Modeling Aerobic Biotransformation of Vinyl Chloride by Vinyl Chloride-assimilating Bacteria, Methanotrophs and Ethenotrophs

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Highlights

- A novel model was developed to describe aerobic groundwater VC biotransformation

- This model considers both aerobic metabolism and cometabolism of VC
- The model well describes VC, methane and ethene dynamics in all microcosms tested
- Interactions between methanotrophs and etheneotrophs enhance aerobic VC degradation
- This model will be a useful tool to support process optimization for VC remediation

Abstract

Recent studies have investigated the potential of enhanced groundwater Vinyl Chloride (VC) remediation in the presence of methane and ethene through the interactions of VC-assimilating bacteria, methanotrophs and ethenotrophs. In this study, a mathematical model was developed to describe aerobic biotransformation of VC in the presence of methane and ethene for the first time. It examines the metabolism of VC by VC-assimilating bacteria as well as cometabolism of VC by both methanotrophs and ethenotrophs, using methane and ethene respectively, under aerobic conditions. The developed model was successfully calibrated and validated using experimental data from microcosms with different experimental conditions. The model satisfactorily describes VC, methane and ethene dynamics in all microcosms tested. Modeling results describe that methanotrophic cometabolism of ethene promotes ethenotrophic VC cometabolism, which significantly enhances aerobic VC degradation in the presence of methane and ethene. This model is expected to be a useful tool to support effective and efficient processes for groundwater VC remediation.

Keywords: Vinyl Chloride (VC); VC-assimilating Bacteria; methanotrophs; ethenotrophs; cometabolism.

1. Introduction

Groundwater is a critically important water source world-wide, and it accounts a large amount of drinking water supplies [1]. Due to tetrachloroethene (PCE) and trichloroethene (TCE) being dumped into the environment as a consequence of intensive industrial use of chloroethene-based solvents and degreasing agents, chloroethene contamination of groundwater has been recognized as a significant environmental problem world-wide [2-4]. PCE and TCE are persistent toxic chemicals and can cause serious health problems in people [5]. However, under favorable anaerobic conditions, dechlorinating bacteria can utilize organic matter/hydrogen as electron donors to reduce PCE and TCE to ethene sequentially. They can do this via intermediates such as cis-dichloroethene (cDCE) and vinyl chloride (VC) [6].

However, anaerobic reductive dechlorination of VC to ethene is the slowest process of all the reductive dechlorination steps due to the possible absence or inactivation of capable microorganisms, thus leading to incomplete dechlorination of chloroethenes and the accumulation of VC [7]. VC is a well-known human carcinogen and its contamination of groundwater is of great concern [8, 9]. For this reason, a maximum VC contaminant level of 2 μg/L in drinking water has been set by the US Environmental Protection Agency (US EPA), which is lower than that of any other volatile organic compound [10].

Alternatively, a following post aerobic polishing process, where VC is generally accepted to be more readily biodegradable [11], is a possible solution to address the

slow and incomplete anaerobic reductive dechlorination of VC. Some aerobic bacteria that can grow using VC as the primary substrate (i.e., VC-assimilating bacteria) have been isolated from environmental samples such as soil, groundwater and activated sludge [12-16]. Other aerobic bacteria which can grow on methane and ethene as primary substrates and produce monooxygenase enzymes, can also degrade VC to nonchlorinated products through cometabolism [17, 18]. Besides VC-assimilating bacteria, methanotrophs and ethenotrophs (ethene-assimilating bacteria) are both good candidates for aerobic VC remediation applications [6], since significant levels of methane and ethene can be generated in the anaerobic zone and further migrate with VC into the aerobic zone [19].

Extensive studies on VC degradation in groundwater have been carried out with either ethene or methane being present [6, 13, 18, 20]. Recent studies have examined enhanced VC remediation linked to methane and ethene oxidation, through the interactions among methanotrophs, ethenotrophs and VC-assimilating bacteria [10]. In the presence of all three substrates and microorganisms, the VC degradation rate was significantly higher than those with the presence of either methane or ethene only. This is likely due to the fact that methanotrophs promoted ethenotrophic VC degradation [10], since methanotrophs can produce epoxyethane, a compound known to stimulate ethene and VC degradation by ethenotrophs, in methane enrichment cultures that are fed ethene [21]. Therefore, advancing our understanding of such a system is of great significance to future strategies for remediating VC.

Mathematical modeling is particularly important toward a full understanding of mechanisms involved in biological VC removal systems, which has been applied to describe metabolic VC degradation [12] and VC cometabolism associated with either methane or ethene presence [11, 13, 17, 21]. However, little effort to date has been

dedicated to modeling the VC dynamics associated with the presence of both methane and ethene, as well as the possible interactions among methanotrophs, ethenotrophs and VC-assimilating bacteria. Thus it is difficult to predict the rate and extent of VC degradation under such conditions.

