Degradation kinetics of chlorinated aliphatic hydrocarbons by methane oxidizers naturally-associated with wetland plant roots

ARTICLE *in* JOURNAL OF CONTAMINANT HYDROLOGY · OCTOBER 2014

Impact Factor: 2.2 · DOI: 10.1016/j.jconhyd.2014.10.001

3 AUTHORS, INCLUDING:

Mark N Goltz
Air Force Institute of Technology
85 PUBLICATIONS **1,496** CITATIONS
SEE PROFILE

Abinash Agrawal
Wright State University
35 PUBLICATIONS **685** CITATIONS
SEE PROFILE

Journal of Contaminant Hydrology 170 (2014) 68–75

Contents lists available at ScienceDirect

Journal of Contaminant Hydrology

journal homepage: www.elsevier.com/locate/jconhyd

Degradation kinetics of chlorinated aliphatic hydrocarbons by methane oxidizers naturally-associated with wetland plant roots

C.L. Powell [a,1], M.N. Goltz [b], A. Agrawal [a,c,*]

[a] Environmental Science Program, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435, United States
[b] Department of Systems Engineering and Management, Air Force Institute of Technology, WPAFB, 2950 Hobson Way, OH 45433, United States
[c] Department of Earth & Environmental Sciences, Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435, United States

ARTICLE INFO

Article history:
Received 21 June 2012
Received in revised form 25 September 2014
Accepted 1 October 2014
Available online 13 October 2014

Keywords:
Trichloroethene
cis-1,2-Dichloroethene
1,1,1-Trichloroethane
Methane oxidizers
Cometabolism
Biodegradation
Wetland

ABSTRACT

Chlorinated aliphatic hydrocarbons (CAHs) are common groundwater contaminants that can be removed from the environment by natural attenuation processes. CAH biodegradation can occur in wetland environments by reductive dechlorination as well as oxidation pathways. In particular, CAH oxidation may occur in vegetated wetlands, by microorganisms that are naturally associated with the roots of wetland plants. The main objective of this study was to evaluate the cometabolic degradation kinetics of the CAHs, *cis*-1,2-dichloroethene (*cis*DCE), trichloroethene (TCE), and 1,1,1-trichloroethane (1,1,1TCA), by methane-oxidizing bacteria associated with the roots of a typical wetland plant in soil-free system. Laboratory microcosms with washed live roots investigated aerobic, cometabolic degradation of CAHs by the root-associated methane-oxidizing bacteria at initial aqueous [CH_4] ~1.9 mg L^{-1}, and initial aqueous [CAH] ~150 μg L^{-1}; *cis*DCE and TCE (in the presence of 1,1,1TCA) degraded significantly, with a removal efficiency of approximately 90% and 46%, respectively. 1,1,1TCA degradation was not observed in the presence of active methane oxidizers. The pseudo first-order degradation rate-constants of TCE and *cis*DCE were 0.12 ± 0.01 and 0.59 ± 0.07 d^{-1}, respectively, which are comparable to published values. However, their biomass-normalized degradation rate constants obtained in this study were significantly smaller than pure-culture studies, yet they were comparable to values reported for biofilm systems. The study suggests that CAH removal in wetland plant roots may be comparable to processes within biofilms. This has led us to speculate that the active biomass may be on the root surface as a biofilm. The *cis*DCE and TCE mass losses due to methane oxidizers in this study offer insight into the role of shallow, vegetated wetlands as an environmental sink for such xenobiotic compounds.

1. Introduction

Chlorinated aliphatic hydrocarbons (CAHs) are common groundwater contaminants that pose threats to human health, as known or suspected carcinogens (ATSDR, 1997). As such, there is a need to understand both natural and engineered processes that can remove CAHs from impacted environments. Natural attenuation of CAHs has been examined as a potential site remediation approach, as it is advantageous economically and environmentally, in comparison to conventional options that are resource intensive. Natural attenuation by biodegradation is especially important because it can occur for a wide range of CAHs by both oxidative and reductive processes. Highly-chlorinated CAHs, such as tetrachloroethene, can undergo biodegradation under strongly reducing conditions, whereas

* Corresponding author at: Department of Earth & Environmental Sciences Wright State University, 3640 Colonel Glenn Highway, Dayton, OH 45435, United States. Tel.: +1 937 775 3452; fax: +1 937 775 4997.
E-mail address: abinash.agrawal@wright.edu (A. Agrawal).
[1] 88 ABW/CEIEA1450 Littrell Rd. Bldg. 22WPAFB, OH 45433.

http://dx.doi.org/10.1016/j.jconhyd.2014.10.001
0169-7722/

less-chlorinated CAHs, such as vinyl chloride, are preferentially biodegraded under oxidizing conditions (Bradley, 2000; Vogel et al., 1987).

Wetlands can provide unique environments for oxidative and reductive transformations of CAHs (Amon et al., 2007; Tawney et al., 2008). Reductive transformation of CAHs (e.g., Perchloroethene) can be facilitated by the abundant natural organic matter in the wetland soil that can provide electron donors, such as hydrogen (H_2) and volatile fatty acids (Conrad, 1999), and support processes such as halorespiration, where the CAHs act as the terminal electron acceptors (Amon et al., 2007; Kassenga et al., 2004). If complete degradation is not achieved through reductive dechlorination, less-chlorinated daughter CAHs, such as trichloroethene (TCE), *cis*-1,2-dichloethene (*cis*DCE), and vinyl chloride may accumulate in the anaerobic zones of wetland environments. However, daughter CAHs can degrade further and become mineralized in the presence of oxygen in the near-surface environment of the wetlands, such as near the sediment–water interface, and in the root zone of wetland plants, in which such CAHs may serve as electron donors for microbial metabolism or by cometabolic processes in the presence of numerous growth substrates (Anderson and Walton, 1995; Bankston et al., 2002). Oxygen transported from plant shoot to root tissues for metabolic functions can diffuse out into the rhizosphere and the immediate soil environment (Colmer, 2003; Justin and Armstrong, 1987), which may create favorable conditions for oxidation of CAHs.

