MATHEMATICAL MODELS FOR BIODEGRADATION OF CHLORINATED SOLVENTS: I. MODEL FRAMEWORK

X. Zhang, S. Banerji, and R. Bajpai[*]*, Department of Chemical Engineering, University of Missouri-Columbia, Columbia, MO, 65211, Phone: 573-882-3708, FAX: 573-884-4940*

ABSTRACT Complete mineralization of chlorinated solvents by microbial action has been demonstrated under aerobic as well as anaerobic conditions. In most of the cases, it is believed that the biodegradation is initiated by broad-specificity enzymes involved in metabolism of a primary substrate. Under aerobic conditions, some of the primary carbon and energy substrates are methane, propane, toluene, phenol, and ammonia; under anaerobic conditions, glucose, sucrose, acetate, propionate, isopropanol, methanol, and even natural organics act as the carbon source. Published biochemical studies suggest that the limiting step is often the initial part of the biodegradation pathway within the microbial system. For aerobic systems, the limiting step is thought to be the reaction catalyzed by mono- and dioxygenases which are induced by most primary substrates, although some constitutive strains have been reported. Other critical features of the biodegradative pathway include: 1) activity losses of critical enzyme(s) through the action of metabolic byproducts, 2) energetic needs of contaminant biodegradation which must be met by catabolism of the primary substrates, 3) changes in metabolic patterns in mixed cultures found in nature depending on the availability of electron acceptors, and 4) the associated accumulation and disappearance of metabolic intermediates. Often, the contaminant pool itself consists of several chlorinated solvents with separate and interactive biochemical needs. The existing models address some of the issues mentioned above. However, their ability to successfully predict biological fate of chlorinated solvents in nature is severely limited due to the existing mathematical models. Limiting step(s), inactivation of critical enzymes, recovery action, energetics, and a framework for multiple degradative pathways will be presented as a comprehensive model.

KEYWORDS: bioremediation, enzyme kinetics, electron acceptors, metabolism

INTRODUCTION

Biodegradation of chlorinated solvents has been addressed excessively during the past decades because of their environmental significance. Complete mineralization has been demonstrated under aerobic and anaerobic conditions. In most of the cases, it is believed that the biodegradation takes place cometabolically with a primary substrate. It has also been demonstrated that the metabolism of chlorinated solvents is a net energy consuming process. Hence, the chlorinated solvents cannot be used as a source of carbon or energy for microbial growth. In-depth mechanisms of biodegradation are being revealed gradually. These include microbial growth and energetics, induction, competitive inhibition, toxic inactivation, recovery action, etc. Kinetic models represent an excellent tool to quantify bioprocesses and have been widely constructed and tested for biodegradation. However, most of them are based on the modification of the Monod model. Their ability to successfully predict biological fate of chlorinated solvents in nature is severely limited due to their simplicity and the complexity of the degradation process. The major problem in application of mathematical models in *in situ* bioremediation results from the diversity of

[*]To whom all correspondence should be addressed (e-mail: bajpai@ecvax2.ecn.missouri.edu).

conditions encountered in nature. The unsteady-state behavior of natural microbial systems often foils the use of models derived from well-defined laboratory results. For example, Semprini and McCarty [1] have reported the results of a two-year controlled-field study and its mathematical analysis using model simulations. It was indicated that the model simulations of degradable chlorinated ethenes such as vinyl chloride (VC) and *trans*-dichloroethylene (*trans*-DCE) agreed well with the observed oscillations in concentration of the chlorinated ethenes resulting from pulse feeding of methane. However, model simulation of relatively less degradable *cis*-dichloroethylene (*cis*-DCE) did not fit the field results well. Furthermore, trichloroethylene (TCE) simulations showed an even greater attenuation in the pulse heights, since it was the most slowly transformed among the chlorinated solvents tested. Similar phenomena were also observed in many other field experiments [2, 3].

Nonetheless, mathematical models are very helpful in developing better understanding of biodegradation and in designing improved operation and control of bioremediation strategies; lack of good mathematical models hampers effective application of biological processes for treatment of chlorinated solvent contamination. The objective of this paper is to map out a model framework for biodegradation of chlorinated solvents. Literature analysis is the method used to integrate the complex mechanisms and existing kinetics. The mechanistic framework should cover both aerobic and anaerobic processes and be of self-organized characteristic in nature. Further derivation of mathematical models will be based on this framework. It is expected that a mathematical model applicable to real situations can be developed this way in the future.

