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KINETICS OF METHANE-OXIDIZING BIOFILMS FOR DEGRADATION OF TOXIC ORGANICS

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ABSTRACT

The kinetics of methane-oxidizing bioreactors for the degradation of toxic organics are modeled. Calculations of the fluxes of methane and toxic chlorinated hydrocarbons were made using a biofilm model. The model simulated the effects of competition by toxics and methane on their enzymatic oxidation by the methane monooxygenase. Dual-competitive-substrate/diffusion kinetics were used to model biofilm co-metabolism, integrating equations of the following form:

$$\frac{d^2S_1}{dx^2} = \frac{-r_1 S_1}{1 + \frac{S_1}{K_1} + \frac{S_2}{K_2}}$$

$$\frac{d^2S_2}{dx^2} = \frac{-r_2 S_2}{1 + \frac{S_1}{K_1} + \frac{S_2}{K_2}}$$

where S_1 and S_2 are the local concentrations of methane and toxic compound, respectively, and r and K are the maximum uptake rates and Monod coefficients, and x is the distance into the biofilm.

KEYWORDS

Water; chlorinated hydrocarbons; co-metabolism; models; kinetics; biofilms; biodegradation; inhibition.

INTRODUCTION

Groundwaters near industrial areas or improperly managed hazardous waste disposal sites are frequently contaminated with chlorinated solvents. The most common chlorinated organic groundwater contaminants, chlorinated solvents such as trichloroethylene and 1,1,1-trichloroethane, are of particular concern due to their carcinogenicity and relative resistance to biological degradation. In this paper we discuss the distribution, fate, and public health consequences of these compounds in the groundwater environment and review the available remedial action technologies. The focus of the remedial action technology section will be on biodegradation of chlorinated aliphatic hydrocarbons by methane-oxidizing bacteria and modeling of their co-metabolic degradation in experimental biofilm reactors presently under development for the destruction of chlorinated solvents.

CHARACTERISTICS OF INDUSTRIAL SOLVENTS

Chlorinated aliphatic hydrocarbons are useful solvents because of their hydrophobic nature, high volatility, low water solubility, relative chemical stability, and nonflammability in air. Total annual production of chlorinated organic solvents in the United States in 1984 exceeded two million tons (Parsons and Lage, 1985). Although none

of these compounds occur naturally in the environment, due to their widespread use they have been detected in many parts of the environment and in human tissues. The chlorinated solvents are not expected to persist in the environment since they do not accumulate in soil or biological tissue due to their low octanol:water partition coefficients, they are highly volatile, and they rapidly photo-oxidized in the atmosphere. Despite these characteristics, accumulation of chlorinated solvents in groundwater has proven to be common in the United States and Europe, and of particular environmental concern since they pose a risk to human health.

Many of the chlorinated solvents are acutely toxic at high levels and carcinogenic at lower levels and therefore have been regulated under the revisions to the Safe Drinking Water Act. The recommended maximum ambient water concentrations of these compounds should be zero based on the non-threshold assumption. But since this is not considered feasible, levels have been set based on incremental increases in cancer risk for humans (EPA, 1980).

OCCURRENCE OF CHLORINATED ALIPHATIC HYDROCARBONS IN GROUNDWATER

Chlorinated solvents are widely used in industrial and household applications. The extent of contamination with chlorinated organic solvents can be seen in the results from a study undertaken by the state of New Jersey. More than one fourth of 670 wells sampled had detectable levels of these chemicals (Tucker, 1981). Concentrations of groundwater chlorinated solvents were as high as several milligrams per liter. These contaminants have been found in wells in virtually all other states.

The US EPA has found that contaminated groundwater normally contains two or more predominant compounds along with others at lower concentrations (Love and Eilers, 1982). This may be due in part to solvent impurities or to the use of several solvents, but it seems primarily to be the result of biological transformations. Within one contaminated well field nearby wells may have different ratios of carbon tetrachloride, TECE, TCE, TCA, DCE, vinyl chloride and other related compounds.

TRANSFORMATIONS IN NATURAL GROUNDWATER ENVIRONMENTS

The occurrence of changing ratios of chlorinated organic compounds with distance away from the source of contamination may indicate some degree of biodegradation. Some authors have reported changing composition of the contaminant plume with some chlorinated compounds disappearing and others appearing as it migrates away from its source (Kobayashi and Rittman, 1982). The typical trend is a decreasing number of chlorine atoms per molecule as the plume moves away from the source.