Few researchers have examined CAH degradation by oxidative processes in shallow, vegetated wetland settings with a focus on root zone processes (Bankston et al., 2002; Tawney et al., 2008). It has been reported (Tawney et al., 2008) that *cis*DCE degradation can be accomplished by native microorganisms associated with *Phragmites australis* roots, perhaps through cometabolic processes that were facilitated by the release of oxygen and exudates by the roots. Aerobic cometabolism of TCE has been reported at bench-scale by methane-oxidizing microorganisms that are naturally associated with roots of wetland plants, *Carex comosa* (Powell and Agrawal, 2011) and *Scirpus atroviren* roots (Powell and Agrawal, 2011; Powell et al., 2011). See Fig. 1 for schematic of TCE degradation with wetland plants. These results suggest that *cis*DCE and other CAHs may also potentially degrade by methane oxidizers that are naturally associated with wetland plants (King, 1996), particularly since CH_4 is readily available in wetland soils due to the abundance of soil organic matter and the ability of methanogenic microorganisms to breakdown the organic matter for CH_4 production.

The methane monooxygenase (MMO) enzymes produced by methanotrophic bacteria catalyze a wide range of oxidative reactions, including epoxidation of alkenes, and hydroxylation of alkanes (Alvarez-Cohen and McCarty, 1991; Colby et al., 1977). Of these, the oxidation of TCE by cometabolic degradation by MMO-producing methanotrophs has been studied in much detail. The cometabolic oxidation of TCE by methanotrophs is mostly an epoxidation process initiated by MMO with NADH as an intermediate energy source (Chang and Alvarez-Cohen, 1995; Fox et al. 1990). With a half-life of 12 s, TCE epoxide may hydrolyze spontaneously in water to form dichloroacetic acid, glyoxylic acid, or one-carbon compounds such as formic acid or carbon monoxide (Alvarez-Cohen and Speitel, 2001; Miller and Guengerich, 1982; Oldenhuis et al., 1989). While methanotrophic bacteria can further oxidize formic acid and CO to CO_2 (Little et al., 1988), the heterotrophic bacteria can mineralize any other non-chlorinated oxidation products. In addition to the epoxide, TCE can less commonly be converted into CO_2 by an alternative pathway, which proceeds through chloral hydrate, and then trichloroethanol and trichloroacetic acid, and finally to CO_2 (Fetzner and Lingens, 1994).

The aim of the current bench-scale investigation was to evaluate the degradation potential and reaction kinetics of the CAHs, *cis*DCE, TCE and 1,1,1-trichloroethane (1,1,1TCA) by methane-oxidizing microorganisms naturally associated with wetland plant roots of *C. comosa* Boott. (longhair sedge). Based on our recent findings of TCE degradation potential by naturally-occurring methane oxidizers with *C. comosa* roots (Powell and Agrawal, 2011; Powell et al., 2011), we expected that the root-associated methane oxidizers had the ability to also degrade *cis*DCE, TCE, and 1,1,1TCA via aerobic cometabolism in the presence of CH_4 as a primary substrate. This approach enabled us to evaluate the degradation potential of additional CAHs in a similar system, and obtain parameters describing degradation kinetics. To our knowledge, this is the first report of *cis*DCE and 1,1,1TCA studied with methane

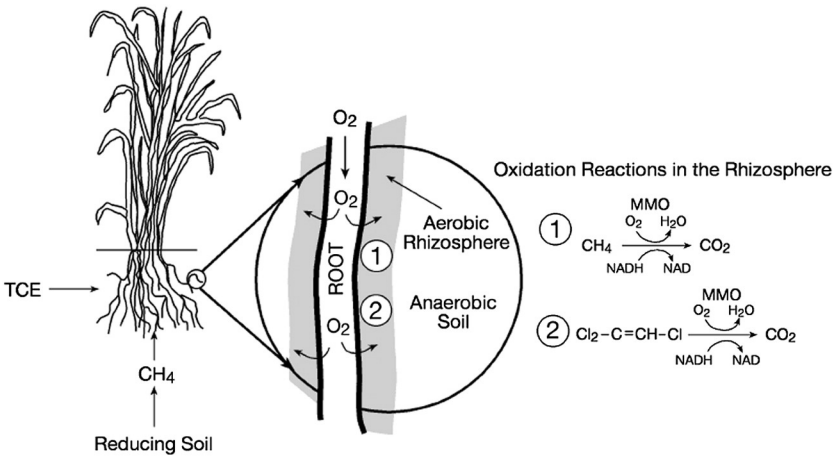

Fig. 1. A schematic showing the potential of aerobic TCE degradation by methane-oxidizing bacteria in the root zone of wetland plants. The shallow depths of a vegetated wetland can be relatively oxidizing due to aerenchymous transport of atmospheric O_2 and its leakage from wetland plant roots into the rhizosphere.

oxidizers in a cometabolic system using live wetland plant roots to determine degradation kinetics.

2. Material and methods

2.1. Experimental setup

Collection of wetland plants (*C. comosa)* from a natural wetland site, microcosm preparation containing washed (soil-free) plant roots, constitution of the growth medium and reagents, gas chromatographic analysis, calculation approaches, and enrichment procedure for methane oxidizers can be found in our earlier work (Powell et al., 2011); details provided in *Supplemental Material (SM)*.

Microcosm experiments with individual CAH treatments (*cis*DCE, and 1,1,1TCA with/without TCE) were completed in this study; a similar study completed only with TCE is described in our earlier work (Powell et al., 2011), but key findings of that study with TCE are included here for comparison and further analysis. Four cycles of microbial enrichment with CH_4 were completed followed by 4 cycles with CH_4 and each of the selected CAH treatments in triplicate microcosms (initial *cis*DCE, or 1,1,1TCA concentrations were nominally ~150 μg L^{-1}; Table 1). In 1,1,1TCA treatments, the microcosms were also amended with ~150 μg L^{-1} of TCE in the last two cycles (i.e., in cycles C3 and C4). The setup was similar to our study of TCE treatment with *C. comosa* roots and native methane-oxidizing bacteria (Powell et al., 2011).

