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MODELING SUBSURFACE BIODEGRADATION OF NON-AQUEOUS PHASE LIQUIDS: A LITERATURE REVIEW

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1.0 INTRODUCTION

Mathematical modeling of in-situ non-aqueous phase liquid (NAPL) biodegradation is potentially useful in the assessment of the transport and fate of contaminants, in the optimization and design of cleanup operations, and in the estimation of the duration of such restoration operations (Chen et al., 1992). Over the past several years, numerous biodegradation models have been proposed. These models take many different approaches to biodegradation modeling and often emphasize a particular aspect of the biodegradation and/or transport problem. The purpose of this report is to summarize the methods by which different researchers model subsurface biodegradation and provide examples of several complete models that represent the variety of approaches.

This report begins by briefly reviewing the physical properties of NAPLs that are important to modeling their transport and biodegradation. In Section 3, an overview of microbiological metabolism is provided for those unfamiliar with the concepts, followed by a summary of NAPL biodegradation. Section 4 describes the factors important in subsurface NAPL biodegradation modeling and describes how different researchers have incorporated these factors into biodegradation models. The application of these methods is demonstrated in Section 5, where five biodegradation models are described and discussed. The report concludes in Section 6 with a discussion of possible approaches to biodegradation modeling.

2.0 PHYSICAL PROPERTIES OF NAPL COMPOUNDS

This section provides a brief overview of NAPL compound physical properties, since these properties are important in establishing a conceptual and mathematical model of NAPL biodegradation. Emphasis is placed on petroleum hydrocarbons and chlorinated solvents, since these compounds are the most ubiquitous NAPL contaminants. A thorough discussion of DNAPL physical properties and a bibliography can be found in Cohen and Mercer (1993).

2.1 Solubility

NAPL compounds vary widely in their solubility. In many cases, NAPL contaminant plumes consist of a mixture of tens or even hundreds of compounds, some of which are very soluble and others that are practically insoluble. Crude oil is an example of this type of mixture. However, a few generalizations about NAPL solubility can be made.

For petroleum mixtures, the most soluble compounds are typically aromatic hydrocarbons such as benzene, toluene, xylenes and ethylbenzene (Fetter, 1993). These compounds will typically leach out of a contaminant plume faster than the less soluble compounds, which tend to stay within the NAPL phase. Solubilities of these aromatics range from 150 mg/L for ethylbenzene to 1,780 mg/L for benzene (Fetter, 1993). Polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) are much less soluble than aromatic hydrocarbons. Solubilities of PCBs range from 0.05 mg/L for PCB-1254 to 1.5 mg/L for PCB-1232 (Cohen and Mercer, 1993). PAH solubilities range from .00026 mg/L for benzo(g,h,i)perylene to 31.7 mg/L for naphthalene (Fetter, 1993).

Chlorinated solvents are typically much more soluble than hydrocarbons. Solubilities of representative chlorinated solvents at 20°C are shown in Table 1 (Fetter, 1993).

Solubility is important to biodegradation because microorganisms typically exist in the aqueous phase (Brock et al., 1984). Compounds with greater solubilities may be

more available to microorganisms, and, all other factors being equal, may be more biodegradable than similar compounds of lesser solubility.

Compound	Water Solubility (mg/L)
dichloromethane	20,000
chloroform	8,000
1,1-dichloroethane	5,500
1,1,1-trichloroethene	4,400
vinyl chloride	1
trichloroethene	1,100
tetrachloroethene	150

Table 1. Chlorinated organic solvent solubilities (Fetter, 1993.)

2.2 Volatility

Volatility is an important factor in determining a compound's potential to migrate in the vadose zone. The combination of a NAPL compound's solubility and vapor pressure will determine its air/water partitioning coefficient (Henry's constant). Henry's constants of NAPL compounds vary widely. For aromatic hydrocarbons, Henry's law constants (in atm-m^3/mol) range from 5.6 x 10^{-3} for benzene to 8.7 x 10^{-3} for ethylbenzene (Brown, 1993). PAHs have much lower Henry's constants, from 4.1 x 10^{-4} for naphthalene to 2.5 x 10^{-5} for phenanthrene (Brown, 1993). Henry's law constants for PCBs range from 3.24 x 10^{-4} for PCB-1221 to 3.5 x 10^{-3} for PCB-1248 (Cohen and Mercer, 1993). Chlorinated hydrocarbons generally have much higher Henry's law constants, ranging from 1.31 x 10^{-3} for dichloromethane to 2.1 x 10^{-2} for carbon tetrachloride (Fetter, 1993). Most other chlorinated hydrocarbons have Henry's law constants in the range of 10^{-2} to 10^{-3} (Fetter, 1993).

2.3 Density

NAPL density determines whether the compound tends to float or sink when it encounters a water bearing zone. Petroleum hydrocarbons are mostly lighter than water and tend to float on the surface. Although PCBs are more dense than water, they are typically mixed with carrier fluids that may be more or less dense than water. Typical carrying fluids include chlorinated benzenes (which are heavier than water) and petroleum mixtures (Cohen and Mercer, 1993). PAHs are also typically mixed with a petroleum-

derived carrier oil, although some mixtures may be heavier than water. Nearly all of the chlorinated solvents have a greater density than water.

2.4 Adsorbability

Adsorption of NAPL compounds could be important in limiting their bioavailability. A relative measure of a compound's adsorbability can be gained by examining its organic carbon partition coefficient (K_{oc} or log K_{oc}). The higher a compound's log K_{oc}, the greater is its tendency to adsorb onto organic matter in the subsurface. For aromatic hydrocarbons, log K_{oc} values range from approximately 2 mL/g for benzene to approximately 3 mL/g for ethylbenzene (Fetter, 1993). PCBs are much more adsorbable than aromatic hydrocarbons, with log K_{oc} values ranging from 2.44 mL/g for PCB-1221 to 5.64 mL/g for PCB-1248 (Cohen and Mercer, 1993). PAHs are also highly adsorbable. Log K_{oc} values for PAHs range from approximately 3 mL/g for naphthalene to approximately 5 mL/g for pyrene (Fetter, 1993). Chlorinated solvents do not adsorb as strongly, with representative log K_{oc} values ranging from approximately 1.2 mL/g for 1,2-dichloroethane to 2.4 mL/g for tetrachloroethene (Cohen and Mercer, 1993).

3.0 NAPL ENVIRONMENTAL DEGRADATION

NAPLs undergo both biotic (biologically mediated) and abiotic (non-biologically mediated) reactions in the subsurface (Vogel et al., 1987). Most abiotic transformations are slow compared to biotic reactions, but they can still be significant on the time scale of groundwater movement (Vogel et al., 1987). Although this report is concerned with modeling biodegradation of NAPLs in the subsurface, ignoring relatively fast abiotic reactions could lead to underestimates of NAPL compound destruction rates. Therefore, the most important abiotic reactions are discussed briefly, followed by a more thorough discussion of biodegradation reactions. A brief review of basic microbial metabolism applicable to NAPL biodegradation is also provided.

3.1 Abiotic NAPL Degradation Reactions

Abiotic reactions may occur independently or as a result of microorganism growth. In addition, microbial reactions may alter the environment's pH and *Eh* and produce agents that can lead to abiotic reactions (Bouwer and McCarty, 1984). Abiotic reactions are most important for chlorinated solvents since abiotic transformations of petroleum hydrocarbons are not expected to be significant in the time scales encountered in biodegradation modeling. Vogel et al. (1987) provide a summary of the current understanding of both abiotic and biotic reactions that these compounds undergo.

The two abiotic reactions of primary concern in biodegradation modeling are substitution reactions and dehydrohalogenation reactions (Vogel, 1993). Hydrolysis reactions, in which water reacts with the halogenated compound to substitute an OH^- for an X^-, create an alcohol (Vogel, 1993) which can then be biodegraded. Hydrolysis reactions occur most rapidly for monohalogenated compounds. As the number of halogen atoms on the molecule increases, the rate of hydrolysis reactions decreases (Vogel, 1993).

Dehydrohalogenation reactions occur when an alkane loses a halide ion from one carbon atom and then a hydrogen ion from an adjacent carbon (Vogel, 1993). A double bond then forms between the carbon atoms to create an alkene. The rate of

Compound	Half-Life (years)	Products
Dichloromethane	1.5 to 704	
Trichloromethane	1.3 to 3,500	
1,2-Dichloroethane	50	
1,1,1-Trichloroethane	0.5 to 2.5	Acetic acid, 1,1-Dichloroethylene
1,1,2,2-Tetrachloroethane	0.8	Trichloroethene
Trichloroethene	0.9 to 2.5	
Tetrachloroethene	0.7 to 6	

Table 2. Environmental half-lives from abiotic reactions of selected chlorinated aliphatic compounds (Vogel et al., 1987).

dehalogenation increases with increasing numbers of halogen atoms on the molecule (Vogel, 1993).

The importance of these reactions is evident from the abiotic hydrolysis or dehydrohalogenation half-lives of some common chlorinated NAPL compounds listed in Table 2 (Vogel et al., 1987). Models that fail to consider these reactions could considerably overestimate contaminant concentrations if the model is attempting to predict contaminant concentrations over a number of years.

3.2 General Principles of Organic Chemical Biodegradation

To survive, microorganisms must have 1) a source of energy, 2) carbon for the synthesis of new cellular material, and 3) inorganic elements (nutrients) such as nitrogen, phosphorous, sulfur, potassium, calcium, magnesium and other inorganic micronutrients (Metcalf and Eddy, 1991; Chapelle, 1993). Electron acceptors are needed to allow the chemical energy contained in biodegradable compounds to be released. Organic nutrients (growth factors) may also be required for cell synthesis (Metcalf and Eddy, 1991).

The process of breaking down compounds to provide energy is called catabolism. The utilization of this energy to synthesize compounds necessary for a microorganism's survival is called anabolism. Collectively, the chemical reactions involved in these two processes are called metabolism (Brock et al., 1984). As shown in Table 3, microorganisms are often classified according to the sources of carbon and energy. In the degradation of NAPLs, chemoheterotrophs are of greatest interest because they utilize organic carbon for both energy and cell growth (Metcalf and Eddy, 1991).

3.2.1 Energetics of Microbial Growth

All reactions involved in the day-to-day processes within microorganisms can be described with established principles of chemistry and thermodynamics (Brock et al., 1984). Therefore, the reactions from which microorganisms obtain energy can be modeled using the same equations used for chemical reactions. Microorganisms obtain energy from oxidation/reduction (redox) reactions in which electrons are transferred from an electron donor to an electron acceptor. The electron donor is oxidized and the electron acceptor is reduced. In biological reactions, the electron donor is often called the energy source or substrate (Brock et al., 1984). Electron acceptors are organic or inorganic compounds that are relatively oxidized compared to the electron donor and are capable of accepting electrons from the electron donor in energetically favorable redox reactions.

Classification	Energy Source	Carbon Source
Autotrophic:		
Photoautotrophic	Light	CO_2
Chemoautotrophic	Inorganic redox reactions	CO_2
Heterotrophic:		
Photoheterotrophic	Light	Organic carbon
Chemoheterotrophic	Organic redox reactions	Organic carbon

Table 3. Classification of microorganisms based on carbon and energy sources (Metcalf and Eddy, 1991).

The tendency of a substance to give up electrons is expressed as the substance's reduction potential. The more negative the reduction potential of a substance, the greater the tendency of the substance to donate electrons. The amount of energy released in any redox reaction depends on both the electron donor and the electron acceptor. The greater the difference between the reduction potentials of the donor and acceptor half reactions, the greater the amount of energy released. Redox pairs can be written in an "electron tower" (Figure 1) to graphically illustrate the potential energy release for coupling of the two redox half reactions (Brock et al., 1984).

The transfer of electrons from the substrate to the electron acceptor usually proceeds in a number of steps, with intermediate electron acceptors and donors carrying electrons to the final or terminal electron acceptor. The total energy available from the

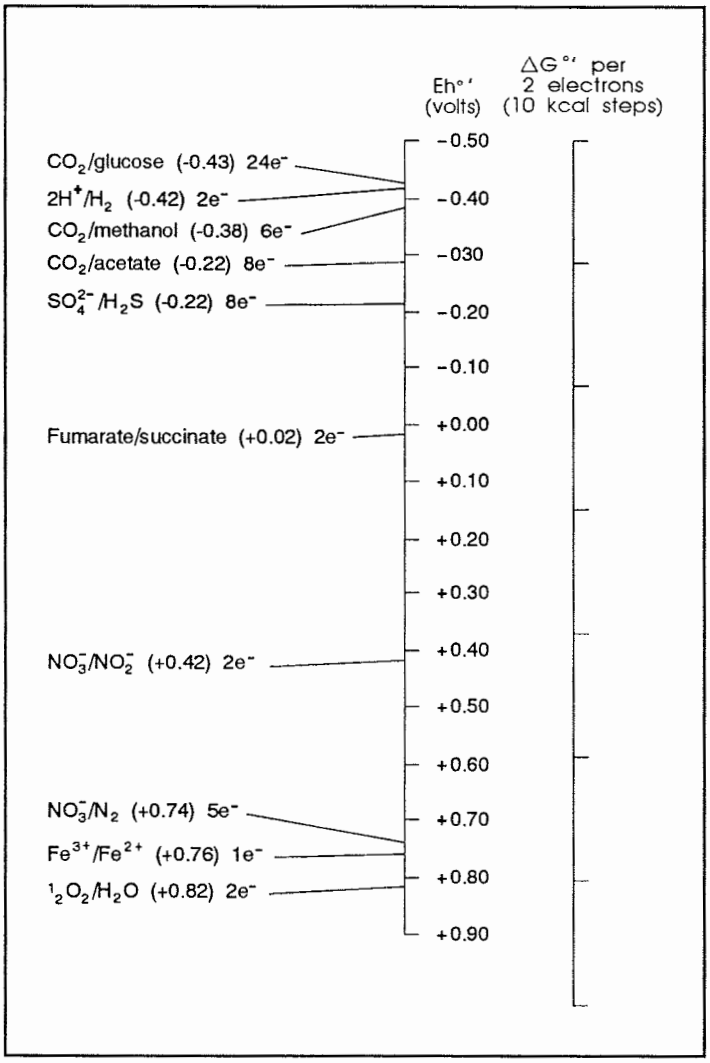


Figure 1. Electron tower (Brock et al., 1984).

substrate oxidation is the energy released when only the original substrate and ultimate electron acceptor are considered. Some of the energy released by the oxidation of substrates is stored as chemical energy (usually in the high-energy phosphate bonds of molecules such as adenosine triphosphate or ATP) so that it can be used to carry out synthesis and other reactions necessary for cell growth and maintenance (Brock et al., 1984).

NAPL compounds are biodegraded because they are substrates (electron donors) for microorganisms. NAPL compounds are oxidized by microorganisms to provide them with energy. Microorganisms also use some fraction of the carbon in NAPL compounds for synthesis of new cells.

Microorganisms utilize substrates for energy through a number of different biochemical pathways. These pathways are defined by the chemical reactions they involve and the terminal electron acceptor. If no *external* electron acceptor (a compound other than the substrate) is utilized in the redox reactions to generate energy, then the process is called fermentation. If an external electron acceptor is used by the microorganism, the process is called respiration. Aerobic respiration utilizes oxygen as the terminal electron acceptor. In anaerobic respiration, microorganisms utilize an external electron acceptor other than oxygen (Brock et al., 1984).

Both respiration and fermentation are potentially important in subsurface biodegradation of NAPL compounds. Although aerobic respiration reactions typically occur much faster than anaerobic respiration and fermentation reactions, oxygen may often be absent in the contaminant plume so that these reactions may be the only significant biotic reactions occurring.

3.2.2 Fermentation

In fermentation, substrates are only partially oxidized. Electrons are "internally recycled," generally yielding at least one product that is more oxidized and one that is more reduced than the original substrate. As a result, only part of the compound can be used to generate energy, and the energy released is less than that released by respiration. An example of a fermentation reaction is the catabolism of glucose by yeast (Brock et al., 1984):

$$C_6H_{12}O_6 \rightarrow 2\,CH_3CH_2OH + 2\,CO_2 \qquad \Delta G^{o'} = -57 \text{ kcal/mole}$$

Note that there is no external electron acceptor in this reaction. The fermentation of glucose actually proceeds in a number of intermediate steps. The collective steps in which glucose is fermented to pyruvate is called glycolysis or the Embden-Meyerhof pathway (Brock et al., 1984). Many compounds other than glucose can be fermented, including sugars, amino acids, organic acids, alcohols, purines, and pyrimidines (Brock et al., 1984). To be fermented, compounds must not be too reduced or too oxidized because part of the compound must transfer electrons to the other part of the compound for energy to be released.

The end products of complete fermentation depend on the initial electron donor and the type of microorganism(s) carrying out the reactions. Typical fermentation end products include (Chapelle, 1993):

- acetic acid
- lactic acid
- formic acid, H_2 and CO_2
- ethanol and CO_2
- 2,3-butanediol and CO_2

- propionic acid and CO_2
- butyric acid
- acetone, butanol, isopropanol and CO_2
- CH_4 and CO_2

Methanogens (methane-producing bacteria) and fermentative bacteria often live together in a symbiotic association (Chapelle, 1993). The fermentative organisms degrade complex sedimentary organic matter to produce CO_2 or acetate and H_2 required by methanogens. In turn, the methanogens use these fermentative products for metabolism, thereby preventing them from accumulating to concentrations inhibitory to the fermentative organisms. There are two common methanogenesis pathways: the CO_2 reduction pathway and the acetate reduction pathway. The overall reactions for these two pathways may be written (Chapelle, 1993):

$$4\,H_2 + CO_2 \rightarrow CH_4 + 2\,H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

As an example, the overall reaction for toluene destruction by methanogenesis can be written (Reinhard, 1993):

$$C_7H_8 + 5\,H_2O \rightarrow 4.5\,CH_4 + 2.5\,CO_2$$

The CO_2 reduction pathway is actually an anaerobic respiration reaction with CO_2 as the electron acceptor. However, because the methanogens live in such a mutually dependent relationship with the fermentative bacteria producing these substances, the processes are usually discussed together. Other anaerobic bacteria also exist with fermentative bacteria in similar associations (Chapelle, 1993).

In addition to acetate and CO_2, methanogens can also convert methanol, formate, methyl mercaptan, and methylamines to methane. The end products of methanogenisis are either methane and water for CO_2 reduction, or methane and CO_2 for organic acid reduction. Methanogens are strict anaerobes so that they cannot function when significant levels of oxygen are present in their environment. Methanogens are also inhibited by sulfate (Brock et al., 1984). Methanogenic reactions are often the predominant metabolic processes in environments lacking other electron acceptors (Chapelle, 1993).

Fermentation to pyruvate or other simple organic compounds is often the first step in the biodegradation of more complex natural organic molecules (Chapelle, 1993). If external electron acceptors are present, the simple products produced from fermentation are channeled into established respiration pathways where the fermentation products can be used to generate far more energy than would be available from fermentation alone.

3.2.3 Respiration

Unlike fermentation, in which substances are only partially oxidized, respiration oxidizes compounds completely to CO_2 and water by using an external electron acceptor. Respiration yields much more energy per mass of substrate metabolized because: 1) compounds are completely oxidized and, 2) the difference in reduction potentials between the initial electron donor and terminal electron acceptor is much higher than in fermentation (Brock et al., 1984).

3.2.3.1 Aerobic Respiration

Conversion of compounds to pyruvate or other central intermediates is often the first step in aerobic respiration. Following generation of pyruvate in glycolysis, pyruvate is completely oxidized to CO_2 through the tricarboxylic acid (TCA) cycle. The TCA cycle is also sometimes called the citric acid or Krebs cycle. As the starting point in the TCA cycle, pyruvate is oxidized to CO_2 in a number of oxidation/reduction reactions in which the electrons from pyruvate are ultimately transferred to oxygen (Brock et al., 1984).

Aerobic respiration is much more efficient than glycolysis. For example, the amount of energy released from the aerobic metabolism of glucose is 686 kcal/mole compared to the 57 kcal/mole released by fermentation (Bailey and Ollis, 1986). Of course, not all of this energy is recovered by microorganisms. Glycolysis actually generates 7.4 kcal/mole of glucose while aerobic respiration generates 266 kcal. Aerobic respiration is both more efficient and more energy-yielding than glycolysis alone. Aerobic respiration yields the most energy per mol of substrate because oxygen has the most positive reduction potential of the common electron acceptors. This can be seen by examining Figure 1. The O_2/H_2O redox pair is further down the "electron tower" from

the CO_2/glucose redox pair than any other electron acceptor redox pair. The end products of aerobic respiration are CO_2 and water.

3.2.3.2 Anaerobic Respiration

Anaerobic respiration involves a terminal electron acceptor other than oxygen. Anaerobic respiration is less efficient than aerobic respiration because the reduction potential of these alternate electron acceptors is less positive than that of oxygen. Therefore, as seen in Figure 1, less energy is released in the oxidation of the substrate (Brock et al., 1984). The most important alternate electron acceptors in groundwater environments are nitrate, sulfate, iron (III), and carbon dioxide.

Microorganisms can use nitrate as a terminal electron acceptor in the degradation of many organic compounds in a process called denitrification. Nitrate is first converted to nitrite, and then to either nitrous oxide or nitrogen gas. The overall reaction, with toluene as the substrate and elemental nitrogen as the final product, is (Reinhard, 1993):

$$C_7H_8 + 7.8\,NO_3^- + 7.2\,H^+ \rightarrow 7\,CO_2 + 3.6\,N_2 + 7.6\,H_2O$$

The end products of denitrification are CO_2, N_2 or N_2O, and water. Nitrate reducing organisms are facultative organisms. They use oxygen as a terminal electron acceptor when it is available and switch to nitrate when oxygen levels become low (Brock et al., 1984).

Like methanogens, sulfate reducing bacteria usually depend on fermentative bacteria to supply them with the principal substrates on which they depend. These substrates are formate, lactate, acetate and hydrogen. The overall process for toluene biodegradation by sulfate reducing bacteria is (Reinhard, 1993):

$$C_7H_8 + 4.5\,SO_4^{2-} + 3\,H_2O \rightarrow 2.25\,H_2S + 2.25\,HS^- + 7\,HCO_3^- + 0.25\,H^+$$

The end products of sulfate reduction are CO_2, H_2 and sulfide. Like methanogens, sulfate reducing organisms are strict anaerobes, i.e., they cannot function when oxygen is present and can even be killed by high oxygen levels.

Ferric iron can also be used as an electron acceptor by many organisms. Ferric iron is reduced to ferrous iron in a process that probably involves the TCA cycle (Chapelle, 1993). The reaction for toluene oxidation by ferric iron reducing bacteria is (Reinhard, 1993):

$$C_7H_8 + 36\,Fe^{3+} + 21\,H_2O \rightarrow 7\,HCO_3^- + 36\,Fe^{2+} + 43\,H^+$$

The relative amount of energy released with these different anaerobic electron acceptors, in decreasing order, is $Fe^{3+} > NO_3^- >> SO_4^{2-} > CO_2$ (Brock et al., 1984).

3.2.4 Cometabolism and Secondary Utilization

Cometabolism is the fortuitous biodegradation of a compound during the biodegradation of another compound that supports microbial growth (Brock et al., 1984). A more rigorous definition is provided by Criddle (1993) as:

> ...the transformation of a nongrowth substrate by growing cells in the presence of a growth substrate, by resting cells in the absence of a growth substrate, or by resting cells in the presence of an energy substrate. A growth substrate is defined as an electron donor that provides reducing power and energy for cell growth and maintenance... An energy substrate is defined as an electron donor that provides reducing power and energy, but does not by itself support growth.

Cometabolism is an important biodegradation mechanism for many compounds that normally cannot be biodegraded, especially chlorinated aliphatic compounds. Cometabolism usually occurs when enzymes generated by microorganisms to degrade a substrate also act on the cometabolite.

Often confused with cometabolism is a process called secondary utilization. Secondary utilization is the metabolism of a compound in the presence of other substrates that supply the microorganism's primary growth needs (Bouwer and McCarty, 1984). The secondary metabolite is typically present at a concentration too low to support growth alone, but is metabolized when other substrates are present. The secondary metabolite may or may not supply the microorganism with energy or carbon needed for growth. Secondary metabolism may be an important biodegradation mechanism for biodegradable NAPL compounds present at concentrations too low to support microbial growth.

The difference between secondary metabolism and cometabolism is that a cometabolite is not inherently biodegradable but is degraded fortuitously by an operating enzyme system, whereas a secondary substrate could be degraded if its concentration were sufficient to support growth.

3.3 NAPL Biodegradation

Most man-made compounds tend to be more resistant to biodegradation than natural compounds. However, most man-made compounds can be biodegraded under the right conditions by microorganisms (Kobayashi and Rittmann, 1982). Extensive literature is available on the biodegradation of particular compounds. References that include good bibliographies are Fetter (1993), Kobayashi and Rittmann (1982), Chapelle (1993), and Environmental Protection Agency (1993). The main pathways of NAPL biodegradation likely to be encountered in groundwater systems are summarized in Table 4.

3.3.1 Petroleum Hydrocarbons

Chapelle (1993) provides a comprehensive discussion of petroleum hydrocarbon biodegradation, and the following discussion is taken largely from this work.

3.3.1.1 Aliphatic Compounds

Aliphatic (non-aromatic, non-cyclic) compounds are primarily biodegraded aerobically. Although anaerobic degradation of hydrocarbons has been demonstrated, biodegradation rates are orders of magnitude less than aerobic rates, so that anaerobic degradation is not considered to be a significant process of removal (Atlas, 1981).

