

MODELING OF MEMBRANE-ATTACHED METHANOTROPHIC BIOFILMS COMETABOLICALLY DEGRADING TRICHLOROETHYLENE

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ABSTRACT

A model was developed to emulate cometabolism of trichloroethylene (TCE) in a membrane attached biofilm. The kinetic considerations of competitive inhibition and byproduct toxicity inherent to TCE cometabolism are incorporated into the models along with an internal recycle regime that allows for the simultaneous growth of methanotrophs and heterotrophs and the production of inert biomass from endogenous decay of the active and secondary biomass. The general model was compared to experimental data so as to facilitate a model capable of simulating real systems. Through parameter estimation for the detachment velocity, the maximum degradation rate and mass transfer coefficient of methane, a model of appreciable comparison to the empirical data was created. This model performed well for the validation procedure, which was composed of comparing model and experimental response to experimental inputs as well as comparing simulated and experimental chloride ion genesis. With the operational, validated model, the scenario of a changed biofilm configuration was explored. Changing the experimental system from simple membrane and biofilm compartments, to membranes and completely mixed biofilms in a series that have plug flow characteristics, this scenario explored the possibility of a better system efficiency with the same TCE inputs.

KEYWORDS

AQUASIM, Cometabolism, Membrane-attached Biofilms, Modeling, TCE.

INTRODUCTION

From Mom and Pop dry cleaners to large industries, trichloroethylene (TCE) is a compound used for a plethora of solvent and degreasing needs. Belonging to the group of compounds known as chlorinated aliphatic hydrocarbons (CAH's), this pollutant is frequently encountered in groundwater, thought to be carcinogenic, migrates quickly and may be considered persistent under aerobic conditions (Arcangeli and Arvin, 1997). TCE finds its way into groundwater through accidental spillage, leaking storage tanks, improper disposal and landfill leachate (Alvarez-Cohen and McCarty, Appl. Env. Microbiol., 1991) and is therefore found in varying concentrations and distribution patterns. While air stripping and activated carbon adsorption has been traditionally used to remove TCE, cometabolism may be used to effectively degrade and not just transfer the pollutant (Alvarez-Cohen and McCarty, Env. Sci. Tech., 1991). Cometabolism focuses on a microorganism's ability to express an enzyme that, through the non-specificity of that enzyme, degrades a substance that may not be used as a viable carbon source for that organism. If the degradation routine is to be sustainable, then a carbon source must be supplied and metabolized along side of TCE and hence the name cometabolism. Methanotrophs are known to express soluble methano monooxygenase (sMMO) under

copper suppression (Oldenhuis et al., 1991). This enzyme mediates oxidation of methane to methanol but also has the ability to oxidize TCE. Under the right conditions, methanotrophs have been able to completely mineralize TCE into chloride ions and carbon dioxide (Clapp et al., 1997).

While cometabolism offers new possibilities in degradation, it is not without its share of associated difficulties. The kinetics of cometabolism are often slower than normal metabolic reactions (Speitel and Segar, 1995). Additionally, competitive inhibition is an issue, meaning that even though sMMO has the ability to degrade TCE, it has a higher affinity for the primary substrate of methane (Smith, Kitanidiis and McCarty, 1997). With high concentrations of methane, it is expected that very little of the available TCE will be degraded. On the other hand, the degradation of TCE creates an intermediate, called TCE epoxide, that binds to cellular proteins resulting in inactivation of the biomass (Clapp et al., 1997). With high concentrations of TCE. This phenomena, called byproduct toxicity, can have a process level effect, decreasing removal efficiencies as well as system stability as "TCE utilization appears to irrevocably harmed the cells"(Alvarez-Cohen and McCarty, 1990).

Cometabolism of TCE presents some unique challenges for designing systems for this type of degradation. Of a very practical matter, methane is scarcely soluble in water, making sufficient transfer of the substrate difficult. Furthermore, the kinetic difficulties alluded to above are inherent to TCE. As a way of overcoming these challenges, biofilms utilizing a counter-diffusional configuration have increased transfer efficiencies of methane and decreased the effect of competitive inhibition and byproduct toxicity (Clapp et al., 1997). The counter diffusional configuration of a system supplies the primary substrate directly to a biofilm's substratum via a silicone membrane creating nearly 100% transfer efficiencies, while dosing the bulk water of the biofilm with TCE. This type of configuration is thought to decrease the effects of competitive inhibition and byproduct toxicity through creating a continual flux of healthy cells grown near the substratum on methane, into an area of the biofilm low in methane but in the presence of TCE. When the cells ability to degrade TCE has been exhausted and turned into secondary or inert biomass, it is further advectively moved out and replaced by a healthy cell from the substratum. In this way, byproduct toxicity has a decreased negative effect on the degradation process. Such a configuration has yielded promising removal efficiencies in the range of 80-90%. It is the purpose of this study to sufficiently model the kinetics of cometabolism, competitive inhibition and byproduct toxicity inclusive, to such a degree as to simulate the real system as presented by Clapp et al. A secondary purpose is to use the finalized working model to experiment with scenarios in hopes of finding ways to even further improve the removal efficiencies.