This study aims to develop a new and generalized model for the prediction of VC remediation under the conditions of each substrate alone (VC, methane and ethene) and combinations of these substrates (mixtures of each two substrates or all three substrates). The model is calibrated and validated using experimental data from a comprehensive study report.

2. Model Development

2.1. Existing aerobic VC degradation models

Metabolic VC degradation has been widely modeled with the Michaelis–Menten kinetics where the concentrations of VC are considered [12]. It was also adapted in our current study to describe the metabolism of VC using VC-assimilating bacteria.

With respect to the cometabolic VC degradation through methane oxidation by methanotrophs, a previous study modeled this process as simultaneous pollutant and growth substrate binding, where the pollutant competed for binding with growth substrate. This links the net rate of methane turnover to the VC turnover rate [21]. However, both methane and VC transformation rates were modeled with the Michaelis–Menten kinetics, which unnecessarily brought in more parameters (i.e., maximum reaction rate of methane oxidation, methane affinity constant for methane oxidation, maximum reaction rate of cometabolic VC degradation, VC affinity constant for cometabolic VC degradation and cometabolic transformation capacity).

Similarly, the cometabolic VC degradation through ethene oxidation by

ethenotrophs that has been described in previous studies was also modeled with complicated differential equations for substrate and pollutant transformation. These considered the competitive inhibition and inactivation of primary substrate and pollutant [11, 13]. Such a model structure would increase the model complexity and the current available dataset may not be enough to calibrate this kind of model. Instead, keeping the model simple can limit the number of model parameters, and consequently make the model's implementation and application easier. Therefore, model simplifications are required for model calibration and actual application purposes. Additionally, previous models only considered metabolic VC degradation and VC cometabolism associated with either methane or ethene, which may not actually work in the presence of both methane and ethene, considering the interaction between methanotrophs and ethenotrophs.

2.2. Development of a generalized aerobic VC biotransformation model

VC is usually generated in groundwater during incomplete anaerobic reductive dechlorination of chloroethenes to ethene [7]. Meanwhile, the strong reducing conditions induce significant methane production in groundwater [19]. Thus, all three substrates (i.e., VC, methane and ethene) can prevail in the following aerobic conditions. The model developed in this work considered the metabolism of VC by VC-assimilating bacteria, and cometabolism of VC by both methanotrophs and ethenotrophs using methane and ethene respectively, under aerobic conditions (Figure 1). One previous experimental study revealed that cometabolic methanotrophic oxidation of ethene to epoxyethane stimulated the activity of ethenotrophs and thus further enhanced ethenotrophic VC removal [10]. This scenario was also included in the generalized model. These biological reaction kinetics were integrated with the

previous cometabolic process-based model [22] to form the proposed aerobic VC biodegradation model, by introducing the transformation coefficient [23] to link VC degradation during cometabolism. Summaries of these are presented in Tables 1 and 2.

The developed model describes the relationships among the following: firstly, three microbial groups, VC-assimilating bacteria (X_{VC}), methanotrophs (X_{CH4}) and ethenotrophs (X_{ETH}); and secondly, five soluble compounds, VC (S_{VC}), methane (S_{CH4}), ethene (S_{ETH}), oxygen (S_{O2}) and epoxyethane (S_{C2H4O}). The units are g-COD m^{-3}. While underpinned by the current scientific knowledge of these processes, the model does not replicate all the known biochemical reactions involved in the system to avoid over-parameterisation. This is because this study aims to develop a practically applicable model that can predict aerobic biotransformation of VC by VC-assimilating bacteria, methanotrophs and ethenotrophs. Instead, these reactions are simplified. There are three key biological reactions contributing to VC biodegradation (Table 2), specifically: VC metabolism by VC-assimilating bacteria (Process 1, Eq. 1); cometabolism of VC and ethene by methanotrophs (Process 2, Eq. 2, which is linked to methanotrophic growth); and cometabolism of VC by ethenotrophs (Process 3, Eq. 3, which is linked to ethenotrophic growth). Kinetic control of these three enzymatic reaction (Processes 1 – 3) rates is described by the Michaelis–Menten equation and the rate of each reaction (Eqs. 1 – 3) is modeled by an explicit function of the concentrations of all substrates involved in the reaction (Table 2). For simplification, cometabolic VC degradation through methane and ethene oxidation is linked to methanotrophic growth and ethenotrophic growth, using transformation coefficients T_1 and T_3, respectively (Table 2). Also, cometabolic ethene degradation through methane oxidation is linked to methanotrophic growth with T_2 (cometabolic

ethene transformation coefficient linked to methanotrophic growth, Table 2).