MECHANISM REVIEW

Diversity of microorganisms

Chlorinated solvents were believed to be biologically undegradable until recently. Wilson and Wilson [4] first reported cometabolic biodegradation of ^{14}C-labeled TCE in unsaturated soil columns exposed to natural gas by the production of $^{14}CO_2$. Since then, numerous studies have been conducted at different scales to explore this dehalogenation process. The active microbes in this process were isolated and characterized as methanotrophs [4-6]. Methanotrophs are aerobic bacteria which grow on methane and utilize it as a sole carbon source and energy source. The enzyme system responsible for both methane and TCE oxidation in methanotrophs has been identified as methane monooxygenase (MMO) [7]. In fact, it catalyzes the oxidation of a wide variety of aliphatic, alicyclic, and aromatic hydrocarbons [8, 9].

Monooxygenases from several other aliphatics-utilizing aerobic-bacteria have also been found to dehalogenate chlorinated solvents. These bacteria include propane [10, 11] and isoprene [12] oxidizers. Propane-oxidizing organisms have a monooxygenase as evidenced by their ability to grow on propane as the sole source of carbon and energy. The propane-oxidizing *Mycobacterium vaccae* has a broad catabolic capacity and can dehalogenate a wide array of chlorinated substances when it is induced for propane oxidation [13, 14].

Besides aliphatics, aromatics have also been found to be primary substrates of some indigenous bacteria that cooxidatively dehalogenate the chlorinated solvents. Such bacteria were first identified by Nelson and

his coworkers as toluene-oxidizing and phenol-oxidizing bacteria [15, 16]. In this system, toluene-dioxygenase was identified as the active TCE oxidizing enzyme [15, 17]. The active microbes isolated include *P. putida* F1 [17], *P. cepacia* G4 [18, 19], *P. putida* B5 [15], *P. putida* UV4 [20], *C. variabilis* SVB74, and *A. radioresistens* SVB65 [21], etc. Haigler, *et al.* [22], even reported an aromatic nonspecific strain which can degrade TCE by utilizing a wide range of substituted aromatic compounds. The mono- or dioxygenases that catalyze the first step in aromatic cometabolism are implicated in the breakdown of chlorinated solvents [23].

In addition, some autrophic microorganisms also degrade chlorinated solvents by cometabolization. Experimental evidence indicated that ammonia monooxygenase (AMO) in cells of *Nitrosomonan europaea* is capable of cooxidizing hydrocarbons [24-26] and aliphatic halogenated hydrocarbons [27-31]. *N. europaea* is an obligate chemolithotrophic nitrifying bacterium which derives its energy for growth exclusively from the oxidation of ammonia to nitrite. Nitrification in *N. europaea* is initiated by the reductant-dependent oxidation of ammonia to hydroxylamine (NH_2OH) through the action of ammonia monooxygenase (AMO). Reductant for AMO-catalyzed reactions is provided by the further oxidation of hydroxylamine to nitrite by hydroxylamine oxidoreductase [32].

On the other hand, a variety of anaerobic microorganisms have also been discovered which can cometabolically but reductively dehalogenate chlorinated solvents. Common microbes often present in *in situ* bioremediation, including obligate methanogens and acetogens, as well as facultative sulfate reducers and nitrate reducers, etc., have dechlorination capabilities for chlorinated solvents. Many studies have been conducted in soil [33, 34], in organic sediment [35, 36], with pure cultures [37-39], and with continuous-flow fixed-film reactors [40-45].

Diversity of cometabolic microbes may reflect the evolutionary ability of organisms in nature [46, 47]. Therefore, there is enough reason to believe that new strains will continue to be isolated.

Key enzymes and limiting step

Behind the diversity of active microorganisms, more and more studies demonstrate that there exists common biodegradative behavior for chlorinated solvents involved in either aerobic or anaerobic microbial systems. In all cases, the biodegradation is initiated by broad-specificity enzymes involved in metabolism of the primary substrate. These key enzymes include mono- and dioxygenases in aerobic systems and reductases with transition-metal cofactors in anaerobic systems. Intermediate metabolites produced depend on the key enzyme type and substrate structure.

Monooxygenases are physiologically active with methane [48], propane [11], isoprene [12], ammonia [27], phenol, and toluene [16]. Experimental results suggest that methane monooxygenase initiates oxidation of haloalkenes by direct attack on the carbon atom in the substrate bond. The immediate reaction products of the enzymatic action on contaminants, e.g., TCE, have been identified as trichloroethylene epoxide and 2,2,2-trichlorocetaldehyde (chloral). Synthetic TCE-epoxide undergoes spontaneous decomposition in aqueous buffers via isomerization and hydrolysis to yield dichloroacetate, glyoxylate, formate, and carbon dioxide [49].