MATERIAL AND METHODS

The kinetic model utilized monod equations to describe the cross competitive effects of methane and TCE and is similar to the kinetics used by Arcangeli and Arvin (1997). Growth of methanotrophs (Equation 1) depended upon the supply of methane, oxygen and TCE. At higher levels of TCE, the growth rate of methanotrophs was modeled to be effected by decreased substrate utilization.

$$\Gamma_{x,m} = \mu_{\max,m} \frac{S_{CH_4}}{(S_{CH_4} + K_{CH_4} (1 + \frac{S_{TCE}}{K_{TCE}}))} * X_m * \frac{S_{O_2}}{(S_{O_2} + K_{O_2})} \quad (1)$$

These organisms resulting from the utilization of substrate at a literature relevant yield had the ability to degrade TCE. Kinetics of TCE degradation were affected by the presence of methane and therefore it is important to have provisions for competitive inhibition (Equation 2). This inhibition is further modeled through the value difference between the half saturation coefficient for TCE and methane, as this difference

reflects the sMMO's higher affinity for methane than for TCE (Clapp, 1996). Monod terms for methane exist both in the numerator and the denominator, first reflecting the fact that methane can act as an electron donor for NADH regeneration (Smith, Kitanidiis and McCarty, 1997) or stimulation effect (Arcangeli and Arvin, 1997) and secondly acting as a competitive inhibitor.

$$r_{\text{TCE}} = k_{\text{TCE}} * \left(\frac{S_{\text{TCE}}}{S_{\text{TCE}} + K_{\text{TCE}} \left(1 + \frac{S_{\text{CH}_4}}{K_{\text{CH}_4}} \right)} \right) * X_m * \frac{S_{\text{CH}_4}}{S_{\text{CH}_4} + K_{\text{CH}_4}} \quad (2)$$

Byproduct toxicity was included in the model through the incorporation of a transformation capacity associated inactivation (Equation 3). The TCE degrading population experienced decay proportional to the amount of TCE transformed and inversely proportional to the amount of TCE transformed per gram X_m .

$$r_{\text{toxicity}} = \frac{1}{T_c} r_{\text{TCE}} \quad (3)$$

The three processes outlined above were implemented to describe cometabolism facilitated through methanotrophs, but Clapp *et al.* did not have a pure culture existing solely of methanotrophic TCE degraders. The internal dynamics common to mixed cultures were modeled to include the growth of secondary biomass upon decay products of the methanotrophs as described by Arcangeli and Arvin, 1997. When the methanotrophs experienced decay, either through endogenous, normal decay or through associated byproduct toxicity, they essentially became possible substrate for other organisms. Ninety percent of the decayed methanotrophs became degradable matter that may be subjected to hydrolysis. After hydrolysis, the now easily degradable material known as readily degradable substrate (Sp) was available to heterotrophic biomass. The other ten percent was assumed to be inert material that was considered non degradable at relevant resident times. The heterotrophs themselves are subjected to endogenous decay after which they follow the same internal recycling regime as the methanotrophs. A complete process matrix is given below, illustrating the connection between the TCE degrading methanotrophs and secondary biomass. The processes outlined in Figure 1 were implemented into AQUASIM, a program developed by EAWAG in Switzerland (Reichert, 1994). This biofilm model is loosely based on a model developed by Wanner and Reichert (1996) and Arcangeli and Arvin (Wat. Sci. Tech., 1997, Environ. Sci. Technol., 1997).

TABLE 1

After implementation of relevant kinetics, the model was calibrated through parameter estimation upon half the experimental input and output data. The split sample calibration/validation technique then used the second half of the experimental data to validate that the model resulting from calibration could appreciably predict effluent concentrations from data not used for calibration. The final model operates upon the assumptions that all methanotrophs present degrade TCE, that the only substrate other than methane available to organisms are products from decay, anoxic and anaerobic conditions are neglected, TCE sorption does not take place within the biofilm matrix and that TCE is completely mineralized.