2.2. Biomass estimation

The microbial biomass in the microcosms was most likely attached to the root surface, or embedded within the plant roots. The microbial biomass in each cycle was estimated using the stoichiometry of O_2 and CH_4 utilization/loss (Jenal-Wanner and McCarty, 1997), which were obtained by subtracting the total O_2 and CH_4 loss in the controls from the live microcosms. The mass loss in the control microcosms was subtracted from the live microcosms to account for O_2 and CH_4 loss in the presence of the plant roots. The theoretical O_2 loss was determined using mass balance (Eq. (1) below), in which the fraction of CH_4 lost is oxidized to CO_2 and used for energy (Eq. (1a)), and the remaining CH_4 fraction is assimilated into biomass ($C_5H_7O_2N$) (Eq. (1b)):

$$f_e CH_4 + 2f_e O_2 \rightarrow 2f_e H_2O + f_e CO_2 \quad (1a)$$

$$f_s CH_4 + {}^3/_5 f_s O_2 + {}^1/_5 f_s\left(H^+ + NO_3^-\right) \rightarrow {}^1/_5 f_s C_5H_7O_2N + {}^7/_5 f_s H_2O \quad (1b)$$

where f_e represents the fraction of CH_4 oxidized for energy, and f_s is the fraction of CH_4 assimilated as biomass (i.e., $f_s + f_e = 1$). The sum of above Eqs. ((1a) and (1b)) is represented in Eq. (1) below:

$$CH_4 + \left({}^7/_5 f_e + {}^3/_5\right)O_2 + {}^1/_5 f_s\left(H^+ + NO_3^-\right) \rightarrow {}^1/_5 f_s C_5H_7O_2N + \left({}^7/_5 f_s + 2f_e\right)H_2O + f_e CO_2 \quad (1)$$

In order to calculate f_e (and f_s), the measured ratio of CH_4 mass to O_2 mass utilized was set equal to the stoichiometric ratio expressed in Eq. (1) (i.e., ${}^7/_5 f_e + {}^3/_5 =$ CH_4 mass loss/TO loss); f_s was then utilized to calculate the biomass yield from CH_4 assimilation, *Y*, as biomass (mmol) per CH_4 (mmol). The biomass concentration, [*X*] (mmol L^{-1}), was calculated by multiplying the amount of CH_4 utilized by the biomass yield, *Y*, divided by the liquid volume in the microcosm (100 mL). The active biomass concentration, [X_a] (mmol L^{-1}), was then estimated based on the biomass growth-and-decay relationship, shown in Eq. (2) (Jenal-Wanner and McCarty, 1997):

$$\frac{dX_a}{dt} = Y\left(-\frac{dS}{dt}\right) - b \bullet X_a \quad (2)$$

where $-dS/dt$ (mmol L^{-1} d^{-1}) is the rate of CH_4 utilization, and *b* (d^{-1}) is the organism decay rate. After the enrichment period, it was assumed that the majority of [X_a] was attached to or within the roots and should reach a steady state value,

Table 1
Average pseudo first-order CAH degradation rate constant, k_{obs} ($=k_{1\text{-CAH}} \times X_{ss}$), steady-state cell biomass concentrations, X_{ss}, transformation yield, T_y, and biomass-normalized degradation rate constant, $k_{1\text{-CAH}}$, for 3 CAH treatments (*cis*DCE, TCE and 1,1,1TCA). The R^2 values are for an exponential fit to the CAH amount vs. time data (in Fig. 2). The data for TCE is included from earlier work (Powell et al., 2011) for comparison.

Treatments	Cycle Id	Pseudo first-order CAH degradation rate constant, $k_{obs\text{-}CAH}$ (d^{-1})	R^2	Steady-state cell biomass concentration, X_{ss} (mmol L^{-1})	Transformation yield, T_y CAH (μmol)/CH_4 (mmol)	$k_{1\text{-CAH}}$ (L $mmol^{-1}$ d^{-1})
*cis*DCE	A1	0.76	0.99	0.96	0.95	0.79
	A2	0.57	0.99	0.64	1.57	0.89
	A3	0.58	1	0.59	1.69	0.98
	A4	0.45	0.99	0.69	1.18	0.65
	Mean	*0.59*	*0.99*	*0.72*	*1.35*	*0.83*
TCE	B1	0.12	0.93	0.5	0.34	0.24
	B2	0.19	0.98	0.30	0.44	0.63
	B3	0.13	0.98	0.47	0.35	0.28
	B4	0.14	1	0.77	0.26	0.18
	Mean	*0.15*	*0.97*	*0.51*	*0.35*	*0.33*
TCE in 1,1,1TCA	C1	–	–	0.58	–	–
	C2	–	–	0.9	–	–
	C3	0.1	0.97	1.07	0.31	0.09
	C4	0.13	0.98	1.14	0.36	0.11
	Mean	*0.12*	*0.98*	*0.92*	*0.34*	*0.10*

$[X_{ss}]$ (mmol L^{-1}), due to balance between biomass growth and decay, as in Eq. (3):

$$X_{ss} = \frac{Y \bullet S_T}{V \bullet T \bullet b} \quad (3)$$

where S_T (mmol) is the total CH_4 loss during a cycle, V (mL) is volume of liquid (100 mL), T (d) is the total cycle length in days; and b is taken to be 0.549 d^{-1} from the literature methanotrophic mixed culture batch system (Chang and Criddle, 1997).

2.3. Data treatment and degradation kinetics

The relative masses (mole fractions), m/m_0, of CH_4 and CAH were calculated for each sampling event, with m_0 being their average mass in the control microcosms, and m being their average mass in the live microcosms (Anderson and McCarty, 1997). Transformation yield (T_y) was calculated by subtracting the average mass loss in CAH and in CH_4 found in control microcosms from the average mass loss in the live microcosms, and then dividing CAH mass (μmoles) degraded by CH_4 mass (mmoles) degraded (Anderson and McCarty, 1997). Since the aqueous medium in each microcosm was discarded after each cycle, T_y was calculated for each cycle individually. Assuming pseudo first-order kinetics, CAH degradation rate-constants, $k_{obs\text{-}CAH}$ (d^{-1}), were obtained as fitting parameters (Suarez and Rifai, 1999), using Eq. (4):

$$[CAH]_i = [CAH]_o e^{-k_{obs-CAH} \bullet t_i} \quad (4)$$

where $[CAH]_i$ (μmol L^{-1}) is the CAH concentration at any time, t_i (days), and $[CAH]_0$ (μmol L^{-1}) is the initial CAH concentration; in Eq. (4) above, $k_{obs\text{-}CAH}$ (d^{-1}) can be further defined in terms of $k_{1\text{-}CAH}$ (L $mmol^{-1}$ d^{-1}), the pseudo first-order degradation rate-constant for CAH that is normalized with respect to active biomass, $[X_{ss}]$ (mmol L^{-1}) using Eq. (5):

$$k_{obs-CAH}/[X_{ss}] = k_{1-CAH} \quad (5)$$

A similar approach was used to obtain the degradation rate-constants for CH_4, $k_{obs\text{-}CH4}$ (d^{-1}), assuming pseudo first-order kinetics in Eq. (6):