With the exception of methane, aliphatic hydrocarbons are usually degraded by converting the compounds to fatty acids. The fatty acids are then broken down primarily by a process called beta-oxidation. In beta-oxidation, straight-chain hydrocarbons are progressively reduced in size by the successive cleavage of terminal ethyl groups. The ethyl groups are removed as acetyl-coenzyme A, which is fed directly into the TCA cycle. Alkenes are degraded by mechanisms similar to alkanes, although some anaerobic pathways may be important. Branched-chain aliphatics are also likely to be degraded by

Compound	Formula	Biodegradation Potential			
		Primary Substrate[a]		Cometabolism[b]	
		Aerobic	Anaerobic	Aerobic	Anaerobic
Carbon tetrachloride	CCl_4			0	4
Methylene Chloride	CH_2Cl_2	Yes	Yes	3	
1,1,1-Trichloroethane	CH_3CCl_3			1	4
1,1-Dichloroethane	CH_3CHCl_2			1	2
1,2-Dichloroethane	CH_2ClCH_2Cl	Yes		1	1
Chloroethane	CH_3CH_2Cl	Yes		2	c
Tetrachloroethene	$CCl_2= CCl_2$			0	3
Trichloroethene	$CHCl=CCl_2$			2	3
cis-1,2-Dichloroethene	CHCl=CHCl			3	2
1,1-Dichloroethene	$CH_2=CCl_2$			1	2
Vinyl Chloride	$CH_2=CHCl$	Yes		4	1
Benzene	C_6H_6	Yes	Yes		
Toluene	C_7H_8	Yes	Yes		
Xylene	C_8H_{10}	Yes	Yes		
Ethylbenzene	C_8H_{11}	Yes	Yes		
Aliphatic Hydrocarbons	N/A	Yes	No		
Alicyclic Hydrocarbons	N/A	Yes	No		
Polynuclear Aromatics	N/A	Yes	Yes		
PCBs	N/A	Yes	Yes		

a No entry means there is not sufficient information available.
b Increasing numbers indicate increasing potential for degradation.
c Readily oxidized abiotically, with half-life on order of one month.

Table 4. Biodegradation pathways for representative NAPL compounds or classes of compounds (Chapelle, 1993; Borden, 1993; McCarty and Semprini, 1993; Atlas, 1981).

beta-oxidation after being transformed into straight-chain fatty acids. Three generalizations with regard to aliphatic organic degradation can be made (Chapelle, 1993; Borden, 1993):

1. Moderate to lower weight hydrocarbons (C_{10} to C_{14}) are most easily biodegraded. As the molecular weight increases, resistance to biodegradation increases.
2. Biodegradability increases with decreasing number of double bonds.
3. Biodegradability increases with decreasing carbon chain branching.

3.3.1.2 Alicyclic Compounds

Alicyclic (non-aromatic cyclic) petroleum hydrocarbons are generally more resistant to biodegradation than non-cyclic compounds (Atlas, 1981), but they are still relatively easily biodegraded. Studies on the biodegradation of cyclohexane indicate that alicyclic hydrocarbons are degraded by two or more organisms working in concert (Chapelle, 1993). Alicyclic hydrocarbons may also be degraded by anaerobic pathways, although this process has received little attention in the literature.

3.3.1.3 Single-ring Aromatic Compounds

Single-ring aromatic hydrocarbons such as benzene, toluene and xylene are readily degraded aerobically. Aromatic compounds with complex side groups are less easily degraded than benzene or simple alkyl substitutions. Biodegradation of these compounds generally proceeds by the formation of catechol (a benzene molecule with two hydroxyl groups attached at adjacent carbon atoms). The aromatic ring is then broken, and further degradation occurs by beta-oxidation or other mechanisms.

Anaerobic degradation of benzene, toluene and xylene has also been documented (Reinhard, 1993). In biodegradation of benzene by methanogenic bacteria (fermentation), phenol appears to be an intermediate. The oxygen forming the hydroxy group is thought to come from water. Toluene and xylene are also degraded by methanogenic bacteria (Reinhard, 1993).

Studies indicate that toluene, ethylbenzene and xylene can be degraded anaerobically with nitrate as the terminal electron acceptor (Reinhard, 1993). Evidence

of benzene biodegradation under denitrifying conditions is not conclusive (Reinhard, 1993). Toluene and xylene have also been biodegraded with sulfate as the electron acceptor, although benzene and ethylbenzene were not degraded (Reinhard, 1993).

Biodegradation of toluene by iron reducing bacteria has been demonstrated (Reinhard, 1993). This finding is especially important since many shallow sand aquifers that are particularly susceptible to contamination from surface spills lack nitrate but contain significant concentrations of iron (III) hydroxides. As a result, biodegradation by iron reducing bacteria may be the first anaerobic process to degrade the hydrocarbons (Chapelle, 1993).

3.3.1.4 Polycyclic Aromatic Hydrocarbons

Polycyclic compounds can be degraded aerobically by mechanisms similar to those used by microorganisms to degrade single-ring aromatic compounds. Resistance to biodegradation generally increases with the number of additional aromatic rings. An increase in branched substitutions may increase biodegradation resistance (Chapelle, 1993).

3.3.2 Chlorinated Aliphatic Compounds

Chlorinated compounds are relatively oxidized, because the chlorine atom withdraws electrons from the carbon-chlorine bond. As a result, chlorinated compounds release less energy than their unchlorinated counterparts in oxidation reactions. This property makes chlorinated compounds less easily degraded than non-chlorinated compounds (Chapelle, 1993). However, chlorinated aliphatic compounds (CAHs) can be degraded either aerobically or anaerobically, although the mechanisms for these two processes differ considerably (Chapelle, 1993).

Only a few chlorinated compounds have been shown to serve as primary energy and growth substrates (McCarty and Semprini, 1993). These compounds include dichloromethane, 1,2-dichloroethane, chloroethane and vinyl chloride. Dichloromethane has been shown to degrade under aerobic or anaerobic conditions, while the other three compounds have been shown to degrade only under aerobic conditions (McCarty and Semprini, 1993). These few studies indicate that the less-halogenated one-and two-carbon

CAHs may be used as primary substrates, but that organisms capable of utilizing them are rare (McCarty and Semprini, 1993).

Cometabolism is the predominant method of transformation of most CAHs (McCarty and Semprini, 1993). In early studies, trichloroethene (TCE) was shown to be degraded to CO_2 by soil microorganisms growing on methane (Chapelle, 1993). The degradation is catalyzed by a methane monooxygenase (MMO), an enzyme that catalyzes the incorporation of molecular oxygen into methane to form methanol (Chapelle, 1993). Current evidence indicates that the process is self-limiting and is inhibited by high methane concentrations (Chapelle, 1993). Microorganisms that oxidize propane, ethylene, toluene, phenol cresol, ammonia, isoprene and vinyl chloride have also been shown to transform CAHs through cometabolism (McCarty and Semprini, 1993). Under aerobic conditions in mixed cultures typical of natural conditions, TCE is mineralized completely to CO_2, water and chloride through cooperation between the TCE oxidizers and other bacteria (McCarty and Semprini, 1993).

CAHs can be cometabolized anaerobically under a variety of environmental conditions (McCarty and Semprini, 1993). The first step in the process is reductive dechlorination. In reductive dechlorination, the CAH is reduced by substituting a hydrogen atom for chlorine or by forming a double bond between two carbon atoms, one or both of which contained a chlorine substitution (Vogel et al., 1987, Kobayashi and Rittmann, 1982). As shown in Figure 2, successive reductive dechlorination can transform CAHs into a series of by-products containing fewer and fewer

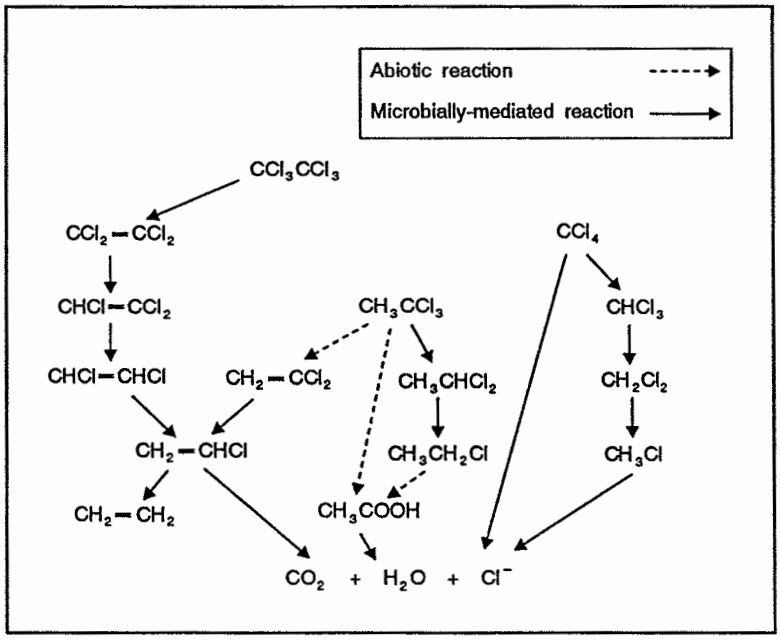


Figure 2. Anaerobic transformation of CAHs (McCarty and Semprini, 1993).

chlorine atoms, and all of these products may be found with the parent CAH (McCarty and Semprini, 1993). In general, the highly chlorinated compounds are degraded more easily than less-chlorinated compounds so that less-chlorinated compounds are more persistent in the environment (McCarty and Semprini, 1993). Biodegradation rates tend to be highest under highly reducing conditions (McCarty and Semprini, 1993).

3.3.3 PCBs

Chapelle (1993) summarizes current knowledge on PCB degradation. PCBs can be degraded aerobically if the molecules contain relatively few chlorine atoms. PCBs with a larger number of chlorine atoms are degraded anaerobically by reductive dechlorination. Thus, a sequence of anaerobic degradation followed by aerobic degradation can degrade PCBs completely to CO_2.

4.0 MODELING SUBSURFACE BIODEGRADATION

Modeling biodegradation in the subsurface is extremely complex. Many of the biological and physical phenomena involved are only partially understood. The difficulty of modeling is compounded by our inability to actually see what is going on in the subsurface. Although we know that the subsurface is generally heterogeneous, we typically do not know the locations and extent of the heterogeneities. We also cannot see the microorganisms in the subsurface and must make assumptions about their distribution and metabolic capabilities. Our lack of knowledge is demonstrated by the relative lack of successful field-scale biodegradation modeling. However, many models have been quite successful at describing biodegradation on a smaller scale in one-dimensional column experiments. In this section, the important factors affecting biodegradation of NAPLs are discussed, and the methods used by other modelers to describe these factors are described.

4.1 General Conceptual Model of Biodegradation

Before attempting to model a complex phenomenon such as subsurface biodegradation, it is often helpful to develop a conceptual model. The conceptual model should include all of the factors affecting the phenomenon. These factors can then be described mathematically and their relative importance assessed. Those factors that do not have a significant effect can be neglected or estimated while those that significantly affect the results of the simulation can be retained.

A conceptual model of the transport and biodegradation of a hypothetical NAPL spill is described below to illustrate the factors that need to be considered in modeling and the complexity of subsurface biodegradation. The conceptual model is derived from a compilation of literature on the subject, and the particular phenomena will be described in greater detail and referenced later.

To begin simply, suppose a spill of LNAPL occurs at the ground surface above a shallow water table. The LNAPL could contain dissolved chlorinated species that may be difficult to biodegrade. What will be the fate of the NAPL? Many of the more

volatile components will vaporize before seeping downward into the soil. The fraction of NAPL that remains is the subject of the conceptual model.

4.1.1 Unsaturated Zone

The LNAPL will follow the basic physical laws of any other fluid and will begin to flow downward through the soil under the force of gravity. Being the non-wetting fluid compared to water, NAPL will tend to occupy the medium-sized pores, while water occupies the smaller pores and air the largest. Assuming the spill is large enough, NAPL will travel downward until it reaches the water table, where it will spread over the surface of the water and form a lens because it is lighter than water. When the spill stops, the lens will continue to spread until the NAPL in the vadose zone has reached residual saturation. Residual saturation is the NAPL saturation at which the NAPL is no longer able to flow as a continuous phase. Fluctuations in the water table elevation can cause the NAPL to smear into the capillary fringe and below the original lens surface.

In the vadose zone, a four-phase system will be established, consisting of NAPL, air, water and solid (soil). The different components of the NAPL will partition among the different phases. If local equilibrium is established, the partitioning can be described with partition coefficients. If the transfer of constituents from one phase to another is rate-limited, then kinetic expressions must be used to predict the change in concentrations of constituents in the different phases. Volatile NAPL components will vaporize into the air phase, more soluble NAPL components will dissolve into the pore water, and highly adsorbable components will adsorb onto organic material in the soil. NAPL will also dissolve from the lens into the groundwater. The composition of the NAPL changes as its components are removed by leaching, dissolution into the groundwater, volatilization and biodegradation, since the chemical and physical properties of each component are affected by these different phenomena at different rates.

Until it reaches residual saturation, the NAPL does not remain static; it moves through the vadose zone under the influence of capillary pressure and gravity. If advection in the vadose zone is neglected, diffusion is the primary transport mechanism once the NAPL reaches residual saturation. NAPL constituent vapors evolve principally from the NAPL phase and diffuse through the air phase. The mobilized vapors dissolve

into pore water as they move and also adsorb onto the soil so that the NAPL constituents tend to spread. NAPL constituent vapors establish equilibrium with the capillary fringe outside of the residual NAPL area. Dissolved NAPL constituents also diffuse through the pore water, which is interconnected throughout the vadose zone. Volatile and soluble NAPL components move through the NAPL phase under induced concentration gradients caused by their loss across the NAPL/air and NAPL/water phase boundaries. As the NAPL constituents spread, some may be lost to the atmosphere by diffusion upward to the ground surface.

The soil contains microorganisms, most of which are attached to small particles and others that are free floating in the pore water. However, even the microorganisms attached to particles are surrounded by water because microorganisms must be in aqueous solutions to be active. Microorganisms are present within pore cavities and at the throats of pores. Most of the bacterial species are adapted to the aquifer conditions, but they have a tremendous variety of metabolic capabilities and nutrient requirements. The microorganism population is in a constant state of flux, with organisms detaching and becoming free-floating, other microorganisms attaching to colloidal particles and moving with the pore water, and other organisms moving along particle surfaces by processes of bacterial motion.

As soon as NAPL is introduced to the ground, the environment experienced by the microorganisms changes. The NAPL components dissolve into the pore water, becoming accessible to microorganisms. Whereas before contamination existed the environment was likely to be substrate-limited, it suddenly becomes limited by electron acceptors or nutrients. Microorganisms immediately begin to adapt to the new environment and begin the processes necessary to biodegrade the NAPL components. If microorganisms possess the necessary metabolic machinery, they may begin to degrade some of the more easily degradable dissolved components of the NAPL. The microorganisms will also begin acclimating to the new conditions created by the NAPL constituents. Because the NAPL components are likely to be different from the substrate the microorganisms normally metabolize, they must begin synthesizing new enzymes capable of acting on the dissolved NAPL constituents. In addition to being chemically different substrates, the NAPL constituents may affect other environmental conditions such as pH, redox potential, and

ionic strength. The NAPL may also contain toxic compounds that cannot be tolerated by the microorganisms present. Microorganisms must adapt to these new conditions to survive.

The microorganisms may accumulate near the NAPL/water interfaces where concentrations of the new substrate are highest. If the NAPL components are not too toxic, the microorganisms will first accumulate directly on the water/NAPL interface. If the NAPL components are toxic, the microorganisms will not be present at the interface, but will metabolize diluted NAPL constituents that diffuse out of the NAPL some distance from the interface. Aerobic bacteria will first utilize the NAPL constituents and will quickly deplete the oxygen in the pore water. In sandy or gravelly strata, diffusion of oxygen and infiltration of oxygenated precipitation from the surface may supply microorganisms with enough oxygen to maintain aerobic conditions. More likely, reaeration will not be fast enough to supply aerobic organisms with oxygen, and microorganisms adapted to anaerobic conditions will begin to metabolize the NAPL constituents. The anaerobic zone will grow as the NAPL constituents migrate with the concomitant switching from aerobic to anaerobic conditions. If the microbial community is relatively diverse and alternate electron acceptors are present, the NAPL constituents may be degraded by successive microbial communities utilizing different electron acceptors, based on their relative availability and energy yield.

The utilization of NAPL components will not be uniform. Larger aliphatic NAPL components will be degraded by aerobic bacteria but not by anaerobic bacteria, so that they will be present in the soil for a very long time. Other NAPL components are degraded only anaerobically as primary substrates. Other NAPL components are degraded both aerobically and anaerobically. Some NAPL components are degraded by cometabolism, while still others are degraded by a complex sequence which may involve aerobic, anaerobic, and cometabolic steps as well as abiotic reactions such as hydrolysis. The NAPL components are not degraded independently of each other, even if cometabolism is not a significant process. Microorganisms may compete for the most easily degraded compounds. One compound may interfere with the degradation of another compound due to its toxicity or binding of a key enzyme site. The products of biodegradation may be toxic and inhibit further degradation of the compound or of other

compounds. These compounds may be biodegraded until their concentration is sufficiently low that it becomes energetically favorable to biodegrade a less easily biodegradable compound. Two compounds may be degraded by the same enzyme so that they are degraded more slowly than if they were present alone.

Individual bacteria may not be capable of completing all of the steps in the biodegradation. One bacteria may dechlorinate a molecule, another may add a hydroxyl group, while another may convert the molecule into a form that can be utilized in one of the many metabolic pathways present in most bacteria. The products from one biochemical reaction may be required by another microorganism so that the two can only exist together. Biodegradation rates of the same organism may differ depending upon whether the organisms are attached to soil surfaces or are free-floating in the pore water.

As the bacteria act on the NAPL constituents, nutrients will be depleted near the interface since they must be supplied by diffusion through the pore water. Microbiological activity may decrease if nutrients cannot be supplied to the interface as fast as they are being consumed, and very little activity may be observed near the interface where nutrient conditions are most limiting due to the high NAPL concentrations. As the NAPL constituents spread in the vadose zone, additional microbial communities begin acclimating to the changing conditions so that the biodegradation processes occurring are constantly changing both with time and position throughout the vadose zone.

4.1.2 Saturated Zone

The biodegradation phenomena occurring in the saturated zone are similar to those in the vadose zone. However, only three phases are present in the saturated zone, air being absent. Transport in the saturated zone also differs from that in the vadose zone. Advection is the dominant transport mechanism in the saturated zone, and physical factors such as dispersion and adsorption play a much larger role.

As in the vadose zone, aerobic microorganisms in the saturated zone will first tend to accumulate at the NAPL/water interface where substrate concentrations are highest. However, oxygen will become rapidly depleted and is not renewed as fast as it is in the vadose zone so that anaerobic conditions are likely to develop quickly. Although flowing

groundwater will resupply oxygen to the microorganisms, this type of transport may be slow relative to the oxygen depletion rate. Therefore, as in the vadose zone, a fringe of aerobic activity develops at the edge of the contaminant plume. In the interior of the plume, anaerobic conditions predominate and electron acceptors other than oxygen must be used by the microorganisms.

Microorganisms in the saturated zone are likely to exist as small colonies. These colonies may be the result of cell division and agglomeration due to extracellular polymers or may be communities of synergistic organisms. Because of the sparing solubility of most typical NAPL contaminants, the colonies are likely to be relatively thin, so that the contaminant concentrations within the colony are the same throughout and perhaps the same as the substrate concentrations dissolved in the bulk aqueous phase. However, if the NAPL constituents, nutrient and electron acceptor fluxes into the colonies are sufficiently high, a thicker biofilm may form so that NAPL constituents must diffuse across not only a liquid boundary layer but also within the biofilm in order to be utilized by microorganisms throughout the biofilm interior. Even if no thick biofilm forms, NAPL constituents may have to diffuse across a stagnant liquid layer to the biomass before they can be biodegraded. If the bacteria are supplied with sufficient substrate and electron acceptor, they may form a continuous film and alter the porosity, permeability and diffusivity of the aquifer.

As NAPL components dissolve out of the NAPL residual, the NAPL phase shrinks, and the dissolved constituents move with the groundwater flow. Because of dispersion, the dissolved constituents at the front edge of the plume are diluted so that more oxygen is available for aerobic respiration. Mixing also occurs along the edges of the plume, promoting higher rates of aerobic respiration there. The more adsorbable components of the NAPL will move through the aquifer at a rate slower than the average groundwater flow. Dissolved oxygen in the groundwater entering the rear edge of the plume will promote aerobic respiration at this edge also, so that adsorption may increase the rate of aerobic respiration there. However, adsorption may decrease biodegradation in other parts of the aquifer by making the adsorbed compounds less available to microorganisms. Reaeration of the aquifer from the vadose zone, if it is significant, will favor aerobic conditions at the water table surface.

As the plume moves through the aquifer, microorganisms first encountering the plume must acclimate to the dissolved NAPL. Since this takes some time, the leading edge of the dissolved NAPL plume may not be biodegraded and a pulse of contamination may move through the aquifer. This effect may be partly mitigated through detachment of microorganisms from acclimated communities within the plume. The detached microorganisms may move as free floating bacteria or move attached to colloid particles at a rate faster than the average groundwater movement. They may become attached to soil ahead of the plume and be able to begin degrading the plume as soon as it reaches them.

On the pore scale, biodegradation may be limited by microorganisms' inability to diffuse into small or dead-end pores so that NAPL contaminants remain separated from microorganisms until the decrease in bulk concentration causes them to diffuse out. If microorganisms can reach these small pore spaces, biodegradation may be limited by the rate of electron acceptor diffusion into these areas. Depending on the pore geometry and location of microorganisms within the pores, biodegradation may be diffusion limited, kinetically limited, diffusion limited in some areas and kinetically limited in others, or limited by both processes to varying degrees throughout the medium.

This conceptual description is obviously very complex, and includes just a few of the many physical, chemical and biological processes that we know are important in subsurface biodegradation. In the next section, these factors are described in more detail and the methods other researchers have used to account for them are presented.

4.2 Transport Equations

This section briefly describes the main equations for multi-phase, multi-component NAPL transport. More complete descriptions of the governing equations and solution methods can be found in other sources (Abriola, 1989; Pinder and Abriola, 1986; Corapcioglu and Baehr, 1987).

Although multi-phase flow and solute transport equations may be written in innumerable ways, most developments begin with basic mass balance equations, supplemented by constitutive relationships to solve the system for all of the variables. This discussion of the flow equations follows the development by Abriola (1989).

4.2.1 Mass Balance Equations

One mass balance equation can be written for each constituent in each phase. The basic form of the mass balance equation is:

$$\frac{\partial}{\partial t}(\theta_\alpha \rho^\alpha \omega_i^\alpha) + \nabla\cdot(\theta_\alpha \rho^\alpha \omega_i^\alpha \mathbf{v}^\alpha) - \nabla\cdot\mathbf{J}_i^\alpha = R_i^\alpha + r_i^\alpha \qquad (1)$$

where:

α = phase

i = component

θ_α = volumetric content of phase alpha (volume of phase α/total volume)

ρ_α = density of phase alpha (M/L^3)

ω_i^α = mass fraction of species i in phase alpha

$\mathbf{v}^\alpha$ = average linear velocity of phase alpha relative to the solid phase (L/T)

$\mathbf{J}_i^\alpha$ = non-advective flux of species i in the α phase (M/L^2T)

R_i^α = rate of exchange of mass of species i due to interphase diffusion and/or phase change (M/L^3T)

r_i^α = rate of creation of species i in phase α (M/L^3T)

In this equation, the product $\theta_\alpha \rho^\alpha \omega_i^\alpha$ has units of mass of i per unit volume of porous media (the concentration of species i). The overall dimensions of the equation are M/L^3T.

The four phases typically modeled are the solid, aqueous, NAPL and air phases. The mass balance equations can be summed over all phases to give a mass balance equation for each species in all phases. When the mass balance equations are summed in this manner so that individual chemical species can be tracked, the approach is often termed compositional. The summation yields an equation of the following form for each chemical species:

$$\sum_\alpha^{np} \left\{ \frac{\partial}{\partial t}(\rho^\alpha \theta_\alpha \omega_i^\alpha) + \nabla\cdot(\rho^\alpha \theta_\alpha \mathbf{v}^\alpha \omega_i^\alpha) - \nabla\cdot\mathbf{J}_i^\alpha \right\} = \sum_\alpha^{np} r_i^\alpha \qquad (2)$$

where np is the number of phases. The first term in the above equation represents the accumulation of component i. The second term accounts for component i advective flux.

The third term represents component i transport by diffusion and mechanical dispersion. The last term represents the rate of production or destruction of component i. Note that the R_i terms drop out of the above equation since all chemical species are conserved across phase boundaries. The non-advective flux term is represented by (Abriola, 1989):

$$\mathbf{J}_i^\alpha = \rho^\alpha \theta_\alpha D^\alpha \cdot \nabla \omega_i^\alpha \tag{3}$$

where D^α is the hydrodynamic dispersion tensor (L^2/T).

The mass balance equations are constrained by the following requirements (Pinder and Abriola, 1986):

$$\sum_i^{nc} \omega_i^\alpha = 1 \qquad \sum_\alpha^{np} \theta_\alpha = 1 \tag{4}$$

A mass balance equation is written for each NAPL constituent, electron acceptor, growth nutrient, and microbial population being modeled. The reaction term (r_i^α) is the part of the mass balance equation of primary interest in this report. Microbial degradation of the NAPL constituents of interest are accounted for through this term by substitution of the appropriate biodegradation kinetic expression. Separate reaction terms can be included to account for non-biological reactions such as rate-limited adsorption and abiotic degradation.

4.2.2 Conservation of Momentum

In order to be solved, the average linear phase velocity must be determined for each phase. The velocities are found through the conservation of momentum equation in the form of Darcy's Law:

$$\mathbf{v}^\alpha = \frac{q}{\theta_\alpha} = -\frac{\mathbf{k}\, k_{r\alpha}}{\mu_\alpha \theta_\alpha} \cdot (\nabla P^\alpha - \rho^\alpha g \nabla z) \tag{5}$$

where:

$\mathbf{v}^\alpha$ = average linear velocity of phase alpha relative to the solid phase (L/T)

q = specific discharge (Darcy velocity, L/T)

$\mathbf{k}$ = intrinsic permeability (L^2)

$k_{r\alpha}$ = relative permeability for phase alpha

μ_α = viscosity of phase alpha (M/LT)

P^α = pressure of phase alpha (M/LT^2)

g = acceleration of gravity (L/T^2)

z = elevation (L)

Relative permeabilities are a function of phase saturations (volume of phase/volume of pore space) and can be predicted from laboratory data or semi-empirical models.

