50 μM, whereas the relative growth rate of pMMO-expressing cells was relatively high (≥ 0.25) at concentrations higher than 50 μM.

Along with the growth experiments, the total amounts of VC, *t*-DCE, and TCE degraded during growth were measured as shown in Table 3. For *M. trichosporium* expressing sMMO, equimolar mixtures of 10 and 30 μM TCE, *t*-DCE, and VC were completely degraded. However, at equimolar concentrations of 50 μM and greater, little degradation of *t*-DCE and TCE was observed, but *c*. 20% of initial amount of VC removed. An abiotic loss of 4–5% was measured for all chlorinated ethenes in negative controls, (data not shown), indicating the losses of *t*-DCE and TCE at high initial concentrations were not due to microbial activity. It is likely that the observed loss of VC was due to the initial activity of the inoculum used to seed these experiments. The inhibition of pollutant degradation was more severe at 20 °C than at 30 °C, as some growth and chlorinated ethene degradation was observed at higher concentrations of chlorinated ethenes at 30 °C (Lee *et al*., 2006).

Methylosinus trichosporium OB3b expressing pMMO was not as effective as sMMO-expressing cells in degradation of these chlorinated ethenes at equimolar concentrations lower than 30 μM, especially for TCE. However, at higher equimolar concentrations, *M. trichosporium* OB3b expressing pMMO was able to continue its growth and thus, degrade the chlorinated ethenes to a greater extent than the same cell expressing sMMO. Thus, bioremediation of sites that are

Table 1. Michaelis–Menten kinetics of chlorinated ethylene degradation by *Methylosinus trichosporium* OB3b at 20 °C expressing either sMMO or pMMO

Enzyme	Substrate	$V_{\max}$ (nmol min^{-1} mg^{-1} protein)	K_s (μM)	$V_{\max}/K_s$ (mL min^{-1} mg^{-1} protein)
pMMO	CH_4	149 (5)	9.4 (1.2)	16
	VC	35 (8)	31 (16)	1.1
	t-DCE	22 (0.8)	55 (5)	0.4
	TCE	1.9 (0.4)	65 (24)	0.029
sMMO	CH_4	83 (3)	23 (4)	3.6
	VC	1370 (343)	485 (213)	2.8
	t-DCE	184 (8)	117 (16)	1.6
	TCE	116 (5)	149 (11)	0.78

The numbers in parentheses are SDs of triplicate samples.

Table 2. Growth of *Methylosinus trichosporium* OB3b at 20 °C expressing either sMMO or pMMO in the presence of varying concentrations of mixture of TCE, *t*-DCE, and VC

Enzyme	Substrate(s)	μ (h^{-1})	μ/μ_0	Max $OD_{600\,nm}$
pMMO	CH_4	0.024 (0.0004)	1.0	0.77
	CH_4+10 μM VC, *t*-DCE, and TCE	0.017 (0.0004)	0.71	0.53
	CH_4+30 μM VC, *t*-DCE, and TCE	0.014 (0.0003)	0.58	0.45
	CH_4+50 μM VC, *t*-DCE, and TCE	0.010 (0.0002)	0.42	0.31
	CH_4+100 μM VC, *t*-DCE, and TCE	0.006 (0.0002)	0.25	0.21
sMMO	CH_4	0.027 (0.0005)	1.0	0.64
	CH_4+10 μM VC, *t*-DCE, and TCE	0.016 (0.0008)	0.59	0.58
	CH_4+30 μM VC, *t*-DCE, and TCE	0.0084 (0.0003)	0.31	0.32
	CH_4+50 μM VC, *t*-DCE, and TCE	0	0	0.08
	CH_4+100 μM VC, *t*-DCE, and TCE	0	0	0.08

The numbers in parentheses indicate the range of growth rates from duplicate samples.

Table 3. Extent of mixed chlorinated ethene degradation by *Methylosinus trichosporium* OB3b grown at 20 °C expressing either sMMO or pMMO

		% Pollutant degraded (range)			
Enzyme	Substrate(s)	VC	*t*-DCE	TCE	Degradation Time (h)
pMMO	CH_4				
	CH_4+10 μM VC, *t*-DCE, and TCE	94 (1)	91 (1)	48 (13)	160
	CH_4+30 μM VC, *t*-DCE, and TCE	94 (3)	95 (1)	52 (0)	175
	CH_4+50 μM VC, *t*-DCE, and TCE	69 (0)	84 (1)	45 (2)	145
	CH_4+100 μM VC, *t*-DCE, and TCE	45 (0)	42 (7)	29 (7)	185
sMMO	CH_4				
	CH_4+10 μM VC, *t*-DCE, and TCE	100 (0)	100 (0)	100 (0)	150
	CH_4+30 μM VC, *t*-DCE, and TCE	100 (0)	100 (0)	98 (3)	230
	CH_4+50 μM VC, *t*-DCE, and TCE	19 (2)	5 (2)	8 (4)	115
	CH_4+100 μM VC, *t*-DCE, and TCE	20 (4)	2 (4)	3 (5)	115

The numbers in parentheses indicate the range of measurements from duplicate samples.

highly contaminated with chlorinated ethenes by methane-oxidizing bacteria expressing sMMO might not be feasible. In many sites contaminated with chlorinated ethenes, these pollutants are found at concentrations exceeding 50 μM (Wiedemeier *et al.*, 1999). Therefore a new approach inducing pMMO expression should be considered for methanotrophic bioremediation of such sites.