$$[CH_4]_i = [CH_4]_0 e^{-k_{obs\ -\ CH4} \bullet t_i} \quad (6)$$

where $[CH_4]_i$ (μmol L^{-1}) is the CH_4 concentration at any time, t_i (days), and $[CH_4]_o$ (μmol L^{-1}) is the initial CH_4 concentration. As above, $k_{obs\text{-}CH4}$ (d^{-1}) can be further defined in terms of $k_{1\text{-}CH4}$ (L $mmol^{-1}$ d^{-1}), the pseudo first-order degradation rate-constant for CH_4 that is normalized with respect to active biomass, $[X_{ss}]$ (mmol L^{-1}) using Eq. (7):

$$k_{obs-CH4}/[X_{ss}] = k_{1-CH4} \quad (7)$$

2.4. Statistical analysis

The effects of methane-oxidizing bacteria treatment (i.e., control vs. live microcosms) were evaluated on each measurement of CH_4 and CAH mass using a one-way repeated analysis of variance (RM ANOVA) with time as the repeated factor (Powell and Agrawal, 2011). Statistical analyses were performed using Statistica 9™ (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. CAH degradation with plant roots

In experiments with *cis*DCE, significant differences in [*cis*DCE] were observed between the live and control microcosms through time in 4 cycles (A1 through A4; Table 1) with CH_4 (one-way RM ANOVAs, treatment effect, time effect, $P < 0.001$; Fig. 2a). *cis*DCE can undergo metabolic oxidative degradation in the environment (Broholm et al., 2005; Coleman et al., 2002); however, in this study, *cis*DCE degradation was attributed to cometabolism by the activity of methane-oxidizing bacteria as its loss corresponded to loss in CH_4 and TO, and production of TIC (Figs. 3, and S1 in *Supplemental Material*). With initial [*cis*DCE] at 150 μg L^{-1}, the percent loss in *cis*DCE mass in each cycle averaged around 90% and the degradation rate constants for *cis*DCE ($k_{obs\text{-}CAH}$) were comparable in cycles A1 though A4 (Table 1); it varied from 0.45 to 0.76 d^{-1} with a mean of 0.59 d^{-1}. Further, $k_{obs\text{-}CAH}$ for *cis*DCE did not show a declining trend through time (Fig. 3, and Table S1), which suggests lack of significant toxicity potentially due to epoxide intermediate towards methanotrophs *cis*DCE degradation process (Arcangeli et al., 1996). This is in contrast to an investigation (Arcangeli et al., 1996) in which *cis*DCE and CH_4 removals were less efficient at initial [*cis*DCE] above 100 μgL^{-1}. The average net T_y for cycles A1 through A4 were 0.95 ± 0.04, 1.57 ± 0.50, 1.69 ± 0.60, and 1.18 ± 0.04 *cis*DCE (μmol) CH_4^{-1} (mmol), which is 2–5 times less than reported values (Anderson and McCarty, 1997; Arvin, 1991), possibly due to the differences in experimental conditions, including [*cis*DCE]. The initial [*cis*DCE] of 1.14 μM in this study was much less in comparison to other studies (e.g., 28 μM (Arvin, 1991), and 86 μM (Anderson and McCarty, 1997), which may have lowered *cis*DCE degradation kinetics ($k_{obs\text{-}CAH}$), in comparison to the kinetics at higher initial [*cis*DCE]. In one study (Chang and Criddle, 1997), greater T_y was reported at high [TCE], which was attributed to a more efficient CH_4 utilization for TCE transformation.

The results of TCE treatments in live and control microcosms with CH_4 in four cycles were reported earlier in Powell et al. (2011), yet key points are discussed here for comparison with the results of this study (Figs. 2b and 3, and S2 in *Supplemental Material*). In Powell et al. (2011), the treatments with initial [TCE] nominally at 150 μg L^{-1}, the average loss in TCE mass was 46 ± 10% for the 4 cycles (B1 through B4; Fig. 2b), and the degradation rate-constants for TCE ($k_{obs\text{-}CAH}$) varied from 0.12 to 0.19 d^{-1} (Table 1) increased in cycles B1 through B4; this suggests that TCE degradation was sustained at initial [TCE] at 150 μg L^{-1} and significant epoxide toxicity towards methane oxidizers did not develop (Alvarez-Cohen and McCarty, 1991; Chang and Criddle, 1997; Han et al., 1999; Henry and Grbic-galic, 1991). The average net T_y for cycles B1 through B4 were from 0.34 ± 0.05, 0.44 ± 0.04, to 0.35 ± 0.11, and 0.26 ± 0.11 TCE (μmol) CH_4^{-1} (mmol), which are considerably less than the reported T_y range 1.5–7.5 TCE (μmol) CH_4^{-1} (mmol) in similar studies (Anderson and McCarty, 1997). A lower T_y for TCE may be attributed to a lower initial [TCE] used in this study as CH_4 is used more efficiently at higher [TCE]