4.2.3 Constitutive Relations

In addition to the constraints on mass fractions and volumetric contents, the mass balance equations are coupled by other constitutive relations. These constitutive relations include:

- capillary pressures = $f(P^\alpha, P^\beta)$
- saturations = f (capillary pressures)
- densities = f (phase pressure, composition of phase)
- viscosity = f (pressure, phase composition)
- equilibrium partition coefficients or kinetic expressions for interphase mass transfer

The above constitutive relationships make the system of equations highly nonlinear so that they must be solved using numerical methods.

4.2.4 Simplifications for Column Studies

Many of the models reviewed in this report describe biodegradation in only one dimension and only account for a single phase – the aqueous phase. In addition, density, viscosity, and permeability are often considered constant and capillary effects do not apply. In this case, the mass balance equations for each constituent reduce to:

$$\frac{\partial C_i}{\partial t} = D_i \frac{\partial^2 C_i}{\partial x^2} - v_x \frac{\partial C_i}{\partial x} - r_i \qquad (6)$$

where C_i is the concentration of the chemical species of interest.

4.3 Physical Phenomena Affecting Biodegradation

The transport equations presented above include terms to account for adsorption and dispersion, two phenomena that are very important in modeling biodegradation. In addition to these phenomena, other physical factors must be considered in biodegradation modeling. These factors can either be explicitly incorporated into the model equations or need to be considered in evaluating the parameters used in the model. Physical factors affecting biodegradation are discussed below. Where appropriate, methods used by other researchers to include these factors are described.

4.3.1 Hydrodynamic Dispersion

Diffusion and mechanical dispersion cause a spreading of the solute front as the front travels through the subsurface in the saturated zone. Diffusion is the movement of a solute from an area of high concentration to an area of less concentration and occurs whenever there is a concentration gradient, regardless of whether or not the fluid is in motion (Fetter, 1993). Mechanical dispersion is mixing caused by differing fluid velocities along the flow path due to three different phenomena (Fetter, 1993):

- solutes travel faster through large pores than through small ones;
- solutes travel over different path lengths for the same displacements because of the tortuous nature of the subsurface matrix;
- frictional forces cause solute velocities in the center of pores to be higher than velocities along the pore walls.

Longitudinal dispersion causes spreading along the direction of flow, while transverse dispersion causes spreading in a direction normal to the flow. In solute transport modeling, diffusion and mechanical dispersion are usually combined into a parameter called the hydrodynamic dispersion tensor, D_{ij}. D_{ij} is defined for longitudinal and transverse dispersion of component β and phase α as follows (Sleep and Sykes, 1993):

$$D^{\alpha}_{\beta ij} = a_T |v_\alpha| \delta_{ij} + (a_L - a_T)\frac{v_{\alpha i} v_{\alpha j}}{|v_\alpha|} + \delta_{ij} D^{\alpha}_{\beta m,ij} \tau_\alpha \qquad (7)$$

where:

$v_{\alpha i}$, $v_{\alpha j}$ = longitudinal and transverse components of the average linear velocity of phase α (L/T)

a_L , a_T = longitudinal and transverse dispersivities (L)

δ_{ij} = Kronecker delta function

$D^{\alpha}_{\beta mij}$ = molecular diffusion coefficient (L^2/T)

τ_{α} = tortuosity

At the laboratory scale, dispersion is well understood and can be readily quantified for a given experiment. In the field, however, dispersivity is scale dependent and is not a characteristic constant for the aquifer (Fetter, 1993). In general, dispersivities tend to increase as solute travel distances increase. The variability in dispersivity causes complications in solute transport modeling. All of the solute transport models reviewed in this study used a constant hydrodynamic dispersion coefficient to account for diffusion and dispersion.

Diffusion of solutes is practically negligible for most saturated aquifers, and mechanical dispersion is usually the dominant cause of solute front spreading. However, diffusion can be important at low flow velocities. The relative importance of these two phenomena can be determined from the Peclet number, defined as:

$$Pe = \frac{v\,d}{D_m} \tag{8}$$

where:

Pe = dimensionless Peclet number

v = average linear velocity (L/T)

d = average aquifer grain diameter (L)

D_m = molecular diffusion coefficient of the solute (L^2/T)

The Peclet number is the ratio of the rate of transport by advection to the rate of transport by diffusion. Longitudinal diffusion is usually not important for $Pe > 6$, and transverse diffusion is usually not important for $Pe > 100$ (Fetter, 1993). However, if solutes diffuse into dead-end pores or small pores where the velocity is much slower than the average velocity, intraparticle diffusion can cause extensive tailing of solute fronts. Intraparticle diffusion is discussed briefly by Valocchi (1985). The effects of intraparticle

diffusion on biodegradation are discussed by Chung et al., (1993). At the pore scale, intraparticle diffusion may cause solutes to be unavailable to microorganisms if the microorganisms are too large to penetrate deep into the pores. Solutes diffusing into the pores can only be degraded when they diffuse back out under induced concentration gradients caused by biodegradation in the bulk liquid phase (Chung et al., 1993).

As pointed out by Lee et al. (1988), intraparticle diffusion can also have important effects on mixing, especially during high flow rates induced by pumping. Solutes may become trapped in dead-end pores or other areas of low permeability during the relatively slow movement of groundwater before remediation begins. When water carrying nutrients for in-situ bioremediation is pumped through the aquifer, the water will tend to flow through the large pores and may not mix with the trapped solute. Models that assume complete mixing of substrates and nutrients without taking diffusion effects into account could substantially overpredict biodegradation.

Although intraparticle diffusion has not been explicitly incorporated into any transport models reviewed for this report, it may be possible to model it as dispersion (Valocchi, 1985). In this case, intraparticle diffusion could be accounted for as an increase in dispersivity. It may also be possible to model intraparticle diffusion as a rate-limited adsorption process (Fetter, 1993). Fetter (1993) identifies work potentially applicable to biodegradation modeling done by Raven et al. (1988) in which diffusion into an immobile fluid zone along fractures is considered. More theoretical work is necessary to determine how to best account for intraparticle diffusion and determine whether or not it is an important process for biodegradation modeling.

On a macroscopic scale, dispersion can be very important to biodegradation modeling. As a result of hydraulic conductivity differences between vertical aquifer layers, flow velocities within different aquifer layers will not be equal. Dissolved NAPL constituents will move with different velocities in these different layers. At any given point, the groundwater may contain contaminants or it may not, depending on the layer's hydraulic conductivity and distance from the source of contamination (Freeze and Cherry, 1979). These aquifer heterogeneities are typically modeled by including areas of high and low permeability in the modeling grid and including a term for dispersion. Since we are often interested in the average concentration of contaminants in the aquifer at a particular

point, this treatment of dispersion adequately accounts for the observed spreading of the contaminant front.

In biodegradation modeling, however, the actual local concentrations of the contaminants are important because the rates of degradation depend on the local concentrations, not vertically averaged concentrations. Models based on spatially averaged concentrations could either over- or under-estimate biodegradation rates depending on whether nutrients, electron acceptors, or substrate were limiting the biodegradation reactions. For example, the extent of biodegradation could be overestimated significantly if a substrate is toxic. In this case, the vertically averaged concentration might indicate that the contaminant concentration in some locations is below some threshold toxicity and that biodegradation will proceed, when the actual concentration is much more than the vertically averaged concentration, and no biodegradation occurs at all. True three-dimensional modeling may be necessary to adequately describe biodegradation, especially when distinct, continuous vertical heterogeneities exist.

Since most of the models reviewed in this report are one-dimensional, the effect of dispersion on biodegradation is not readily apparent. The effect of dispersion on biodegradation was investigated by Borden and Bedient (1986) using their two-dimensional model. They concluded that transverse dispersion was the dominant source of oxygen for biodegradation as a result of mixing of hydrocarbon plumes with oxygenated formation water. Longitudinal dispersion had little effect. Since aerobic biodegradation can be the dominant biodegradation mechanism in some aquifers, the increased mixing caused by dispersion is very important. If a constant dispersion coefficient is used to describe dispersion, however, the mixing can be considerably overestimated with a corresponding overestimation of biodegradation rates. Borden and Bedient (1986) reported that transverse dispersion causes greater aerobic biodegradation at a hydrocarbon plume's sides and causes the plume to appear much narrower than expected.

4.3.2 Adsorption

Adsorption is "the process in which matter is extracted from the solution phase and concentrated on the surface of the solid material" (Weber, 1972). Adsorption results in the distribution or partitioning of solutes between the solid and fluid phases. Adsorption can be modeled as an equilibrium process or as a kinetic process. If the rate of adsorption and desorption is fast relative to other processes occurring in the aquifer, the solute(s) can be assumed to be at equilibrium between the fluid and solid phases. This assumption is called the local equilibrium assumption (LEA). The applicability of the LEA has been studied for some time by a number of researchers (Valocchi, 1985; Bahr and Rubin, 1987; Harmon et al., 1992).

If the LEA is applicable, then the equilibrium partitioning of solutes between the solid and liquid phases can be described by an isotherm in which the solid phase concentration is some function of the solute concentration in the bulk liquid. The most common isotherm relationships are the linear isotherm, Freundlich isotherm and Langmuir isotherm (see Fetter, 1993). The simplest partitioning relationship is the linear isotherm where the solute concentration on the solid is a linear function of the bulk fluid solute concentration. In this case, the mass of solute adsorbed onto the solid is (Fetter, 1993):

$$C^* = K_d C \tag{9}$$

where:

C^* = mass of solute sorbed per mass of solid (M solute/M solid)

C = fluid phase solute concentration (M/L^3)

K_d = distribution coefficient (L^3/M solid)

This description of adsorption is used in most of the transport models reviewed in this report. It is usually valid at low solute concentrations. At higher substrate concentrations, the equilibrium partitioning between solute and solid phase is often nonlinear. In this case, the Freundlich and Langmuir nonlinear equilibrium adsorption isotherms could be more accurate than the linear adsorption isotherm.

For situations where the LEA is not applicable, kinetic expressions must be used. The reversible linear kinetic sorption model describes the rates of sorption and desorption as first-order according to (Fetter, 1993):

$$\frac{dC^*}{dt} = k_f C - k_r C^* \qquad (10)$$

where:

k_f = forward (sorption) rate constant

k_r = backward (desorption) rate constant

This expression was used by Semprini and McCarty (1992) to model biodegradation of dissolved chlorinated organics at the Moffet Naval Air Station field site. Semprini and McCarty (1992) reported that their model did not accurately predict the observed concentrations with a simple linear equilibrium model, but predicted the data well when adsorption was treated as a first-order reversible reaction.

The first-order reversible adsorption model reduces to the linear equilibrium sorption expression if equilibrium is assumed ($dC^*/dt = 0$). Similar rate expressions can be developed from the Freundlich or Langmuir isotherm equations. If adsorption is assumed to be controlled by diffusion, then a diffusion-controlled rate expression can be used to described adsorption as described by Fetter (1993).

Surface diffusion (movement of a sorbed compound over the solid surface) can also affect adsorption. If surface diffusion occurs slowly relative to liquid diffusion, it may dominate the adsorption process.

Adsorption is important in modeling biodegradation, and could either increase or decrease the biodegradation rate (McCarty, 1988). Adsorption could increase biodegradation by concentrating nutrients in the subsurface, by immobilizing substrates so that microorganisms have more time to degrade them, or by immobilizing nutrients so that water-borne nutrients flow into the area (Borden and Bedient, 1986; Lee et al., 1988). Conversely, adsorbed solutes may reduce biodegradation by making the solute unavailable to microorganisms in the water phase, or by reducing the rate of biodegradation in the water phase by reducing the fluid phase concentration (Lee et al., 1988; McCarty, 1988).

Speitel and DiGiano (1987) modeled the regeneration of activated carbon by biofilms and determined that the substrate flux into the biomass from the sorbed phase was greater than the substrate flux from the liquid phase. This suggests that contaminants adsorbed on particles before significant biomass growth occurs can be substantially biodegraded by biomass growing on the particles at later times. Lee et al. (1988) reported

studies in which adsorption increased or decreased biodegradation and postulated that sorption may increase biodegradation under oligotrophic (nutrient poor) conditions by concentrating nutrients, but may decrease biodegradation under nutrient-rich conditions by competing with microorganisms for substrate. Simulations of BTEX degradation performed by Borden and Bedient (1986) indicated that adsorption may enhance biodegradation by allowing oxygen to continuously move into the retarded contaminant plume. This would supply more oxygen and increase biodegradation under oxygen limited conditions. From these and other studies it is apparent that the effects of adsorption are complex and could increase or decrease the biodegradation rate. More research on the effects of adsorption on biodegradation is needed.

Adsorption could also have important implications for both microorganism and NAPL movement. Migration of highly sorbed compounds has been observed to exceed the expected migration rates as predicted by the compounds' retardation factor (Corapcioglu and Jiang, 1993). The unexpectedly high migration rate may be due to the compounds' adsorption onto colloidal particles (including bacteria) that travel through the aquifer much faster than the average groundwater velocity (Corapcioglu and Jiang, 1993). Column studies have verified this effect (Lindqvist and Enfield, 1992; Jenkins and Lion, 1993).

4.3.3 Reaeration

Reaeration from the ground surface could be a major oxygen source for aerobic microorganisms and could be important in modeling biodegradation. A significant mass of NAPL vapors can vaporize from contaminated pore water and groundwater, entering the air phase of the vadose zone where they can dissolve into pore water near the surface. The oxygen-rich conditions near the surface could accelerate removal by biodegradation. Oxygenation of the groundwater from reaeration could provide substantial oxygen to oxygen-poor groundwater when the vapor pressures of the NAPL components are low. Borden and Bedient (1986) reported that vertical exchange of oxygen and hydrocarbon with the unsaturated zone may significantly enhance the rate of biodegradation. In multi-phase, multi-component models, reaeration should be accounted for as an additional mass transfer process.

4.3.4 Temperature

The biochemical reactions that microorganisms carry out are affected by temperature just like non-biological reactions. Growth of *pseudomonad* bacteria, a genus known to degrade a variety of organic compounds, is usually optimal at temperatures between 25°C and 30°C (Focht, 1988), whereas groundwater temperatures can be significantly lower. Dibble and Bartha (1979) found that biodegradation of oil sludge in soil was negligible at 5°C, occurred only after a 2-week lag period at 13°C, but was significant above 20°C. Focht (1988) reports that the Q_{10} (difference in reaction rate for a 10°C difference in temperature) for most biological systems is 1.5 to 3. In a review, Atlas (1981) reported that the rate of oil biodegradation was affected by temperature, although the ultimate extent of transformation of petroleum compounds was not. In some cases, Atlas (1981) reported a greater extent of biodegradation at low temperatures than at high temperatures. Atlas (1981) points out that temperature affects the composition of petroleum mixtures through volatilization and dissolution as well as the rates of biodegradation.

Since biodegradation modeling is still in an early stage of development, many models attempt only to describe biodegradation in simple laboratory column studies so that temperature effects are not relevant. In simulations of biodegradation in actual aquifers, the incorporation of temperature effects is not always clear. For example, Borden and Bedient (1986) modeled the migration of a creosote plume using data from rate studies conducted with actual aquifer material. However, the temperature of the test conditions and in-situ groundwater were not explicitly given, and some parameters were taken from the literature. Sykes et al. (1982) used a maximum growth rate value of ½ the measured value to account for lower temperatures in the aquifer being modeled. MacQuarrie et al. (1990) assumed that the biodegradation kinetic parameters were independent of temperature and used values determined from laboratory experiments.

Two points regarding temperature for biodegradation modeling in the subsurface are important. First, because the rates of biodegradation are dependent on temperature, caution must be used in extrapolating results of biodegradation experiments carried out at typical laboratory temperatures to actual biodegradation in the subsurface. Not only are reaction rates slower at lower temperatures, but the Arrehenius relation may not hold

below temperatures of 10°C, making predictions of reaction rates at lower temperatures difficult (Focht, 1988). Second, the effects of temperature on biodegradation are complex, since temperature affects not only biochemical reactions but also NAPL phase transfer and transport (Atlas, 1981). These points must be considered when modeling NAPL biodegradation.

4.3.5 pH

Bacteria live in subsurface environments under a wide pH range. Bacteria have been reported in environments with pH ranging from 3 or below to over 10 (Chapelle, 1993). However, bacteria living in these pH extremes are usually adapted to the environment. Most bacteria prefer a pH in the neutral range (Chapelle, 1993). Natural waters tend to buffer the pH so that it remains around neutral. However, the pH in contaminated environments can be drastically altered by contaminants (Chapelle, 1993).

The pH of an aquifer has at least two important effects on subsurface biodegradation. First, the pH affects the type of microorganisms present and will select for those most adapted to the pH environment. Second, the pH, together with the reduction potential, will determine the ionic form of ionizable species in the groundwater. Both of these factors affect the type of biodegradation that can occur. For example, nitrification is inhibited at pH values less than 6 (Lee et al., 1988). Biodegradation reactions can change the pH of aquifers if the amount of oxidized material is large enough (Bouwer and McCarty, 1984). Generally, biodegradation is optimum at a pH of neutral to slightly alkaline (Lee et al., 1988; Focht, 1988).

The pH can be controlled in laboratory experiments so that biodegradation modeling is relatively unaffected by pH. As a consequence, most models of biodegradation do not explicitly consider pH. In field systems, the pH should be determined before modeling begins. If biodegradation reactions are expected to significantly alter pH, then pH shifts should be included in the model, especially if electron acceptors other than oxygen are involved.

4.3.6 Reduction Potential

The reduction potential (*Eh*) is an indication of the degree of oxidation of dissolved constituents in an aqueous solution. Positive *Eh* values indicate that most NAPL constituents are oxidized and are typical of aerobic aquifers. Negative *Eh* values indicate that most constituents are in reduced form so that there is a high potential for redox reactions to occur. Another way of thinking about *Eh* is that at very positive *Eh* values, there are many chemical species present which can accept electrons and few donors, while at low values of *Eh* there are many electron donors but few acceptors. Low *Eh* values also indicate that the electron acceptors present will yield less energy in redox reactions than the electron acceptors present at high *Eh* values.

The *Eh* value of an aquifer is important because it reflects the thermodynamic potential for different redox reactions to occur. As each electron acceptor, beginning with oxygen, is consumed in biodegradation reactions, the *Eh* decreases. As the *Eh* decreases, redox reactions in which other electron acceptors participate become thermodynamically favored (Freeze and Cherry, 1979). This process results in a sequential utilization of electron acceptors (see Section 4.4.3).

Eh is an important variable when modeling anaerobic biodegradation with multiple electron acceptors because it is an indicator of when anaerobic biodegradation reactions are thermodynamically favored. None of the models reviewed in this report account for changes in *Eh*, although researchers did consider *Eh* when determining the potential for denitrification or desulfurization reactions to occur (Frind et al., 1990). The *Eh* value of the aquifer can be modeled by tracking the concentrations of all dissolved species that could participate in redox reactions.

4.4 Microbial Community

Most models of subsurface biodegradation assume that a population of microorganisms exists that can degrade the contaminants under study. These microorganisms are implicitly assumed to occur in subsurface locations where the contaminant is accessible. While studies have shown that bacteria in shallow aquifers are capable of degrading a variety of organic compounds (Chapelle, 1993), the distribution and composition of the subsurface microbial community can have a significant effect on

the rates at which biodegradation occurs. This section describes the occurrence of microorganisms in the subsurface and explains the importance of the microbial community on modeling biodegradation. The methods by which some of these factors have been incorporated into biodegradation models are contained in the next section.

4.4.1 Number and Distribution of Subsurface Microorganisms

4.4.1.1 Macroscopic Scale

The vast majority of microorganisms in subsurface soils are bacteria, and their numbers decrease with increasing depth (Chapelle, 1993). The upper 1 to 2 meters of the vadose zone, called the soil zone, is the most biologically active and varied subsurface microbial habitat due to its proximity to vegetation at the ground surface. Fewer microbiological studies of deeper soil zones have been made, but the few studies completed indicate that microorganisms exist at appreciable numbers down to the water table. Total bacterial counts of 10^6 to 10^7 cells per gram of dry sediment were measured in one study (Chapelle, 1993). Very deep soils in dry environments may contain less than 10^5 cells/g, and these cells may contain less than 100 viable cells/g (Chapelle, 1993). The low viable cell counts in these relatively dry environments could be due to a lack of moisture, which all microorganisms require to survive.

The total number of microorganisms in the saturated zone usually depends on the type of flow system being studied. Three types of flow systems can be defined (Chapelle, 1993). A local flow system has its recharge area at a topographic high and its discharge area at a topographic low located adjacent to the topographic high. Local flow systems are typically water table aerobic aquifers with short hydraulic residence times. Because of their proximity to the surface, local flow systems may contain most of the cases of water pollution. In intermediate flow systems, recharge and discharge areas are separated by one or more topographic highs. Intermediate flow systems are typically anaerobic and often consist of confined aquifers less than 300 m in depth (Chapelle, 1993). Regional flow systems are characterized by a recharge area at a water divide and a discharge area at the bottom of the basin. Regional aquifers are characterized by very low groundwater flow rates, anaerobic conditions, and very high dissolved mineral concentrations (Chapelle, 1993).

The three different flow systems tend to support different types and numbers of microorganisms. Microorganism concentrations of 10^6 to 10^7 cells/g have been reported in local flow systems (Lee et al., 1988), and these bacteria are typically aerobic (Chapelle, 1993). In intermediate flow systems, microbial counts are typically lower, and concentrations of 10^5 to 10^6 cells/g have been reported. Little data are available on the numbers of microorganisms in regional flow systems, probably because of their relative unimportance as drinking water sources and their distance from surface contamination sources.

The concentration of bacteria is an important parameter in biodegradation modeling because substrate utilization rates depend on the biomass concentration (see Section 4.5). In the saturated zone, most modelers assume that microorganisms are present at a concentration of around 10^6 cells/gram of aquifer material (Chen et al., 1992; Wood et al., 1994). However, the concentration of microorganisms varies tremendously between the soil zone and the saturated zone, so that a distribution of microorganism concentrations must be used to accurately model biodegradation in all portions of an aquifer. A realistic model would account for both the lateral and vertical initial distribution of biomass.

4.4.1.2 Pore Scale

Little data are available on the distribution of microorganisms at the pore scale. Studies have indicated that most bacteria tend to be attached to solid particles, and that a majority of the attached bacteria are associated with particles less than 20 μm in diameter (Harvey et al., 1984). The data by Harvey et al. (1984) also indicate that bacteria tend to be present as small colonies rather than as continuous films. The lack of continuous films is most likely due to the oligotrophic environment; there are not enough nutrients and growth substrates present to support continuous biofilms. However, when contaminants enter the subsurface, the environment is changed drastically. Continuous films could develop where there is a sufficient concentration of substrate, electron acceptor and nutrients, such as at injection wells. Whether microorganisms are attached as continuous and potentially thick biofilms or as microcolonies has important

implications for biodegradation modeling. The methods by which the microorganism configuration is accounted for in various models are discussed in Section 4.11.

The location of attached microorganisms at the pore scale could also be important for reasons of transport (see Section 4.13) and biodegradation rates. If many bacteria are free-floating, then they may be more easily transported than attached bacteria. For attached bacteria, the location of attachment could have important effects on biodegradation rates. If most bacteria are attached at pore throats, then they would have greater accessibility to substrates and nutrients flowing through the aquifer than bacteria attached at the walls of pore cavities. In pore cavities, substrates and nutrients would have to diffuse from pore centers to the pore walls before they could be utilized by bacteria growing there. This phenomenon could be described with equations similar to those used to describe diffusion-controlled adsorption (Fetter, 1993). Theoretical analyses could indicate whether the physical location of microorganisms has an appreciable effect on subsurface biodegradation rates.

4.4.1.3 Location within Phases and at Interfaces

Microscopic evidence indicates that microorganisms grow only in the water phase (Brock et al., 1984; Atlas, 1981). Therefore, biodegradation can occur only where water is present: in the water phase of the vadose zone and in the saturated zone. Although biodegradation can only occur in the water phase, this does not mean that attached bacteria cannot biodegrade contaminants. On the contrary, since most bacteria are attached to surfaces, biodegradation by attached bacteria may be significant. However, microorganisms' requirement for water does preclude their growth when attached to particles in direct contact with the air or NAPL.

When pure-phase NAPL that can be degraded by microorganisms is introduced in the subsurface, microorganisms will accumulate in the water phase at the NAPL/water interface where substrate concentrations are highest (Atlas, 1981). Thus, the higher the available surface area, the faster biodegradation will proceed (Atlas, 1981). Microorganisms may increase the available surface area and increase NAPL dissolution by releasing emulsifying agents (Atlas, 1981).

Biodegradation at NAPL/water interfaces is typically limited by nutrient availability (Atlas, 1981). If the NAPL constituents are toxic, then microorganisms may not exist at the interface, but may accumulate at a distance at which the toxic constituent concentration is low enough that they can survive.

Biodegradation activity at NAPL/water interfaces may have at least three important implications for modeling. First, if the NAPL is toxic to microorganisms, modeling could overpredict NAPL biodegradation if biodegradation is assumed to occur everywhere within the water phase. Second, NAPL toxicity or nutrient limitations could cause sharp concentration gradients near NAPL phases, which could require very fine spatial grids in computer simulations, resulting in long computer run times. Third, if the NAPL is not toxic, bacteria may release emulsifying agents that could break the NAPL up into small droplets, creating a high NAPL/water interfacial area (Atlas, 1981). This could cause biodegradation rates to be much higher than would be predicted if the total surface area available to microorganisms is assumed to be the surface area of a single continuous interface, such as in an oil slick floating on a water surface.

