CHAPTER 2

CHEMICAL AND BIOLOGICAL PROCESSES: THE NEED FOR MIXING

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2.1 INTRODUCTION

Except for spontaneous reactions such as radioactive decay, chemical transformations often require that two or more substances be brought together for the transformation to occur. Examples of particular interest in groundwater are oxidations of inorganic or organic species, which require the presence of some oxidant, such as diatomic oxygen (O_2), nitrate, sulfate, or ferric iron (Fe(III)). In biological reactions, three entities generally are required, the compound being oxidized (electron donor), the oxidant (electron acceptor), and the microorganism carrying out the transformation. At times, the required entities are already present together, and then transformation occurs based simply on normal reaction kinetics. However, this is often not the case in groundwater remediation, and then the missing reactants must be supplied through some means and mixed with the substance or substances targeted for removal. The speed of the reaction is then likely to be governed primarily by the rate at which the required substances can be brought together. Natural attenuation for transformation of materials may require mixing brought about by the diffusion of oxygen into an aquifer from the vadose zone above, or from an adjacent groundwater flow stream. The process of adding and mixing needed substances for desired transformation is one of the most challenging and costly aspects of *in situ* remediation of contaminated groundwater and soil. This is a much more difficult process than with an aboveground reactor because of complex and often undefined hydrogeology and the general uncertainty of the exact location of the contaminants.

Some form of mixing may also be required for processes other than chemical oxidations. Included are the addition of reducing compounds for chemical reductions; acids or bases for pH control; chemicals that promote precipitation for in-place stabilization; detergents, solvents, or other chemicals that promote solubilization of the compound of interest for easier removal; addition of a separate phase such as air; use of thermal treatment to enhance vaporization; as well as chemical changes resulting from groundwater-surface water interactions that are driven by variability in rates of precipitation, extraction, and aquifer recharge. All such processes involve mixing in one form or another. The emphasis in this chapter is not on the mixing processes themselves, but on the chemical and biological requirements for contaminant transformation, destruction, or removal. A few examples of field studies where mixing has been used to bring the reactants together are provided for illustration, and many others are provided in other chapters of this volume.

2.2 GROUNDWATER CONTAMINANTS

The most frequently found chemicals in groundwater at hazardous waste sites are listed in Table 2.1 (NRC, 1994). Among organic contaminants, the chlorinated solvents, trichloroethene (TCE), perchloroethene (PCE), methylene chloride (dichloromethane or MC), and

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Table 2.1. Most Frequently Detected Groundwater Contaminants at Hazardous Waste Sites (after NRC, 1994)

Organic contaminants		Inorganic contaminants	
Rank	**Chemical**	**Rank**	**Chemical**
1	Trichloroethene (TCE)	1	Lead
2	Tetrachloroethene (PCE)	2	Chromium
3	Benzene	3	Zinc
4	Toluene	4	Arsenic
5	Methylene chloride (MC)	5	Cadmium
6	1,1,1-Trichloroethane (TCA)	6	Manganese
7	Chloroform	7	Copper
8	1,1-Dichloroethane (1,1-DCA)	8	Barium
9	1,2-Dichloroethene (1,2-DCE)	9	Nickel
10	1,1-Dichloroethene (1,1-DCE)		
11	Vinyl chloride (VC)		
12	1,2-Dichloroethane (1,2-DCA)		
13	Ethylbenzene		
14	Di(2-ethylhexyl)phthalate		
15	Xylenes		
16	Phenol		

1,1,1-trichloroethane (TCA) are among the six most frequently found organic chemicals. These chemicals are denser than water such that when spills of the liquid solvents reach groundwater, they continue downward under the force of gravity, often penetrating deeply into a groundwater aquifer. They are poorly biodegradable and represent the most difficult and costly chemicals for remediation. It is for this reason that so much attention has been paid to them. Others among the list of frequently found organic chemicals are degradation products of these four chlorinated solvents, including 1,1-dichloroethane (1,1-DCA), 1,2-dichloroethene (1,2-DCE), 1,1-dichloroethene (1,1-DCE), and vinyl chloride (VC). Thus, 8 of the 11 most frequently found organic chemicals are chlorinated solvents themselves and their degradation products.