At spill sites, and hazardous waste disposal sites there is frequently sufficient Chemical Oxygen Demand (COD) in the spilled material or leachate to produce anaerobic groundwater conditions. Studies on anaerobic degradation of chlorinated organics in simulated groundwater environments by Wilson, Smith and Rees (1986) and Parsons and Lage (1983) showed that after a lag period anaerobic transformations would be carried out. TCE was degraded to 1,2-DCE and DCE to vinyl chloride. These anaerobic transformations are important but unfortunately produce products such as dichloroethylenes or vinyl chloride which are as or more toxic than the parent compounds.

REMEDIAL ACTION FOR CONTAMINATED AQUIFERS

Current and emerging technologies for remediation of groundwaters contaminated with chlorinated aliphatic hydrocarbons include air stripping, activated carbon adsorption, and biodegradation. Air stripping and activated carbon adsorption are both effective and widely accepted environmental engineering unit processes which have been proven capable of removing TCE and related compounds down to the low levels required to meet drinking water standards (Love and Eilers, 1982).

However, both of these physical-chemical processes involve the transfer of the contaminant from the liquid phase to another phase without destroying it. In the case of air stripping, the contaminant is volatilized into the atmosphere where it may cause further environmental degradation. It is expected that the forthcoming revisions to the Clean Air Act will include limitations to discharges of volatile organic carbon compounds (VOC) which will eliminate the air stripping option in many cases. Spent activated carbon must be either land-filled or thermally regenerated. As a result activated carbon adsorption may be two to four times as expensive as air stripping (Knox *et al.*, 1986). These difficulties with physical/chemical processes have stimulated research into lower cost methods of biologically destroying chlorinated solvents.

Biological treatment of contaminated groundwaters can be performed above ground by first pumping out the water or in-situ by adding the necessary materials such as nutrients, an inoculum bacterium culture if the indigenous population is not adequate, or primary substrates for co-metabolism. Above ground treatment involves the use of conventional suspended growth or attached growth systems similar to those used for domestic and industrial wastewater.

In-situ biodegradation eliminates the expense of pumping out the groundwater and constructing treatment facilities, but adds a host of complexities related to the subsurface environment and the hydrogeology of the site. For example, in many northern locales low groundwater temperatures will slow or prevent biological reactions (EPA, 1985). Additional difficulties include transporting substrates such as nitrogen, phosphorus, and oxygen in the required concentrations throughout the highly variable subsurface environment. In-situ bioreclamation of contaminated aquifers presents many challenges but has proven feasible for cleaning up aquifers contaminated with gasoline, acrilonitrile, formaldehyde, methylene chloride, phenols, chlorinated benzenes and a host of other compounds (Knox *et al.*, 1986; EPA, 1985). Nutrients can be added through injection wells or trenches and oxygen can be added through blowers, ozone or hydrogen peroxide (EPA, 1985).

AEROBIC CO-METABOLISM BY METHANE-OXIDIZING BACTERIA

Co-metabolism refers to the fortuitous transformation of a compound by an organism which relys upon another compound for its primary energy source. Co-metabolic transformations occur as a result of non-specific enzymes intended for other purposes. Co-metabolism is the predominant mechanism of biodegradation at low concentrations (below 10-100 µg/L) (Law and Button, 1977; Schmidt *et al.*, 1985; Shehata *et al.*, 1971; Scow *et al.*, 1986). At low carbon levels there may be insufficient energy available to supply maintenance energy requirements (Powell, 1967). Certainly high-rate, high-efficiency bioreactors cannot be operated using as a growth substrate the low concentrations of toxic compounds in polluted groundwaters. Additional carbon sources must be added to sustain the high amounts of biomass that are required for high activities.

Methanotrophic organisms occur in nature wherever both methane and oxygen are present. Methanotrophs oxidize methane to methanol using the enzyme methane monooxygenase (MMO) and molecular oxygen. MMO is a highly non-specific enzyme able to oxidize and dehalogenate a large number of compounds (Haber *et al.*, 1983). Some of the reactions catalyzed by the MMO enzyme are hydroxylations of n-alkanes, epoxidations of n-alkenes, and dechlorinations of aliphatic and aromatic hydrocarbons (Dalton and Stirling, 1982).