In contrast to the MMO, it has been shown that toluene dioxygenase initially catalyzes addition of activated O_2 across a bond of the aromatic ring to form a cis-dihydrodiol [50]. In the case of TCE, such an intermediate could lead to dehalogenation, but it is unlikely to evoke epoxide formation or chloride migration. By use of purified toluene dioxygenase obtained from two overproducing recombinant *E. coli* strains, Li and Wackett [51] demonstrated that dioxetane and dihydroxy intermediates were produced after the reaction.

A plausible mechanism for reductive dechlorination of chlorinated solvents by methanogens and other anaerobes involves reactions mediated by reduced transition metal cofactors. Gantzer and Wackett [52] observed that the bacterial transition-metal coenzymes, including vitamin B_{12}(Co), coenzyme F_{430}(Ni), and hematin(Fe), catalyze the reductive dechlorination of polychlorinated ethylenes and benzenes. Vitamin B_{12} and related corrinoids are abundant in methanogens and other anaerobes, especially acetogens. Coenzyme F_{430} is prosthetic in the "methyl reductase" enzyme complex in methanogens. Both of these cofactors were capable of reductive dechlorination of chlorinated ethenes using the strong reductant Ti^{3+} as the electron donor. Transition metal cofactors can catalyze many other reductive dehalogenations including dihaloelimination from 1,2-dibromoethane and 1,2-dichloroethane [53], forming ethylene (ETH). Trichloroethanes can also be dehalogenated by transition metal cofactors, but at a relatively low rate [54]. Thus, anaerobes rich in reduced transition metal cofactors, most notably methanogens and acetogens, may catalyze reductive dechlorination in anaerobic habitats.

Most studies indicate that the key enzymes responsible for initiating the dechlorination reaction chain are inducible [55] and are where the limiting step locates [56]. This means that such enzymes can be formed in appreciable amounts only when the growth substrate is present, and they are usually involved in the early steps in the breakdown of substrate molecule. Other constitutive enzymes affect the metabolization only within a limited range [56]. For example, the addition of methanol or formate (instead of methane) enhanced the TCE degradation in methanotrophs; unfortunately, TCE degradation stopped within several days even though methanol or formate was still present in the system [57, 58]. This indicated that methane was a better inducer than methanol for biodegradation of TCE; formate is not an inducer for the MMO. Similar results were obtained with toluene-utilizing bacteria as well. The addition of methanol and ethylene stimulated TCE degradation in soil by toluene-utilizing microorganisms only moderately [59]. This is probably due to the existence of an active ethanol dehydrogenase involved in the process which presumably produced $NADH_2$, which is required in the initial reaction induced by toluene dioxygenase [50].

Competitive inhibition

In all the cases known to date, degradation of chlorinated solvents by both aerobic and anaerobic microorganisms occurs mainly through the so-called fortuitous activity of key enzymes. The chlorinated molecules bind to, and are oxidized or reduced at, the active site of the enzymes that are intended for the primary substrates. This leads to the competition between the growth-supporting substrate and the chlorinated molecule. In other words, the two substrates compete with each other and competitive inhibition

occurs. It has been observed, for example, that the TCE-degradation rate reduces under active methanotrophic growth due to methane saturating the catalytic site of the MMO enzyme responsible for TCE oxidation [60, 61]. Thus, addition of reducing equivalents in the form of formate enhances the rate of TCE degradation in pure cultures [61] since formate does not compete with TCE for the active site of MMO. On the contrary, the cometabolizing compound can also inhibit microbial utilization of the primary substrate. For example, addition of TCE decreased the oxidation rate of ammonia in an ammonia-utilizing bacterial system [62].

For anaerobic cometabolic processes also, competitive inhibition characteristics have been observed. In a report of TCE degradation under natural sediment conditions [63], it was found that the concentration of total carbon in sediment competitively inhibit the dechlorination of TCE. Also, Corapcioglu, *et al.* [64], observed the inhibition and toxicity phenomena due to too high concentration of secondary substrates in anaerobic processes. They considered a critical inhibitory concentration of the chlorinated compounds, above which biotransformation ceases due to the inhibition to microorganisms.