RESULTS AND DISCUSSION

Calibration ensued upon three parameters, namely biofilm detachment coefficient, maximum TCE degradation rate and mass transfer coefficient for methane. The justification for using the mass transfer coefficient for methane is due to its dependence upon biofilm thickness. Though pilot trials using 1,2 dichloroethane (DCE) and *Xanthobacter autotrophicus*, it has been found that the pollutant flux through the membrane could be slightly enhanced, as a function of changing mass transfer coefficients with biofilm thicknesses up to 100 micros, and then markedly reduced as biofilms grew thicker (Freitas dos Santos and Livingston, 1995). The resulting parameters from calibration are presented in the following table.

Table 2. Table of Calibration parameters

Parameter		Value before Calibration	Value after Calibration
Detachment Velocity Coefficient	m/s	0.799022	0.8789242
Mass Transfer Coefficient	m/d	2.49	1.5
Maximum TCE degradation rate	$\text{gTCE}(\text{gX}\cdot\text{d})^{-1}$	0.377	0.87377

Comparing measured effluent TCE concentration to simulated concentrations, the model performed well for the both calibration and validation experimental data (Figure 1).

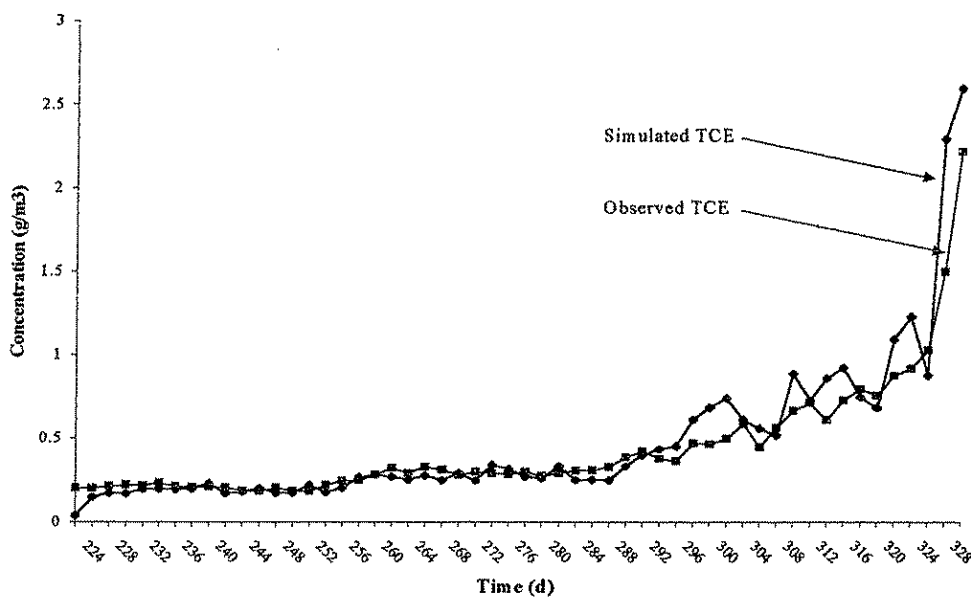


Figure 1 Simulated versus Measured TCE concentration. Day 224 – 298: Calibration, Day 299-330: Validation

The model performed best for TCE inputs in the middle range of 1.37-2.13 gTCE/m^3 . At TCE concentrations lower than this range (1.21-1.3 gTCE/m^3) and higher than this range (2.13-10.11 gTCE/m^3), the model performed less well. Alzare-Cohen and McCarty has found that it is difficult for co-metabolism to be described by TCE alone. In this model, TCE effluent concentrations were well matched for TCE concentrations in the middle range while zero and first order kinetics were not sufficient to describe degradation phenomenon at low and high TCE influent concentrations. In the validation of a model for the cometabolic biodegradation of TCE using toluene oxidizing bacteria, Arcangeli and Arvin (1997) suggested

that an imperfect fit could be due to TCE inlet concentrations being outside the calibrated range of inlet concentrations. Since higher TCE inlet concentration occurred later in the series and the second half of the series was used for validation and not calibration, the model could suffer from an imperfect match such as experienced by Arcangeli and Arvin.