Co-metabolism by methane-oxidizing bacteria has be used successfully to degrade 1,1,1-trichloroethylene, 1,1,1-trichloroethane, and chloroform by microbial communities growing on methane and propane (Wilson and Wilson, 1985; Fogel *et al.*, 1986; Strand and Shippert, 1986). Wilson and Wilson (1985) measured TCE degradation in an unsaturated soil column which was exposed to a mixture of natural gas and air in the headspace. Fogel, Taddeo, and Fogel (1986) found that a mixed culture of methanotrophic organisms could completely degrade vinyl chloride, 1,2-dichloroethylene, vinylidene chloride, and 1,1,1-trichloroethylene. Degradation rates decreased as the degree of chlorination increased. Strand and Shippert (1986) found that the rate of chloroform degradation by a methane-enriched soil decreased as methane concentration increased.

In order to efficiently use the phenomenon of co-metabolism, the properties of the methane monooxygenase and the interactions between the toxic contaminant and the primary substrate must be understood. The toxic compound competes with methane for access to the methane monooxygenase. Above ground reactors for degradation of toxic compounds are likely to use biofilms because of the simplicity and efficient retention of biomass on biofilm reactors. As will be seen below the effects of competitive inhibition on biofilm uptake rates of methane and toxic compounds are increased. Thus a rational model of competitive kinetics in the biofilm environment is important in analysis of the results of methanotrophic biofilm studies.

ANALYSIS OF BIOFILM FLUX WITH COMPETITIVE INHIBITION

The following analysis presents a model of the competitive kinetics for the consumption of methane, oxygen and toxic compound in a biofilm reactor. The model simulated the effects of competition by toxics and methane on their enzymatic oxidation by the methane monooxygenase. Dual-competitive-substrate/diffusion kinetics were used to model biofilm co-metabolism, integrating the following equations (Bailey and Ollis, 1977):

$$\frac{d^2S_m}{dx^2} = -\frac{\mu_{max}X}{Y_m D_m}\left(\frac{S_{ox}}{K_{ox}+S_{ox}}\right)\left(\frac{S_m/K_m}{1+\frac{S_m}{K_m}+\frac{S_t}{K_t}}\right) \quad (1)$$

$$\frac{d^2S_t}{dx^2} = -\frac{k_t X}{D_t}\left(\frac{S_{ox}}{K_{ox}+S_{ox}}\right)\left(\frac{S_t/K_t}{1+\frac{S_m}{K_m}+\frac{S_t}{K_t}}\right) \quad (2)$$

$$\frac{d^2S_{ox}}{dx^2} = -\frac{\mu_{max}X}{Y_{ox}D_{ox}}\left(\frac{S_{ox}}{K_{ox}+S_{ox}}\right)\left(\frac{S_m/K_m}{1+\frac{S_m}{K_m}+\frac{S_t}{K_t}}\right) \quad (3)$$

S_m = local concentration of methane
S_{ox} = local concentration of oxygen
S_t = local concentration of the toxic compound
μ_{max} = maximum specific growth rate
X = local methane-oxidizer biomass level in the biofilm
Y = yield, substrate per biomass
K = kinetic coefficients (half-saturation coefficients)
D = diffusivity in the biofilm
x = distance into the biofilm
k_t = maximum specific uptake rate of the toxic compound per biomass
subscripts m, ox, and t designate methane, oxygen and the toxic compound, respectively.

Biofilm depth was assumed to be limited by either methane or oxygen. The effect of oxygen and methane on rates were modeled by multiplicative dual-substrate kinetics. As a first approximation, parameters such as diffusivity, biomass concentration, kinetic constants, and the yields were assumed to be constant and homogeneous.

Internal diffusion of methane, oxygen, and the toxic compound were included. The effects of hydraulic boundary layer resistance external to the biofilm were not considered. These effects will be added by an iterative solution of the implicit equations for the concentrations that equate the boundary layer fluxes to the fluxes into the biofilm surface due to biological activity.