Under competitive cometabolism, the rates of degradation and the formation of intermediates are relevant to the structure of chlorinated solvents. Although a complete agreement has not arrived, some clear trends have emerged [65]. However, quantitative correlations of key enzyme activity with substrate property have not been found in literature until now.

Toxic inactivation and recovery action

This pair of effects may play a central role in sustainable biodegradation of chlorinated solvents. Since they are unable to be incorporated into central metabolic pathways, intermediate metabolites accumulate and may result in toxic inactivation of key enzymes directly or indirectly. For example, chloroethene epoxides are chemically unstable and are transformed to a variety of products, some of which may be toxic to the transforming organisms [6, 48, 66, 67]. Results presented by Fox, *et al.* [48], strongly imply that neither TCE nor the immediate enzyme-catalyzed oxidation products, TCE epoxide and chloral, are responsible for the reactive inactivation. This was further examined by acetylene-treated methanotrophic cells [66]. Acetylene selectively arrests the activity of methane monooxygenase in the cells without affecting the activity of formate dehydrogenase. The toxicity of chloroform (CF), TCE, and their transformation products to whole cells was evaluated by comparing the rate of formate oxidation, with and without exposure to CF or TCE, by acetylene-treated cells to that of non-acetylene-treated cells. Exposure to either CF or TCE by cells not treated with acetylene diminished the rate of formate oxidation significantly compared to cells that were treated with acetylene. This suggested that the solvents themselves were not toxic under the experimental conditions, but their oxidation products were. The most suspect transformation products include either radical or carbonation intermediates, resulting from the thermal rearrangement of the epoxides or from atomic migration while still on the active site of the enzyme. In both enzymatic and whole cell studies, the degree of toxicity was found to be proportional to the amount of chloroethene transformed.

Transformation product toxicity also exists with chloroethene cometabolic transformation by toluene [68, 69], ammonia [26, 30], alkene [70], and isoprene utilizers [12]. For example, Rasche, *et al*. [28, 30], have observed a turnover-dependent inactivation of AMO in *N. europaea* cells inoculated with TCE and several other halogenated hydrocarbons. The results showed that inactivation by TCE was accompanied by covalent modification of cellular proteins and loss of O_2 uptake activity associated with both ammonia and hydrazine oxidation.

Similarly, losses of toluene dioxygenase activity in *P. putida* F1 was responsible for growth inhibition in the presence of TCE. In a ^{14}C radiotraced TCE degradation experiment with *P. putida* F1 [68], about 16.6% of the radioactivity added to the culture was accounted for in the total cell fractions; most of the ^{14}C incorporated was found in the protein fraction. The inhibitory effects may stem from metabolic activation of TCE by toluene dioxygenase to form reactive intermediates that modify intracellular molecules. The results with *P. cepacia* G4 presented by Heald and Jenkins [20] are consistent with the above report.

Therefore, both mono- as well as di-oxygenases mediate the production of toxic intermediates which inactivate the enzymes. As a result, the controlling point in the microbial cells is on such key enzymes, their activity, and production.

Consequently, it is logical to suspect that the microbial cell will initiate recovery function from the enzyme inactivation resulting from toxic intermediates. Ely, *et al.* [62, 71], defined the recovery action as the ability of cells to recover from the damage caused by the toxicity associated with contaminant transformation. Such ability will direct the microbial system to fight the inhibition or toxicity effects and to sustain itself. Some toxic intermediates may be degraded, some inactivated enzymes may be repaired, or new enzymes may be produced to compensate for the inactivated ones. Indeed, recovery action has been observed in both aerobic and anaerobic processes [72, 73]. Kanazawa and Filip [72] have conducted enzyme assays for β-glucosidase, β-acetylgluco-saminidase, phospharase, phosphodiesterase, and proteinase in soil samples collected two months after contamination with tetrachloroethylene (TCE), dichloroethane (DCA), and TCE. The results indicated that TCE, PCE, and DCA inhibited activities of all enzymes tested at a concentration of 1,000 µg per 100 g soil initially. However, after two months the enzymatic activities, especially in soil samples contaminated with PCE and DCA, were found to be at the same or higher level than in the control samples.

The recovery action has not been addressed on a mechanistic level in researches dealing with biodegradation to chlorinated solvents. However, it is noteworthy that such a characteristic represents a major difference between biochemical reactions within living microbial cells and pure chemical reactions. Study of such mechanisms is urgently needed in order to provide insight into the biodegradation process, especially for the refractory and toxic chlorinated solvents.