These transformation coefficients were adapted from Alvarez-Cohen and McCarty [24]. Considering the enhanced ethene oxidation rate due to stimulation from methane oxidation [10], a factor that promotes ethenotrophic growth (P_{ETH}, Eq. 4) from epoxyethane generated during cometabolic methanotrophic oxidation of ethene was added to Process 3. Our simplification with the ignorance of other minor epoxyethane production pathways can well predict substrate dynamics with a relatively simple model structure and limited number of model parameters. In this way, the implementation, application, and comprehension of the model becomes easier.

$$r1 = \mu_{VC} \frac{S_{VC}}{S_{VC}+K_{VC}} \frac{S_{O2}}{S_{O2}+K_{O2}^{VC}} X_{VC} \quad (1)$$

$$r2 = \mu_{CH4} \frac{S_{CH4}}{S_{CH4}+K_{CH4}} \frac{S_{O2}}{S_{O2}+K_{O2}^{CH4}} X_{CH4} \quad (2)$$

$$r3 = \mu_{ETH} \frac{S_{ETH}}{S_{ETH}+K_{ETH}} \frac{S_{O2}}{S_{O2}+K_{O2}^{ETH}} X_{ETH} P_{ETH} \quad (3)$$

$$P_{ETH} = 1 + \frac{S_{C2H4O}}{K_P} \quad (4)$$

Where μ_{VC}, μ_{CH4} and μ_{ETH} are maximum reaction rates of Processes 1, 2 and 3, respectively; K_{VC}, K_{CH4} and K_{ETH} are substrate affinity constants of VC, methane and ethene for Processes 1, 2 and 3, respectively; K_{O2}^{VC}, K_{O2}^{CH4} and K_{O2}^{ETH} are oxygen affinity constants in Processes 1, 2 and 3, respectively; P_{ETH} indicates the promotion effect of epoxyethane (S_{C2H4O}) on the ethenotrophic growth; and K_p is the promotion constant of S_{C2H4O}.

In addition, biomass decay of each species (Processes 4 – 6) was included. The gas/liquid mass transfer of VC, methane, ethene and oxygen was also considered in the model. The liquid-phase substrate concentration was calculated based on gas-phase substrate concentration and gas-liquid transfer coefficient [25]. Table 1 lists the

definitions, values, units, and sources of all parameters used in the developed model.

3. Model Calibration and Validation

3.1. Experimental data for model evaluation

Experimental data from Findlay et al. [10] are used for the model calibration and validation. Totally 19 microcosms (containing VC-assimilating bacteria, methanotrophs and ethenotrophs) were incubated at 22 °C in 160-mL sealed bottles, with 100-mL liquid phase and 60-mL headspace. Duplicate microcosms were prepared for the addition of VC only, methane only, and ethene only. Triplicate microcosms were prepared for the presence of methane and VC, ethene and VC, methane and ethene as well as the presence of methane, VC and ethene together [10]. Diluted VC gas was injected into the corresponding microcosms to initiate a VC concentration of 0.08 μmol per bottle. Pure methane and/or ethene were added into the corresponding microcosms to initiate a methane/ethene concentration of both 1.6 μmol per bottle. Initial liquid phase substrate concentrations were calculated with Henry's Law, namely 0.47 μM VC, 1.0 μM methane, and 3.0 μM ethene. Bottles were periodically monitored during the batch tests for methane, ethene and VC analysis. More detailed batch experimental setup and analysis methods can be found in Findlay et al. [10]. The biomass concentrations were not experimentally measured in the study that the obtained data were applied for model development. In this study, it was found that epoxyethane generation from cometabolic methanotrophic oxidation of ethene enhanced the activity of ethenotrophs in microcosms when VC, methane and ethene were present. This in turn enhanced ethenotrophic VC removal [10].

3.2. Parameter estimation and model validation

The developed model contains 19 stoichiometric/kinetic parameters (Table 1). Most of these model parameter values (e.g., 15) are well established in previous studies. Therefore, previous reported literature values were adopted for these 15 parameters. The remaining 4 parameters, i.e., T_1 (cometabolic VC transformation coefficient linked to methanotrophic growth), T_2 (cometabolic ethene transformation coefficient linked to methanotrophic growth), T_3 (cometabolic VC transformation coefficient linked to ethenotrophic growth) and K_p (promotion constant on ethenotrophic growth), which are unique to the developed model and also are the key parameters associated with the VC, methane and ethene dynamics during aerobic biodegradation of VC, were calibrated with experimental data. Parameter values were estimated by minimizing the sum of squares of the deviations between the measured data and the model predictions in all cases, using the secant method embedded in AQUASIM 2.1d [26]. Experimental datasets were developed to calibrate the mode as follows: (VC, methane, and/or ethene) derived from microcosms for adding VC only, methane only, and ethene only, as well as for adding methane and VC, ethene and VC, as well as methane and ethene.