TABLE 1 Parameters Used in the Model of Uptake of Methane and Toxics by Methane-Oxidizing Biofilms

Biological Parameters		Value Used in Sensitivity Study	
X, methane-oxidizer biomass level in the biofilm		20. (mg biomass) ml^{-1}	
μ_{max}, maximum specific growth rate		1.81 d^{-1}	
k_t, maximum specific toxic degradation rate		1.82 (mg toxic) (mg biomass•d)$^{-1}$	
K_m, half-saturation coefficient for methane		0.48 mg L^{-1}	
K_{ox}, half-saturation coefficient for oxygen		0.032 mg L^{-1}	
K_t, half-saturation coefficient for toxic compound		0.48 mg L^{-1}	
Y_m, yield for methane		0.996 (mg biomass)(mg methane)$^{-1}$	
Y_{ox}, yield for oxygen		0.384 (mg biomass)(mg oxygen)$^{-1}$	
Chemical/Physical Parameters	Oxygen	Methane	Toxic
D, Biofilm Diffusivity, cm^2 d^{-1}	2.07	1.50	6500.
S, Baseline Bulk fluid conc., mg L^{-1}	8.	4.	1.

Parameters used in the model are shown in Table 1. All parameters were assumed to be constant and uniform in the biofilm. Most of the parameters were taken from published data (Akgerman and Gainer, 1972; Drozd and Linton, 1981), but some parameters were less certain. The biomass of methane-oxidizers in the biofilm was estimated to be 20 mg/ml based on analogy to the levels of nitrifying bacteria observed in wastewater treatment biofilms (Stratta and Long, 1981; Strand, 1986). Values for the kinetic constants for methanotrophic co-metabolic degradation of toxic compounds have not been reported for whole cell systems representative of those expected in biofilms. Colby *et al.* (1977) observed that the oxidation rate of chloroform in cell-free extracts of methane-oxidizing bacteria was about one third that of methane. As a first approximation for the purposes of this study the inhibition constant and maximum uptake rate for the toxic compound (K_t and k_t) were assumed to be equal to those for methane.

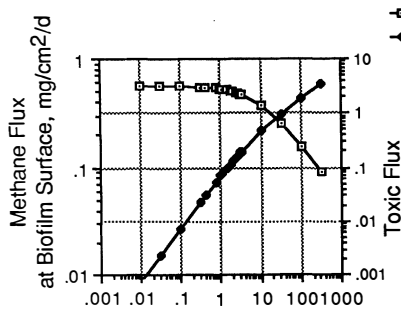

Fig. 1. Sensitivity to Surface Concentration, Toxic

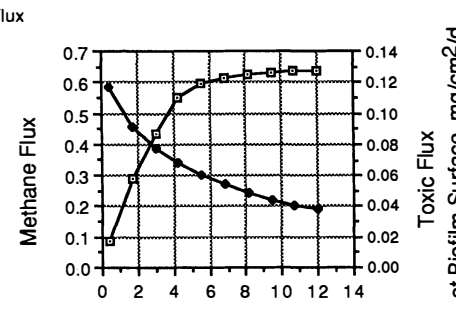

Fig. 2. Sensitivity to Surface Concentration, Methane

Equations (1-3) were solved by numerical integration. A variable-step Runge-Kutta technique (Press *et al.*, 1986) was used to integrate the finite difference equation with iterative solution to satisfy the desired boundary conditions (shooting method): zero flux at the biofilm wall, and the specified surface concentrations for methane, oxygen and the toxic compound at the biofilm surface.

The effects of variations in the biofilm surface concentrations of toxic compound, methane, and oxygen on the calculated methane and toxic biofilm fluxes was determined as shown in Figures 1-3. The effects of variations in the magnitudes of the kinetic constants, the toxic diffusivity, and biomass concentration were also determined (Figures 4-8).