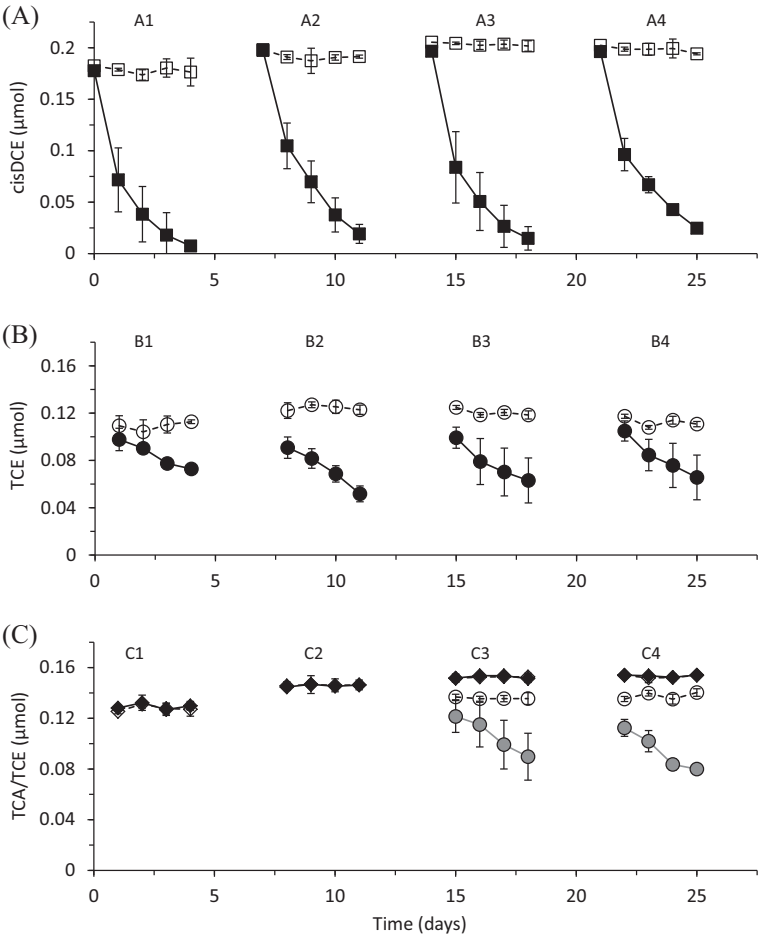

Fig. 2. Variations in the amount of *cis*DCE, TCE, and 1,1,1TCA in live (black symbols) and control (white symbols) microcosms with CH_4 (mean ± SD, $n = 3$ per treatment). The symbols in the bottom chart in cycles C3 and C4 represent variations in TCE in live (gray circles) and control (white circles) microcosms. Cycle numbers (A1 through A4, B1 through B4 and C1 through C4) are shown at the top of figures (A), (B) and (C). The data for TCE is included from earlier work (Powell et al., 2011) for comparison. The average pseudo first-order degradation rate constants of *cis*DCE and TCE for individual treatments and cycles are included in Table 1.

(Chang and Alvarez-Cohen, 1995; Chang and Criddle, 1997), where CH_4 has a greater chance to occupy/bind with active MMO sites than TCE (Chang and Alvarez-Cohen, 1995; Chang and Criddle, 1997). The ratio of initial [CH_4] to initial [TCE] in the study was ~104, in comparison to 0.34–12 found in previous studies (Jenal-Wanner and McCarty, 1997).

In 1,1,1TCA treatments, its concentrations were not significantly different between the live microcosms and killed controls in cycles C1 through C4 with CH_4 (one-way RM ANOVAs, treatment effect, time effect, $P > 0.05$, Fig. 2c). However, significant changes were observed through time in terms of loss in CH_4 and TO, and TIC production in the live microcosms in comparison to the controls (one-way RM ANOVAs, treatment effect, time effect, $P < 0.01$, Figs. 3, and S3 in *Supplemental Material*). Recent studies have indicated conflicting evidence concerning cometabolic 1,1,1TCA degradation by methane oxidizers; a few studies reported no 1,1,1TCA degradation (Broholm et al., 1990; Henson et al., 1989) while others indicated potential for 1,1,1TCA degradation (Chang and Alvarez-Cohen, 1996; Strand et al., 1990). The results of the present study are consistent with the investigation in which little or no 1,1,1TCA degradation was observed (Broholm et al., 1990; Henson et al., 1989), and CH_4 degradation wasn't influenced by initial [1,1,1TCA] ~103 mg L^{-1}. However, Strand et al. (1990) found 1,1,1TCA degradation due to the activity of methane oxidizers at initial [1,1,1TCA] at or below 4.47 mg L^{-1}, and an increase in initial [CH_4] in excess of 0.25 mg L^{-1} negatively influenced 1,1,1TCA degradation. In the present study, aqueous [CH_4] was maintained at ~1.9 mg L^{-1}, which may have been too high for 1,1,1TCA degradation to occur.

The TCE amendments in the last 2 cycles (C3 and C4) showed significant differences in [TCE] between the live and control microcosms over time (one-way RM ANOVAs, treatment effect, time effect, $P < 0.01$, Fig. 2c). In a similar study, the observed TCE degradation rates were lower with both TCE and 1,1,1TCA present than with TCE only and 1,1,1TCA degradation was inhibited in the presence of TCE (Strand et al., 1990). The observed rates for TCE degradation, in the treatments including 1,1,1TCA and TCE, were similar to the TCE only experiment (Fig. 3, and Table 1). The average TCE loss in cycle C3 and C4 was ~40%. The average net T_y were 0.31 ± 0.11 and 0.36 ± 0.03 TCE (µmol) CH_4^{-1} (mmol) in the last two cycles, respectively,