Sensitivity analyses were conducted to evaluate the model structure and to investigate the biokinetic parameters that most determined the system's ability to function using AQUASIM built-in algorithms. The most sensitive parameters are transformation coefficients (T1, T2 and T3, Figure S1). These parameters directly regulate the cometabolic processes which determine the system's performance. It is not practical to measure all of the numerous biokinetic parameters involved. Accurate determination of these parameters in combination with reported values of other parameters could significantly reduce the calibration efforts while generating reliable results. Furthermore although the promotion constant (K_p) shows the lowest

sensitivity (Figure S1), it requires model parameter calibration due to the lack of literature value. It should be noted that K_p may still affect other model output despite its low sensitivity to the studied output with available experimental data.

A two-step procedure was used for model calibration/parameter estimation. Firstly, the individual kinetics of VC-assimilating bacteria, methanotrophs and ethenotrophs were tested using experimental data from microcosms for adding VC only, methane only, and ethene only (Figure 2a, b and c). Then, the cometabolism-related parameters (T_1, T_2 and T_3) and enhancements of ethenotrophic growth from cometabolism of methanotrophs (K_p) were further calibrated/estimated with the experimental results from microcosms with the presence of methane and VC, ethene and VC, as well as methane and ethene (Figure 2d, e and f).

The modeling results demonstrated that the previously reported kinetics values can reproduce the experimental data from microcosms for adding VC only (R^2=0.96), methane only (R^2=0.98), and ethene only (R^2=0.95) well (Figure 2a, b and c). VC, methane and ethene were degraded gradually in the individual test over time, confirming the presence of native VC-assimilating bacteria, methanotrophs and ethenotrophs in the sampled microcosms. Despite the initial lag phase, 50% degradations of VC, methane and ethene were observed in ca. 43 d, 19 d and 39 d for microcosms fed with only one substrate, respectively.

The developed cometabolic process-based model was then calibrated with experimental data from microcosms fed with two substrates (Figure 2d, e and f), which involved estimating four key parameter values (T_1, T_2, T_3 and K_p) by fitting the simulation to the experimental results. These were as follows: T_1 (cometabolic VC transformation coefficient linked to methanotrophic growth) using microcosms with VC and methane (Figure 2d), T_2 (cometabolic ethene transformation coefficient

linked to methanotrophic growth) using microcosms with ethene and methane (Figure 2f), and T_3 (cometabolic VC transformation coefficient linked to ethenotrophic growth) as well as K_p (promotion constant on ethenotrophic growth) using microcosms with VC and ethene (Figure 2e). The estimated T_1, T_2, T_3 and K_p values that generate the best model fittings with experimental results are summarized in Table 1.

Generally, VC degradation in microcosms with methane (Figure 2d) was quicker than those with VC only (Figure 2a), i.e., with the time for reaching 50% of VC degradation being ca. 30 d (30% shorter than that of VC alone), suggesting the significant contribution of methanotrophs to cometabolic VC degradation. The proposed model captured VC and methane dynamics well in this case (R^2=0.99 and R^2=0.98, respectively). Similarly, VC degradation in microcosms with ethene (Figure 2e) was also quicker than those with VC only (Figure 2a). The observed time for 50% of VC degradation was ca. 38 d (12% shorter of time than that of VC alone), indicating the important role of cometabolic VC degradation by ethenotrophs. The developed model reproduced VC and ethene profiles reasonably well (R^2=0.98 and R^2=0.95, respectively). The slight difference between simulated and measured ethene data was likely due to the relative high standard deviations on triplicate ethene measurement. In addition, the data from ethene oxidation in microcosms with methane but without VC was also applied to test cometabolic ethene transformation linked to methanotrophic growth. Subsequently, a consensus between model predictions and experimental data (Figure 2f, R^2=0.97 and R^2=0.99, respectively) was revealed. The time for reaching 50% of ethene degradation was ca. 21 d, much shorter (46% less time) than that of ca. 39 d for microcosms with ethene alone. Overall, the good match between the modeled and measured data meant that the proposed model

properly describes the cometabolic relationships involved in aerobic VC transformation.

Model validation was then done using the estimated model parameters with the experimental data that was not used for model calibration, which was conducted by comparing the model simulation results (using the same model parameters summarized in Table 1) and experimental data from microcosms containing all three substrates (methane, VC and ethene). The model predictions along with experimental data are presented in Figure 3 and illustrate the good match between model predictions and measured experimental results in the validation experiment (R^2=0.97 for VC, R^2=0.97 for CH_4 and R^2=0.94 for ethene). The validity of the proposed cometabolic model for aerobic VC biodegradation associated with methane and ethene oxidation is supported. With the presence of methane and ethene, the time for reaching 50% of VC degradation (ca. 20 d) was much shorter than those of microcosms with VC alone (ca. 43 d), VC and methane (ca. 30 d), as well as VC and ethene (ca. 38 d). Also, the time for reaching 50% of ethene degradation (ca. 19 d) was comparable to that of microcosms with methane and ethene but without VC (ca. 21 d). Therefore, these results predicted that methanotrophs promote ethenotrophic degradation of VC, in addition to the regular cometabolic VC degradation by methane and ethene. The model captured all these trends reasonably well.