It is not clear how these implications would actually affect predicted biodegradation rates. Further research in this area is needed.

4.4.2 Acclimation

When placed in a new environment, bacteria usually take time to adjust or *acclimate* to the new environmental conditions. Acclimation is characterized by a lag time during which microorganisms do not grow significantly. The length of the lag period depends on many factors, including the type of organism, the organism's growth rate, the magnitude of the changing conditions, and the nature of the environmental change (Bailey and Ollis, 1986). When the lag period is over, bacteria begin to grow and reproduce normally. Acclimation mechanisms are discussed briefly below. Modeling of the lag period is discussed in more detail in Section 4.7.5.

Microorganisms adjust to changing conditions by three mechanisms (Chapelle, 1993):

1. Induction of specific enzymes not present (or present at low levels) before exposure to the new conditions;

2. Selection of new metabolic capabilities produced by genetic changes; and,
3. Increase in the number of organisms able to metabolize newly available substrates.

All of these mechanisms are important in subsurface biodegradation, where environmental changes can be dramatic with the introduction of contamination to the subsurface environment. Aerobic organisms typically consume nearly all available oxygen very quickly, so that surviving organisms must adapt to the use of other electron acceptors as well as to the changing environmental conditions (Chapelle, 1993). Acclimation is affected by the kinds of organic compounds and their relative concentrations, the time of exposure to the compounds, and the similarity of the compounds to those the microorganisms are accustomed to using as substrates (Chapelle, 1993). Some xenobiotics typically are metabolized without any lag time at all, while others are metabolized only after a significant lag time (Lee et al., 1988).

Acclimation can have important effects on biodegradation modeling. When a spill occurs and contamination is introduced to the subsurface, it will take time for the microorganisms present to begin biodegradation. As the contaminant plume moves in the saturated zone, the edge of the contaminant front may move past microorganisms before they have time to acclimate to the contaminants as substrates. Thus, a pulse of contamination may spread out from the spill source even though microorganisms are capable of using the contaminants as substrates (Wood et al., 1994). Models that do not take acclimation into account may not accurately predict the spatial distribution of the plume concentration. Dispersion may help to mitigate this effect. Additional research is needed to quantify the effects of acclimation on contaminant plume movement in biodegradation models.

Of the models reviewed in this report, only the model of Wood et al. (1994) accounted for acclimation. The method by which a lag period was accounted for by this model is discussed in Section 4.7.5.

4.4.3 Microbial Community Composition and Capabilities

Studies indicate that the subsurface microbial community is metabolically active, nutritionally diverse, and usually oligotrophic because of the low substrate concentrations

and refractory nature of substrates in the subsurface environment (Lee et al., 1988). As a result, subsurface microorganisms are capable of utilizing a variety of substrates, including xenobiotic compounds. Some strains of microorganisms are capable of degrading NAPL compounds alone, while other biodegradation reactions can only be completed by microorganisms with the aid of other microbial strains.

The interdependence of some strains of bacteria that carry out biodegradation reactions is important for biodegradation modeling, particularly when conditions are anaerobic. Under anaerobic conditions, microorganisms utilize alternate electron acceptors in an order of those yielding the most energy from redox reactions to those yielding the least. Thus, in groundwater systems, electron acceptors will usually be used in the following order: nitrate, iron(III), sulfate, CO_2, and conditions become increasingly reducing (Lee et al., 1988; Freeze and Cherry, 1979). As the contaminant plume travels, different electron acceptors and redox conditions may prevail downstream from the initial point, and each particular environment may tend to favor the transformations of different constituents of the NAPL plume (Bouwer and McCarty, 1984). This phenomenon, depicted graphically in Figure 3, was shown to exist in studies of the Middendorf aquifer in South Carolina (Chapelle, 1993). In order to model these successive reactions successfully, the aquifer *Eh*, pH and electron acceptor concentrations

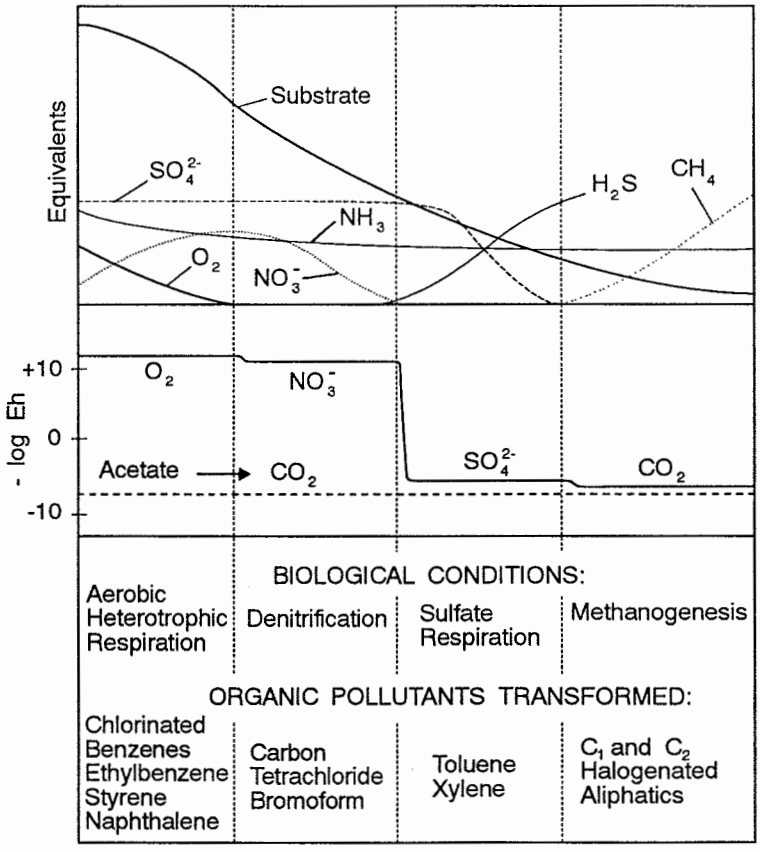


Figure 3. Potential microbially mediated reactions in an aquifer (Bouwer and McCarty, 1984).

magnitude of the changing conditions, and the nature of the environmental change (Bailey and Ollis, 1986).

At the end of the lag period, the population of microorganisms is well adapted to its new environment. During this exponential period, microorganisms reproduce rapidly according to the expression (Bailey and Ollis, 1986):

$$\mu = \frac{1}{X}\frac{dX}{dt} = \mu_{max} \tag{11}$$

where:

X = concentration of cells (M/L^3)

μ = specific growth rate of cells (T^{-1})

μ_{max} = maximum specific growth rate (T^{-1})

t = time (T)

Exponential (first-order) growth continues until some nutrient necessary for growth becomes limiting, at which time the population enters the stationary period, where the cell concentration reaches its maximum. In the stationary period, nutrient limitations prevent further increase of the microbial concentration. Some cells die and lyse, and other cells utilize the nutrients released by the lysed cell for growth. Eventually, due to severe nutrient depletion or toxic build-up, the population begins to decline and the death period begins (Bailey and Ollis, 1986). The death period is usually described as an exponential decay, where the number of cells that die at any time is a constant fraction of those living.

In the subsurface, microorganisms can go through each of these periods. In the saturated zone in front of a contaminant plume, for example, the indigenous microflora are probably in the stationary period or simply dormant since substrates are in scarce supply. When the contaminant plume encounters the microorganisms, the microorganisms must adapt to the new conditions and substrates so that there is a lag before they begin growing. When they are adapted to the new conditions, the microorganisms begin growing exponentially until some nutrient limits their growth. The exponential period may be quite short or nonexistent in most aquifers, where electron acceptors are likely to severely limit growth regardless of substrate concentrations. After the plume has passed, the microorganisms enter the stationary period, where there is no longer a supply

must be known so that the appropriate biodegradation reactions can be modeled (Bouwer and McCarty, 1984).

Although no models reviewed in this report model *Eh*, pH and electron acceptor concentrations, several models account for multiple electron acceptors (Frind et al., 1990; Widdowson et al., 1988; Kindred and Celia, 1989; Kinzelbach and Schafer, 1991; Chen et al., 1992). The way in which the multiple electron acceptors are accounted for is discussed in the section 4.7.9.

4.5 Microorganism Growth Periods

Since microorganisms in the subsurface experience varying concentrations of substrate and electron acceptor, their growth rate and stages may be constantly changing. Therefore, the growth cycles of subsurface micro-organisms may show some similarity to microbial growth in batch cultures. Microorganisms in batch culture experience four distinct growth periods (Bailey and Ollis, 1986). These periods, shown graphically in Figure 4, are the lag period, exponential growth period, stationary period and death period.

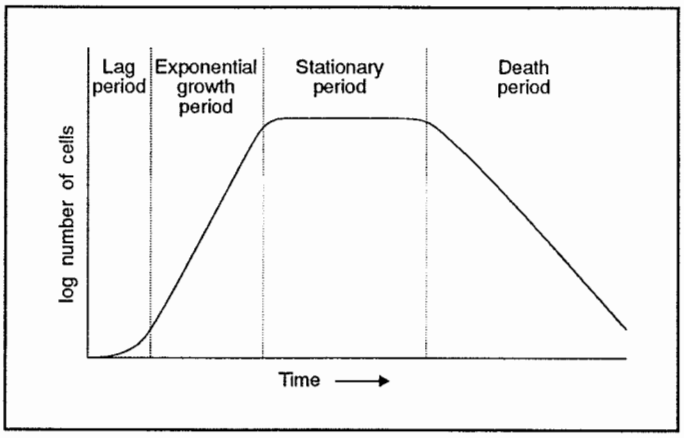


Figure 4. Microorganism growth periods (Bailey and Ollis, 1986).

In the lag period, micro-organisms adapt to changing environmental conditions while growing little or none at all. The lag period is thought to be due to the microorganisms' synthesis of new enzymes and other necessary chemicals used in the metabolic pathways necessary to survive in the new conditions. A lag in growth may also occur when microorganisms are transferred into an environment of different ionic strength. Multiple lag periods are sometimes observed when a culture is fed two substrates, one of which is preferentially utilized. A lag occurs when the organism switches from the exhausted preferred substrate to the second substrate. This phenomenon is called diauxic growth. The length of the lag period depends on many factors, including the type of organism, the organism's growth rate, the

Unstructured and unsegregated models have the advantage of simplicity over structured models. In unstructured, unsegregated models, only one variable need be considered. This variable can be treated similar to a solute in a solution, and no consideration of different cell components is necessary. The unstructured, unsegregated assumption is common in the environmental field. In wastewater treatment, for example, the cells are typically represented by an average mass concentration. All of the biodegradation models reviewed in this study are unstructured, unsegregated models. Therefore, in all of these models, biomass is described as a single component, although it may exist in more than one phase.

Structured models may be able to describe batch microbial growth more effectively than unstructured models, but at the expense of considerable added complexity. Three types of structured models are briefly discussed below.

In compartmental models, the microbial population is assumed to be unsegregated, but the biomass is compartmentalized into a small number of components. The components can be cellular components important to the cells' growth, such as the concentration of DNA or a particular protein of interest. The model is justified on the basis of system dynamics. All of the processes occurring within a cell have a characteristic relaxation time, or time to reach equilibrium after a perturbation. The relaxation time can be used to determine the rate at which cellular processes occur with respect to environmental changes. Components depending on processes that have very long relaxation times can be taken as constant. Components depending on processes that have very fast relaxation times can be assumed to be at quasi-steady state. However, components that depend on processes that occur at the same time scale as environmental changes are included in the model. Since relaxation times vary by many orders of magnitude, the number of compartments is typically only two or three in these models. Compartmental models have potential application in biodegradation modeling.

Metabolic models describe growth based on the metabolic processes cells undergo. These models typically require a fairly detailed knowledge of the important metabolic processes in order to describe experimental data accurately. The models are both structured and segregated, since different cellular components and the change of these components with time are both modeled. Metabolic models are most useful when a single

of substrate for growth. After a time, the population begins to die off once nutrients are completely exhausted.

All of these growth periods are important in biodegradation modeling. The lag period is typically called acclimation and is discussed further in Section 4.7.5. The growth and death periods are central to biodegradation modeling and are discussed further in Section 4.7 in the context of microbial growth kinetics.

4.6 Models of Microbial Growth

A given microbial population consists of individual cells, each carrying out a complex array of chemical reactions necessary to survive. However, a description of all of the processes occurring in the microbial population is not practical, so models have been developed to describe how the average population behaves. Microorganism population growth can be described in several ways, depending on the assumptions made about the population as a whole. The descriptions of the different growth models given below are based on the treatment by Bailey and Ollis (1986).

Microbial growth can be described by unstructured or structured models. Unstructured models assume that the microbial population can be characterized by a single variable; for example, cell number or mass concentration. In structured models, cells are recognized to consist of multiple interacting components that can be described separately, e.g. DNA, ATP, or some key enzyme (Bailey and Ollis, 1986)

The collection of cells that make up a microbial population can also be viewed as either single entities, which they are, or as a single "average" cell. If the population is modeled as individual cells, the model is called segregated. If the population is viewed as a collection of cells with the same average characteristics, the model is called unsegregated. Unsegregated models can be applied when growth is balanced, i.e., when cell components are constant with time as in exponential growth. During the lag and stationary periods of batch growth, balanced growth generally does not occur, and different cells in the population may have very different characteristics, depending on their age, location, or other factors. As a result, unsegregated models may not be a good approximation to bacterial growth during these periods.

substrate concentration is reduced by the stoichiometric amount that can be oxidized by the available electron acceptor. The reaction is assumed to occur in each gridblock where substrate and electron acceptor are both present. This treatment of biodegradation reactions is used by Borden and Bedient (1986) and was adopted by Corapcioglu and Baehr (1987) for the case where biodegradation is limited by oxygen transport into the contaminant plume.

The instantaneous reaction model has two important advantages over models in which biodegradation kinetics are explicitly considered. First, no estimate of kinetic parameters is needed. Since kinetic parameters are difficult to estimate accurately, especially for field modeling, this is a significant advantage. Second, biomass is assumed to be constant. As a result, the equations governing the transport and biodegradation of contaminants are much easier to solve because the equation for microbial growth is eliminated and there are no nonlinear kinetic expressions.

The instantaneous reaction model may be applicable to when biodegradation kinetics are fast relative to the transport of oxygen into the contaminant plume (Rifai and Bedient, 1990). In cases where groundwater velocities are fast or biodegradation reaction rates are slow, the assumption of an instantaneous reaction is not a good approximation of the physical situation. To help determine when the instantaneous reaction model is appropriate, Rifai and Bedient (1990) compare model results from two runs of the same model in which instantaneous reaction kinetics is used in one run and biodegradation kinetics in the other. For the example problem, Rifai and Bedient provide quantitative data on when the instantaneous reaction assumption approximates the more rigorous kinetic approach in terms of a Damköhler number, Da_1, and a dimensionless concentration product, π_2. The Damköhler number is the ratio of a chemical reaction rate to the rate of advective transport. Rifai and Bedient defined Da_1 as:

$$Da_1 = \frac{kL}{v} \tag{12}$$

where:

k = maximum specific rate of substrate utilization (T^{-1})

L = length of the modeling domain (L)

v = average velocity (L/T)

metabolic pathway controls metabolic rates, or when the interaction of all metabolic rates is well understood. The complexity of metabolic models probably makes their application to subsurface biodegradation modeling impractical.

Cybernetic growth models describe the effects of cellular regulatory processes as the outcome of an optimization strategy (Bailey and Ollis, 1986). These models can be used to describe the growth dynamics of a population on multiple substrates. The advantage of these models is that kinetic parameters can be determined based on kinetic studies on single substrates, since the interaction of multiple substrates is accounted for in the optimization modeling. Cybernetic models are potentially useful in subsurface biodegradation modeling, but much more research is needed before they can be applied.

4.7 Substrate Biodegradation Kinetic Expressions

Expressions for microorganism growth and substrate utilization, together with the transport equations described in Section 4.2, form the basis for biodegradation models. A number of kinetic expressions exist to describe decrease in substrate concentration. The particular kinetic expressions used depend on assumptions about the microbial population and growth, since in most cases substrate utilization is assumed to result in a biomass increase. The form and complexity of these expressions depend on the type of growth model (unstructured/structured; segregated/unsegregated) and factors specifically included in the model such as inhibition. Three basic types of substrate utilization kinetics are typically used in the biodegradation models reviewed in this report: 1) instantaneous reaction, 2) Monod kinetics, and 3) first-order kinetics.

4.7.1 Instantaneous Reaction

The assumption of an instantaneous reaction is equivalent to assuming that the reaction rate is infinitely fast so that kinetics can be ignored altogether. In this case, sufficient biomass is assumed to be present so that substrate and electron acceptor react stoichiometrically. If the electron acceptor is in excess, then all of the substrate in contact with the electron acceptor is assumed to react, and the electron acceptor concentration is reduced by the stoichiometric amount required to oxidize the substrate to CO_2. If substrate is in excess, then all of the electron acceptor is assumed to react and the

The Monod equation expresses the microbial growth rate as a function of the nutrient that limits growth. The expression is of the same form as the Michaelis-Menton equation for enzyme kinetics but was derived empirically. The limiting nutrient can be a substrate, electron acceptor, or any other nutrient such as nitrogen or phosphorous that prevents the cells from growing at their maximum (exponential) rate. The nutrient limitation is expressed in the form of a Monod term multiplying the maximum growth rate. The Monod equation is:

$$\mu = \mu_{max}\left(\frac{S}{K_s + S}\right) \tag{14}$$

where:

μ = specific growth rate (T^{-1})

S = substrate concentration (M/L^3)

μ_{max} = maximum specific growth rate (T^{-1})

K_s = half saturation constant (value of S at which μ is ½ μ_{max}, M/L^3)

The term in parentheses is the Monod term. Note that equation (14) is simply the expression for exponential cell growth multiplied by a Monod or growth limiting term. The functional form of this expression for batch growth, and the effects of the Monod parameters K_s and μ_{max}, are shown in Figure 5. The maximum specific growth rate (μ_{max}) and K_s must be determined experimentally for each substrate and microbial culture.

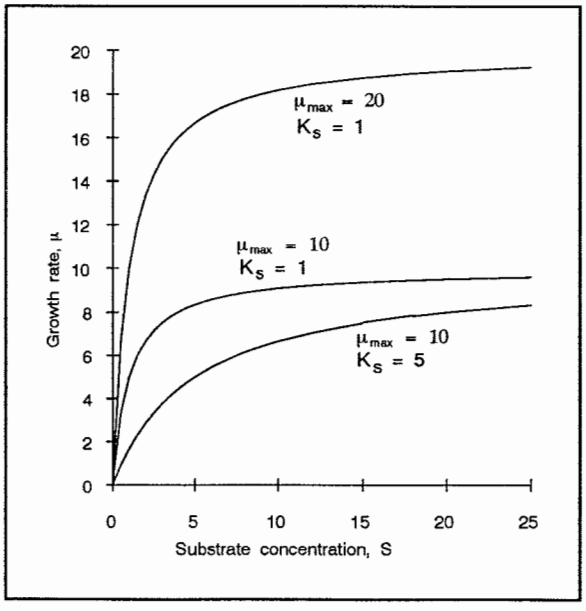


Figure 5. Functional form of Monod kinetics and effect of K_s and μ_{max} on reaction rates.

Studies have shown that the Monod expression underpredicts the cell concentrations in continuous flow reactors at low dilution rates (long hydraulic residence times) (Bailey and Ollis, 1986). This phenomenon can be explained considering endogenous decay. Endogenous decay consists of internal

The dimensionless concentration product was defined as:

$$\pi_2 = \left(\frac{C_o}{K_c + C_o}\right)\left(\frac{O_o}{K_o + O_o}\right) \qquad (13)$$

where:

C_o, O_o = initial substrate and oxygen concentrations (M/L^3)

K_c, K_o = substrate and oxygen half-saturation constants (M/L^3)

For the particular situation that they examined, the instantaneous reaction model approached the kinetic model as Da_1 increased and as π_2 approached 1. The differences in the results of the two approaches were also a function of the half-saturation constants of the substrate and oxygen, and of the initial oxygen concentration. For $Da_1 > 2{,}500$, the instantaneous reaction model differed from the kinetic model by approximately 20%.

Although the instantaneous reaction model may be applicable in some situations, Rifai and Bedient (1990) make the important observation that its applicability varies with time and space in the modeling domain. Because biodegradation reaction rates are generally a function of both microorganism concentration and limiting nutrient concentrations (including substrate, electron acceptor, or other nutrients) and because these concentrations vary spatially, different biodegradation reaction rates will typically be observed at different points in the modeling domain and at different times. Therefore, the instantaneous reaction rate model may apply only to part of the domain only part of the time. If the instantaneous reaction rate model is used, constant checks on its applicability would have to be made to ensure that the assumption was valid where it was being used. The kinetic biodegradation model would have to be used at locations where the instantaneous model was not valid. The mixing of the two kinetics could add complexity to a biodegradation model. This is an important disadvantage of the instantaneous reaction model.

4.7.2 Monod Kinetics

The Monod equation is the most popular kinetic expression applied to modeling groundwater biodegradation. This discussion is based on the treatment by Bailey and Ollis (1986).

cellular reactions that consume cell substance. The endogenous decay term is also sometimes conceived of as a cell death rate or maintenance energy rate and represents cells in the death period of the microbial growth cycle. Endogenous decay is accounted for by adding a decay term to the Monod expression:

$$\mu = \mu_{\max}\left(\frac{S}{K_s + S}\right) - b \tag{15}$$

where b is the endogenous decay rate constant (T^{-1})

Under oxygen or other electron acceptor limited conditions, the endogenous decay term can be multiplied by a Monod term for the limiting electron acceptor. This approach is taken by Molz et al. (1986), Widdowson et al. (1988), and Semprini and McCarty (1991).

Substrate utilization is determined by dividing the Monod expression by a yield coefficient, $Y_{x/s}$. The yield coefficient must also be determined experimentally. Substitution of the yield coefficient into the Monod expression for microbial growth results in the following expression for substrate utilization:

$$r_s = \frac{dS}{dt} = -\frac{r_x}{Y_{x/s}} = -\frac{\mu_{\max} X}{Y_{x/s}}\left(\frac{S}{K_s + S}\right) \tag{16}$$

where:

X = biomass concentration (M/L^3)

r_s = rate of substrate utilization (M/L^3T)

r_x = rate of biomass growth (M/L^3T)

S = substrate concentration (M/L^3)

$Y_{x/s}$ = biomass yield coefficient (mass of cells formed/mass of substrate consumed)

The constant quotient $\mu_{\max}/Y_{x/s}$ is often called k, the maximum specific substrate utilization rate, so that the Monod equation for substrate utilization becomes:

$$r_s = -kX\left(\frac{S}{K_s + S}\right) \tag{17}$$

Two limiting conditions of the Monod equation should be noted. First, when the substrate concentration is sufficiently low that $K_s >> S$, then the Monod equation becomes $dS/dt = -k'XS$ where $k' = k/K_s$. In this situation, the Monod equation predicts that the substrate utilization is linearly dependent on S (first-order with respect to S). When all nutrients are present in great excess so that $K_s << S$, the substrate utilization rate is independent of S and equal to $-kX$ (zero order with respect to S). It is important to note that the substrate utilization is first-order with respect to the biomass concentration, X, regardless of the substrate concentration.

Most of the models reviewed in this report use Monod kinetics to describe subsurface biodegradation. Monod kinetics may not be applicable to all biodegradation reactions, however, and use of the Monod expression should be justified based on some other information before it is used. In particular, Monod kinetics may not be applicable when substrate concentrations are very low (Bailey and Ollis, 1986).

4.7.3 First-Order Kinetics

Some substrate biodegradation rates follow reaction kinetics in which the biodegradation rate is first-order with respect to the substrate concentration:

$$r_s = -kXS \tag{18}$$

Note that the biodegradation rate in this expression is also first-order with respect to biomass concentration so that the reaction is second-order overall. Wood et al. (1994) found that this type of first-order kinetic expression best described the disappearance of quinoline from a groundwater system, although they modified the first-order kinetics with a Monod term for oxygen limitation. In this case, first-order kinetics were justified by experiments.

Brusseau et al. (1992) used pseudo first-order kinetics in which the biodegradation rate is first-order with respect to substrate concentration only to describe the disappearance of an arbitrary substrate, and applied the equations to the disappearance of 2,4,5-T in soil columns. They justified the use of pseudo first-order kinetics by assuming that microbial growth was negligible and that no nutrient, substrate or electron acceptor limitations existed. Under these assumptions, X can be treated as a constant in equation

(17), and the growth rate is $r_s = -k'S$ where $k' = kX/K_s$. Brusseau et al. (1992) determined the parameters for their simulations independently of the data being simulated. Their simulations matched data obtained from the literature quite well.

As discussed above, Monod substrate utilization kinetics reduce to pseudo first-order kinetics in S for very low substrate concentrations if X is assumed to be constant. The advantage of using pseudo first-order kinetics is that the kinetic expressions are linear and can be solved more easily than the nonlinear equations that Monod kinetics produces. When data indicate that pseudo first-order kinetics are applicable throughout the range of expected concentrations, pseudo first-order kinetics should be used. If pseudo first-order kinetics cannot be justified throughout the entire range of expected concentrations, then Monod kinetics or some other kinetic expression should be used. Use of first-order or pseudo first-order kinetics as an approximation to Monod kinetics when Monod kinetics are applicable would tend to over-predict substrate destruction.

4.7.4 Other Growth Kinetics

Many other forms of growth kinetics are provided in the literature. Three of the most common alternative expressions include (Bailey and Ollis, 1986):

Tessier:

$$\mu = \mu_{max}(1 - e^{-S/K_s}) \tag{19}$$

Moser:

$$\mu = \mu_{max}(1 + K_s S^{-\lambda})^{-1} \tag{20}$$

Contois:

$$\mu = \mu_{max}\left(\frac{S}{BX + S}\right) \tag{21}$$

The Tessier equation is based on the assumption of a diffusion-controlled substrate supply (Luong, 1987). The Moser expression is similar to the Monod equation except that the substrate concentration is raised to the power λ. The Contois expression contains

an apparent Michaelis constant that is proportional to biomass concentration (X). The maximum growth rate diminishes as X increases, eventually leading to $\mu \propto 1/X$ (Bailey and Ollis, 1986). Sarkar et al. (1994) used Contois kinetics to describe the anaerobic degradation of glucose by *B. licheniformis* JF-2 in a multi-phase microbial transport model.