The second group of organic chemicals includes benzene and toluene, the third and fourth most frequently found on the list. These aromatic hydrocarbons are the more soluble components of gasoline that partition into groundwater from gasoline spills. Gasoline itself is lighter than water and so tends to spread out over the surface of the groundwater, rather than penetrating into it. Two other aromatic hydrocarbon components of gasoline are also on the list, ethylbenzene and xylenes (of which there are three different isomers). These four aromatic hydrocarbons are collectively known as the BTEX compounds (benzene, toluene, ethylbenzene, xylene).

Only 4 of the top 16 organic chemicals are not among the chlorinated solvent or BTEX groups. These include chloroform, generally formed from the chlorination of water through its interaction with humic materials; 1,2-dichloroethane (1,2-DCA), a chlorinated compound used widely in chemical synthesis and as a solvent; di(2-ethylhexyl)phthalate, a chemical used in plastics manufacture; and phenol and its derivatives, including the chlorinated phenols used in treating wood.

Other organic chemicals of importance as groundwater contaminants but not included on this list are carbon tetrachloride (CT), another widely used solvent in the past; methyl tertiary-butyl ether (MTBE), an oxygenate additive of gasoline; 1,4-dioxane, an industrial chemical commonly used as a solvent stabilizer; and chlorinated benzenes and benzoates, which have a wide variety of industrial and commercial uses. These chemicals are all persistent organic pollutants (POPs) that need to be addressed in groundwater remediation.

Table 2.1 includes nine inorganic chemicals. These substances are not destroyed chemically or biologically. Consequently, their remediation is through removal from the groundwater by extraction or immobilization. Five of the inorganic chemicals in Table 2.1 (lead, zinc, cadmium, barium and nickel) are metals that exist primarily as stable cations and so are not susceptible to oxidation and reduction, but can be removed from water by adsorption or chemical precipitation. The other metals in Table 2.1 are directly susceptible to oxidation-reduction reactions that alter their solubility and thus mobility in groundwater. As shown in Table 2.2, chromium (Cr), arsenic (As), selenium (Se), and uranium (U) are present as either cations or oxyanions depending upon oxidation state and pH. Under neutral to basic conditions, hexavalent chromium exists as the highly soluble and toxic chromate oxyanion (CrO_4^{2-}). Under acidic conditions, however, it exists as dichromate ($Cr_2O_7^{2-}$). It is also readily reduced to a trivalent state that is nontoxic and precipitates as $Cr(OH)_3(s)$, a solid with low solubility in water and low toxicity. Arsenic can be found in the soluble trivalent (AsO_3^{3-}) or pentavalent (AsO_4^{3-}) states. The relative solubility and mobility of soluble arsenic species depends on interactions with the solid phase. Selenium is a metalloid that is naturally present in some groundwaters where it may be present as the soluble oxyanions selenite (SeO_3^{2-}) or selenate (SeO_4^{2-}). These species can be

Table 2.2. Regulated Metals and Metalloids That Are Susceptible to Changes in Solubility Through Microbial or Chemically Mediated Redox Reactions (adapted from Nyman et al., 2005)

Metal or metalloid	Oxidation state	Oxidized species	Reduced species (often less soluble)	Common sources
As[a]	-II		AsS	Erosion of natural deposits; runoff from orchards; runoff from glass & electronics production
	0		FeAsS, As	
	III	H_2AsO_3, $H_2AsO_3^-$, $HAsO_3^{2-}$, AsO_3^{3-}	As_2O_3	
	V	H_3AsO_4, $H_2AsO_4^-$, $HAsO_4^{2-}$, AsO_4^{3-}		
Cr	III		Cr_2O_3	Steel and pulp mills; erosion of natural deposits
	VI	H_2CrO_4, $HCrO_4^-$, CrO_4^{2-}, $Cr_2O_7^{2-}$		
Se	-II	H_2Se, HSe^-, Se^{2-}		Refineries; natural deposits; mines
	0		Se	
	IV	H_2SeO_3, $HSeO_3^-$, SeO_3^{2-}	SeO_2	
	VI	H_2SeO_4, $HSeO_4^-$, SeO_4^{2-}		
	VII	SeO_4^-		
U	IV		UO_2, $USiO_4$	Mine tailings; atomic bomb fabrication sites; weapons use; erosion of natural sources
	VI	UO_2^{2-}, $UO_2(CO_3)$, $UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{4-}$		

[a]The normal valence states of arsenic are III and V. As(III) can be more mobile and toxic than As(V)

biologically reduced to zero-valent selenium (low solubility) or to selenium hydride (H_2Se). Finally, uranium is a radionuclide that is often present in nature in the +IV oxidation state as uraninite UO_2, a sparingly soluble mineral. During extraction and refining operations, the U(IV) is oxidized to toxic, soluble, and mobile complexes. At low pH, the uranyl cation UO_2^{2+} is dominant; at near neutral pH and above, carbonate complexes dominate.