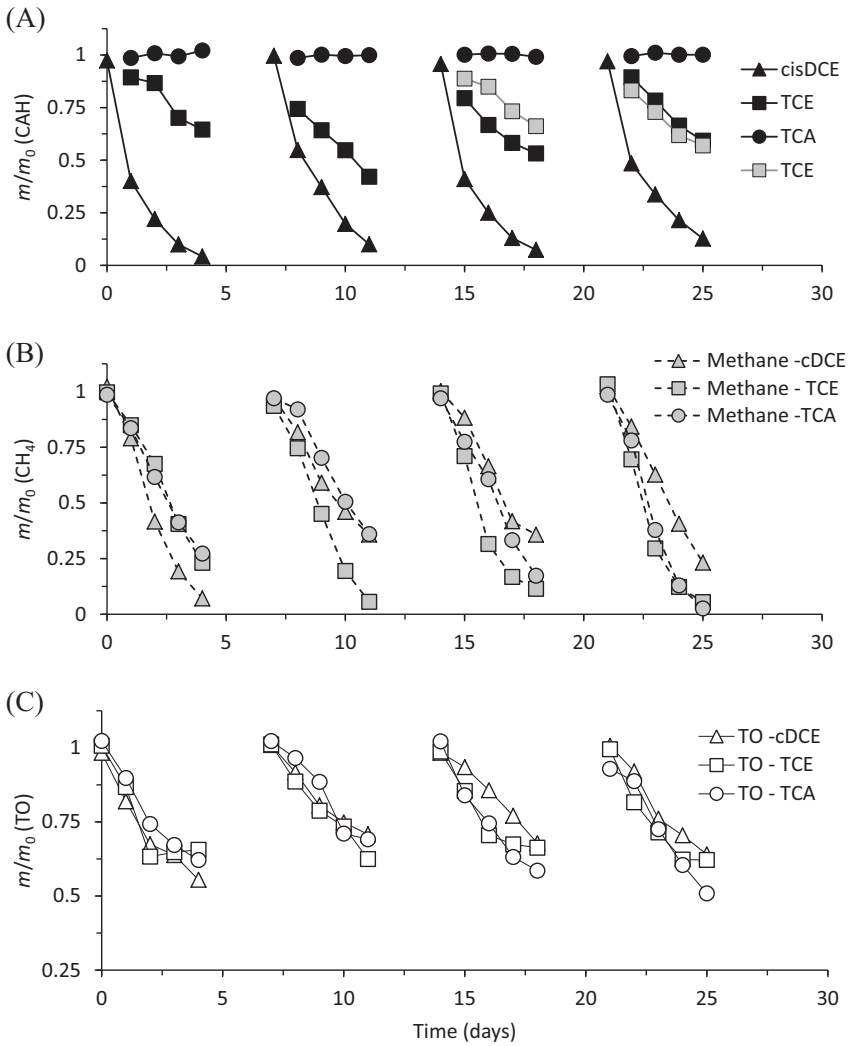

Fig. 3. Mean relative changes in the amount of (A) CAHs, (B) CH_4, and (C) O_2 (TO) in the microcosms during the experiments with *cis*DCE, TCE, and 1,1,1TCA ($n = 3$ per treatment). The gray symbols in chart 3(A) in the last 2 cycles (3 and 4) show TCE amendments in the microcosms originally with 1,1,1TCA treatment. The data for TCE is included from earlier work (Powell et al., 2011) for comparison.

which are similar to T_y reported above for TCE only treatments (Table 1). The observed trend showing TCE degradation in cycles C3 and C4 suggests that MMO was produced, and the lack of 1,1,1TCA degradation may have been due to high [CH_4]. The degradation of CH_4 exhibited pseudo first-order kinetics with average $k_{obs\text{-}CH4}$ of 0.49 ± 0.29, 0.52 ± 0.26, and 0.64 ± 0.15 d^{-1} for *cis*DCE, 1,1,1TCA, and TCE treatments, respectively. Further, the average pseudo first-order degradation rate-constants, $k_{obs\text{-}CAH}$, for *cis*DCE, and TCE were 0.59 ± 0.07 and 0.12 ± 0.01 d^{-1}, respectively (Table 1). In comparison, the $k_{obs\text{-}CAH}$ summarized in the literature for bench-scale studies by Suarez and Rifai (1999) indicate median values of 0.26 d^{-1} for TCE and 0.434 d^{-1} for DCE isomers. The degradation of *cis*DCE was faster than TCE in the present study, which is consistent with data of (Arvin, 1991) thus indicating *cis*DCE transformation kinetics to be 50% greater than that for TCE. It is generally believed that the oxidation kinetics of chlorinated ethenes increase as the degree of chlorination decreases; for example, Wiedemeier et al. (1999).

3.2. Parameters of degradation kinetics

Using the mass balance in Eq. (1), Y, [X], and [X_{ss}] were estimated (Table 2), where [X] represents the total biomass concentration, and [X_{ss}] represents the steady-state active biomass concentration resulting from continuous growth-and-decay, such that [X_{ss}] is less than [X]. The biomass yield, Y, reported here (Table 2) is slightly greater than literature values possibly because Jenal-Wanner and McCarty (1997) compared the ratio of stoichiometric O_2 to CH_4 loss with actual values in the experiment. The same study (Jenal-Wanner and McCarty, 1997) further indicated that the biomass estimate by their method can be higher because of losses in both soluble and particulate biomolecules, which in this study may have occurred as the supernatant was discarded after each cycle.

The biomass-normalized degradation rate-constant values ($k_{1\text{-}CAH}$) for *cis*DCE and TCE obtained are compared to similar

Table 2
Average kinetic parameters for *cis*DCE and TCE degradation. See Table 1 for $k_{obs\text{-}CAH}$, X_{ss} and $k_{1\text{-}CAH}$ values for each cycle. The data for TCE is included from an earlier work (Powell et al., 2011) for comparison. The unit conversions assume the formula weight of microbial biomass to be ~113 g mol^{-1}.

	*cis*DCE	TCE	TCE (in 1,1,1TCA)
TO/CH_4 (mmol $mmol^{-1}$) *Measured*	1.19 ± 0.04	1.09 ± 0.14	1.42 ± 0.13
TO/CH_4 (mmol $mmol^{-1}$) *Theoretical*	$(^3/_5 + {}^7/_5 f_e)$		
f_e	0.42 ± 0.03	0.35 ± 0.10	0.59 ± 0.09
f_e Literature values	0.44 (Chang and Criddle, 1997)		
Y (mmol $mmol^{-1}$)	0.12 ± 0.01	0.13 ± 0.02	0.08 ± 0.02
Y (mmol $mmol^{-1}$) Literature values	0.05–0.07 (Alvarez-Cohen and McCarty, 1991; Chang and Criddle, 1997; Strand et al., 1990)		
X (mmol L^{-1})	1.58 ± 0.36	2.03 ± 0.55	1.12 ± 0.43
X_{ss} (mmol L^{-1})	0.72 ± 0.17	0.92 ± 0.25	0.51 ± 0.19
$k_{obs\text{-}CAH}$ ($=k_{1\text{-}CAH} \times X_{ss}$) ($d^{-1}$) *Measured*	0.59 ± 0.07	0.15 ± 0.02	0.12 ± 0.01
$k_{1\text{-}CAH}$ (L $mmol^{-1}$ d^{-1}) *Measured*	0.83 ± 0.14	0.17 ± 0.04	0.19 ± 0.03
$k_{1\text{-}CAH}$ (L $mmol^{-1}$ d^{-1}) Literature values (cultures)	994.4 (Oldenhuis et al., 1991); 971.8 (Aziz et al., 1999) (*M. trichosporium* OB3b)	8.81 (Chang and Criddle, 1997) (mixed); 5.65 (Lontoh and Semrau, 1998); 327.7 (Oldenhuis et al., 1991) (*M. trichospoium* OB3b)	
$k_{1\text{-}CAH}$ (L $mmol^{-1}$ d^{-1}) Literature values (biofilms)	0.963 (Arcangeli et al., 1996) (mixed)	0.33 (Arvin, 1991) (mixed)	