An additional source of imperfect matching is due to TCE diffusing into the membrane at high concentrations in the experimental system. In the simulated system TCE had only two options: to be converted into carbon dioxide and chloride ion or transported out of the system with the effluent. Allowing for discrepancies and limitations, the model preformed well. The model found that the active biofilm layer was similar to the substrate penetration within the biofilm matrix, which is in agreement with a model for the cometabolic biodegradation of TCE with toluene oxidizing bacteria (Arcangeli and Arvin, 1997). As Clapp theorized, the counter diffusional effect of TCE and methane, created an efficient degradation area within the biofilm that was both low in methane concentration but sufficiently high in active biomass (not shown). While the methanotroph distribution in TCE-exposed biofilms could not be evaluated due to sample fixation problems, the model suggests that methanotrophs and heterotrophs were concentrated near the substratum, while inert material was concentrated on the outer most layer of biofilm where it was subjected to detachment processes (Figure 2).

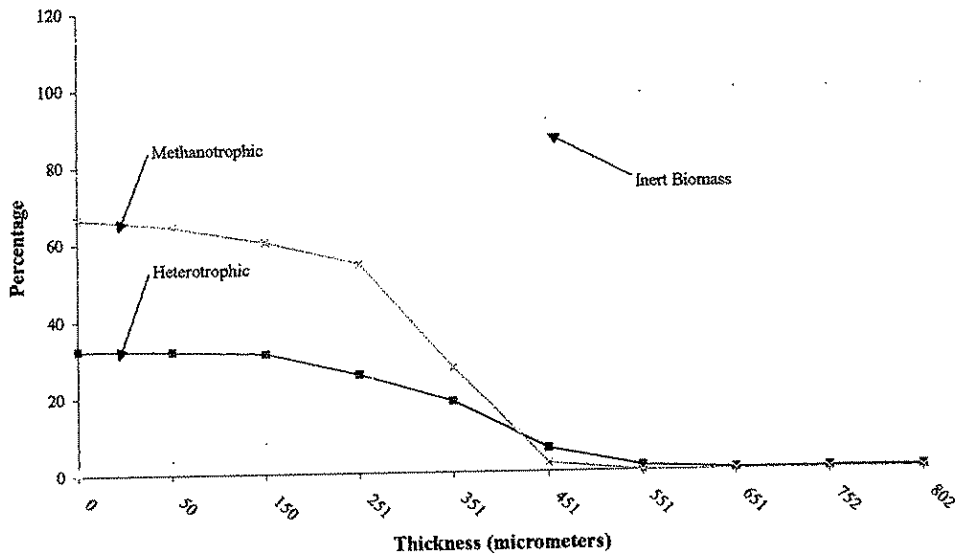


Figure 2. The composition of methanotrophs, heterotrophs and inert material as a function of biofilm depth

Using the validated model, the system was reconfigured from an ideally mixed membrane tube acting as the substratum of a biofilm reactor that is ideally mixed to membranes and biofilms in a series. The system was divided into three membrane-attached biofilms where the silicone membrane tubing was described by three ideally mixed reactors in a series. The combined tubing and biofilm reactors had the combined surface area and volume as the original system. By creating a system that was more similar to plug flow, it was hoped that even higher TCE removal efficiencies could be experienced. Due to the gradient changes in concentrations from bioreactor to bioreactor, the model did perform better as an advectively linked system. From the model simulations, it appears that the counter diffusional method performs even better when individually implemented into ideally mixed reactors in a series. Typical TCE removal efficiencies for the

experimental system were in the range of 80-90%. Through reconfiguration, the model predicts that TCE removal efficiencies could possibly be increased to the 90-100% range (Figure 3).