4.7.5 Lag Period

Most researchers ignore the lag period in biodegradation modeling either because the systems being modeled are acclimated to the contaminants in advance (in column studies, for example) or because the phenomenon is not well understood (Borden and Bedient, 1986). However, as discussed in Section 4.4.2, the lag period can be important in modeling biodegradation. Wood et al. (1994) modeled the lag period with a metabolic potential function given by:

$$\lambda = 0 \qquad t < \tau_L$$

$$\lambda = \frac{\tau - \tau_L}{\tau_E - \tau_L} \qquad \tau_L \leq t \leq \tau_E \tag{22}$$

$$\lambda = 1 \qquad t > \tau_E$$

where:

λ = metabolic potential function (dimensionless)

τ = time that microorganisms in a given volume have been in contact with the inducing substrates (T)

τ_L = lag time (length of time for significant growth to begin; T)

τ_E = length of time required to reach exponential growth (T)

The function λ multiplies the biomass growth and substrate utilization terms that depend on electron acceptors. λ increases from 0 to 1 over the acclimation period τ_L to τ_E. After the acclimation period is over, λ no longer limits biomass growth or substrate utilization.

Wood et al. (1994) determined the lag time parameters τ_L and τ_E in separate experiments. Inclusion of this expression for lag time in the simulations of quinoline

degradation in soil columns indicated that a pulse of quinoline would travel through the column before the microorganisms became acclimated to the substrate and began degrading it. The pulse predicted by the model matched the experimental data well. The authors noted that dispersion had a significant effect on this pulse. Because dispersion causes spreading of the substrate, the trailing edge of the substrate pulse was in contact with the microorganisms longer than the front edge. Because of the longer contact time, microorganisms in contact with the trailing edge of the pulse acclimated to the substrate and began biodegrading it. This caused a sharpening of the trailing edge of the pulse.

Incorporation of lag into biodegradation models is likely to be important when groundwater contaminants move fast relative to their rate of disappearance from the bulk liquid phase. This might occur when contaminants are very slowly biodegrading or when groundwater velocities are very high. High dispersivities should tend to decrease the effects of acclimation by increasing contact time for the trailing edge of the leading edge of the plume and by decreasing the concentration of the leading edge of the plume, reducing the concentration of any pulse that may develop. The need for including acclimation is therefore dependent on both the flow conditions and factors affecting biodegradation rates.

4.7.6 Inhibition Kinetics

Many xenobiotic compounds are toxic to microorganisms at higher concentrations. Other organic compounds degrade into toxic intermediates or final products. Kinetic expressions have been developed to incorporate this toxicity, and some of these kinetic expressions have been used by researchers to model subsurface biodegradation. The kinetic parameters for these expressions can be determined from laboratory experiments.

4.7.6.1 Substrate Inhibition

A very simple method of accounting for substrate inhibition is to assume that no biodegradation occurs when the substrate concentration is above some critical level. This method was used by Corapcioglu et al. (1991) in modeling the cometabolism of tetrachloroethene (PCE) and TCE in laboratory columns under methanogenic conditions.

A popular kinetic expression for substrate utilization with substrate inhibition is (Grady, 1990):

$$r_s = -kX\left(\frac{S}{K_s + S + S^2/K_i}\right) \tag{23}$$

where:

K_s = substrate half saturation constant (M/L^3)

K_i = inhibition coefficient M^2/L^6)

This expression is similar to the expression for Haldane enzyme inhibition kinetics (Luong, 1987) and can be derived from enzyme kinetics considerations (Bailey and Ollis, 1986). As the substrate concentration increases, this equation predicts Monod behavior until the substrate concentration reaches a maximum. The rate then decreases because of the S^2 term in the denominator. When K_i is very large, the equation predicts Monod behavior for the entire range of substrate concentration. The expression has been used to successfully model substrate inhibition by other researchers (Bailey and Ollis, 1986).

The above equation predicts that some growth occurs for inhibitory substrates even at very high concentrations. However, it has been observed that growth ceases altogether at sufficiently high concentrations of inhibitory substrates (Grady, 1990). Grady (1990) identifies equations proposed by Luong (1987) and Han and Levenspiel (1988) that account for the cessation of growth at high inhibitory substrate concentrations:

$$r_s = -kX\left(1 - \frac{S}{S^*}\right)^n \frac{S}{S + K_s\left(1 - \frac{S}{S^*}\right)^m} \tag{24}$$

where:

S^* = critical substrate concentration above which growth stops (M/L^3)

m = exponent depicting the impact of the substrate on K_s (M/L^3)

n = exponent depicting the impact of the substrate of μ_{max}.

Luong's equation is the same, except that $m = 0$ (Grady, 1990). Other expressions for substrate inhibition are given by Luong (1987). The Luong (1987) model represented the inhibition of a batch culture growing on butanol better than three other models tested.

4.7.6.2 Product Inhibition

Product inhibition occurs when biodegradation end products inhibit biodegradation of the original substrate. An equation for modeling product inhibition substrate utilization is:

$$r_s = -kX\left(\frac{S}{K_s + S}\right)\left(\frac{K_p}{K_p + P}\right) \tag{25}$$

where P is the product concentration and K_p is a product inhibition coefficient (Bailey and Ollis, 1986). Sarkar et al. (1994) used a product inhibition term of this form to model anaerobic growth on glucose when lactic acid and 2,3-butanediol were expected to accumulate. This expression could also be used to account for substrate inhibition.

Luong (1987) identifies the following equation for product inhibition:

$$r_s = -kX\left(\frac{S}{K_s + S}\right)e^{-p/K_i} \tag{26}$$

where the terms have the same meaning as in equation (25). Many of the expressions for substrate inhibition may also be used for product inhibition as discussed by Luong (1987).

Kindred and Celia (1989) present a method of accounting for any type of inhibition, including product inhibition, through an inhibition factor $I(i)$ defined as:

$$I(i) = 1 + \frac{Q_i}{k_i} \tag{27}$$

where:

i = subscript indicating the inhibiting substance

Q_i = concentration of inhibiting substance (M/L^3)

k_i = inhibition constant (M/L^3)

If the product or substrate is inhibiting, then the biodegradation rate is divided by this expression. In this case, the expression is identical to the inhibition term in equation (25). The biodegradation rate of an inhibiting substrate could then be expressed as:

$$r_s = -\frac{kX}{I(s)}\left(\frac{S}{K_s + S}\right) = -\frac{kX}{(1 + S/k_s)}\left(\frac{S}{K_s + S}\right) \qquad (28)$$

where S = substrate concentration. At low substrate concentrations, $I(s)$ (= $1 + S/k_s$) is approximately equal to 1, and no inhibition is observed. At high substrate concentrations, $I(s)$ becomes larger and larger, and the biodegradation rate approaches 0 as S grows large.

4.7.6.3 Competitive Inhibition

When two or more compounds serve as substrates for a microbial population, the compounds can be degraded simultaneously, sequentially, or simultaneously with competition (Chang et al., 1993). Competitive inhibition may be observed in this situation if the same enzymes are required for degradation of more than one compound. Competitive inhibition may also be observed in cometabolic processes where the cometabolites compete with the primary growth substrate for enzyme sites (Semprini and McCarty, 1992). Grady (1990) points out that a different fraction of biomass could be responsible for degradation of the different compounds so that experiments may be necessary to accurately predict substrate utilization.

The most popular kinetic expression for competitive inhibition in the groundwater modeling literature is of the general form (Bailey and Ollis, 1986):

$$r_{s_1} = \frac{ds_1}{dt} = -\frac{k_1 X S_1}{K_{s_1} + S_1 + K_{s_1} S_2 / K_{s_2}} \qquad (29)$$

$$r_{s_2} = \frac{ds_2}{dt} = -\frac{k_2 X S_2}{K_{s_2} + S_2 + K_{s_2} S_1 / K_{s_1}} \qquad (30)$$

where:

r_{s1}, r_{s2} = utilization rates of substrates 1 and 2 (M/L^3T)

k_1, k_2 = maximum specific substrate utilization rates of substrates 1 and 2 (T^{-1})

S_1, S_2 = concentrations of substrates 1 and 2 (M/L^3)

K_1, K_2 = half-saturation constants of substrates 1 and 2 (M/L^3)

An alternative way of writing (29) is (Alvarez-Cohen and McCarty, 1991):

$$r_{s_i} = \frac{ds_i}{dt} = -\frac{k_i X S_i}{K_{s_i}\left(1 + \frac{S_j}{K_{s_j}}\right) + S_i} \tag{31}$$

This expression has been used by a number of researchers to describe competitive inhibition in cometabolism of multiple substrates (Chang et al., 1993; Alvarez-Cohen and McCarty, 1991; Semprini and McCarty, 1992); and by Kindred and Celia (1989) to model competitive inhibition for aerobic biodegradation of multiple substrates. An expression of this type was used by Chang et al. (1993) to accurately describe competitive inhibition and cometabolism of benzene, toluene, and p-xylene biodegradation by two *pseudomonas* isolates in batch laboratory cultures. Alvarez-Cohen and McCarty (1991) found that this expression accurately predicted TCE and chloroform (CF) biodegradation by methanogenic resting cells in batch cultures when the expression was coupled to a more complex cometabolism model.

A modified form of the expression given above was used by Semprini and McCarty (1992) to model transport and cometabolic biodegradation of VC, t-DCE, c-DCE and TCE at the Moffett Naval Air Station field site. The expression was modified by multiplying by a Monod term to account for oxygen limitations as the electron acceptor. The inhibiting compound for the model was methane, as the concentrations of the chlorinated compounds were negligible in comparison to the methane concentration. The model was able to accurately predict the breakthrough curves of the substrates only when competitive inhibition was included, which demonstrates the importance of competitive inhibition in some systems.

Malone et al. (1993) modeled the transport and biodegradation of benzene, toluene and xylene in a gasoline mixture with a competitive inhibition expression similar to the one above. The substrate utilization expression in their model was:

$$r_{s_i} = -\left[\frac{k_i X S}{\left(K_i + p_{int} S_{int}^{\ a} + \sum_{j=1}^{M} p_{ij} S_i^{\ a}\right)}\right]\left(\frac{O}{K_o + O}\right) \tag{32}$$

where:

S_i = concentration of substrate i

k_i = maximum specific substrate utilization rate of component i (T^{-1})

p_{ij} = Yoon's inhibition constant ($p_{ii} = 1$)

K_i, K_{int} = half-saturation constant for compounds i and intermediate (M/L^3)

O = oxygen concentration (M/L^3)

K_o = oxygen half-saturation coefficient (M/L^3)

In this model, the substrates are first converted to intermediate compounds (S^a_{int}). The intermediates are then biodegraded to CO_2. Malone et al. (1993) were able to accurately simulate the laboratory column biodegradation of a benzene, toluene and xylene mixture using the full model.

4.7.7 Cometabolism

The kinetics of cometabolism have been addressed by a number of researchers (Anderson and McCarty, 1994; Criddle, 1993; Chang et al., 1993; Corapcioglu et al., 1991; Alvarez-Cohen and McCarty, 1991; Bae et al., 1990; Bouwer and McCarty, 1984; Semprini and McCarty, 1992; Kindred and Celia, 1989). The simplest method to account for cometabolism is to model the disappearance of the cometabolite as a first-order process with respect to the cometabolite. Such a method can be viewed as a simplification of the Monod equation for low substrate concentrations. The disappearance of the cometabolite is modeled by the expression (Bouwer and McCarty, 1984):

$$\frac{dS}{dt} = -\frac{kX}{K_s}S \tag{33}$$

where:

S = concentration of secondary substrate undergoing cometabolism (M/L^3)

X = biomass concentration (M/L^3)

k = specific secondary substrate utilization rate (T^{-1})

K_s = half-saturation constant of secondary substrate (M/L^3)

Bouwer and McCarty (1984) used this expression to model the steady-state cometabolism of chlorobenzene and 1,4-dichlorobenzene in laboratory columns under

methanogenic conditions where the biomass was assumed to exist as a biofilm. The model correctly predicted the disappearance of these compounds and correlated the disappearance of acetate with the disappearance of the chlorinated compounds. Bouwer and McCarty concluded that a first-order expression was adequate when the cometabolite concentration is very low. Corapcioglu et al. (1991) also used first-order kinetics to model the successive cometabolic conversion of PCE to TCE, DCE and finally VC by a methanogenic culture in laboratory columns. Corapcioglu et al. (1991) assumed that first-order kinetics were adequate because the influent PCE and TCE concentrations were very low. The data of Vogel and McCarty (1985) were used to test the model. The model parameters were determined by fitting the experimental data to a linear plot. The model did an adequate job of matching the experimental data from Vogel and McCarty's (1985) experiments.

Other researchers have used an unmodified Monod expression to model cometabolism. Bae et al. (1990) used a Monod expression to model the steady-state utilization of several halogenated compounds in laboratory columns by denitrifying biofilms. Bae et al. (1990) conducted the experiments at three different flow rates. The kinetic parameters were determined from one run at one flow rate, and these same parameters were used to predict cometabolic removal of the chlorinated compounds at the other two flow rates. The model slightly overestimated the chlorinated organics substrate profiles but successfully predicted the decrease in substrate concentration to a steady-state concentration. A general Monod expression modified by the incorporation of competitive inhibition was used by Anderson and McCarty (1994) to model the cometabolic degradation of TCE by methanogenic biofilms. No verification studies were performed, but the simulation results were consistent with published data.

Semprini and McCarty (1992) used a modified form of the Monod expression to model methanotrophic cometabolism of TCE, DCE and VC in groundwater at the Moffet Naval Air Station field site. In this study, methane and oxygen were pulsed into the aquifer to prevent excessive biofilm growth at the injection well and to ensure that the biomass in the entire aquifer was stimulated. Semprini and McCarty (1992) included a deactivation process in their model because studies have shown that cometabolic transformations of these compounds stop when methane injection stops, and that MMO

enzyme activity is deactivated when methane is absent. Biodegradation would be overestimated without accounting for deactivation because the total amount of biomass is not capable of cometabolizing the substrates when methane is not present. The pulsing creates such zones where methane is not present. The kinetic expression used by Semprini and McCarty (1992) in this model is:

$$\frac{dC_2}{dt} = -F_a X k_2 \left(\frac{C_2}{K_{S2} + C_2 + \frac{C_D}{K_i}} \right) \left(\frac{C_A}{K_{SA} + C_A} \right) \tag{34}$$

where:

C_2 = concentration of substrate undergoing cometabolism (M/L^3)

C_D = methane concentration (M/L^3)

C_A = concentration of electron acceptor (M/L^3)

K_{SA} = half-saturation constant of electron acceptor (M/L^3)

K_{SD} = half-saturation constant of methane (M/L^3)

K_{S2} = half-saturation constant for substrate undergoing cometabolism (M/L^3)

K_i = an inhibition constant = K_{SD}/K_{S2}

F_a = fraction of the total microbial population active towards the cometabolic transformation

The above expression includes inhibition in the C_D/K_i term and the limitations of the electron acceptor in a Monod term. This type of inhibition expression is identical to the type in equation (31). The deactivation process is embodied in F_a. When biomass is growing (because methane is present at sufficient amounts to promote growth), F_a = 1, and all of the biomass is capable of cometabolizing the substrates. When the biomass is decaying ($dS/dt < 0$), F_a decreases with time according to:

$$\frac{dF_a}{dt} = -b_d F_a \tag{35}$$

where b_d is the rate constant for a first-order deactivation process. Semprini and McCarty were able to accurately model biodegradation of TCE, DCE and VC using this kinetic expression.

A thorough discussion of even more advanced methods of accounting for deactivation is provided by Criddle (1993). These methods incorporate a transformation capacity that serves to limit the capacity of biomass to cometabolize substrates. These more advanced methods have not generally been incorporated into biodegradation models, although they have been used successfully to predict batch biodegradation reactions (Chang et al., 1993).

4.7.8 Multiple Limiting Substrates and/or Nutrients

It is possible and perhaps even likely that microorganisms in the subsurface are growth limited by more than a single substrate, nutrient, or electron acceptor. In this case, substrate utilization rate limitations can be accounted for by adding additional Monod terms to the expression for substrate utilization (Bailey and Ollis, 1986):

$$r_s = -kX\left(\frac{S_1}{K_{s_1} + S_1}\right)\left(\frac{S_2}{K_{s_2} + S_2}\right)\cdots\left(\frac{S_n}{K_{s_n} + S_n}\right) \tag{36}$$

where S_1, S_2, . . . S_n are the concentrations of limiting substrates, electron acceptors or other nutrients. This expression is the most common method of accounting for multiple nutrient limitations in the groundwater modeling literature, and nearly all of the models reviewed in this report rely on it.

An alternate method of accounting for multiple nutrient limitations is to assume that the most limiting nutrient controls the growth rate so that the rate of substrate utilization is (Widdowson et al., 1988):

$$r_s = -kX\left[min.\left\{\left(\frac{S_1}{K_{s_1} + S_1}\right); \left(\frac{S_2}{K_{s_2} + S_2}\right); \cdots\right\}\right] \tag{37}$$

Use of the latter expression requires that the identity of the limiting nutrient in the single Monod term incorporating the nutrient limitation be changed as the limiting nutrient changes, and could be different in different areas of the modeling domain. This approach was used by Kindred and Celia (1989) to model biodegradation of arbitrary substrates. The advantage of this method is that numerical computations may be easier because the transport equations are less strongly coupled. However, a method of

determining which nutrient is limiting in each model grid must be added and the switching could add considerable complexity to the model.

Other kinetic expressions exist to account for multiple substrate utilization (Roels, 1983). There is no consensus on which kinetic expression for modeling multiple limiting substrates is most accurate (Widdowson el al., 1988), and more research is needed in this area.

4.7.9 Multiple Electron Acceptors

As discussed in Section 4.4.3, some microorganisms are capable of using more than one electron acceptor to oxidize groundwater contaminants. Often, many different strains of microorganisms are present, each with its own particular ability to use one or more electron acceptors. Therefore, as suggested by Bouwer and McCarty (1984), the most sophisticated models should account not only for growth limitations due to one particular electron acceptor, but also for the presence and changes of multiple electron acceptors.

The substrate utilization rate dependence on electron acceptor concentration is usually accounted for by multiplying the substrate utilization rate expression by a Monod term for the electron acceptor concentration as discussed above (equation 36). This is the approach taken by all of the biodegradation models reviewed in this report.

The loss of the electron acceptor is commonly accounted for through the use of a yield coefficient defined as E = *(mass of electron acceptor consumed/mass of substrate consumed)*. The yield coefficient can be determined by considering the stoichiometric requirement for conversion of the substrate being modeled to its end products by the electron acceptor in question. Some electron acceptors are used only for catabolism (e.g. SO_4^{2-}) while other electron acceptors are also used in building biomass (e.g. O_2). Therefore, the energy and growth reactions must both be taken into account in calculating E. The rate of electron acceptor use is then calculated as (Chen et al., 1992):

$$r_i = E_j^i r_{sj}^i \tag{38}$$

where:

r_i = utilization rate of electron acceptor i (M/L^3T)

E_j^i = use coefficient of electron acceptor i under j-based respiration

r_{sj}^i = substrate utilization rate under j-based respiration (M/L^3T)

This approach is taken by, for example, Borden and Bedient (1986), Kindred and Celia (1989), and Chen et al. (1992). Widdowson et al. (1988) also include the loss of oxygen from endogenous decay using the expression:

$$r_o = \gamma Y_o r_{so} + \alpha_o k_o \left[\frac{o}{K_o' + o} \right] \tag{39}$$

where:

r_o = specific oxygen utilization rate (M/L^3T)

γ = oxygen use coefficient for synthesis of biomass

Y_o = cell yield coefficient under oxygen-based respiration

r_{so} = substrate utilization rate under oxygen-based respiration (M/L^3T)

α_o = oxygen use coefficient for endogenous metabolism

k_o = endogenous decay rate constant for aerobic decomposition (T^{-1})

o = microorganism colony/stagnant liquid layer interfacial oxygen concentration (M/L^3)

K_o' = oxygen half-saturation coefficient for endogenous decay (M/L^3)

When multiple electron acceptors may be used by the same population of microorganisms, one electron acceptor usually inhibits respiration using the other available electron acceptor. This type of inhibition is called non-competitive inhibition. For example, some facultative microorganisms are capable of utilizing either oxygen under aerobic conditions or nitrate under anaerobic conditions. The presence of a significant oxygen concentration inhibits denitrification (use of nitrate as an electron acceptor). When the microorganisms exhaust the available oxygen, they switch to nitrate. The inhibition of nitrate respiration can be modeled with an inhibition function such as that in equation (27) as follows (Widdowson et al., 1988):

$$r_n = \eta Y_n r_{sn} + \alpha_n k_n \left[\frac{n}{K_n' + n} \right] I(o) \qquad (40)$$

where:

r_n = specific nitrate utilization rate (M/L^3T)

Y_n = cell yield coefficient under nitrate-based respiration

r_{sn} = substrate utilization rate under nitrate-based respiration (M/L^3T)

η = nitrate use coefficient for biomass synthesis

α_n = oxygen use coefficient for endogenous metabolism

k_n = endogenous decay rate constant for aerobic decomposition (T^{-1})

n = microorganism colony/stagnant liquid layer interfacial oxygen concentration

K_n' = oxygen half-saturation coefficient for endogenous decay (M/L^3)

$I(o)$ = inhibition function (= $1 + O/k_o$; k_o = oxygen inhibition constant)

This approach can be extended to multiple electron acceptors by specifying an inhibition function for each type of respiration based on the concentration of any other electron acceptors that inhibit that type of respiration. This approach is used by Chen et al. (1992) and Kindred and Celia (1989). The inhibition function approach can be used to model the biodegradation of many different compounds, each of which degrade only under certain conditions. The inhibition functions "switch on" the ability of the biomass in any local model grid section to degrade the particular compound, based on the concentration of other compounds that inhibit its respiration.

4.7.10 Incorporation of Kinetic Expressions into Transport Equations

Regardless of the form of the kinetic expressions, they are incorporated into the mass balance equations in a sink term. If no mass transfer resistances are modeled, then the kinetic expressions can be directly substituted into the mass balance equations. For Monod kinetics, the biomass mass balance equation (in one dimension for saturated conditions and ignoring adsorption) is written (Baveye and Valocchi, 1989):

$$\frac{\partial X}{\partial t} = \mu_{max} X \left(\frac{S}{K_s + S} \right) - bX \qquad (41)$$

Examination of this mass balance equation for biomass reveals an important point. The biomass will continue to grow until the substrate concentration drops below some threshold concentration for which the decay of biomass due to endogenous decay equals growth due to substrate utilization (Rittmann and McCarty, 1980). In some cases, the amount of biomass predicted by this equation could exceed the porosity because there is no upper bound on the concentration of biomass. Furthermore, since biomass is expressed as a concentration (dimensions = M/L^3) and not as a volume fraction multiplied by a density, it may not be obvious from the model output whether or not realistic biomass concentrations are being predicted. Since substrate utilization is proportional to biomass concentration, unrealistic biomass concentrations also result in unrealistic substrate utilization rates. In column studies, for example, a model might predict that all of the substrate was utilized at the column inlet if the simulation were run long enough for the biomass to get unrealistically high. Methods of establishing limits on biomass growth are discussed in Section 4.12.

4.8 Multiple Microorganism Populations

Often a single microbial population may not be able to use more than one electron acceptor, but several electron acceptors are available for respiration. In this case, since substrate utilization is a function of both biomass and substrate concentration, multiple microorganism populations must be accounted for to accurately model biodegradation. Multiple populations are handled by writing a separate mass balance equation for each microbial population. The different populations can only grow under the particular type of respiration of which they are capable. Their growth is controlled in the model by using inhibition functions similar to those used to control electron acceptor utilization. This method of modeling multiple microbial populations was used by Chen et al. (1992) and Kindred and Celia (1989). It is usually assumed that growth of each microbial population occurs independently of the others.

4.9 Incomplete Destruction/Multiple Reactions

In most biodegradation models, it is implicitly assumed that the substrate is completely mineralized in a single reaction, even though the actual process consists of a complex sequence of multiple reactions. Electron acceptor utilization and nutrient requirements are then calculated based on this assumption. In some cases, however, the initial substrate may be only partially degraded, either because further degradation requires different electron acceptors or because the product of initial biodegradation is resistant to further microbial attack. If complete mineralization is assumed in model development while only partial degradation physically occurs, then the model will underestimate the degree of disappearance of the primary substrate and overestimate electron acceptor and nutrient requirements. For example, if oxygen is limiting in an aerobic aquifer, then most of the oxygen may be used in the initial stages of biodegradation but may be depleted before the intermediates formed are biodegraded (Malone et al., 1993). In other cases, a sequence of reactions is required to model the conversion of an initial substrate to its intermediate products, some of which may be of particular interest due to their persistence or toxicity. An example would be the sequential transformation of PCE to TCE, DCE and finally VC. Sequential reactions may also be required if the initial substrate produces intermediates that biodegrade further under different types of kinetics, or if the intermediate compounds differ in adsorbability from the initial substrate.

Partial biodegradation is accounted for by adjusting the electron acceptor and other nutrient utilization rates to account for only partial degradation of the primary substrate. Experiments can be performed to measure the rate of disappearance of the primary substrate with time to determine the kinetic parameters. Utilization of electron acceptors and other nutrients can be calculated from the reaction stoichiometry. Multiple reactions, whether they result in one or more intermediates, are handled in the same general way. A mass balance equation is written for the initial substrate and each intermediate of interest. The mass balance equation for the initial substrate includes only a sink term, while the mass balance equations for the intermediates include a sink term as well as a generation term from the generating reactions. These reaction rates can be multiplied by

an inhibition function to control the conditions under which they occur. In this way, multiple reactions can be modeled even if they require different electron acceptors.