Inorganic chemicals of concern that are not listed in Tables 2.1 or 2.2 include nitrate, perchlorate (ClO_4^-), and ferrous iron. Nitrate is a common contaminant from agricultural operations and from the use of nitric acid for mineral extraction. It is also a common electron acceptor for bacteria, and can be removed from water by denitrification. Perchlorate is used in rocket fuel, fireworks, and road flares. Like nitrate, it can serve as an electron acceptor for microbial growth, and as such can be biologically reduced to harmless chloride. Although iron and manganese are not listed in Table 2.1 as prevalent contaminants, they can be present at high levels in solution, often formed from natural aquifer minerals through biological reduction.

Knowledge of the physical properties of contaminants (Tables 2.3, 2.4) is of interest to help better understand processes that affect their movement and fate in groundwater. As already indicated and as Table 2.3 illustrates, the chlorinated solvents, which are liquid at room temperature, have densities greater than water (1.0 gram per cubic centimeter [g/cm^3]) and thus tend to penetrate deeply into groundwater. BTEX compounds have densities lower than water and so will not penetrate downward into groundwater, but will remain in the capillary fringe above. Water solubility of chemicals indicates the extent to which the free phase liquid of the solvent can dissolve in water. Solubilities of most chemicals listed in Table 2.3 are in the gram per liter (g/L) range or less, and are thus called "sparingly soluble."

Table 2.3. Physical and Chemical Properties of Chlorinated Solvents and Their Transformation Products at 25 Degrees Celsius (°C) (after Yaws, 1999)

Compound	Density (g/cm^3)	Henry's law constant, *H* (atm/M)	Water solubility (mg/L)	Octanol-water partition coefficient (log K_{ow})
Methanes:				
Carbon tetrachloride (CT)	1.59	29	790	2.83
Trichloromethane	1.48	4.1	7,500	1.97
Methylene chloride (MC)	1.33	2.5	19,400	1.25
Chloromethane	0.92	8.2	5,900	0.91
Ethanes:				
1,1,1-Trichloroethane (1,1,1-TCA)	1.34	22	1,000	2.49
1,1-Dichloroethane (1,1-DCA)	1.18	5.8	5,000	1.79
1,2-Dichloroethane (1,2-DCA)	1.24	1.2	8,700	1.48
Chloroethane	0.90	6.9	9,000	1.43
Ethenes:				
Tetrachloroethene (PCE)	1.62	27	150	3.4
Trichloroethene (TCE)	1.46	12	1,100	2.42
cis-1,2-Dichloroethene (*cis*-DCE)	1.28	7.4	3,500	1.85
trans-1,2-Dichloroethene (*trans*-DCE)	1.26	6.7	6,300	2.09
1,1-Dichloroethene (1,1-DCE)	1.22	23	3,400	2.13
Vinyl chloride (VC)	0.91	22	2,700	1.62

(*continued*)

Table 2.3. (continued)

Compound	Density (g/cm^3)	Henry's law constant, *H* (atm/M)	Water solubility (mg/L)	Octanol-water partition coefficient (log K_{ow})
Aromatic compounds:				
Benzene	0.88	5.6	1,760	2.13
Toluene	0.87	6.4	540	2.73
Ethylbenzene	0.86	8.1	165	3.15
o-xylene	0.88	4.2	221	3.12
m-xylene	0.86	6.8	174	3.20
p-xylene	0.86	6.2	200	3.15
Methyl tertiary-butyl ether (MTBE)	0.74	0.54	51,000	0.94
Chlorobenzene	1.10	4.5	300	2.84
1,2-Dichlorobenzene	1.30	2.8	92	3.43
Phenol[a]	#	0.00076	80,000	1.46

Note: *atm*/*M* atmosphere liters per mole, *mg*/*L* milligrams per liter
[a]Solid at room temperature

Table 2.4. Mineral Solubility Products (from Nyman et al., 2005)