Energetics

Since there is no energy production during further metabolization of chlorinated solvents and their intermediate metabolites, another exogenous carbon/energy source is needed to continue the degradation. This source is the primary substrate, whose metabolism results in nutrients and energy for microbial maintenance and growth.

Cometabolism often requires a supplemental source of $NADH_2$ [74, 75]. In the case of methanotrophs, formate has been shown as the energy source that replenishes the supply of $NADH_2$ for methanotrophic transformations of TCE [58, 60, 76].

Under anaerobic situation, it seems that much low energetic efficiency for cometabolism exists. Based on the electron balance data, Pavlostathis and Zhuang [77] found that a very small fraction (0.06 to 0.25%) of the reducing equivalents used were actually utilized toward dechlorination. In another previous study of TCE dechlorination at 35ºC by mixed sulfate-reducing, actively-growing cultures in liquid media supplemented with acetate and lactate, the fractions of the total reducing equivalents used for sulfate reduction, methane production, and dechlorination of TCE to *cis*-dichloroethylene (*cis*-DCE) were 98.66, 1.29, 0.05%, respectively [78]. Due to this disproportionate distribution of electron equivalents between the primary metabolism (e.g., sulfate reduction or methanogenesis) and dechlorination, biological reductive dechlorination of chlorinated alkenes requires a continuous supply of the primary substrate to maintain the requisite enzyme(s) in an active stage. As a result, the nutrient requirements for successful dechlorination are considerably higher compared to what would have been if the target contaminants were used as primary substrate (i.e., as both the carbon and energy source). In addition, the low electron transfer efficiency will result in increased costs (both chemical and pumping) to supply sufficient nutrients, especially electron donors.

Obviously, cometabolic energetics are directly associated with bioremediation scenarios and process operations. Cometabolism complicates the energetics of the microbial system. Although systematic investigation for energetics of primary substrate metabolism have been reported under both aerobic and anaerobic conditions [56, 79, 80], complete investigation of cometabolism during degradation of chlorinated solvents has not been reported.

ANALYSIS OF CURRENT MATHEMATICAL MODELS

A number of mathematical models have been proposed for the aerobic degradations of chlorinated solvents in the past several years. Although the detailed mechanistic pathways for chlorinated solvents are different from each other, the mathematical models have several common characteristics. Relatively very few models are available for the anaerobic degradation of chlorinated solvents. All the models are constructed based on the basic microbial kinetic theories.

Basic model

Monod kinetics are often used as a starting-point to relate the transformation rate of a compound to its concentration and microbial concentration [56].

$$-\frac{dC}{dt} = kX\frac{C}{K+C} \quad (1)$$

where C = substrate concentration, t = time, X = microbial amount, k = maximum specific utilization rate of the substrate, and K = half-saturation constant for the substrate.

This equation is based on the assumption that the one substrate is a rate-limiting factor. It is only suitable for a single, nontoxic substrate in solution under steady state conditions. In practice, this assumption is not always true. As a result, the unsuitability of basic Monod kinetics alone

hinders process design attempts for biotransformation of chlorinated solvents.

Multisubstrate models

For *in situ* bioremediation of chlorinated solvents, organic or mineral nutrients often become a limiting factor. Widdowson, *et al.* [81], proposed a model to simulate organic carbon biodegradation by facultative bacteria using oxygen-based respiration patterns in aerobic conditions and/or nitrate-based respiration patterns in anaerobic conditions. The substrate degradation rate for aerobic patterns is presented according to Monod kinetics as the following:

$$-\left(\frac{dC}{dt}\right)_{so} = k_o\ X \frac{C}{K_C + C} \frac{C_{EA}}{K_{EA} + C_{EA}} \frac{C_A}{K_A + C_A} \qquad (2)$$

where k_o = maximum specific utilization rate of substrate under aerobic condition, K_C = half-saturation constant for substrate, C_{EA}= concentration of electron acceptor, K_{EA} = half-saturation constant for electron acceptor, C_A = nutrient concentration, and K_A = half-saturation constant for nutrient.

Similarly, the substrate degradation rate under anaerobic conditions can be expressed as:

$$-\left(\frac{dC}{dt}\right)_{sn} = k_n\ X \frac{C}{K_C + C} \frac{C_N}{K_N + C_N} \frac{C_A}{K_A + C_A} I\left(C_{EA}\right) \qquad (3)$$

where k_n = maximum specific utilization rate of the substrate under anaerobic condition, K_N = half-saturation constant for electron donor, C_N = electron donor concentration, and $I(C_{EA})$ = the inhibition effect of oxygen to the anaerobic process.