4. Discussion

Recently, selecting effective remediation strategies for treatment of residue VC generated during incomplete anaerobic reductive dechlorination of chloroethene contamination in groundwater has attracted more attention [6, 11, 12, 27-31]. Since significant levels of methane and ethene can be generated in the anaerobic process

[19], aerobic cometabolic degradation of VC by methanotrophs and ethenotrophs has proven to be a promising technology for complete VC remediation [6, 10, 13, 18, 20, 32]. In this study, a generalized cometabolic process-based model considering the interactions among VC-assimilating bacteria, methanotrophs and ethenotrophs was developed for the first time based on the known metabolisms. In this model, apart from the conventional metabolism of VC by VC-assimilating bacteria and cometabolism of VC by both methanotrophs and ethenotrophs, the methanotrophic cometabolism of ethene can stimulate ethenotrophic VC degradation, leading to enhanced aerobic VC degradation. In this work, the experimental conditions for the dataset used for model calibration (i.e., the presence of 1 – 2 substrates among methane, VC and ethene) were clearly different from those for model validation (i.e., the combined presence of methane, VC and ethene). The good predictions of the model for all the datasets applied under different conditions strongly suggest the validity of the developed model.

The set of best-fit parameter values (T_1, T_2, T_3 and K_p) are summarized in Table 1 which are robust in their ability to predict VC, methane and ethene dynamics under various initial conditions, indicating the wide applicability of the proposed model. T_1 (cometabolic VC transformation coefficient linked to methanotrophic growth) with a value of 5.1 m^3 g COD $^{-1}$, is slightly higher than T_2 (cometabolic ethene transformation coefficient linked to methanotrophic growth) with a value of 4.5 m^3 g COD $^{-1}$. Together with the higher reaction rate of aerobic methane oxidation process than that of ethene oxidation, this explains the shorter time required for 50% VC degradation by the combination of metabolism and cometabolism in the presence of methane (ca. 30 d) than that of ethene (ca. 38 d) [10]. Also, T_3 (cometabolic VC transformation coefficient linked to ethenotrophic growth) with a value of 7.0 m^3 g

COD $^{-1}$ and K_p (promotion constant on ethenotrophic growth) with a value of 0.03 g COD m^{-3} further explain the accelerated ethenotrophic growth and thus ethenotrophic VC cometabolism. In turn this leads to significantly rising VC degradation in microcosms with the presence of VC, methane and ethene [10]. In addition, the modeling results in Figure 2a (adding VC only) demonstrated that VC-assimilators are present, based on the observation of VC degradation as a sole substrate. Model validation using the same model parameters in Figure 3 further validated the presence of VC-assimilators. These agreed with the findings reported by Findlay et al. [10].

Modeling of aerobic cometabolic VC degradation is of great importance for understanding and predicting VC variations in groundwater, thus becoming a powerful tool to support effectively working mitigation operations [7, 11, 13, 16]. The model proposed in this study can well predict the cometabolic process of aerobic VC degradation in the presence of VC, methane and/or ethene (Figures 2 and 3) with a relatively simple model structure and limited number of model parameters. Such a simplification will not only reduce model calibration efforts but also ensure the model can be easily integrated with existing models for more comprehensive simulations, and in turn make the new model more applicable in practice. This refers to readily integrating the existing widely applied Anaerobic Digestion Model No. 1 (ADM1)-based [33] and/or Aerobic Sludge Model (ASM)-based models [34] to describe overall substrate dynamics in groundwater remediation [35]. For example, the function of VC in inhibiting methanotrophic and ethenotrophic growth is not considered, which is reasonable due to the relatively low VC concentrations (i.e., 2 – 27 μg/L) as reported in targeted groundwater [11]. This model may not be applicable for high VC concentration conditions. However, the proposed model would be revised to contain these inhibitory effects if necessary for future applications. Also, the

possible existence of ethenotrophs using both ethene and VC as primary substrates is not included [36, 37], because they are lumped into the individual metabolism of VC-assimilating bacteria and ethenotrophs in this model for simplification purposes. This assumption can be modified in the future if more information is available.

5. Conclusions

In summary, a new mathematical model was developed based on the cometabolic process-based model to describe for the first time the aerobic metabolic VC degradation by VC-assimilating bacteria as well as cometabolic VC degradation by both methanotrophs and ethenotrophs in groundwater. The proposed model has been successfully calibrated and validated to reproduce experimental data from microcosms with different conditions (VC, methane and/or ethene), and clearly demonstrated its wide applicability. The modeling results predict that methanotrophic cometabolism of ethene stimulates ethenotrophic VC cometabolism, which significantly enhances aerobic VC degradation in the presence of VC, methane and ethene.

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Figure Captions

Figure 1. Schematic representation of the proposed aerobic VC biodegradation model concept in the presence of VC-assimilating bacteria, methanotrophs and ethenotrophs.