Using the kinetic parameters measured at 20 °C (Table 1) and the model developed earlier (Lee *et al.*, 2006), Δ values were calculated to determine if this model qualitatively described the effect of mixed chlorinated solvents on growth and biodegradation capacity of *M. trichosporium* OB3b when grown at 20 °C. In calculating Δ values, the maximum rate of methane degradation was assumed. As can be seen in Fig. 1, the Δ values for *M. trichosporium* OB3b expressing sMMO were reduced much more rapidly with increasing pollutant concentration than for pMMO-expressing cells. Even in the presence of relatively high concentrations ($\geq$100 μM) of chlorinated ethenes, a relatively high positive Δ value was measured for *M. trichosporium* OB3b expressing pMMO. For sMMO-expressing cells, however, negative Δ values were predicted at a relatively low concentrations (*c.* 20 μM), indicating that the rate of chlorinated ethene degradation was faster than the methane oxidation.

A relationship was observed between the Δ values and the relative growth rates of *M. trichosporium* OB3b expressing either pMMO or sMMO (Fig. 1 and Table 2). Although quantitatively accurate correlations are not possible, a qualitative comparison is. Specifically, the Δ value appears to be a good indicator in estimating the severity of growth inhibition by the presence of chlorinated ethenes, because conditions with negative values of Δ resulted in no growth at 20 °C as was also observed at 30 °C (Lee *et al.*, 2006). Because the effectiveness of Δ model has been verified under different temperatures, it may be applied for *in situ* bioremediation efforts to estimate the effectiveness of methanotrophic biodegradation of various pollutants.

The findings here extend the conclusions of earlier work (Lee *et al.*, 2006) that methanotrophs expressing pMMO have a distinct advantage over those cells expressing sMMO in mixed waste systems and may be more numerous, particularly at elevated concentrations of cometabolites. Again, the 'tortoise', or pMMO-expressing cell, by selectively binding its growth substrate, methane, is ultimately able to

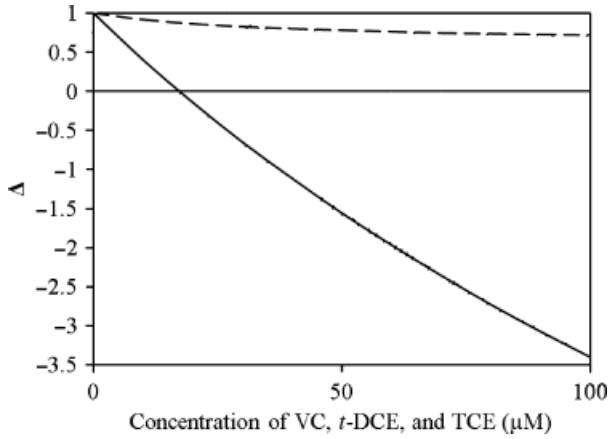

Fig. 1. The specificity of *Methylosinus trichosporium* OB3b expressing either pMMO (dashed line) or sMMO (solid line) for methane grown at 20 °C and in presence of TCE, *t*-DCE, and VC as estimated using the Δ model.

degrade cometabolites to a greater extent than the 'hare' or sMMO-expressing cell that exhausts itself from its rapid, yet counter-productive oxidation of cometabolites.

The data collected here raise an interesting question that is still unresolved – under what conditions do sMMO-expressing cells have a selective advantage over pMMO-expressing cells, particularly in pristine environments? It is well known that copper strongly regulates sMMO/pMMO expression by those cells that can express both forms (Stanley *et al.*, 1983), and copper affects the activity of pMMO-expressing cells (Lontoh & Semrau, 1998). From the data collected here when juxtaposed with earlier work (Oldenhuis *et al.*, 1991; Lee *et al.*, 2006) it appears sMMO-expressing cells have a selective growth advantage at elevated temperatures (i.e. at 30 °C) as maximal methane uptake rates are greater than for pMMO-expressing cells, but the inverse appears to be true at 20 °C. Future work should examine in more detail how temperature affects MMO expression, particularly by mixed methanotrophic cultures. Such information may provide insights as why some, but not all methanotrophs can express sMMO, as well as help provide strategies to control the composition and activity of mixed methanotrophic cultures.

Acknowledgements

Support from the Department of Energy (DE-FC26-05NT42431) and Office of the Vice President for Research (University of Michigan) is gratefully acknowledged.

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