studies (Arcangeli et al., 1996; Arvin, 1991; Chang and Criddle, 1997; Lontoh and Semrau, 1998; Oldenhuis et al., 1991); see Table 2. The $k_{1\text{-}CAH}$ values for *cis*DCE and TCE in this study were less than the literature values for pure culture studies by three orders of magnitude for *cis*DCE, and one to three orders of magnitude for TCE (Table 2). This may be attributed to factors such as differences in the experimental design (e.g., temperature; mixed vs. pure cultures; addition of formate, etc.), and the approach to estimate active biomass. Further, the smaller $k_{1\text{-}CAH}$ values obtained in this study may be attributed to mass-transfer effects, since the microbial biomass was presumably localized on or within the roots and not dispersed/suspended throughout the batch reactors. While the $k_{1\text{-}CAH}$ values in this study were significantly different than pure-culture studies (Aziz et al., 1999; Chang and Criddle, 1997; Lontoh and Semrau, 1998; Oldenhuis et al., 1991), they were comparable to values reported for biofilm systems (Table 2). It can be argued that $k_{1\text{-}CAH}$ values that are comparable to biofilms may be due to the similarity between the two microbial systems, where the active biomass may be considered a biofilm growing on the root surface. This study suggests that the removal of CAHs within wetland plant roots may be mechanistically comparable to processes within the biofilms. This is consistent with other studies that have investigated degradation of contaminants by wetland plants (McBride and Tanner, 2000; Pollard, 2010). The biofilm thicknesses in those studies were on the order of tens of μm (McBride and Tanner, 2000; Pollard, 2010). For *cis*DCE and TCE respectively, Arvin (1991) and Arcangeli et al. (1996) reported degradation rates in biofilms comparable to the rates found in this study (Table 2). In their studies, biofilm thicknesses were reported to increase from tens to hundreds of μm over the course of the study (Arcangeli et al., 1996; Arvin, 1991). Thus, based on these earlier reports, it is likely that the biofilms in this study are on the order of tens to hundreds of μm thick.

4. Conclusions

This bench-scale study examined the role of aerobic bacteria native to the wetland plant rhizosphere in the natural attenuation of chlorinated aliphatic hydrocarbons that may move from contaminated soil/water to atmosphere during plant transpiration. This investigation clearly demonstrated that TCE and *cis*DCE degradation is facilitated by methane-oxidizing bacteria naturally associated *C. comosa* roots. 1,1,1TCA did not degrade under the experimental conditions. The degradation of methane TCE and *cis*DCE exhibited pseudo first-order kinetics, and the biomass-normalized degradation rate-constant values for TCE and *cis*DCE were much less than the values obtained for systems with pure-cultures, which may be attributed to several factors relating to experimental design or biomass estimate calculations. However, these values were comparable to rate-constant reported for biofilm systems, possibly due to the similarity of the microbial systems, where the active biomass may be considered a biofilm growing on the root surface. The *cis*DCE and TCE removal by methane-oxidizing bacteria in this study offers insights into the environmental fate of CAHs in vegetated wetlands if a contaminant plume may discharge into a natural or constructed treatment wetland.

Acknowledgments

The research described in this article has been funded in part by the United States Environmental Protection Agency (EPA) (MA-91684601-0) through Greater Research Opportunities (GRO) fellowship to C.L. Powell during 2007–10. EPA has not officially endorsed this article and the views expressed herein may not reflect the views of the EPA. This research is part of a larger study to characterize chlorinated ethene biodegradation

in an experimental wetland, and it has been supported by a continuing annual grant from Air Force Institute of Technology, Wright-Patterson Air Force Base (FA8601-07-P-0370, FA8601-08-P-0358 and FA8601-09-P-0415), through M.L. Shelley. The views expressed in this manuscript are those of the authors and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government. We thank J.P. Amon for advice and help with wetland plants and S.J. Tritchler for support with data collection.

Appendix A. Supplementary data

The details of calculations, and variations in mass of total CH_4, TO, and TIC plots for each experiment, and a table with $k_{obs\text{-}CAH}$ and X_{ss} for each cycle are available electronically. Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jconhyd.2014.10.001.

References

Alvarez-Cohen, L., McCarty, P.L., 1991. A comebolic biotransformation model for halogenated aliphatic-compounds exhibiting product toxicity. Environ. Sci. Technol. 25 (8), 1381–1387.

Alvarez-Cohen, L., Speitel, G.E., 2001. Kinetics of aerobic cometabolism of chlorinated solvents. Biodegradation 12 (2), 105–126.

Amon, J.P., et al., 2007. Development of a wetland constructed for the treatment of groundwater contaminated by chlorinated ethenes. Ecol. Eng. 30 (1), 51–66.

Anderson, J.E., McCarty, P.L., 1997. Transformation yields of chlorinated ethenes by a methanotrophic mixed culture expressing particulate methane monooxygenase. Appl. Environ. Microbiol. 63 (2), 687–693.

Anderson, T.A., Walton, B.T., 1995. Comparative fate of C-14 trichloroethylene in the root-zone of plants from a former solvent disposal site. Environ. Toxicol. Chem. 14 (12), 2041–2047.

Arcangeli, J.P., Arvin, E., Mejlhede, M., Lauritsen, F.R., 1996. Biodegradation of cis-1,2-dichloro-ethylene at low concentrations with methane-oxidizing bacteria in a biofilm reactor. Water Res. 30 (8), 1885–1893.

Arvin, E., 1991. Biodegradation kinetics of chlorinated aliphatic-hydrocarbons with methane oxidizing bacteria in an aerobic fixed biofilm reactor. Water Res. 25 (7), 873–881.

ATSDR, 1997. Toxicological Profile for Trichloroethylene (Update). AfTSaDR, Atlanta, GA.

Aziz, C.E., Georgiou, G., Speitel, G.E., 1999. Cometabolism of chlorinated solvents and binary chlorinated solvent mixtures using M-trichosporium OB3b PP358. Biotechnol. Bioeng. 65 (1), 100–107.

Bankston, J.L., Sola, D.L., Komor, A.T., Dwyer, D.F., 2002. Degradation of trichloroethylene in wetland microcosms containing broad-leaved cattail and eastern cottonwood. Water Res. 36 (6), 1539–1546.

Bradley, P.M., 2000. Microbial degradation of chloroethenes in groundwater systems. Hydrogeol. J. 8 (1), 104–111.

Broholm, K., Jensen, B.K., Christensen, T.H., Olsen, L., 1990. Toxicity of 1,1,1-trichloroethane and trichloroethene of a mixed culture of methane oxidizing bacteria. Appl. Environ. Microbiol. 56 (8), 2488–2493.

Broholm, K., Ludvigsen, L., Jensen, T.F., Ostergaard, H., 2005. Aerobic biodegradation of vinyl chloride and cis-1,2-dichloroethylene in aquifer sediments. Chemosphere 60 (11), 1555–1564.