The net rate is the sum of the substrate degradation due to aerobic and/or anaerobic activities in which oxygen may inhibit the anaerobic process to some extent [81].

Semprini and McCarty [1] used this concept for oxygen supply as a limiting factor and have developed relevant mathematical models used in pilot scale field tests. In their model, all of the oxygen demand was attributed to cell growth or the primary substrate utilization, and none to the degradation of the secondary substrate. This assumption may be valid under conditions of high growth substrate to contaminant ratio. Apparently, such high ratios cannot be guaranteed for all *in situ* operating scenarios, and other conditions need to be incorporated in the model.

Competitive inhibition model

Considering the characteristics of cometabolism reviewed above, the competition effects have been incorporated into the basic Monod model. Currently, competitive inhibition effects are quantified based on the Dixon expression [82] as the following:

$$-\frac{dC_1}{dt} = k_1 X \frac{C_1}{K_1 + C_1 + \frac{K_1}{K_2} C_2} \qquad (4)$$

$$-\frac{dC_2}{dt} = k_2 X \frac{C_2}{K_2 + C_2 + \frac{K_2}{K_1} C_1} \qquad (5)$$

where the subscripts 1 and 2 represent the primary and secondary substrates, respectively.

Many degradation simulations on cell level have been conducted by use of the two basic equations above, including methane and TCE [83, 84], aromatics and TCE [16, 85, 86], methane and trichloroethane (TCA) [87], natural organics and TCE [63], toluene and TCE [88], methane and CF, as well as TCE [66]. Usually it is assumed that the inhibition constant of the secondary substrate, such as TCE, on the primary substrate, such as toluene, is equal to the

half-saturation constant for TCE conversion, and vice versa. But the results of Landa, *et al*. [88], indicated that this was not the case with simultaneous toluene and TCE conversion. This may be caused by the fact that the measurements were conducted with whole cells and not with purified enzymes. Thus, factors other than competition for the active site of the enzyme (e.g., reductant supply or substrate transport to the enzyme) may have influenced the effect of toluene on TCE conversion and vice versa.

In the case of anaerobic processes of chlorinated solvents, similar competitive inhibition characteristics are exhibited. Barrio-Lage, *et al*. [63], investigated the depletion of TCE in microcosms containing water and three types of natural sediment ranging in composition from highly organic to a calcareous sedimentary rock. It was found that the kinetic rates varied slightly in the different sediments. Depletion was found to follow nonlinear forms of the Michaelis-Menten kinetics in the organic sediments. An improved model similar to the Dixon expression could fit the results obtained in separate situations with the same type of sediment and water microcosms. The kinetic coefficient values were found to be dependent on the percent of total organic carbon (primary substrate) in the sediment. In the case of chloroaromatics, it was also found that the parent substrate such as 3,5-dichlorobenzoate [89] competitively inhibited the dehalogenation, causing a deviation from first-order kinetics.

The theoretical meaning for inhibition constants in the above equations has not yet been fully understood on the cell level. It is believed that such inhibition constants must depend on the "fortuitous" mechanisms in the cometabolic degradation of chlorinated solvents. In addition, the distribution of electron acceptor and the other nutrients must also be related to the "fortuitous" mechanisms. Therefore, the "fortuitous" function is one of the most important to model the competitive inhibition effect during the cometabolic degradation of chlorinated solvents. However, very little quantitative information has been available in this field. Ely, *et al*. [71], recently discussed this problem in deriving mathematical models for TCE degradation by ammonia-oxidizing bacteria. They assumed that the ratio of specific oxidation rates of ammonia and TCE is a constant. That means the cometabolic action is taking place in a constant ratio of ammonia and TCE. Unfortunately, their experimental results demonstrated that this ratio may be some kind of function rather than a constant. Obviously, there is a need to understand this "fortuitous" constant or "fortuitous" inhibition function under specific conditions.

Inactivation model

Toxic inactivation also plays a crucial role in the kinetics of biotransformation processes of chlorinated solvents. The toxicity may result from the primary substrate such as phenol or from the cometabolized substrates (the contaminants) or their metabolic intermediates. Folsom, *et al*. [16], observed that high phenol concentration would inhibit the degradation process. The toxicity from the substrate itself usually depends on its concentration in solution and can be expressed based on the classic enzyme Haldane equation [82] as the following:

$$-\frac{dC_1}{dt} = k_1 X \frac{C_1}{K_1 + C_1 + \frac{C_1^{\,2}}{K_I}} \qquad (6)$$

where K_I = substrate inhibition constant.