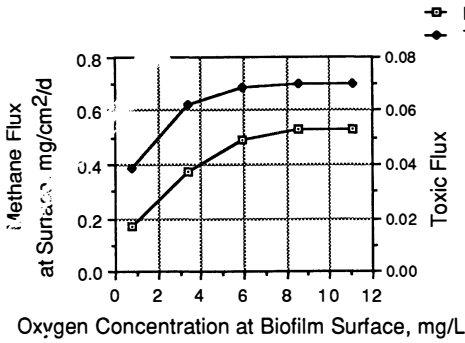

Fig. 3. Sensitivity to Surface Coeff.of Oxygen

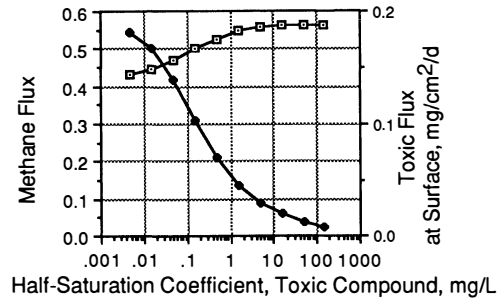

Fig. 4. Sensitivity to Half-Saturation Coeff.for Toxic

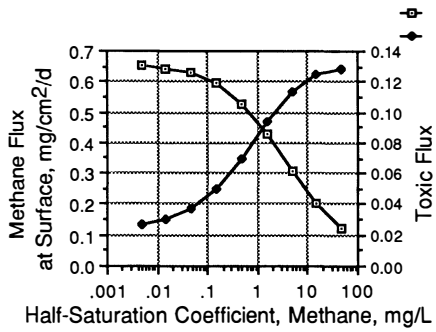

Fig. 5. Sensitivity to Half-Saturation Coeff., Methane

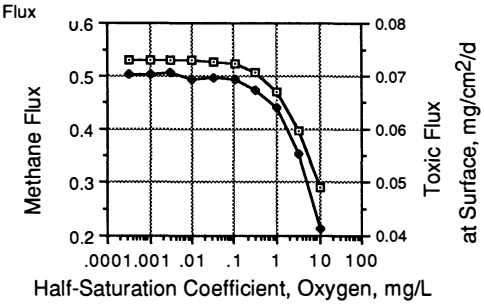

Fig. 6. Sensitivity to Half-Saturation Coeff., Oxygen

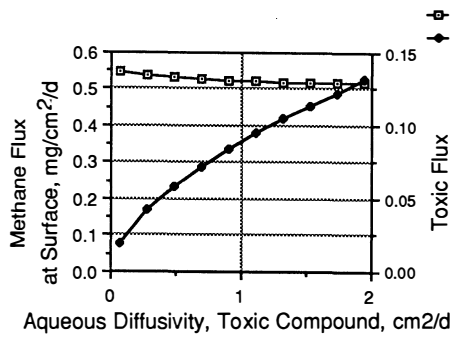

Fig. 7. Sensitivity to Aqueous Diffusivity of Toxic

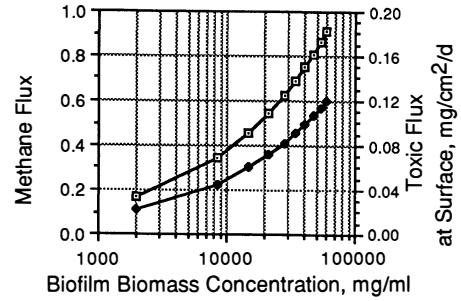

Fig. 8. Sensitivity to Biomass Concentration

DISCUSSION

These simulations suggest that competitive inhibition of methanotrophic degradation of toxic compounds by methane will have a significant negative effect on toxic degradation rates at concentrations of methane greater than the half-saturation concentration for methane (Figure 2). This negative effect will be increased by diffusional effects in the biofilm. Since high methane levels will be necessary to maintain high biomass levels and deep active layers in the biofilm, biofilm reactors may have to be operated cyclically with toxic degradation proceeding during periods of low methane.

The model predicts that methane fluxes will be relatively unaffected by surface toxic concentrations less than five times the half-saturation constant for the toxic (Figure 1). The rates were found to be moderately sensitive to variations in the diffusivity of the toxic compound and less sensitive to methanotrophic biomass, half-saturation coefficients for methane and the toxic. The response of the model biofilm to the half-saturation constant for oxygen and to the surface oxygen concentration was as expected from conventional biofilm kinetics (Williamson and McCarty, 1976): no effect until oxygen fell low enough to limit the biofilm depth (Figure 3).

It should be noted that this model does not include effects which may be important in predicting actual methanotrophic biofilm kinetics: direct inhibitory effects on bacterial metabolism by toxic effects of the chlorinated hydrocarbons or their metabolites, depletion of cell energy at low methane levels, and the effect of bacterial carbon reserves on cell energy depletion.

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