Chang, H.L., Alvarez-Cohen, L., 1995. Transformation capacities of chlorinated organics by mixed cultures enriched on methane, propane, toluene, or phenol. Biotechnol. Bioeng. 45 (5), 440–449.

Chang, H.L., Alvarez-Cohen, L., 1996. Biodegradation of individual and multiple chlorinated aliphatic hydrocarbons by methane-oxidizing cultures. Appl. Environ. Microbiol. 62 (9), 3371–3377.

Chang, W.K., Criddle, C.S., 1997. Experimental evaluation of a model for cometabolism: prediction of simultaneous degradation of trichloroethylene and methane by a methanotrophic mixed culture. Biotechnol. Bioeng. 56 (5), 492–501.

Colby, J., Stirling, D.I., Dalton, H., 1977. Soluble methane mono-oxygenase of methylococcus-capsulatus-(bath)—ability to oxygenate normal-alkanes, normal-alkenes, ethers, and alicyclic, aromatic and heterocyclic-compounds. Biochem. J. 165 (2), 395–402.

Coleman, N.V., Mattes, T.E., Gossett, J.M., Spain, J.C., 2002. Biodegradation of cis-dichloroethene as the sole carbon source by a beta-proteobacterium. Appl. Environ. Microbiol. 68 (6), 2726–2730.

Colmer, T.D., 2003. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, paddy and deep-water rice (*Oryza sativa* L.). Ann. Bot. 91 (2), 301–309.

Conrad, R., 1999. Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. FEMS Microbiol. Ecol. 28 (3), 193–202.

Fetzner, S., Lingens, F., 1994. Bacterial dehalogenases—biochemistry, genetics, and biotechnological applications. Microbiol. Rev. 58 (4), 641–685.

Fox, B.G., Froland, W.A., Dege, J.E., Lipscomb, J.D., 1989. Methane monooxygenase from Methylosinus trichosporium OB3b: purification and properties of a three-component system with high specific activity from a type II methanotroph. J. Biol. Chem. 266, 540–550.

Han, J.I., Lontoh, S., Semrau, J.D., 1999. Degradation of chlorinated and brominated hydrocarbons by Methylomicrobium album BG8. Arch. Microbiol. 172 (6), 393–400.

Henry, S.M., Grbic-galic, D., 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 (1), 236–244.

Henson, J.M., Yates, M.V., Cochran, J.W., 1989. Metabolism of chlorinated methanes, ethanes, and ethylenes by a mixed bacterial culture growing on methane. J. Ind. Microbiol. 4 (1), 29–35.

Jenal-Wanner, U., McCarty, P.L., 1997. Development and evaluation of semicontinuous slurry microcosms to simulate in situ biodegradation of trichloroethylene in contaminated aquifers. Environ. Sci. Technol. 31 (10), 2915–2922.

Justin, S., Armstrong, W., 1987. The anatomical characteristics of roots and plant—response to soil flooding. New Phytol. 106 (3), 465–495.

Kassenga, G., Pardue, J.H., Moe, W.M., Bowman, K.S., 2004. Hydrogen thresholds as indicators of dehalorespiration in constructed treatment wetlands. Environ. Sci. Technol. 38 (4), 1024–1030.

King, G.M., 1996. In situ analyses of methane oxidation associated with the roots and rhizomes of a bur reed, *Sparganium eurycarpum*, in a Maine wetland. Appl. Environ. Microbiol. 62 (12), 4548–4555.

Little, C.D., et al., 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54 (4), 951–956.

Lontoh, S., Semrau, J.D., 1998. Methane and trichloroethylene degradation by *Methylosinus trichosporium* OB3b expressing particulate methane monooxygenase. Appl. Environ. Microbiol. 64 (3), 1106–1114.

McBride, G.B., Tanner, C.C., 2000. Modelling biofilm nitrogen transformations in constructed wetland mesocosms with fluctuating water levels. Ecol. Eng. 14 (1–2), 93–106.

Miller, R.E., Guengerich, F.P., 1982. Oxidation of trichloroethylene by liver microsomal cytochrome-P-450—evidence for chlorine migration in a transition-state not involving trichloroethylene oxide. Biochemistry 21 (5), 1090–1097.

Oldenhuis, R., Vink, R., Janssen, D.B., Witholt, B., 1989. Degradation of chlorinated aliphatic-hydrocarbons by methylosinus-trichosporium Ob3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. 55 (11), 2819–2826.

Oldenhuis, R., Oedzes, J.Y., Vanderwaarde, J.J., Janssen, D.B., 1991. Kinetics of chlorinated hydrocarbon degradation by methylosinus-trichosporium Ob3b and toxicity of trichloroethylene. Appl. Environ. Microbiol. 57 (1), 7–14.

Pollard, P.C., 2010. Bacterial activity in plant (*Schoenoplectus validus*) biofilms of constructed wetlands. Water Res. 44 (20), 5939–5948.

Powell, C.L., Agrawal, A., 2011. Cometabolic degradation of trichloroethene by methane oxidizers naturally associated with wetland plant roots: investigation with *Carex comosa* and *Scirpus atrovirens*. Wetlands 31 (1), 45–52.

Powell, C.L., Nogaro, G., Agrawal, A., 2011. Aerobic cometabolic degradation of trichloroethene by methane and ammonia oxidizing microorganisms naturally associated with *Carex comosa* roots. Biodegradation 22 (3), 527–538.

Strand, S.E., Bjelland, M.D., Stensel, H.D., 1990. Kinetics of chlorinated hydrocarbon degradation by suspended cultures of methane-oxidizing bacteria. Res. J. Water Pollut. Control Fed. 62 (2), 124–129.

Suarez, M.P., Rifai, H.S., 1999. Biodegradation rates for fuel hydrocarbons and chlorinated solvents in groundwater. Biorem. J. 3 (4), 337–362.

Tawney, I., Becker, J.G., Baldwin, A.H., 2008. A novel dual-compartment, continuous-flow wetland microcosm to assess *cis*-dichloroethene removal from the rhizosphere. Int. J. Phytoremediation. 10 (5), 455–471.

Vogel, T.M., Criddle, C.S., McCarty, P.L., 1987. Transformation of halogenated aliphatic compounds. Environ. Sci. Technol. 21 (8), 722–736.

Wiedemeier, T.H., Rifai H.S., Newell, C.J., Wilson, J.T., 1999. Natural Attenuation of Fuels and Chlorinated Solvents in the Subsurface. John Wiley, New York (619 pp.).