Figure 2. Model calibration with experimental data from aerobic biodegradation of VC, methane, and/or ethene in groundwater by the culture containing VC-assimilating bacteria, methanotrophs and ethenotrophs. (a) VC dynamics with only VC added; (b) methane dynamics with only methane added; (c) ethene dynamics with only ethene added; (d) VC and methane profiles in the presence of both VC and methane; (e) VC and ethene profiles in the presence of both VC and ethene; (f) ethene and methane profiles in the presence of both ethene and methane.

Figure 3. Model validation with experimental data from aerobic biodegradation of VC in the presence of both methane and ethene in groundwater by the culture containing VC-assimilating bacteria, methanotrophs and ethenotrophs.

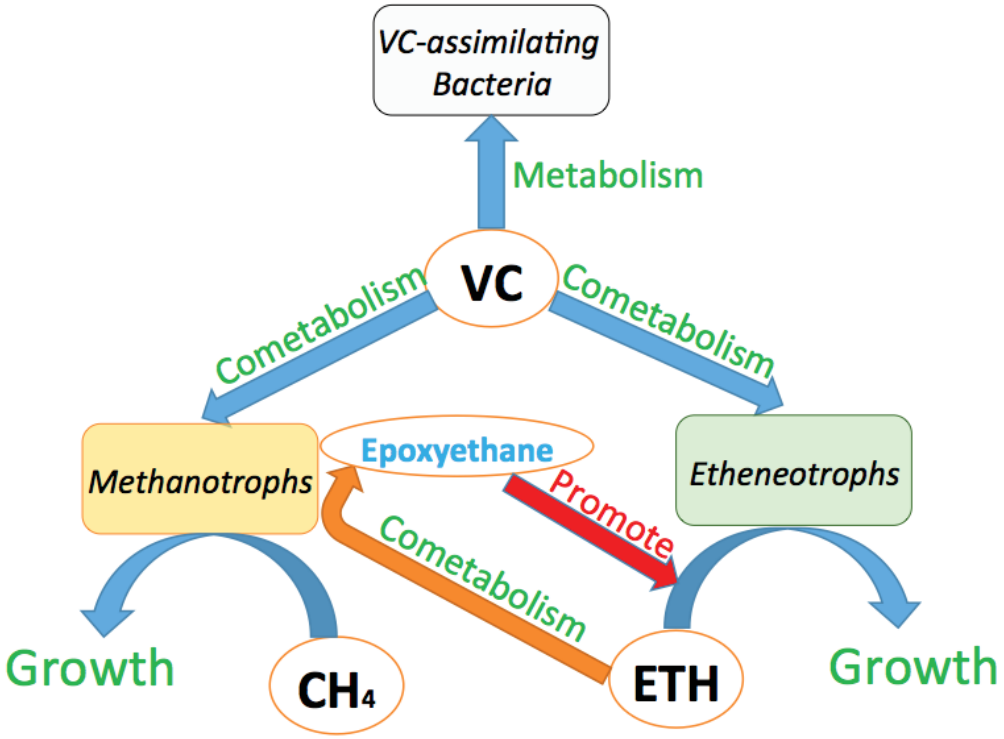

Figure 1. Schematic representation of the proposed aerobic VC biodegradation model concept in the presence of VC-assimilating bacteria, methanotrophs and ethenotrophs.