Compound	Formula	pK_{sp}	K_{sp}	Reference
Arsenic(III) sulfide	As_2S_3	21.68	2.1×10^{-22}	Dean, 1999
Cadmium sulfide	CdS	26.10	8.0×10^{-27}	Dean, 1999
Chromium(III) hydroxide	$Cr(OH)_3$	30.20	6.3×10^{-31}	Dean, 1999
Cobalt sulfide	CoS	20.40	4.0×10^{-21}	Dean, 1999
	CoS	24.70	2.0×10^{-25}	
Copper(I) sulfide	Cu_2S	47.60	2.5×10^{-48}	Dean, 1999
Copper(II) sulfide	CuS	35.20	6.3×10^{-36}	Dean, 1999
Ferrihydrite	$Fe(OH)_3$	39.5	3.16×10^{-40}	Cornell and Schwertmann, 1996
Goethite	FeOOH	40.7	2.00×10^{-41}	Cornell and Schwertmann, 1996
Hematite	Fe_2O_3	42.75	1.78×10^{-43}	Cornell and Schwertmann, 1996
Iron(II) sulfide	FeS	17.20	6.3×10^{-18}	Dean, 1999
Lead sulfide	PbS	27.10	8.0×10^{-28}	Dean, 1999
Manganese hydroxide	$Mn(OH)_2$	12.72	1.9×10^{-13}	Dean, 1999
Mercury(II) sulfide	HgS red	52.4	4×10^{-53}	Dean, 1999
	HgS black	51.8	1.6×10^{-52}	
Nickel α-sulfide	NiS	18.5	3.2×10^{-19}	Dean, 1999
β-sulfide	β-NiS	24.0	1.0×10^{-24}	
γ-sulfide	NiS	25.70	2.0×10^{-26}	
Technicium	TcO_2	8	10^{-8}	Rard et al., 1999
Uraninite	UO_2	60.6	2.5×10^{-61}	Langmuir, 1978
Zinc sulfide: sphaelerite	ZnS	23.8	1.6×10^{-24}	Dean, 1999
wurtzite	ZnS	21.6	2.5×10^{-22}	

Note: K_{sp} solubility product constant; $pK_{sp} = -\log K_{sp}$

The Henry's Law constant (H) indicates the potential of a compound to partition between water and air and, therefore, the tendency of a compound to be removed from water by air stripping, the higher the value the easier it is to be removed such as by air sparging. Ionic (i.e., charged) compounds and compounds with H less than about 0.2 atm/M are not likely to be removed readily by air stripping. The octanol-water partition coefficient (K_{ow}) indicates the potential of a compound to partition from water onto aquifer solids, and particularly into the organic portion of aquifer solids. This partitioning impacts on the compound's rate of movement through an aquifer and on the ease with which a chemical injected into the aquifer can move and interact with a contaminant. Compounds with log K_{ow} in the range of 2 or above will partition moderately onto aquifer solids, depending upon the organic content, and this applies to most of the chemicals listed in Table 2.3. Compounds such as DDT (dichlorodiphenyltrichloroethane) and PCBs (polychlorinated biphenyls) have log K_{ow} values around 6 or higher and thus sorb very strongly to aquifer solids. It is for this reason that they are not major groundwater contaminants as they sorb so strongly to soils that they rarely penetrate sufficiently downward to contaminate groundwater.

2.3 REACTION AND MASS TRANSFER PROCESSES

2.3.1 Overview

The emphasis in this chapter is on chemical or biological transformations or reactions that require the bringing together of two or more chemical or biological species for the reaction to occur. Mass transfer refers to the process or processes by which they come together. The discussion of these processes is rather brief, more detailed information can be found in environmental chemistry textbooks (Benjamin, 2002; Morel and Hering, 1993; Sawyer et al., 2003; Stumm and Morgan, 1996). Reaction stoichiometry, i.e., the relative amounts of chemicals needed for transformations to go to completion, is an important component of reaction and mass transfer analyses that is needed for the design of a delivery system or for analysis of natural attenuation. Stoichiometry also makes it possible to quantify the products of a transformation, which sometimes are also contaminants of concern. Examples of products include methane, sulfide, the soluble reduced forms of iron and manganese, and partially reduced or oxidized contaminant species. Reaction stoichiometry depends to some extent on the type of reaction involved, so this requires some consideration. Next comes understanding of mass transfer and reaction kinetics to determine when the rate of a reaction will be controlled primarily by the intrinsic kinetics of the reaction itself and when it will be controlled by the rate at which reactants are brought into contact with one another.