Malone el al. (1993) modeled the biodegradation of benzene, toluene and xylene under the assumption that biodegradation occurred in two steps, with the formation of a general intermediate. The system was described by writing one mass balance equation for the partial degradation of the initial substrate and a second mass balance equation for the degradation of the intermediate to carbon dioxide. The model accurately predicted the disappearance of benzene, toluene and xylene from laboratory columns in the high concentration range and captured the general trend of disappearance in the low concentration range.

Wood et al. (1994) used a two-step reaction model to describe the aerobic biodegradation of quinoline in a layered porous media. Laboratory studies indicated that the biodegradation of quinoline to 2-hydroxy-quinoline (2HQ) was first-order, while the biodegradation of 2HQ to CO_2 was assumed to follow Monod kinetics. Model parameters were determined independently of the simulation runs in other laboratory studies. The model successfully predicted breakthrough curves of quinoline, 2HQ and oxygen.

Corapcioglu et al. (1991) modeled the sequential transformation of PCE to TCE, DCE and VC in methanogenic columns using first-order kinetics for all of the reactions. They justified first-order kinetics based on the low substrate concentrations and assumed constant biomass. The model results were fit to the data of Vogel and McCarty (1985) from a separate study and successfully predicted column profiles of these compounds.

4.10 Diffusional Resistances to Mass Transfer

Under some conditions, diffusion resistances to mass transfer can be important in modeling biodegradation. In order to become available to microorganisms, substrates, electron acceptors and other nutrients may first have to diffuse across stagnant liquid layers at the bulk liquid/biomass interface or diffuse deep into the biomass. These diffusion resistances can reduce the chemical concentrations experienced by microorganisms to below the bulk fluid concentration, affecting the rate of biodegradation reactions and biomass growth. In general, biodegradation models have been developed that include either: 1) no diffusional resistances, 2) diffusional resistance across a stagnant

liquid layer adjacent to the biomass, or 3) diffusional resistance in both a stagnant liquid layer and within the biomass itself. Concentration profiles that result from each of these three assumptions are shown in Figure 6. The effect of these different assumptions on the biodegradation model mass balance equations are discussed in the following sections.

4.10.1 No Diffusion Resistances

If no diffusion resistances exist, then the concentration of substrates, electron acceptors and nutrients within the biomass is the same as the concentration of these substances in the bulk fluid (Figure 6a). In this case, the loss of substrate from the bulk liquid is equal to its rate of biodegradation. The biodegradation kinetic expression can then be substituted directly into the bulk liquid substrate mass balance equation (Baveye and Valocchi, 1989). Assuming a one-dimensional, single-phase, saturated porous media with one limiting nutrient (the substrate) for illustrative purposes, the transport and biodegradation of the substrate and microbial growth can be represented by the following system of equations:

$$\frac{\partial S_m}{\partial t} = \frac{\partial}{\partial x}\left(\frac{D\partial S_m}{\partial x} - vS_m\right) - \mu_{\max} X\left(\frac{S_m}{K_s + S_m}\right) \tag{42}$$

$$\frac{\partial X}{\partial t} = \mu_{\max} X\left(\frac{S_j}{K_{s_j} + S_j}\right) - bX \tag{43}$$

where:

S_m = substrate concentration in the bulk (mobile) fluid (M/L^3)

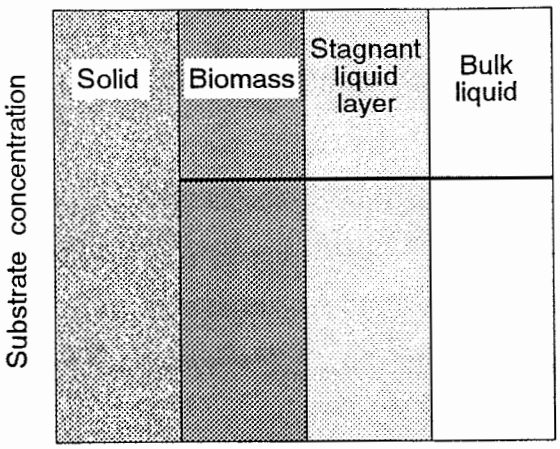
D = dispersion coefficient (L^2/T)

v = average linear groundwater velocity (L/T)

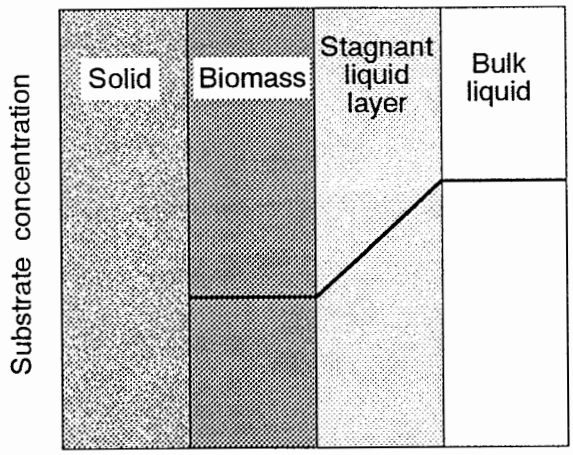
X = biomass concentration (M/L^3)

b = endogenous decay coefficient (T^{-1})

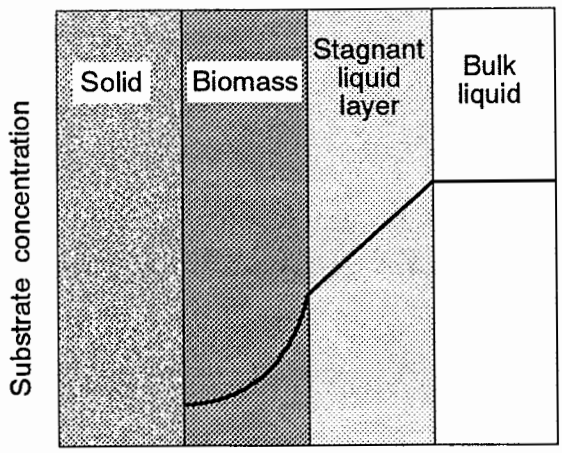
Substrate biodegradation is therefore described with two equations in two unknowns (S_m and X). This approach is taken by Borden and Bedient (1986), Corapcioglu and Haridas (1984), and Kindred and Celia (1989). In effect, the pore



a) No diffusion resistance.



b) Stagnant liquid layer diffusion resistance only.



c) Intra-biomass and stagnant liquid layer diffusion resistances.

Figure 6. Substrate concentration profiles for different diffusion resistance assumptions.

volume in each modeling cell is assumed to be equivalent to a completely mixed reactor containing a homogeneous mixture of substrate, electron acceptor, nutrients and biomass.

4.10.2 Diffusion Resistance from a Stagnant Liquid Layer

In the second approach, chemicals must diffuse from the bulk liquid to the biomass across a stagnant liquid layer that offers resistance to mass transfer. This approach is commonly used in the chemical engineering field and is generally referred to as the "stagnant film model" of mass transfer resistance (Bailey and Ollis, 1986). With this assumption, the system of equations describing substrate biodegradation and biomass growth becomes (Baveye and Valocchi, 1989):

$$\frac{\partial S_m}{\partial t} = \frac{\partial}{\partial x}\left(\frac{D\partial S_m}{\partial x} - vS_m\right) - C(S_m - S_{im}) \tag{44}$$

$$\frac{\partial S_{im}}{\partial t} = C(S_m - S_{im}) - \frac{\mu_{\max} X}{Y_{x/s}}\left(\frac{S_m}{K_s + S_m}\right) \tag{45}$$

$$\frac{\partial X}{\partial t} = \mu_{\max} X\left(\frac{S_j}{K_{s_j} + S_j}\right) - bX \tag{46}$$

where:

C = a mass transfer coefficient expression (L/T)

S_{im} = substrate concentration at the stagnant liquid layer/biomass interface (M/L^3)

In these equations, diffusion within the biomass is neglected so that all of the biomass experiences the same local substrate concentration S_{im}. Unless there are no biological reactions occurring, S_{im} will be less than S_m. As a result, the substrate concentration in the biomass will then be less than the bulk liquid substrate concentration (Figure 6b). The last term in equation (44) is the substrate flux through the stagnant liquid layer. If no diffusional resistances are accounted for, then $S_{im} = S_m$, and the system reduces to the two equations shown in the previous section.

The magnitude of the mass transfer resistance depends on the mass transfer coefficient expression C. The mass transfer coefficient expression C is a function of the biomass/bulk fluid interfacial area, the substrate diffusion coefficient, and the boundary

layer thickness. The value of C can be constant or variable, depending on the conceptualization of the biomass configuration (see Section 4.11).

4.10.3 Diffusion Resistances from the Biomass and a Stagnant Liquid Layer

If the biomass becomes sufficiently thick, there may be diffusion resistances within the biomass itself as well as in a stagnant liquid layer. In this case, the outer layers of the biomass will experience higher substrate concentrations than the inner layers because substrate is degraded before it can diffuse deep into the biomass. The substrate profile that develops is shown in Figure 6c.

Consideration of biomass diffusion resistances results in a system of equations much more complicated than those that consider only a stagnant liquid layer diffusion resistance. These equations are described below in the context of the biomass as a continuous film.

Rittmann and McCarty (1980) presented a model of steady-state biofilm kinetics, which defined many important biofilm concepts. Figure 7 shows the concentration profiles that result under different assumptions about biofilm mass transfer. In a "fully penetrated biofilm," the concentration of a constituent within the biofilm is the same as the concentration at the interface between the biofilm and the stagnant liquid layer. This case corresponds to the case where all mass transfer resistance is contained in the stagnant liquid layer. If no stagnant liquid layer is assumed to exist, then the concentration within the biofilm is equal to the bulk liquid concentration. This corresponds to the assumption of no mass transfer resistance.

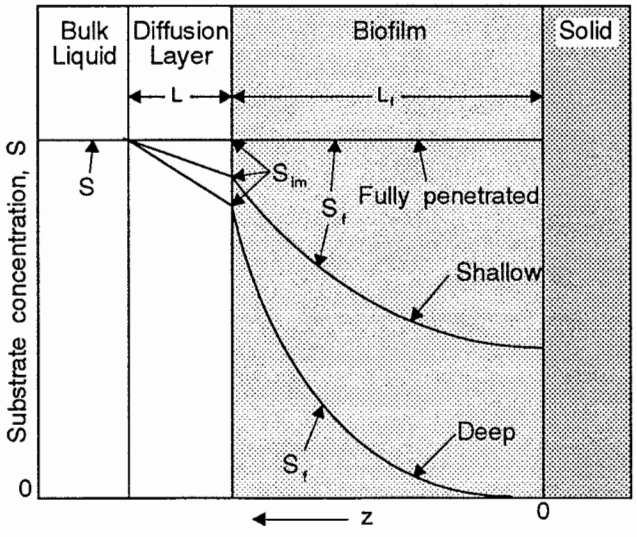


Figure 7. Biofilm substrate concentrations for different assumptions (Rittmann and McCarty, 1980).

If the concentration of the rate limiting chemical reaches 0 at or before the biofilm/solid surface interface is reached, the biofilm is termed "deep". The substrate con-

centration profile is nonlinear because the substrate is being biodegraded in the biofilm, resulting in a continuously changing concentration gradient with distance into the biofilm. In the case of a deep biofilm, the steady-state substrate flux into the biofilm is at its maximum, and the steady-state substrate flux into the biofilm can be calculated (Rittmann and McCarty, 1980).

Biofilms of intermediate thickness between deep and fully penetrated are termed "shallow." To calculate the substrate flux into a shallow biofilm, a mass balance is performed on a differential volume element within the biofilm. The mass balance results in a second order, nonlinear differential equation for the substrate concentration within the biofilm (Rittmann and McCarty, 1980):

$$\frac{\partial S_f}{\partial t} = D_f \frac{d^2 S_f}{dz^2} - k X_f \left(\frac{S_f}{K_s + S_f} \right) \qquad 0 \le z \le L_f \tag{47}$$

where:

D_f = diffusion coefficient of substrate within the biofilm (L^2/T)

S_f = substrate concentration within the biofilm (M/L^3)

X_f = biofilm density (M/L^3)

L_f = biofilm thickness (L)

and z = 0 at the biofilm/solid surface interface. The biofilm substrate profile is usually assumed to be at steady-state because the concentration profile within the biofilm changes rapidly with respect to the biofilm thickness (Rittmann and McCarty, 1981). Therefore, the $\partial S_f/\partial t$ term is usually taken to equal 0. The boundary conditions used to solve this equation are (Sáez and Rittmann, 1988):

$$S_f = S_{im} \qquad at\ z = L_f \tag{48}$$

$$\frac{dS_f}{dz} = 0 \qquad at\ z = 0 \tag{49}$$

The substrate flux through the stagnant liquid layer is assumed to be equal to the substrate flux into the biofilm. This is expressed mathematically as:

$$J_{im} = J_f \tag{50}$$

The stagnant liquid film substrate flux is given by:

$$J_{im} = k_m(S_m - S_{im}) \tag{51}$$

where k_m is a mass transfer coefficient (L/T). The flux into the biofilm is given by:

$$J_f = D_f \frac{\partial S_f}{\partial z} \qquad at\ z = L_f \tag{52}$$

where J_f = substrate flux into the biofilm (M/L^2T). The system of equations is augmented by an additional equation for the change in biofilm thickness:

$$\frac{dL_f}{dt} = \int_0^{L_f} \left(\frac{YkS_f}{K_S + S_f} - b' \right) dz \tag{53}$$

where:

dz = local biofilm thickness (L_f, L)

b' = total mass loss coefficient (including decay and shear, T^{-1})

This is a system of five equations (47, 50, 51, 52 and 53) and five unknowns (S_f, S_{im}, J_{im}, J_f and L_f). If the biofilm thickness is assumed to be at steady-state (the biofilm thickness remains constant), the term on the left-hand side of (53) is 0, and L_f can be calculated directly:

$$L_f = \frac{J_f Y}{bX_f} \tag{54}$$

The solution to the steady-state model is typically found by putting the equations in dimensionless form and solving them by iterative methods (Rittmann and McCarty, 1980). Pseudo-analytical solutions to steady-state biofilms are also available (Sáez and Rittmann, 1988, 1992) in which the numerical solutions to the differential equations are accurately approximated with algebraic equations.

In modeling substrate utilization with intra-biomass diffusional resistances, the substrate flux expression is the sink term in the substrate transport equation. Additional complexity is added because the intra-biomass mass balance equation must be solved

simultaneously with the expression for mass flux into the biomass. Additionally, the substrate may not be the limiting nutrient for microbial growth. In the case of two potentially limiting nutrients, diffusion of one may limit growth in the outer portion of the biomass, and the diffusion of the other may limit growth in the inner portion, or either may limit growth in the entire thickness of the biomass. These limitations may change with time and space in the modeling domain. For additional limiting nutrients, the situation becomes even more complicated.

4.10.4 Biofilms in Biodegradation Modeling

Because of the complexity of intra-biomass diffusion, a key question is: when is it necessary to consider intra-biomass mass transport resistance when modeling contaminant transport and biodegradation? This question has been addressed by a number of researchers, including Rittmann (1993).

Rittmann (1993) presents the following equation for utilization of substrate by biofilms:

$$r_f = -(X_f L_f a)\eta k\left(\frac{S_f}{K_s + S_f}\right) \tag{55}$$

where:

S_f = substrate concentration within the biofilm (M/L^3)

r_f = rate of substrate utilization in the biofilm (M/L^3T)

X_f = biofilm density (M/L^3)

L_f = biofilm thickness (L)

a = specific surface area of the biofilm (area of biofilm/volume of porous media, L^{-1})

η = effectiveness factor, defined as the substrate flux into the biofilm divided by the substrate flux into a fully penetrated biofilm of equal thickness

k = maximum specific rate of substrate utilization (T^{-1})

When $\eta = 1$, the biofilm is fully penetrated and $S_f = S_{im}$, the substrate concentration at the biofilm/stagnant liquid layer interface. As the biofilm thickness increases, S_f becomes less than S_{im} and η becomes less than 1. The question then becomes: when is $\eta = 1$ so that the biofilm can be assumed to be fully penetrated? This

question was investigated by Odencrantz et al. (1990), who found that $\eta = 1$ for all reasonable values of groundwater flow velocity and substrate utilization (Rittmann, 1993). In experiments to determine how permeability was affected by biofilm growth, Taylor and Jaffé (1990b) determined that η was only slightly less than 1, even though the permeability of the porous media in their experiments was reduced by 3 orders of magnitude because of microbial growth.

From these sources, it appears that intra-biomass diffusion is probably not important for modeling transport and biodegradation of contaminants under natural conditions. However, near injection wells where both substrate and electron acceptor are being injected, intra-biomass diffusion could have a more noticeable effect on biodegradation rates if the pore sizes were large enough for thick biofilms to form. Suidan et al. (1987) give rigorous criteria of when biofilms can be assumed to be fully penetrated in terms of a dimensionless biofilm thickness. Suidan et al. (1987) also provide quantitative criteria of when external mass transfer is important. Rittmann (1993) suggests that if biofilms are assumed to be fully penetrated, then the assumption should be checked in areas of the modeling domain where formation of shallow biofilms is possible.

4.11 Biomass Conceptualization and Mass Balance Equations

In an evaluation of subsurface biodegradation and transport models, Baveye and Valocchi (1989) describe three schools of thought regarding the configuration of biomass in the subsurface. The three configurations are depicted in Figure 8. In the first school, no particular assumption about the configuration of the microorganisms is made. The biomass could exist as either a continuous biofilm or as scattered, small microbial colonies of arbitrary shape. This is referred to as the "strictly macroscopic" viewpoint. In the second school, biomass is assumed to exist as small, discontinuous, geometrically defined colonies. This viewpoint is called the "microcolony" concept. The third school assumes that biomass exists as a continuous film over all of the particles in the subsurface (the "biofilm" concept). The choice of the biomass configuration conceptual model affects the interpretation of the parameters used in the models to account for diffusional

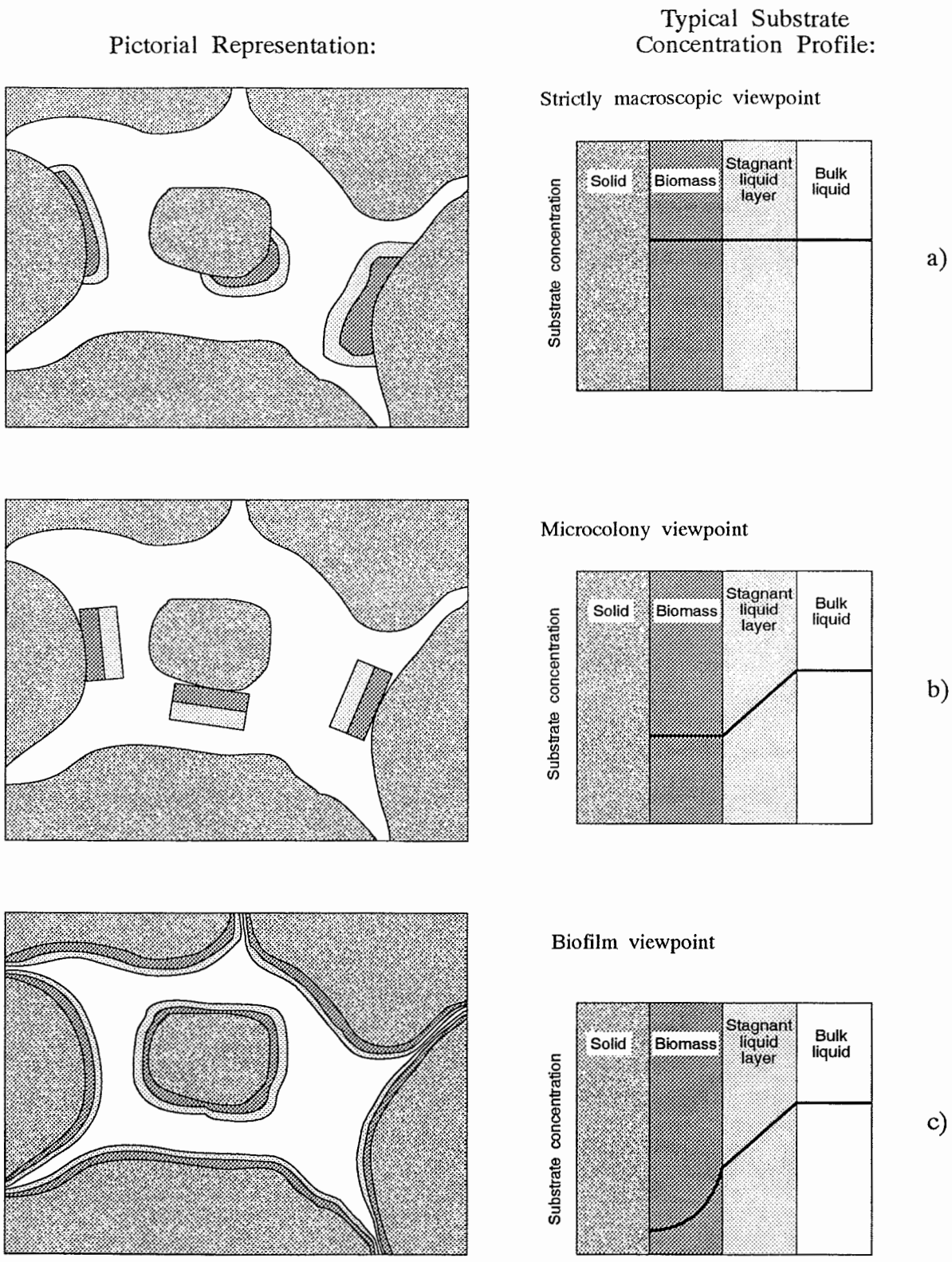


Figure 8. Three conceptualizations of biomass configuration in porous media (Baveye and Valocchi, 1989; Odencrantz et al., 1990).

limitations and affect the models' ability to incorporate these diffusional effects (Baveye and Valocchi, 1989).

4.11.1 Strictly Macroscopic Viewpoint - No Biomass Configuration Assumptions

In the models of Borden and Bedient (1986), Corapcioglu and Haridas (1984), and Kindred and Celia (1989), biomass is conceptualized as being attached to subsurface particles in some unspecified configuration (see Figure 8a). The chemical concentrations experienced by the biomass are assumed to be the same as the average bulk fluid concentrations, and the biomass is represented by a concentration similar to another chemical component. No diffusional resistances are explicitly assumed to exist. This is the simplest conceptual model because bulk fluid concentrations can be used directly in the kinetic expressions for substrate utilization. The kinetic expressions can then be substituted directly into the mass balance equations, resulting in the set of two equations (42) and (43).

As Baveye and Valocchi (1989) point out, the lack of a conceptual depiction of biomass does not have to eliminate any possibility of diffusional limitations. The equations (44) through (46) can be used to describe diffusion through a stagnant liquid layer into attached and immobile biomass, or equations (47) through (53) can be used (with some modifications) to describe both stagnant liquid layer diffusion and intra-biomass diffusion resistances. However, the assumption of no diffusional resistance is typically invoked with this biomass configuration. If diffusion is considered with this biomass conceptualization, the mass transfer coefficient expression C is usually an empirical constant used to match experimental data. If the substrate diffusion coefficient and stagnant liquid layer thickness are known or calculated from literature correlations, then the true fitting parameter is the biomass/bulk liquid interfacial area. This area is constant for all times and positions in the modeling domain.

4.11.2 Microcolony Viewpoint

The microcolony concept was introduced by Molz et al. (1986) and is incorporated into the models of Widdowson et al. (1988) and Chen et al. (1992). In this conceptual model, the biomass is assumed to exist in disk-shaped microcolonies of thickness τ and

radius r_c, with a stagnant liquid (diffusion) layer of thickness δ on the flat face facing the bulk fluid (see Figures 8b and 9). Biomass growth is modeled as an increase in the *number* of microcolonies; the *size* of each microcolony remains constant. This conceptualization is typically coupled with the assumption that all mass transfer resistance occurs across the stagnant liquid layer so that the concentration of substrate and electron acceptor within the colony is equal to the concentration at the colony/stagnant layer interface. However, as in the strictly macroscopic viewpoint, either no diffusion resistance or intrabiomass diffusion resistances can be considered.

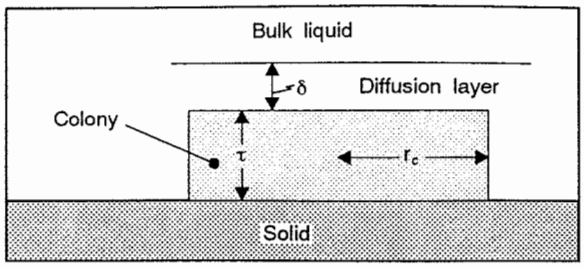


Figure 9. Microcolony dimensions (Molz et al., 1986).

Conceptualization of the biomass as microcolonies results in a different mass transfer expression than the empirical constant in the strictly macroscopic model. Under substrate-limiting conditions, the microcolony conceptual model equations describing substrate utilization and biomass growth in one dimension are (Molz et al., 1986):

$$\frac{\partial S_m}{\partial t} = \frac{\partial}{\partial x}\left(\frac{D\,\partial S_m}{\partial x} - v S_m\right) - \frac{D_{im}}{\theta}\left(\frac{S_m - S_{im}}{\delta}\right) N_c \pi r_c^2 \qquad (56)$$

$$\frac{\partial S_{im}}{\partial t} = D_{im}\left(\frac{S_m - S_{im}}{\delta}\right)\pi r_c^2 - \frac{\mu_{\max} m_c}{Y_{x/s}}\left(\frac{S_{im}}{K_s + S_{im}}\right) \qquad (57)$$

$$\frac{1}{N_c}\frac{\partial N_c}{\partial t} = \mu_{\max}\left(\frac{S_{im}}{K_s + S_{im}}\right) - b \qquad (58)$$

where:

D_{im} = molecular diffusion coefficient of substrate in the stagnant liquid layer (L^2/T)

m_c = mass of a single microorganism colony (M)

N_c = number of bacterial colonies per volume of porous media (L^{-3})

Note that the mass transfer coefficient expression and biomass concentration are (Baveye and Valocchi, 1989):

$$C = N_c m_c = D_{im} N_c \pi r_c^2 / \delta$$

$$X = N_c \rho_c \pi r_c^2 \tau$$

The mass transfer coefficient expression is no longer a constant, but depends on the biomass concentration. This is a consequence of the assumption that microcolonies of fixed size increase in number; the interfacial area available for mass transfer into the biomass increases with an increasing number of microcolonies. Since the colonies are assumed to have a fixed mass, the interfacial area increase is proportional to the biomass increase. As a result, substrate is removed at an increasing rate as biomass grows.