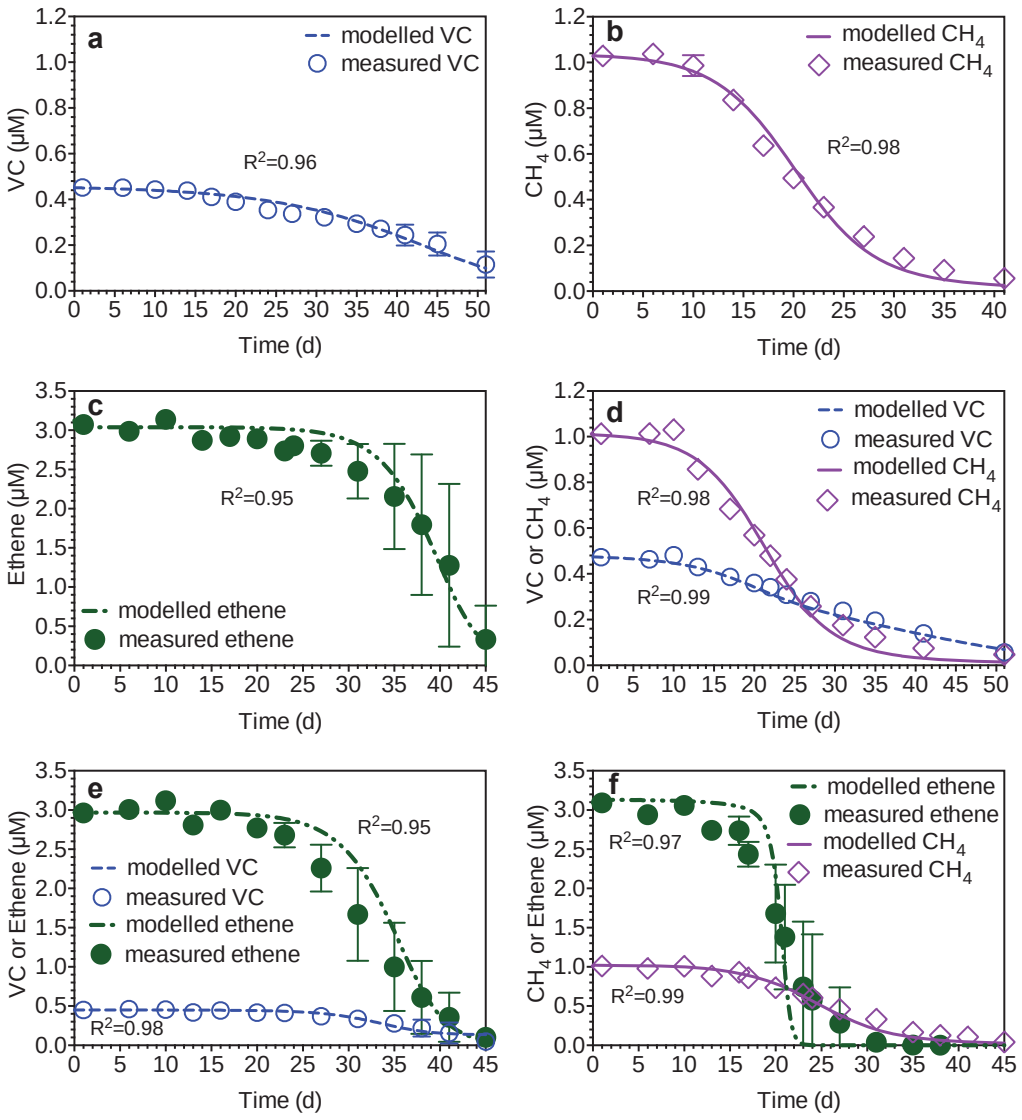

Figure 2. Model calibration with experimental data from aerobic biodegradation of VC, methane, and/or ethene in groundwater by the culture containing VC-assimilating bacteria, methanotrophs and ethenotrophs. (a) VC dynamics with only VC added; (b) methane dynamics with only methane added; (c) ethene dynamics with only ethene added; (d) VC and methane profiles in the presence of both VC and methane; (e) VC and ethene profiles in the presence of both VC and ethene; (f) ethene and methane profiles in the presence of both ethene and methane.

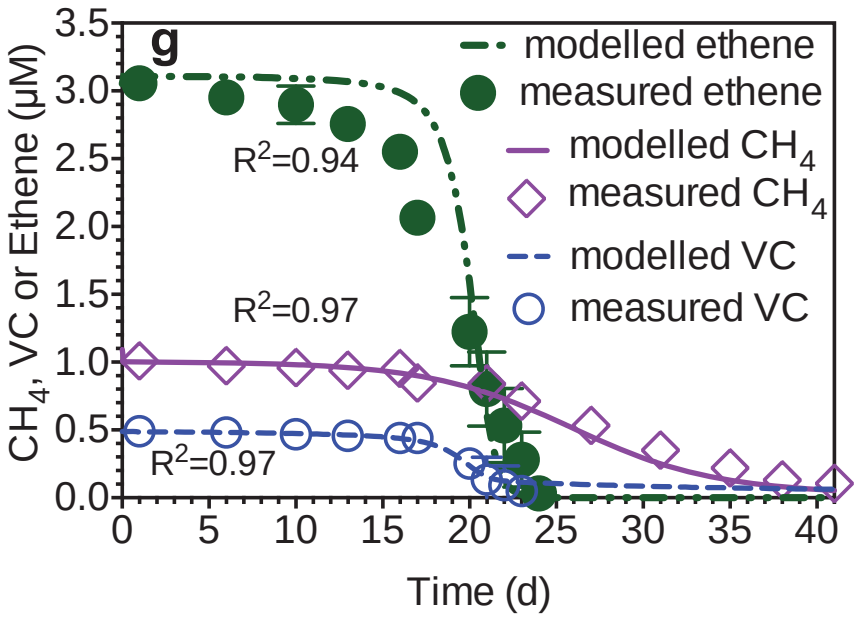

Figure 3. Model validation with experimental data from aerobic biodegradation of VC in the presence of both methane and ethene in groundwater by the culture containing VC-assimilating bacteria, methanotrophs and ethenotrophs.

Table 1. Stoichiometric and Kinetic Parameters of the Generalized Model.