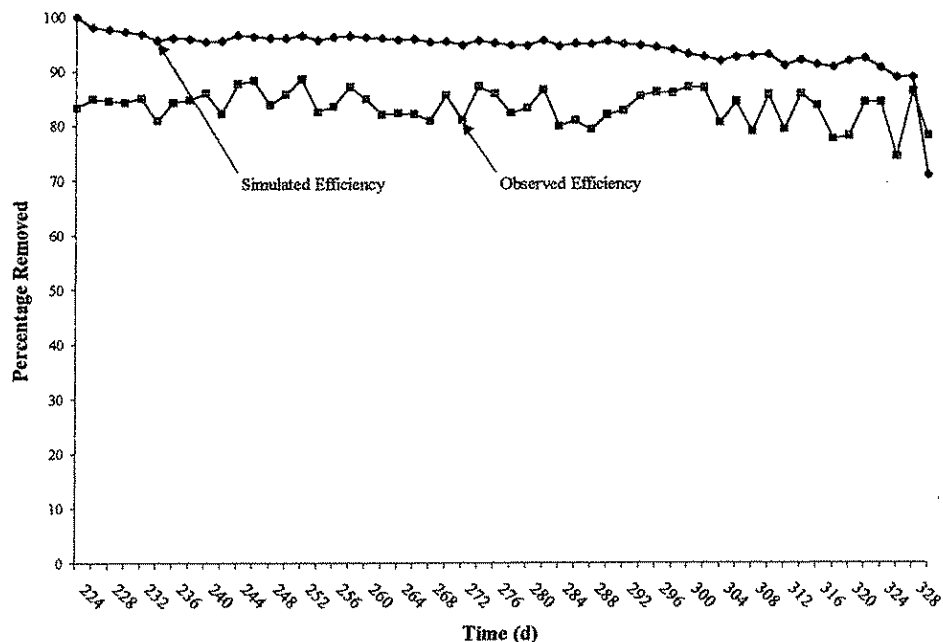


Figure 3. TCE Removal Efficiency after the third bioreactor in the series and the observed data from the experimental system

CONCLUSION

Monod kinetics describing the degradation of TCE with the associated byproduct toxicity and competitive interactions between substrate and TCE functioned well inside the model to emulate the experimental data. At very high concentrations of TCE, the model was not able to respond and predict as well as the intermediate range, which probably reflects the limitation created through the assumptions of the model (i.e. no diffusion of TCE through the membrane as well as lack of calibration at higher concentrations). Having recognized and delineated the model's limitations, the AQUASIM program is a worthy tool for improving environmental design for cometabolism of TCE. Not only may the model be used for giving some credence to theories, such as the composition of biomass as a function of biofilm depth, but it may also be used to explore ways of improving the efficiency of cometabolism through the use of scenario experimentation, instead of costly experimental reconfigurations. While this model has the capability to give some insight into cometabolic processes for TCE, TCE is rarely found in groundwater alone. More information is needed about the interactions of TCE with other, perhaps similar compounds as well as how a plethora of different compounds could simultaneously be degraded in a similar system configuration

NOMENCLATURE

- $r_{x,m}$ = Reaction rate for methanotrophic growth
- $\mu_{max,m}$ = Maximum growth constant
- S_{TCE} = Concentration of TCE
- K_{TCE} = Half saturation coefficient for TCE
- S_{CH_4} = Concentration of Methane
- K_{CH_4} = Half saturation coefficient for CH_4

S_{O_2}	= Concentration of Oxygen
K_{O_2}	= Half saturation coefficient for O_2
X_m	= Concentration of methanotrophic biomass
k_{TCE}	= First order degradation rate
r_{TCE}	= Reaction rate for TCE degradation
$r_{Toxicity}$	= Reaction of byproduct toxicity inactivation
T_c	= Transformation capacity
k_h	= Hydrolysis constant

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		Particulates			Dissolved								
j	Process	X_M	X_H	X_I	1 S_{CH_4}	2 S_{O_2}	3 S_{TCE}	4 S_{CO_2}	5 S_{CI}	6 S_H	7 S_{org}	8 S_p	
Methanotrophic Organisms													
1	Methanotrophic Growth	+1			-1/ Y_m	1-1/ Y_m							$\mu_{maxM}(S_{org}/(S_{CH_4} + K_{dM}(1+(S_{org}/K_{rO_2}))))$ * $(S_{O_2}/(S_{O_2} + K_{dO_2}))^*X_M$
2	Methanotrophic Decay	-1		+0.1							+0.9		$b_M^*X_M$
3	TCE Degradation					-0.366	-1	+0.67	+0.81	+0.203			$K_{rO_2}^*(S_{rO_2}/(S_{rO_2} + K_{rO_2}(1+(S_{CH_4}/K_{CH_4}))))^*X_m$ * $(S_{CH_4}/(S_{CH_4} + K_{CH_4}))$
4	Byproduct Toxicity	-1		+0.1							+0.9		$r_{rO_2}^*I/T_c$
5	Hydrolysis										-1	+1	$K_h^*S_{org}$
Heterotrophic Organisms													
6	Heterotrophic Growth		+1				1-1/ Y_H				-1/ Y_H		$\mu_{maxH}(S_{sp}/(S_{sp} + K_{sp}))^*(S_{O_2}/(S_{O_2} + K_{dO_2}))^*X_H$
7	Heterotrophic Decay		-1	+0.1							+0.9		$b_H^*X_H$