Although the microcolony concept has the advantage of a physical basis, it requires that the biomass exist in a predetermined configuration, which is obviously a great simplification, whereas the strictly macroscopic viewpoint does not. However, in the model of Widdowson et al. (1988), an alternative approach is used that precludes this restriction. Instead of defining a definite shape with an accompanying interfacial area to each microcolony, an interfacial area is assigned to each microcolony or unit of biomass. Although the choice of what interfacial area to assign must still be made, the microcolonies are no longer envisioned as geometrically simple structures. This approach retains the advantage of a changing interfacial area with biomass growth while eliminating the application of a definite shape to the biomass (Widdowson, 1991).

4.11.3 Biofilm Viewpoint

In the biofilm conceptual model, biomass is assumed to cover the entire surface of the solid in a continuous biofilm. As in the strictly macroscopic and microcolony viewpoints, mass transfer resistances can be ignored, assumed to exist across a stagnant liquid layer, or assumed to exist both within the biofilm as well as across a stagnant liquid layer. However, the biofilm viewpoint is most useful when intra-biomass mass transfer is assumed to exist.

If mass transfer resistance is assumed to occur only across a stagnant liquid layer for purposes of comparison with the other two conceptual models, then the equations for substrate utilization and biomass growth for the biofilm model are:

$$\frac{\partial S_m}{\partial t} = \frac{\partial}{\partial x}\left(\frac{D\partial S_m}{\partial x} - vS_m\right) - \frac{D_{im}}{\theta}\left(\frac{S_m - S_{im}}{\delta}\right)A \tag{59}$$

$$\frac{\partial S_{im}}{\partial t} = D_{im}\left(\frac{S_m - S_{im}}{\delta}\right)A - \frac{\mu_{\max}}{Y_{x/s}}\left(\frac{S_{im}}{K_s + S_{im}}\right) \tag{60}$$

$$\frac{\partial L_f}{\partial t} = \mu_{\max}\left(\frac{S_{im}}{K_s + S_{im}}\right)L_f - bL_f \tag{61}$$

where:

X_f = biomass density (M/L^3)

A = specific surface area of porous medium (L^{-1})

L_f = thickness of continuous biofilm (L)

These equations are formally the same as those for the strictly macroscopic viewpoint. In this system of equations, the specific surface area A must be known, and the change in biomass is reflected by changes in the biofilm thickness L_f. The advantage of this model is that the specific surface area A can usually be measured so that no assumption about the interfacial area must be made. The disadvantage of this viewpoint is that studies have shown that the biomass is not usually uniformly distributed on the porous media so that the assumption of a continuous biofilm may be unrealistic. In addition, the assumption of a continuous biofilm leads to excessive mass transfer into the biomass if the diffusion coefficient and stagnant liquid layer thickness are obtained independently.

4.11.4 Summary of Biomass Configuration Conceptualization

Since biomass has generally been observed to exist as scattered colonies in oligotrophic environments (Harvey et al., 1984), the microcolony conceptualization appears to be the most realistic model. However, in some cases, the choice of conceptual model does not make any difference because the governing equations are identical. For example, if no diffusion resistance is assumed to exist, or if mass transfer is assumed to be rapid relative to biodegradation rates, then all three models collapse into a system of

two equations and will yield the same results. The only difference is in how biomass is defined; as a concentration, number of microcolonies, or biofilm thickness.

4.12 Biomass Growth Limitations

As discussed in Section 4.7.10, the biomass mass balance equation does not have a built-in mechanism for limiting the amount of biomass that can exist to physically possible quantities. When substrates or other nutrients are low, biomass growth can be limited by the low concentrations of these chemicals through Monod terms. However, at locations where there are no substrate, electron acceptor, or other nutrient limitations, most models predict continued growth of biomass, even beyond the volume of biomass equal to the pore volume of the porous media. Therefore, methods are required to limit the growth of biomass independent of nutrient concentrations. In general, biodegradation models have controlled the growth of biomass by one of three methods: 1) explicit consideration of mass transfer through stagnant liquid layers and within the biomass itself; 2) biomass inhibition functions, and; 3) consideration of biomass as a separate phase with losses from the biofilms due to sloughing and shearing. Each of these methods is discussed below.

4.12.1 Mass Transfer Resistances

As discussed in Section 4.10, diffusion resistances across a stagnant liquid layer reduce the substrate concentration within the biomass. The biomass grows at a rate slower than it would grow if the substrate concentration within the biomass were the same as the bulk liquid concentration. Although the stagnant liquid layer diffusive resistances reduce the substrate concentration available to the biomass, these resistances to mass transfer may not limit biomass growth sufficiently. Additional biomass growth limitations are imposed when intra-biomass diffusion is considered. If the biomass grows so thick that the substrate concentration becomes 0 at or before the biomass/solid interface (a deep biofilm), the biomass cannot continue to grow (or at least become thicker) because it is already receiving the maximum flux of substrate possible from the bulk liquid.

Intra-biomass diffusional limitations may be sufficient to limit the ultimate biomass concentration if the pore volume is large enough for a deep biofilm to form.

However, the pores in some media may be too small for deep biofilms to form. In these media, the predicted biomass volume could exceed the porosity even with intra-biomass diffusional resistances.

4.12.2 Biomass Inhibition Functions

Kindred and Celia (1989) used a biomass inhibition function of the following form to limit biomass growth and model intra-biomass diffusional resistances:

$$I_b = (1 + X/k_b) \tag{62}$$

where:

I_b = biomass inhibition factor

X = biomass concentration (M/L^3)

k_b = biomass inhibition constant (M/L^3)

This biomass inhibition term is identical to the product inhibition term (equation 25) if the numerator and denominator of the product inhibition term are divided by K_p. The inhibition function is incorporated into the Monod expression for biomass growth:

$$\frac{dX}{dt} = \mu_{max} X \left(\frac{S}{(K_s + S)(1 + X/k_b)} \right) - bX \tag{63}$$

If $X << k_b$, then $1 + X/k_b = \sim 1$ and the basic Monod equation results. If $X >> k_b$, then $1 + X/k_b = \sim X$ and the expression for biomass growth becomes:

$$\frac{dX}{dt} = \mu_{max} k_b \left(\frac{S}{K_s + S} \right) - bX \tag{64}$$

This expression is the same as the Monod equation except that X is replaced by k_b. The result is that biomass growth is no longer first-order with respect to X and depends only on the substrate concentration. As the biomass continues to utilize substrate, X will continue to increase. However, equation (64) predicts that the growth rate will tend to 0 as X continues to increase because of the second term for endogenous decay, which depends on the increasingly large X. As a result, biomass concentration will reach a maximum for some given substrate concentration. Kindred and Celia (1989) point

out that the above expression corresponds to a situation where a biofilm is fully penetrated and the total thickness of the biofilm is irrelevant since only the outer layer degrades the substrate.

Two points about this approach should be emphasized. First, the inhibition function is empirical. Experiments would be necessary to determine a realistic value of k_b. Since porous media are usually not homogeneous, this value would have to be an average or effective value for the media as a whole. Second, depending on the value of the inhibition constant, the inhibition function could limit biomass growth even at biomass concentrations far below the concentrations that would significantly restrict pores. For small pore volumes, this limitation may be realistic. However, for media with large pore sizes, biomass growth could be unjustifiably restricted.

4.12.3 Sloughing and Shearing Losses

Sloughing is the detachment of biomass sections caused by the death of cells at the biomass/solid interface. The cell death is in turn caused by lack of sufficient nutrients at the interface due to their complete utilization in the outer portion of the biomass. Sloughing is difficult to predict quantitatively and is not well understood.

Biomass shearing is more amenable to quantitative treatment. As pores become restricted from biomass growth, the groundwater velocity through the pore openings increases. These increased velocities could cause shearing of biomass from the pore walls. Since the shearing loss is a function of the velocity, biomass losses could increase with increasing biomass growth until a steady state is reached between biomass growth and shearing loss. Equations for biomass shearing losses are shown in Section 4.14 from the work of Taylor and Jaffé (1990b).

4.13 Importance of Boundary Conditions on Biomass Growth

Under most natural settings, the substrate and electron acceptor concentrations in an aquifer are probably not sufficient to support growth of biomass in quantities that would cause its volume to comprise a significant fraction of the pore space. This is not true when injection wells inject substrate and electron acceptor into aquifers in large quantities, or when substrate and electron acceptor are continuously injected at a column

inlet in the laboratory. Under these conditions, the selection of the inlet boundary conditions can have a large impact on the model predictions of biomass growth at the column inlet (or adjacent to the well screen for injection wells).

The two most common inlet boundary conditions for laboratory column studies are (Fetter, 1993):

$$S = S_o \qquad x = 0,\ t \geq 0 \qquad \text{first type}$$
$$-D\frac{\partial S}{\partial x} + vS = vS_o \qquad x = 0,\ t \geq 0 \qquad \text{third type} \tag{65}$$

The first-type boundary condition provides the biomass with an unlimited supply of substrate and electron acceptor by holding the concentration of these species constant at the column inlet (Chen et al., 1992). Thus, the biomass can continue to grow unbounded according to equation (41) because no substrate limitations exist (Chen et al., 1992). Models that use this boundary condition will, therefore, predict excessive biomass growth at the column inlet. However, if the third-type boundary condition is used, then the flux of substrate and electron acceptor is limited at the column inlet, a much more realistic situation (Chen et al., 1992). Under this boundary condition, the concentration at the inlet can be reduced by the biomass growing there so that much more realistic estimates of biomass growth will be simulated. Chen et al. (1992) demonstrated this boundary condition effect in simulations of biodegradation in laboratory columns.

4.14 Microorganism Transport and Effect on Porous Media

Microorganism transport is important to biodegradation modeling for a number of reasons. First, contaminants have been shown to migrate when adsorbed on colloidal-sized bacteria (Lindqvist and Enfield, 1992; Jenkins and Lion, 1993). Second, bacteria themselves may be transported when attached to colloidal particles. If this type of transport occurs, then an acclimated population of microorganisms could develop in advance of a contaminant plume and significantly reduce the acclimation period. Finally, it may be desirable to introduce microorganisms acclimated to a particular contaminant or genetically engineered to degrade a contaminant into a subsurface environment to

enhance bioremediation (Lindqvist and Enfield, 1992). The transport and attachment of bacteria to subsurface particles is also important in estimating permeability reductions.

4.14.1 Important Considerations and Mechanisms

The movement of bacteria in the subsurface is governed by transport processes, attachment phenomenon, and detachment phenomenon. Transport is generally assumed to occur by advection, diffusion (for small bacteria), and chemotaxis (Corapcioglu and Haridas, 1984). Chemotaxis is the directed movement of bacteria in response to chemical gradients (Corapcioglu and Haridas, 1984). Through chemotaxis, bacteria move toward areas of higher nutrient concentrations (Corapcioglu and Haridas, 1984).

Many interacting factors govern the transport and attachment of bacteria to surfaces. These factors include physical, chemical and biological properties of both the bacteria and surfaces and are summarized in Table 5. Removal of bacteria from the flowing liquid phase generally occurs by filtration (Yates and Yates, 1988), adsorption (Lindqvist and Bengtsson, 1991) and cell death (Camper et al., 1993). Detachment in subsurface environments is most likely to occur by desorption, erosion, or sloughing (Stewart, 1993). The various attachment and detachment mechanisms are affected by one or more of the factors listed in Table 5, among others.

4.14.2 Methods of Modeling Bacterial Transport and Attachment

Bacterial transport and elucidation of the methods by which bacteria attach to porous media is a very large area of active research. This section provides only a brief summary of a few of the factors known to affect bacterial transport and several relatively simple methods of modeling the phenomenon.

All of the models of bacterial transport reviewed in this report are based on the advection-dispersion equation. Some models account for chemotaxis by lumping a chemotactic dispersion coefficient into the overall dispersion coefficient (Corapcioglu and Haridas, 1985), while other models include a separate chemotactic dispersion coefficient (Sarkar et al., 1994). The major differences in the models are in the method by which bacterial attachment and detachment are modeled. Removal of bacteria from the liquid

FACTOR	EFFECT ON TRANSPORT OR ATTACHMENT
pH	Low pHs favor attachment (Yates and Yates, 1988).
Ionic strength	High ionic strength increases attachment by reducing the size of the particle double layer (Yates and Yates, 1988; Fontes et al., 1991).
Clay content	Increasing clay content favors increasing attachment due to a greater specific area for adsorption (Teutsch et al., 1991) and possible filtering effects (Yates and Yates, 1988; Fontes et al., 1991).
Oxygen limitations	Oxygen-limited biofilms exhibit lower shear removal rates but higher sloughing possibly due to high extracellular polymer production (Applegate and Bryers, 1991).
Charge on media	Positive charges on media tend to increase attachment by negatively-charged bacteria (Lindqvist and Bengtsson, 1991).
Flowrate	Higher flowrates reduce attachment of bacteria (Yates and Yates, 1988).
Nutrient concentrations	Higher nutrient concentrations reduce bacterial size (Camper et al., 1993).
Bacterial size	May decrease or increase attachment. Smaller bacteria may interact with the media less and may not be removed by filtration as easily as large bacteria (Camper et al., 1993). On the other hand, larger bacteria have been shown to move faster than small bacteria, possibly due to size exclusion (Fontes et al., 1991; Yates and Yates, 1988).
Cell concentration	Attachment is favored when the cell density (mass concentration) in the liquid is decreased (Lindqvist and Enfield, 1992). Also, bacteria tend to move from areas of high concentration to areas of low concentration by a tumbling diffusive flux (Sarkar et al., 1994).
Bacterial motility	Motile bacteria may migrate faster than non-motile bacteria through chemotaxis.
Water content	Bacteria move faster through unsaturated soils at higher water contents (Yates and Yates, 1988).

Table 5. Factors affecting bacterial attachment and transport.

phase is generally modeled as an adsorption process, a filtration process, or a combination of both. Two methods of modeling detachment are desorption and removal by shearing.

4.14.2.1 Adsorption Models

The simplest method of modeling bacterial transport is to assume that bacteria are adsorbed according to the linear equilibrium model. This approach is taken by Borden and Bedient (1986) and MacQuarrie et al. (1990). In fact, these researchers were the only ones to account for bacterial transport in models whose primary objective was to model contaminant transport. All of the other methods of accounting for bacterial transport discussed below are incorporated into models specifically designed to describe bacterial transport, although some also describe contaminant transport since they include terms for growth and decay of the biomass.

The next level of complexity is to model bacterial detachment as a reversible, first-order adsorption process. The equations used are the same as the equations used to model first-order reversible adsorption of a chemical constituent (equation 10). This method was adopted by Corapcioglu and Haridas (1984, 1985) in a model to describe bacterial transport. Hornberger et al. (1992) used a modified version of Corapcioglu and Haridas's model to describe the data obtained by Fontes et al. (1991) in laboratory columns. The growth term was eliminated from the model since experiments were conducted by resting cells. The model was compared to two other models, one ignoring detachment and one ignoring dispersion. The model of Corapcioglu and Haridas (1985) best predicted the data, as the effects of both detachment and dispersion were important. The model performed reasonably well and captured the general shape of the bacterial breakthrough curves. The model seemed to perform best for the larger bacteria and finer grained soils.

Lindqvist and Bengtsson (1991) described the transport of bacteria through sand columns with both a linear equilibrium isotherm adsorption model and a two-site model. The two-site model assumes that a fraction of the adsorbing solute adsorbs to soil instantaneously while the adsorption of the remaining fraction is kinetically limited. The two-site model is also used to describe adsorption of chemical constituents. Lindqvist and Bengtsson (1991) accounted for both growth and decay of biomass where growth was

described with a Monod term and decay was described as first-order. The equations used for the two-site model were:

$$\left(1 + \frac{f\rho_b K_d}{\theta}\right)\frac{\partial C_a}{\partial t} + \frac{\rho_b}{\theta}\frac{\partial C_s^{II}}{\partial t} = D\frac{\partial^2 C_a}{\partial x^2} - v\frac{\partial C_a}{\partial x} - bC_a + \mu C_a \qquad (66)$$

$$\frac{\partial C_s^{II}}{\partial t} = k_m[(1 - f)K_d C_a - C_s^{II}] \qquad (67)$$

where:

f = fraction of instantaneous adsorption sites

ρ_b = bulk soil density (M/L^3)

C_a = concentration of bacteria in the bulk liquid (M/L^3)

θ = porosity

b = first-order rate coefficient for bacterial loss by all mechanisms (T^{-1})

μ = Monod growth rate (T^{-1})

C_s^{II} = mass fraction of bacteria adsorbed onto kinetically limited adsorption sites (M/M solid)

k_m = mass transfer coefficient (T^{-1})

K_d = adsorption partition coefficient (L^3/M)

Lindqvist and Bengtsson (1991) determined that the two-site model described the breakthrough of bacteria better than a linear equilibrium model.

4.14.2.2 Filtration and Combined Adsorption/Filtration Models

Harvey and Garabedian (1991) investigated bacterial transport in an organically contaminated groundwater plume in Cape Cod, Massachusetts. Harvey and Garabedian (1991) used filtration theory coupled with either linear equilibrium adsorption or first-order reversible kinetic adsorption to describe bacterial removal from the bulk liquid. Their version of the advection-dispersion equation was:

$$\theta\frac{\partial C}{\partial t} + \rho_b\frac{\partial C^*}{\partial t} = D\,\theta\frac{\partial^2 C}{\partial x^2} - v\theta\frac{\partial C}{\partial x} - k_p C \qquad (68)$$

where C^* is the adsorbed bacterial concentration and k_p (T^{-1}), the irreversible adsorption constant, is:

$$k_p = \frac{3}{2}\frac{(1-\theta)}{d}\alpha\eta \qquad (69)$$

where α is the collision efficiency factor and η is the single-collector efficiency. $\partial C^*/\partial t$ is equal to $K_d\partial C/\partial t$ for linear adsorption and to $k_f C - k_r C^*$ for first-order reversible kinetic adsorption. Harvey and Garabedian (1991) fitted the model parameters to the elution curves and concluded that their model fit the data reasonably well. Because adsorption was not a major factor in their studies, the linear equilibrium model and first-order reversible kinetic adsorption models performed equally well.

Lindqvist and Enfield (1992) compared the two-site adsorption model to a filter model in which the concentration of bacteria were described as:

$$\frac{dC}{dx} = -\lambda C \qquad (70)$$

where C is the aqueous bacterial concentration and λ is a filter coefficient (T^{-1}). The filter coefficient is essentially the same as the irreversible adsorption constant k_p used by Harvey and Garabedian (1991). However, if an empirical fit to the data is desired and if the filtration parameters are assumed to remain constant, the function can be lumped into the filter coefficient. Lindqvist and Enfield (1992) determined that this description of bacterial attachment did not perform as well as the two-site adsorption model.

Sarkar et al. (1994) present a model for bacterial transport and growth using a different approach to filtration. This multi-phase, multi-component model uses an empirical fractional flow curve to simulate the data from column experiments. The fractional flow curve is represented by:

$$C_{fD} = \frac{AC_{TD}}{1 + BC_{TD}} \qquad (71)$$

where:

$$C_{fD} = \frac{C_f}{C_T} \qquad and \qquad C_{TD} = \frac{C_T - C^*}{C_T} \tag{72}$$

and:

C_f = flowing bacterial concentration (M/L^3)

C_T = total bacterial concentration (M/L^3)

C_{fD}, C_{TD} = dimensionless flowing bacterial and total bacterial concentrations

A, B, C^* = retention parameters determined experimentally

The trapped bacterial concentration is $\hat{C}_k = C_T - C_f$. This filtration model was successful in simulating the bacterial elution curves and in describing reductions in permeability of the experimental columns.

Taylor and Jaffé (1990b) use a first-order model for bacterial deposition that differs slightly from the other filtration models discussed above. They assume that the biomass exists as a continuous film and model bacterial removal from the water phase as:

$$R_d = (c_1\theta^f + c_2)\theta^w C_b^w \tag{73}$$

where:

R_d = rate of removal of bacteria (M/L^3T)

θ^f = volume fraction of biomass phase (volume biomass/volume of porous medium)

θ^w = volume fraction of water phase (volume water/volume of porous medium)

C_b^w = concentration of bacteria in water phase (M/L^3)

c_1, c_2 = experimentally determined constants

The simplest method of modeling biomass detachment is to assume that it is first-order with respect to adsorbed biomass concentration (Taylor and Jaffé, 1990b). The first-order rate constant may be a function of the shear stress, which is itself a function of fluid viscosity, seepage velocity, and permeability (Taylor and Jaffé, 1990b).

Taylor and Jaffé (1990b) modeled biomass detachment with a biofilm shearing model based on the work of Speitel and DiGiano (1987). Incorporating the effectiveness factor concept into the biofilm shearing model, Taylor and Jaffé expressed the loss of biomass from the biofilm as:

$$R_s = b_s \theta^f \rho^f + b_s' \left(\frac{Y \eta k C_s^w}{K_s + C_s^w} \right) A L_f \rho^f \qquad (74)$$

where:

L_f = biofilm thickness (L)

A = specific surface area of the water/biofilm interface (L^{-1})

θ^f = volume fraction of biofilm phase (volume biofilm/total volume of porous media)

η = biofilm effectiveness factor

b_s = specific shear loss coefficient (a function of shear stress and L_f)

b_s' = dimensionless parameter describing biological aspects of shearing

C_s^w = concentration of substrate in the bulk phase (M/L^3)

ρ^f = biomass density (M/L^3)

Taylor and Jaffé (1990b) incorporate the expressions for biomass deposition and detachment into a comprehensive model that accounts for bacterial transport and substrate utilization in both the biomass and in the bulk fluid where free-floating bacteria are assumed to contribute to substrate biodegradation.

4.15 Effect of Microorganism Growth on Porous Media

In many realistic field situations, substrate and electron acceptor are not present in sufficiently high concentrations or for sufficient time for growing biomass to occupy a significant fraction of the pore space. In these cases, biomass growth may have an insignificant effect on the porous medium properties. However, in column studies and in bioremediation projects where high concentrations of substrate and electron acceptor coexist for long periods, biomass growth may cause changes in the porosity, permeability and dispersivity of the porous medium, and these changes must be considered.

Only four models reviewed in this study addressed the effects of biomass growth on properties of the porous media (Sarkar et al., 1994; Corapcioglu and Haridas, 1985; Taylor and Jaffé, 1990b; and Sarkar et al., 1992). Sarkar (1992) and Sarkar et al. (1994) used effective medium theory (EMT) to estimate permeability losses due to the retention

of bacteria. A complete description of the theory can be found in other sources (Sharma and Yortsos, 1987), and only a summary of the important relationships are provided here.

The basic premise of EMT is that as bacteria are retained by the porous media, the pore size distribution is changed (Sarkar, 1992). Some pores become plugged and cannot transmit fluid while others remain unaffected. The initial undamaged mean conductance is defined as (Sarkar, 1992):

$$g_{mi} = \frac{\int_0^\infty \frac{r^n f(r)}{r^n + \alpha g_m} dr}{\int_0^\infty \frac{f(r)}{r^n + \alpha g_m} dr} \tag{75}$$

where:

g_{mi} = initial undamaged mean conductance (L^3)

r = pore throat radius (L)

$f(r)$ = pore throat radius distribution function

g_m = effective hydraulic conductance (L^3)

n = exponent between 3 and 4

α = $z/2 - 1$

z = coordination number (of pore throats that join at each interior pore body)

The damaged mean conductance is calculated from (Sarkar, 1992):

$$g_{mD} = \frac{(1-y) \int_0^\infty \frac{r^n f_D(r)}{r^n + \alpha g_{mD}} dr}{\frac{y}{\alpha g_m} + (1-y) \int_0^\infty \frac{f_D(r)}{r^n + \alpha g_{mD}} dr} \tag{76}$$

where:

y = fraction of nonconductive pores (a function of the effective particle radius)

$f(r)$ = damaged medium pore size distribution function

g_{mD} = mean conductance for the damaged medium (L^3)

The permeability of the damaged medium is then found from:

$$\frac{k_D}{k_i} = \left(\frac{g_{mD}}{g_{mi}}\right)^{2/n} = \sqrt{\frac{g_{mD}}{g_{mi}}} \qquad (77)$$

where k_i is the intrinsic permeability of the medium. The EMT model in this form assumes zero permeability for the nonconductive pores, but in actuality the permeability will have some very low value (Sarkar, 1992). In this case, the damaged permeability is expressed by the empirical relation:

$$\frac{k_D}{k_{D_c}} = e^{d(a-a_c)}$$

where:

a_c = percolation threshold

k_{Dc} = permeability at a_c

d = critical damage parameter

Sarkar (1992) and Sarkar et al. (1994) used the model to simulate the permeability reduction and bacteria breakthrough curves for saturated water flow and NAPL/water flow. In the experiments, permeability was reduced to 70 to 80 percent of the initial permeability. The simulation matched the experimental data reasonably well. The model underpredicted permeability reduction at early times but matched the leveling of permeability reduction.