Parameter	Definition	Value	Unit	Source
Stoichiometric parameters				
Y_{VC}	Yield coefficient for X_{VC}	0.18	g COD g^{-1} COD	[16] [a]
Y_{CH4}	Yield coefficient for X_{CH4}	0.19	g COD g^{-1} COD	[38]
Y_{ETH}	Yield coefficient for X_{ETH}	0.275	g COD g^{-1} COD	[16] [b]
T_1	Cometabolic S_{VC} transformation coefficient linked to X_{CH4} growth	5.1	m^3 g COD $^{-1}$	This study
T_2	Cometabolic S_{ETH} transformation coefficient linked to X_{CH4} growth	4.5	m^3 g COD $^{-1}$	This study
T_3	Cometabolic S_{VC} transformation coefficient linked to X_{ETH} growth	7.0	m^3 g COD $^{-1}$	This study
Kinetic parameters				
μ_{VC}	Maximum reaction rate of Process 1	0.22	d^{-1}	[16] [a]
μ_{CH4}	Maximum reaction rate of Process 2	1.50	d^{-1}	[38]
μ_{ETH}	Maximum reaction rate of Process 3	0.76	d^{-1}	[16] [b]
b_{VC}	Decay rate coefficient of X_{VC}	0.011	d^{-1}	[16] [c]
b_{CH4}	Decay rate coefficient of X_{CH4}	0.075	d^{-1}	[38] [c]
b_{ETH}	Decay rate coefficient of X_{ETH}	0.038	d^{-1}	[16] [c]
K_{VC}	S_{VC} affinity constant for Process 1	0.04	g COD m^{-3}	[16] [a]
K_{O2}^{VC}	S_{O2} affinity constant for Process 1	0.17	g COD m^{-3}	[16] [a]
K_{CH4}	S_{CH4} affinity constant for Process 2	0.24	g COD m^{-3}	[38]
K_{O2}^{CH4}	S_{O2} affinity constant for Process 2	0.20	g COD m^{-3}	[38]
K_{ETH}	S_{ETH} affinity constant for Process 3	0.38	g COD m^{-3}	[16] [b]
K_{O2}^{ETH}	S_{O2} affinity constant for Process 3	0.25	g COD m^{-3}	[16] [b]
K_P	Promotion constant on Process 3	0.03	g COD m^{-3}	This study

[a] The values were selected from *Mycobacterium JS60*.
[b] The values were selected from *Mycobacterium JS61*.
[c] Decay coefficients were calculated as 1/20 of maximum growth rate [39].

Table 2. Stoichiometric Matrix and Process Kinetic Rate Equations for the Aerobic Biotransformation of Vinyl Chloride.

Variable Process	S_{VC} COD	S_{CH4} COD	S_{ETH} COD	S_{O2} COD	S_{C2H4O} COD	X_{VC} COD	X_{CH4} COD	X_{ETH} COD	Kinetics rates expressions
1	$-\frac{1}{Y_{VC}}$			$-\frac{0.2-Y_{VC}}{Y_{VC}}$ [a]		1			$\mu_{VC}\frac{S_{VC}}{S_{VC}+K_{VC}}\frac{S_{O2}}{S_{O2}+K_{O2}^{VC}}X_{VC}$
2	$-T_1 * S_{VC}$	$-\frac{1}{Y_{CH4}}$	$-T_2 * S_{ETH}$	$-\frac{1-Y_{CH4}}{Y_{CH4}}$ [a]	$\frac{5T_2*S_{ETH}}{6}$ [b]		1		$\mu_{CH4}\frac{S_{CH4}}{S_{CH4}+K_{CH4}}\frac{S_{O2}}{S_{O2}+K_{O2}^{CH4}}X_{CH4}$
3	$-T_3 * S_{VC}$		$-\frac{1}{Y_{ETH}}$	$-\frac{0.33-Y_{ETH}}{Y_{ETH}}$ [a]				1	$\mu_{ETH}\frac{S_{ETH}}{S_{ETH}+K_{ETH}}\frac{S_{O2}}{S_{O2}+K_{O2}^{ETH}}X_{ETH}P_{ETH}$
4						-1			$b_{VC}X_{VC}$
5							-1		$b_{CH4}X_{CH4}$
6								-1	$b_{ETH}X_{ETH}$

[a] Values 0.2, 1 and 0.33 are dependent upon the state of mineralization of carbon source considering electron balance [34], while 1 means complete oxidation of carbon (methane to carbon dioxide, C: +4,), i.e., (4-(-4))/(4-(-4))=1, and 0.2 and 0.33 mean partial oxidation of carbon, VC (C: -1) to Acetyl-S-CoA (C: 0) instead of CO_2, i.e., (0-(-1))/(4-(-1))=1/5, and ethene (C: -2) to Acetyl-S-CoA (C: 0) instead of CO_2, i.e., (0-(-2))/(4-(-2))=1/3, respectively.

[b] 5/6 is the COD equivalent of C_2H_4O to ethene