2.3.2 Stoichiometry

In the design of a system involving chemical transformation, the making of a mass balance is critical for determining how much chemical must be added to bring about a given amount of change and what will be the products of the reaction. These quantities can be provided through use of a stoichiometric equation that describes the overall reaction of interest (Sawyer et al., 2003). If a stoichiometric equation for the reaction of interest cannot be written because of inadequate information, then knowledge of the reaction is insufficient to make a good judgment on chemical requirements. In such a case, more study is needed before *in situ* remediation is attempted, or else costly mistakes may be made, either in adding too much of a needed substance or too little. In order to address stoichiometry, knowledge of reaction and mass-transfer processes is useful.

2.3.3 Reaction and Mass-Transfer Processes

Table 2.5 summarizes important reaction and mass-transfer processes involved in contaminant movement and fate in water. The significance of acid–base reactions is that they change the active species of a chemical under given chemical conditions in water. They also dominate the acid–base buffering of a system.

Table 2.5. Examples of Reaction and Mass-Transfer Processes of Interest in Groundwater Remediation

Reaction process	Description	Examples
Acid–base	Change in an element in solution from one chemical form to another without a change in the valance state – generally in response to pH conditions.	$H^+ + OH^- = H_2O$
		$HCO_3^- = H^+ + CO_3^{2-}$
		$CO_2 + H_2O = H^+ + HCO_3^-$
		$H_2S = H^+ + HS^-$
		$Zn_2^+ + OH^- = ZnOH^+$
		$Cr_2O_7^{2-} + H_2O = 2CrO_4^{2-} + 2H^+$
Oxidation-reduction	Change in the oxidation state of an element in a chemical, generally requires change in oxidation state of two elements, one is oxidized, the electron acceptor, and the other reduced, the electron donor.	$4Fe(OH)_2 + O_2 + 2H_2O = 4Fe(OH)_3$
		$4Cr^{3+} + 3O_2 + 8H_2O = 2Cr_2O_7^{2-} + 16H^+$
		$CH_3COOH + 2O_2 = 2CO_2 + 2H_2O$
		$CH_3COOH + SO_4^{2-} = 2CO_2 + H_2S + 2OH^-$
Precipitation	Formation of a solid phase from reaction between chemicals in solution.	$Ca^{2+} + CO_3^{2-} = CaCO_3\ (s)$
		$Cr^{3+} + 3OH^- = Cr(OH)_3\ (s)$
		$2Fe^{3+} + 6OH^- = Fe_2O_3\ (s) + 3H_2O$
		$Fe^{2+} + S^{2-} = FeS\ (s)$
		$Zn^{2+} + S^{2-} = ZnS\ (s)$
Mass transfer process	**Description**	**Examples**
Solubilization	May represent dissolution of a chemical from a solid phase into a soluble form, the reverse of precipitation. It may also represent the partitioning of a chemical from a non-miscible liquid phase into the aqueous phase.	$CaCO_3\ (s) = CaCO_3\ (aq)$
		$Fe_2O_3\ (s) = Fe_2O_3\ (aq)$
		TCE (*l*) = TCE (*aq*)
		benzene (*l*) = benzene (*aq*)
Volatilization	The movement of a chemical from an aqueous phase to a gaseous phase.	TCE (*aq*) = TCE (*g*)
		benzene (*aq*) = benzene (*g*)
Sorption	The partitioning of a chemical from the aqueous phase onto or into a solid phase.	TCE (*aq*) = TCE (*sorbed*)
		$Fe^{3+} = Fe^{3+}$ (*sorbed*)
Advection	The transport of a chemical by being carried along in a moving fluid such as water or air.	
Diffusion-dispersion	Diffusion is the net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion. Dispersion is similar but is a faster process brought about in addition by dynamic mixing of the fluid in which the chemical is contained.	