The experiments conducted by Sarkar (1992) and Sarkar et al. (1994) were for relatively high flow velocities: 25 and 100 ft/day. Taylor and Jaffé (1990a) conducted experiments of aerobic methanol biodegradation in laboratory columns with lower flow rates of approximately 10 and 33 ft/day. They found that permeability was reduced by three orders of magnitude over the initial permeability, but that the permeability was not reduced below a threshold of about three orders of magnitude. The experimental permeability data were found to be a function of the biomass volume fraction:

$$\frac{k}{k_o} = \exp\left(a[BOC] + b[BOC]^2\right) \qquad [BOC] \le 0.4\ mg/cm^3$$
$$\frac{k}{k_o} = c \qquad [BOC] > 0.4\ mg/cm^3 \tag{79}$$

where:

k = permeability at the end of the experiment (L^2)

k_o = initial permeability (L^2)

$[BOC]$ = bacterial organic carbon (M/L^3)

Taylor and Jaffé (1990b) used a cut-and-random-rejoin type of model to simulate the porosity and permeability reduction observed in their experiments:

$$n^w = \beta\left(\frac{L_f}{R}\right)^{\lambda}\left[I_2\left(\frac{R}{L_f} - 1,\ \lambda\right) - I_2\left(\frac{r_0}{L_f} - 1,\ \lambda\right)\right] \tag{80}$$

$$k = \frac{\kappa\sqrt{n^w}\,\beta^2 L_f^2}{8}\left(\frac{L_f}{R}\right)^{2\lambda}\left[I_3\left(\frac{R}{L_f} - 1,\ \lambda\right) - I_3\left(\frac{r_0}{L_f} - 1,\ \lambda\right)\right]^2 \tag{81}$$

$$I_n(u,\ \lambda) = \int_0^u \frac{x^n}{(x+1)^{3-\lambda}}\,dx \tag{82}$$

$$a_L = \frac{(n^w)^2\langle L_b\rangle\langle T_b^*\rangle^2\beta}{k}\left(\frac{L_f}{R}\right)^{\lambda}\left(\frac{L_f^2}{8}\right)^2\left\{\left[I_6\left(\frac{R}{L_f} - 1,\lambda\right) - I_6\left(\frac{r_0}{L_f} - 1,\lambda\right)\right]\right.$$
$$\left. - \beta\left(\frac{L_f}{R}\right)^{\lambda}\left[I_6\left(\frac{R}{L_f} - 1,\lambda\right)^2 - I_4\left(\frac{r_0}{L_f} - 1,\lambda\right)^2\right]\right\} \tag{83}$$

where:

R = maximum pore radius (L)

κ, β = dimensionless constants

L_f = biofilm thickness (L)

λ = pore size distribution index

a_L = longitudinal dispersivity (L)

$\langle L_b \rangle$ = average length of an elemental pore channel (L)

$\langle T_b^* \rangle$ = tortuosity

k = permeability (L^2)

Taylor and Jaffé (1990b) were successful in predicting biofilm thicknesses on a laboratory column. Taylor and Jaffé (1990b) also compared the effect of three test cases to determine whether such a complex model is justified. In case 1, dispersivity, porosity and permeability were allowed to change with biomass growth (the most complex case for which the above model applies). In case 2, these parameters were considered constant and equal to their values when no biomass is present. Case 3 was the same as case 2 except that interphase biomass transfer were not allowed, i.e., there was no exchange of biomass from the biofilm and bulk liquid.

At low substrate loadings, cases 1 and 2 predicted an increase in biomass concentration with distance through the column followed by a decrease and gradual decline with distance. This prediction matched the observed experimental results. The case 3 scenario predicted excessive biomass growth at the column inlet since no biomass shearing was taken into account. This effect is the same effect seen with other models which do not establish an upper limit on biomass growth. At high substrate loading, case 1 still predicted realistic biomass distributions. However, the higher substrate loadings caused the case 2 simulation to overpredict biomass accumulation at the column inlet because aquifer properties were not taken into account. In fact, the case 2 solution became unstable because of very high predicted advective fluxes, whereas in case 1, where dispersivity was allowed to vary with biomass growth, the solution remained stable. Thus, accounting for changes in the porous media may not only lead to more realistic predictions of biomass distributions, but also assist in the stability of the numerical solutions (Taylor and Jaffé, 1990b).

5.0 DISCUSSION OF REPRESENTATIVE MODELS

Table 6 summarizes the features of the biodegradation models reviewed in this literature review. The objective of this section is to describe the performance of representative models reviewed. To accomplish this objective, the following information is provide for each model:

- brief general description
- key model features
- important assumptions
- method of validation
- comments on the model's advantages and disadvantages

5.1 Widdowson et al., 1988

The Widdowson et al. (1988) model simulates the biodegradation of generic organic carbon in laboratory columns. This is one of the first models to incorporate multiple electron acceptors. The concentration of two electron acceptors and one additional nutrient are simulated. Distinguishing features of the model include:

- Conceptualization of the microorganisms as microcolonies,
- Inhibition functions to "switch" from oxygen to nitrate metabolism,
- Consideration of a stagnant liquid film diffusion layer.

5.1.1 Important Assumptions

- Adsorption is described as a linear equilibrium process.
- Monod kinetics apply.
- Bacterial transport is not significant.
- Microbial community consists of a single bacterial species.

5.1.2 Validation

The model solution for a conservative tracer was compared to the one-dimensional analytical solution of the advection-dispersion equation (van Genutchten and Alves,

AUTHOR(S):	Sykes et al.	Bouwer & McCarty	Borden & Bedient	Molz et al.	Baehr and Corapcioglu	Rifai et al.	Widdowson et al.	Chiang et al.
YEAR PUBLISHED:	1982	1984	1986	1986	1987	1988	1988	1989
GENERAL:								
Maximum Dimensions	2	1	2	1	1	2	1	2
Maximum Phases (Excluding the solid phase)	1	1	1	1	3	1	1	1
Numerical Solution Method	FE	IT	FD/MOC	FD	FD	FD/MOC	FD	FD/MOC
Multiple Substrates?	N	N	N	N	Y	N	N	N
Multiple Electron Acceptors?	N	N	N	N	N	N	Y	N
Multiple Reactions?	N	N	N	N	N	N	N	N
GROWTH KINETICS:								
Microbial Growth Included?	Y	Y	Y	Y	N	N	Y	Y
Kinetics: Monod (M), First-Order (FO), Other (O)	M/FO	FO	M/O	M	O	M	M	FO/O
Aerobic (AR), Anaerobic (AN), or Both (B)	AN	B	AR	AR	AR	AR	B	AR
Cometabolism?	N	Y	N	N	N	N	N	N
Inhibition Included?	N	N	N	N	N	N	N	N
Acclimation Included?	N	N	N	N	N	N	N	N
BIOFILM/DIFFUSION LIMITATIONS:								
Stagnant Liquid Layer Diffusion Limitation?	N	Y	N	Y	N	N	Y	N
Intra-Biofilm Diffusion Limitation?	N	Y	N	N	N	N	N	N
ADSORPTION:								
Included?	N	N	Y	Y	Y	Y	Y	N
Linear (LN) or Non-linear (NL)	N/A	N/A	LN	LN	LN	LN	LN	N/A
Equilibrium (E) or Kinetic (K)	N/A	N/A	E	E	E	E	E	N/A
MICROORGANISM CONCEPT								
Biofilm (BF), Microcolony (MC), Not Specified (NS)	NS	BF	NS	MC	NS	NS	MC	NS
Microbial Transport Included?	N	N	Y	N	N	N	N	N
Multiple Microorganism Populations?	N	N	N	N	N	N	N	N
Upper Limit on Biomass Volume?	N	N	N	N	N	N	N	N
Growth Effects on Porous Media Included?	Y	N	N	N	N	N	N	N
TESTING/VERIFICATION:								
Compared to Analytical Solution?	N	N	Y	Y	N	Y	Y	N
Laboratory Tested?	N	Y	N	N	N	N	N	N
Field Tested?	Y	N	Y	N	N	Y	N	Y

Table 6. Features of selected biodegradation models included in this review.

AUTHOR(S):	Kindred & Celia	MacQuarrie et al.	Odencrantz et al.	Taylor and Jaffe	Corapcioglu et al.	Kinzelbach et al.	Taylor and Jaffe	Angley et al.
YEAR PUBLISHED:	1989	1990	1990	1990a	1991	1991	1991	1992
GENERAL:								
Maximum Dimensions	1	2	2	1	1	2	1	1
Maximum Phases (Excluding the solid phase)	1	1	1	2	1	3	2	1
Numerical Solution Method	FE	FE	FD	FE	FD	?	FE	FD
Multiple Substrates?	Y	N	N	N	Y	N	N	N
Multiple Electron Acceptors?	Y	N	N	N	N	Y	N	N
Multiple Reactions?	N	N	N	N	Y	N	N	N
GROWTH KINETICS:								
Microbial Growth Included?	Y	Y	Y	Y	N	Y	Y	N
Kinetics: Monod (M), First-Order (FO), Other (O)	M	M	M	M	M	M	M	FO
Aerobic (AR), Anaerobic (AN), or Both (B)	B	AR	AN	N/A	AN	B	AR	AR
Cometabolism?	Y	N	N	N	Y	N	N	N
Inhibition Included?	Y	N	N	N	N	N	Y	N
Acclimation Included?	N	N	N	N	N	N	N	N
BIOFILM/DIFFUSION LIMITATIONS:								
Stagnant Liquid Layer Diffusion Limitation?	N	N	Y	N	N	Y	N	N
Intra-Biofilm Diffusion Limitation?	N	N	Y	N	N	N	N	N
ADSORPTION:								
Included?	Y	Y	N	N	Y	?	N	Y
Linear (LN) or Non-linear (NL)	LN	LN	N/A	N/A	LN	?	N/A	LN
Equilibrium (E) or Kinetic (K)	E	E	N/A	N/A	E	?	N/A	K
MICROORGANISM CONCEPT								
Biofilm (BF), Microcolony (MC), Not Specified (NS)	NS	NS	NS/BF	BF	NS	NS	BF	NS
Microbial Transport Included?	N	Y	N	Y	N	N	Y	N
Multiple Microorganism Populations?	Y	N	N	N	N	N	N	N
Upper Limit on Biomass Volume?	N	N	N	Y	N	N	Y	N
Growth Effects on Porous Media Included?	N	N	N	Y	N	N	Y	N
TESTING/VERIFICATION:								
Compared to Analytical Solution?	N	Y	N	N	N	?	N	N
Laboratory Tested?	N	Y	Y	Y	Y	?	N	Y
Field Tested?	N	N	N	N	N	?	N	N

Table 6. Features of selected biodegradation models included in this review.

AUTHOR(S):	Brusseau et al.	Chen et al.	Semprini and McCarty	Malone et al.	Wood et al.
YEAR PUBLISHED:	1992	1992	1992	1993	1994
GENERAL:					
Maximum Dimensions	1	1	1	1	2
Maximum Phases (Excluding the solid phase)	1	3	1	1	1
Numerical Solution Method	FD	FE	FD	FD	FE/MOC
Multiple Substrates?	N	Y	Y	Y	N
Multiple Electron Acceptors?	N	Y	N	N	N
Multiple Reactions?	N	N	N	Y	Y
GROWTH KINETICS:					
Microbial Growth Included?	N	Y	Y	Y	Y
Kinetics: Monod (M), First-Order (FO), Other (O)	FO	M	M	M	FO/M
Aerobic (AR), Anaerobic (AN), or Both (B)	N/A	B	AR	AR	AR
Cometabolism?	N	N	Y	N	N
Inhibition Included?	N	Y	Y	Y	N
Acclimation Included?	N	N	Y	N	Y
BIOFILM/DIFFUSION LIMITATIONS:					
Stagnant Liquid Layer Diffusion Limitation?	Y	Y	N	N	N
Intra-Biofilm Diffusion Limitation?	N	N	N	N	N
ADSORPTION:					
Included?	Y	Y	Y	Y	N
Linear (LN) or Non-linear (NL)	LN	LN	LN	LN	N/A
Equilibrium (E) or Kinetic (K)	K	E	K	E	N/A
MICROORGANISM CONCEPT					
Biofilm (BF), Microcolony (MC), Not Specified (NS)	NS	MC	NS	NS	NS
Microbial Transport Included?	N	N	N	N	N
Multiple Microorganism Populations?	N	Y	N	N	N
Upper Limit on Biomass Volume?	N	N	N	N	N
Growth Effects on Porous Media Included?	N	N	N	N	N
TESTING/VERIFICATION:					
Compared to Analytical Solution?	Y	Y	Y	N	Y
Laboratory Tested?	Y	Y	N	Y	Y
Field Tested?	N	N	Y	N	N

Table 6. Features of selected biodegradation models included in this review.

1982) for a conservative tracer. The numerical solution matched the analytical solution extremely well for Peclet numbers of 1 and 100.

5.1.3 Comments

Widdowson et al. (1988) ran a number of simulations designed to mimic conditions expected to develop in laboratory columns. Substrate, oxygen, nitrate and biomass concentration profiles in the column were shown for several different times under different limiting conditions.

The lack of any experimental validation of this model is an important drawback. However, the model demonstrates several important simulation methods. First, the model simulates biodegradation under any combination of limiting conditions. Substrate, oxygen, nitrate, a fourth limiting nutrient or a combination of these can control the rate of biodegradation. The biodegradation rates in each modeling grid are controlled by the local concentration of these nutrients. This multiple limitation model is probably physically realistic in that multiple zones, each characterized by its own chemical conditions, are expected to develop in a contaminant plume.

Second, the model illustrates how inhibition functions can be used to switch between oxygen and nitrate limiting conditions. The inhibition factor multiplies the rate expressions for substrate utilization and microbial growth under nitrate metabolism. At high oxygen concentrations, this factor is nearly zero. As oxygen is depleted, the function approaches 1 and nitrate metabolism steadily increases. These type of inhibition functions can be used to model multiple microbial populations under multiple types of respiration or fermentation.

A key part of the model is the modeling of a stagnant liquid layer between the microcolony and the bulk fluid. A linear concentration profile across the diffusion layer is assumed and a mass transfer coefficient controls the chemical flux. The concentration of nutrients experienced by the microorganisms is less than the concentration of nutrients in the bulk fluid. The lower concentration tends to reduce the growth rate of biomass at the column inlet. However, the simulations were not run long enough to determine whether or not the reduced concentrations of nutrients in the microcolonies were sufficient

to reach a steady-state biomass concentration below the maximum physically possible. The structure of the model does not limit unbridled growth of biomass.

5.2 Semprini and McCarty, 1992

The model of Semprini and McCarty (1992) demonstrates how a relatively simple model can accurately simulate a real contaminant plume. The one-dimensional model simulates the pulsing of methane and oxygen into an aquifer at the Moffet Field Naval Air Station to stimulate a methanotrophic culture into biodegrading chlorinated hydrocarbons by cometabolism. Key features of the model include:

- Biomass is modeled as a concentration.
- Biodegradation is inhibited by high primary substrate (methane) concentrations.
- First-order deactivation of the methanotrophic culture's biodegradation ability is simulated when it is decaying due to lack of oxygen or methane or both.
- Adsorption is modeled as a first-order non-equilibrium process.

5.2.1 Important Assumptions

- Monod kinetics apply.
- Bacterial transport is not significant.
- A single bacterial species is present.
- Biodegradation is aerobic only.
- Cometabolism is assumed to follow Monod kinetics.
- Biodegradation rates are limited by either oxygen, methane, or both.

5.2.2 Validation

The model was used to simulate the cometabolism of vinyl chloride, trans-dichloroethylene, cis-dichloroethylene, and trichloroethylene between an injection well and two downgradient sampling wells. The solution containing these compounds was injected continuously into the aquifer until the concentrations at the sampling well were nearly equal to the concentrations at the injection well. Methane and oxygen were then pulsed into the aquifer to stimulate the methanotrophic population to begin biodegrading the

contaminants. The main fitting parameter for the model was k_2, the TCE transformation rate constant. The model successfully simulated the oscillating concentrations of methane and the chlorinated contaminants at the sampling well.

5.2.3 Comments

This model illustrates the importance of accounting for inhibition when the primary substrate, products, or other substrates interfere with biodegradation of the compound of primary interest. Simulations of inhibition kinetics were compared to simulations where Monod kinetics without inhibition were used. Semprini and McCarty (1992) found that inhibition kinetics were necessary to accurately represent the methane and contaminant concentrations seen at the sampling wells.

This model is also one of only a few models that used non-equilibrium reactions to model adsorption and illustrates how adsorption can influence biodegradation predictions. Simulations using linear equilibrium adsorption did not match the data as well as simulations where rate-limited adsorption was included. Simulations using rate-limited adsorption correctly modeled the extensive tailing observed at the sampling wells, whereas equilibrium adsorption predicted more rapid declines in contaminant concentrations.

5.3 Chen et al., 1992

Chen et al. (1992) present a model of considerably greater complexity than the two models discussed above. Most of the complexity comes from the fact that this model is a multi-phase model, accounting for four phases: solid, water, NAPL and air. The model accounts for mass exchange between these phases, two substrates, two electron acceptors, one additional limiting nutrient, and two microbial populations. Other chemicals can be added if necessary. The two substrates modeled are benzene and toluene, and the two electron acceptors are oxygen and nitrate. The model is applied to biodegradation of these chemicals in laboratory columns with only an aqueous phase present. Key features of the model include:

- Biomass is modeled as microcolonies with fully penetrated biofilms.

- A stagnant liquid film diffusion layer exists between the bulk fluid and the microcolonies.
- Inhibition functions switch from oxygen to nitrate respiration.

5.3.1 Important Assumptions

- Local equilibrium exists for mass transfer between phases.
- Monod kinetics apply.
- Bacterial transport is insignificant.
- Biodegradation rates are limited by any substrate, electron acceptor, or nutrient.
- The air phase is immobile.
- Biodegradation occurs only in the aqueous phase.
- No adsorption occurs on particles exposed only to the air phase.
- One microbial population degrades only benzene, only aerobically.
- The second microbial population degrades only toluene either aerobically or anaerobically (with nitrate as the electron acceptor).

5.3.2 Validation

The model was validated in three ways. First, the model numerical solution for a conservative tracer was compared to the analytical solution of Ogata and Banks (1961) for transport under steady-state flow conditions in a homogeneous, water-saturated, soil-packed column. Second, the model solutions were compared to the solutions obtained by Molz et al. (1986). Finally, simulated breakthrough curves were compared to breakthrough curves from laboratory experiments.

An attempt was made to measure all parameters independent of the experiments so that the model parameters represented physical quantities and not fitting parameters. Monod kinetic parameters were determined from aquifer slurry experiments, and other parameters were determined by experiments with the soil columns prior to the biodegradation experiments. The only parameter that was adjusted to provide a better fit to the data was the fraction of benzene and toluene degrading microorganisms in the

reactor at the beginning of the experiments, since these proportions could not be determined prior to the experiment.

The columns were continuously fed a mixture containing substrate(s), electron acceptors and sufficient nutrients and the breakthrough curves of benzene and toluene were recorded. The breakthrough curves were successfully simulated by the model under substrate-limited aerobic conditions. The model successfully simulated the toluene breakthrough curve under nitrate-based respiration and oxygen-limited conditions, but did not simulate the benzene breakthrough curve well.

5.3.3 Comments

This model illustrates how multiple electron acceptors, nutrients, and microbial populations can be simulated. Although the model was not applied to a multi-phase or 3-dimensional modeling domain, the model equations are general so that modification of the model to account for this greater complexity is relatively straightforward. The model contains nearly all of the elements that are likely to be important in biodegradation modeling including inhibition kinetics, multiple nutrient/electron acceptor growth limitations, inhibition functions to switch between methods of substrate utilization, and diffusional resistances. This model forms a good basis on which to build for future modeling efforts.

5.4 Taylor and Jaffé, 1990b

One shortcoming of the three previous models is their inability to model bacterial transport and the effect of biomass growth on permeability, porosity and dispersivity. The model presented by Taylor and Jaffé (1990b, 1991) includes methods to account for these changes. The single-phase model is typical of models in the groundwater literature (as opposed to models in the petroleum literature) in the manner in which concentrations and phases are expressed. Taylor and Jaffé (1990b) used the model to simulate the growth and transport of bacteria in laboratory columns under conditions where only one nutrient (the substrate) was limiting. Taylor and Jaffé (1991) then used the model to investigate the effectiveness of different nutrient addition strategies during a simulation of in-situ bioremediation. Key features of the model include:

- Biomass is modeled as a separate phase (a fully penetrated biofilm).
- Biomass transport is modeled with an advection-dispersion equation.
- Biofilm removal is by shearing, and deposition is modeled as a first-order process.
- Porosity, permeability and dispersivity change with biomass growth.

5.4.1 Important Assumptions

- Monod kinetics apply.
- Biodegradation rate is limited by a single substrate.
- No diffusional limitations exist.
- No adsorption of the substrate or microorganisms occurs.

5.4.2 Validation

Taylor and Jaffé compared the modeled biofilm thicknesses to observed biofilm thicknesses in an experimental reactor. Model parameters were determined from these laboratory experiments or literature sources. The model was calibrated by adjusting the dimensionless parameter describing the biological aspects of shearing appearing in the expression for biomass decay, and the depositional parameters that appear in the biomass deposition expression. The model was then used to predict substrate concentration profiles and biofilm thicknesses in a second laboratory column. The substrate profiles and biomass thicknesses were predicted well at early and late times, although the predictions at intermediate times were not as good.

5.4.3 Comments

This model is probably the most sophisticated model for predicting changes in the porous media from biomass growth. With the exception of the model of Corapcioglu and Haridas (1985), this model is the only one reviewed that treats biomass as a separate phase. It may be possible to incorporate the expressions used to model biomass growth and its effects on porous media into other models that are focused more on contaminant biodegradation.

5.5 Sarkar et al., 1994

The model of Sarkar et al. (1994) is based on the sophisticated platform of the multi-component, multi-phase, three-dimensional model UTCHEM. The method of expressing concentration, particularly adsorbed phase concentrations, differs slightly from the models in the groundwater and soils literature. However, the results are the same. The primary purpose of the Sarkar et al. (1994) model is to simulate microbial-enhanced oil recovery (MEOR) processes. However, the flow principles are the same as those in multi-phase groundwater flow. The model is used to simulate bacteria and substrate effluent concentrations, as well as the changes in the permeability of the porous media, in laboratory columns. Bacterial growth is anaerobic with glucose as the substrate. Key features of the model include:

- Biomass is modeled as a concentration.
- Adsorption is modeled as a reversible process with a Langmuir isotherm.
- Transport and retention of bacteria are modeled with a fractional flow function.
- Permeability reduction is modeled using effective medium theory (EMT).
- Contois kinetics are used to model substrate utilization and biomass growth.
- Inhibition kinetics are used to model the effect of toxic metabolites on bacterial growth.

5.5.1 Important Assumptions

- No diffusional resistances exist.
- Biodegradation rate is limited by one substrate.
- Biodegradation occurs only in the aqueous phase.
- Bacteria, nutrients and produced metabolites exist only in the aqueous phase.

5.5.2 Validation

Model simulations of effluent bacteria and glucose concentrations were compared to laboratory experiment effluent concentrations. Simulated permeability histories were also compared to laboratory experiments. Experiments were performed for both single-phase (water only - no NAPL) and multi-phase flow. Effluent bacteria concentrations

were predicted by the model with good accuracy at low flow rates in single-phase flow. Predictions of effluent bacteria concentrations were not as good for higher flow rates. Effluent bacteria concentration simulations of the two-phase experiments was very good. Simulations of permeability reduction and effluent glucose concentration histories were also fairly good.

5.5.3 Comments

This model demonstrates how bacterial transport and multi-phase flow can be integrated into the same model. It also illustrates an alternative to the first-order removal process used to model bacterial deposition in the model of Taylor and Jaffé. Like the model of Chen et al. (1992), this model could also serve as a good platform for incorporation of other biodegradation processes, which could be important in predicting subsurface biodegradation.

6.0 CONCLUSIONS AND RECOMMENDED MODELING APPROACH

From the variety of approaches other researchers have taken to model subsurface biodegradation, it is apparent that much work remains before the process can be modeled with a high degree of accuracy in the field. However, our understanding of the processes has increased immeasurably over the last few years, and many models have been successful in simulating biodegradation in laboratory studies. These modeling efforts help identify the factors that are important in biodegradation modeling and suggest expanded approaches to model the phenomenon.

From the extensive review of biodegradation models and the related engineering principles completed for this report, it appears that a comprehensive model can be developed that incorporates the best aspects of the other models reviewed. A successful comprehensive model should:

1. Account for changes in porosity, permeability and dispersivity due to biomass growth.
2. Treat adsorption as a nonlinear process.
3. Model multiple phases.
4. Account for reaeration from the surface through diffusion and/or infiltration.
5. Base biodegradation rates on local, not spatially averaged, concentrations.
6. Account for diffusion through a stagnant liquid layer adjacent to the biomass.
7. Model growth and decay of biomass as a separate phase.
8. Model multiple microbial populations.
9. Account for transport of microorganisms.
10. Allow different growth rates for free-floating and adsorbed bacteria.
11. Limit biomass growth to what is physically possible.
12. Include a lag phase for microorganisms to adapt to contamination.
13. Include inhibition kinetics if necessary.
14. Accommodate cometabolism.
15. Accommodate multiple substrates with substrate inhibition functions.

16. Model multiple electron acceptors.
17. Account for sequential reactions of substrates.
18. Track the concentration of all potential redox reaction participants.
19. Account for multiple nutrient limitations on biomass growth.
20. Model fermentative, anaerobic and aerobic metabolism simultaneously.

Also from the literature review, it appears that the following assumptions are reasonable:

1. The biophase is a fully penetrated biofilm (no intra-film diffusional limitations exist), except possibly at injection wells where substrate and electron acceptor concentrations are kept very high.
2. Biodegradation occurs only in the aqueous phase.
3. Diffusion across a stagnant liquid layer can be modeled by Fick's Law.
4. Biomass can be modeled with an unstructured, unsegregated approach.

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