The kinetics of inactivation of degradation processes are complicated by yet unclear direct and indirect pathways. Many

researchers [58, 66, 84, 85] considered that inactivation is correlated with the rate, i.e., transformation rate of chlorinated solvents. The most usual expression is as follows:

$$\frac{dX}{dt} = Y\left(-\frac{dC_1}{dt}\right) - \beta X - \xi\left(-\frac{dC_2}{dt}\right) \quad (7)$$

where Y = cell yield constant, β = cell maintenance constant, and ξ = constant related with secondary substrate biodegradation.

In this equation, Criddle [85] defined ξ as "true" biomass transformation capacity, while Alvarez-Cohen and McCarty [66] and Alexander and Roch [84] defined it as toxicity constant explicitly. As a matter of fact, the parameter (ξ) in the above equation holds all the effects resulting from the secondary substrate biodegradation including that discussed above. When the cometabolic degradation is under steady-state, ξ may appear as a constant. Under unsteady-state, however, it will change unceasingly, leading to failure of the model.

Since the toxic intermediates may accumulate in cells, Ely, *et al*. [62, 71], believed that inactivation may be associated with the amount of intermediates and such actions could lead to an intrinsic unsteady-state of the microbial system. In their report with ammonia-utilizing bacteria, an inactivation function was related to the amount of nongrowth substrate oxidized. Through dimensional analysis, they believed that inactivation effect was in proportion to the amount of intermediate from the oxidation of nongrowth substrate oxidation. Thus, a specific inactivation rate constant could be derived as the following:

$$E^* = k_{inact} P_2 \quad (8)$$

where E^* = inactivated enzyme, k_{inact} = specific inactivation constant, and P_2 = products of secondary substrate oxidation.

However, there was a significant difference between model predictions and the experimental results. Apparently, the mechanisms of inactivation have not been modeled satisfactorily to date [62]. Indirect toxicities from intermediates further complicate such problems because they usually affect the relevant processes to the key enzymes such as the protein synthesis, gene expression, enzyme production, etc.

Model of recovery action

Although many authors have observed recovery of cells from the damaging effects of toxic contaminants and their degradation products, this factor had not been considered in modeling efforts until very recently. Hyman, *et al*. [90], and Ely, *et al*. [62], have addressed this problem by proposing the concept of inhibition/inactivation/recovery in microbial systems. According to this concept, the maximal sustainable degradation rate of chlorinated solvents is likely to be achieved as a result of a balance between the ability of the microbial cells to oxidize contaminants and their ability to repair and recover from the concurrent cellular damage caused by the contaminants and their oxidation products. Ely, *et al*. [71], suggested that this concept provides a reasonable basis for understanding the effects of chlorinated solvents such as TCE on the enzyme and bacterial response. Hence, they modeled the processes involving inhibition/inactivation/recovery as occurring under pseudo-steady-state conditions. The model simulation provided positive results by incorporating the recovery effect, giving better fit to the experimental data than those obtained

without incorporating recovery effect. The recovery function is presented here:

$$E_{new} = k_{rec} P_1 \quad (9)$$

where E_{new} = new synthesized enzyme, k_{rec} = specific recovery constant, and P_1 = products of primary substrate oxidation.

Unfortunately, the most significant departure from the experimental data occurred in their most innovative concept, i.e., recovery action. More than 76.3% deviation for the specific recovery constant was noted, even after discarding the lowest and highest estimates. This failure may result from the following reasons: 1) The assumptions used to construct models were not verified by experiments. For example, it was assumed that "the nongrowth substrate may also bind to the enzyme/growth-substrate complex." This, in fact, implies that the oxygenase can associate simultaneously with the growth and nongrowth substrates, which is obviously contradictory to the general enzyme mechanism of competitive inhibition. 2) The recovery function was very simplistic. The assumption made was that "synthesis of new enzyme is a function of oxidation of the growth substrate." As a result, no explicit relationship between recovery action and toxic inactivation was brought into the model. After analyzing the model prediction and experimental results, the authors themselves recognized that the toxic effect should itself be one of the driving forces for recovery action.

The toxicity and recovery are complex processes which are controlled in a living cell through a hence unknown mechanism, perhaps in a nonlinear and unsteady-state manner. The conventional methods based on pseudo steady-state modeling (similar to the Monod model derivation) to address this unconventional process may not be adequate. This is especially important when the models are to be applied to *in situ* bioremediation where several different environments may be encountered at different locations and at the same location at different times. Therefore, a considerable amount of work awaits to be done in order to appropriately model the cometabolic degradation of chlorinated solvents.

MODEL FRAMEWORK AND DISCUSSION

In conclusion, the all-around picture within a microbial system during biodegradation of chlorinated solvents is emerging, although many mechanisms need in-depth study. In spite of the difference in the detailed pathways under aerobic and anaerobic conditions, the kinetic behavior of the key enzymes seems similar including competitive inhibition, inactivation, and recovery action. Therefore, a universal mathematical model may be constructed in which the specific coefficients are related to the process characteristic such as aerobic or anaerobic, molecular structures of primary and secondary substrates, as well as environmental conditions, etc.

The key problem in developing mathematical models is how to appropriately express the effects such as competitive inhibition, toxic inactivation, and recovery action related with the biodegradation of chlorinated solvents. To help understand the relationships among these factors, a logical physical model is constructed here based on the key enzyme activities on the cell level (Figure 1).

This model framework clearly expresses the logical relationships among several actions around the key enzymes. It can be seen that the reactions of either the primary substrate or the secondary substrate with key enzymes

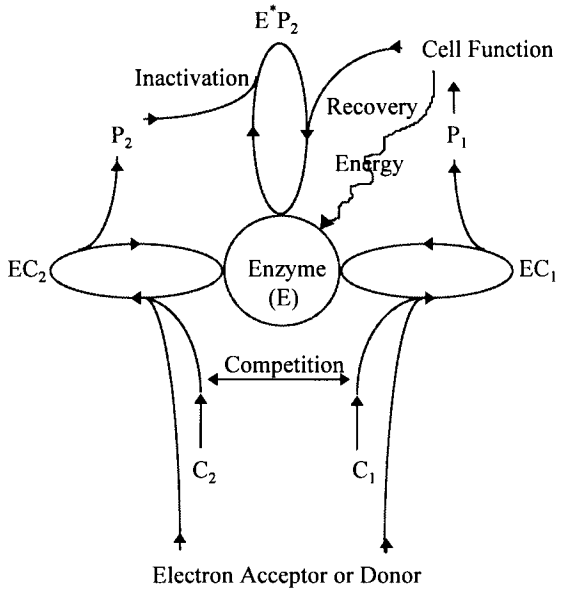

FIGURE 1. DIAGRAM OF MODEL FRAMEWORK FOR BIODEGRADATION OF CHLORINATED SOLVENTS.

should fit the Michaelis-Menten model. Competition between primary and secondary substrates for active enzymes will also result in relevant sharing of electron acceptor or donor and energy. For biodegradation of chlorinated solvents, according to the models discussed above, the inactivation of key enzymes is mainly mediated by metabolic intermediates directly or indirectly. Genetic regulations include induction and recovery action functions on the cell level and are associated with metabolization of the primary substrate and the extent of inactivation. Microbial growth and energetics are mainly supported by primary substrate metabolism but should be influenced by cometabolism of the secondary substrate.

Integration of all the important factors together in this model framework makes it possible to simulate the *in situ* biodegradation process of chlorinated solvents. It should be noted that all the factors in the model framework are involved in several action/anti-action loops. Under steady-state conditions, e.g., continuous reactor, the loops other than enzyme reaction with substrates can match well with reaction loops and can be considered as a constant term in mathematical models. However, under unsteady-state, e.g., batch reactor or pulse supply of substrates, those loops will go with different steps with reaction loops. This situation will result in the complex behavior of a microbial system. Such complex behaviors have been observed by McCarty and his coworkers in their *in situ* pilot-scale field tests where the substrate is supplied in a pulse way [1, 91].

This model framework properly represents the self-organized characteristic of a microbial system. It is expected that mathematical models based on this framework may be used in real bioremediation processes. Currently, a detailed mathematical model is being developed based on the state of the art of microbial kinetics and specific data available in literature.

SUMMARY

1. Successful mathematical models for biodegradation of chlorinated solvents should be integrative and self-organized in nature;
2. Empirical modifications of the Monod model cannot deal with the complicated biodegradation process of chlorinated solvents;
3. All the major factors including induction, competitive inhibition, inactivation, recovery action, microbial growth, and energetics, etc., should be properly organized in a logical network;
4. Inactivation and recovery action are crucial for mathematical model

development, especially under unsteady-state conditions.

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