Note: (*aq*) aqueous phase, (*g*) gas phase, (*l*) liquid phase, (*s*) solid phase

Oxidation-reduction reactions are perhaps the most important reactions used in groundwater remediation. Here, the chemical being oxidized is termed the electron donor as electrons are removed from it in the process. The chemical being reduced is the electron acceptor because it accepts the electrons. This electron exchange is illustrated by the half reactions shown in Table 2.6 for typical electron donors and Table 2.7 for typical electron acceptors. Stoichiometric equations for oxidation-reduction reactions can be written by adding a given electron donor half-reaction to that of an electron acceptor half reaction. For example, the oxidation of the electron donor ethanol with the electron acceptor carbon dioxide (CO_2) results in the following stoichiometric equation for the conversion of ethanol into methane:

$$\frac{1}{12}CH_3CH_2OH = \frac{1}{8}CH_4 + \frac{1}{24}CO_2 \qquad \text{(Eq. 2.1)}$$

Table 2.6. Electron Donor Half Reactions

Electron donor		End product	Half reaction
Hydrogen	H_2	H^+	$\frac{1}{2}H_2 = H^+ + e^-$
Zero-valent iron	Fe^0	Fe^{2+}	$\frac{1}{2}Fe(s) = \frac{1}{2}Fe^{2+} + e^-$
Acetate	CH_3COO^-	CO_2	$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$
Lactate	$C_3H_5O_2^-$	CO_2	$\frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O = \frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^-$
Fatty acid	$C_{18}H_{31}O_2^-$	CO_2	$\frac{1}{100}C_{18}H_{31}O_2^- + \frac{7}{20}H_2O = \frac{17}{100}CO_2 + \frac{1}{100}HCO_3^- + H^+ + e^-$
Methanol	CH_3OH	CO_2	$\frac{1}{6}CH_3OH + \frac{1}{6}H_2O = \frac{1}{6}CO_2 + H^+ + e^-$
Ethanol	CH_3CH_2OH	CO_2	$\frac{1}{12}CH_3CH_2OH + \frac{3}{12}H_2O = \frac{1}{6}CO_2 + H^+ + e^-$
Carbohydrate	$C_6H_{12}O_6$	CO_2	$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^-$
Benzene	C_6H_6	CO_2	$\frac{1}{30}C_6H_6 + \frac{2}{5}H_2O = \frac{1}{5}CO_2 + H^+ + e^-$
Toluene	$C_6H_5CH_3$	CO_2	$\frac{1}{36}C_6H_5CH_3 + \frac{7}{18}H_2O = \frac{7}{36}CO_2 + H^+ + e^-$
Ethylbenzene	$C_6H_5C_2H_5$	CO_2	$\frac{1}{42}C_6H_5C_2H_5 + \frac{8}{21}H_2O = \frac{4}{21}CO_2 + H^+ + e^-$
Xylene	$C_6H_4(CH_3)_2$	CO_2	$\frac{1}{42}C_6H_4(CH_3)_2 + \frac{8}{21}H_2O = \frac{4}{21}CO_2 + H^+ + e^-$
TCE	$CHCl{=}CCl_2$	$CO_2 + Cl^-$	$\frac{1}{6}CHCl{=}CCl_2 + \frac{2}{3}H_2O = \frac{1}{3}CO_2 + \frac{1}{2}Cl^- + \frac{3}{2}H^+ + e^-$
DCE	$CHCl{=}CHCl$	$CO_2 + Cl^-$	$\frac{1}{8}CHCl{=}CHCl + \frac{1}{2}H_2O = \frac{1}{4}CO_2 + \frac{1}{4}Cl^- + \frac{5}{4}H^+ + e^-$
VC	$CH_2{=}CHCl$	$CO_2 + Cl^-$	$\frac{1}{10}CH_2{=}CHCl + \frac{2}{5}H_2O = \frac{1}{5}CO_2 + \frac{1}{10}Cl^- + \frac{11}{10}H^+ + e^-$
Chlorobenzene	C_6H_5Cl	$CO_2 + Cl^-$	$\frac{1}{28}C_6H_5Cl + \frac{3}{7}H_2O = \frac{3}{14}CO_2 + \frac{1}{28}Cl^- + \frac{29}{28}H^+ + e^-$
Dichlorobenzene	$C_6H_4Cl_2$	$CO_2 + Cl^-$	$\frac{1}{26}C_6H_4Cl_2 + \frac{6}{13}H_2O = \frac{3}{13}CO_2 + \frac{1}{13}Cl^- + \frac{14}{13}H^+ + e^-$

Table 2.7. Electron Acceptor Half Reactions

Electron acceptor		End product	Half reaction
Oxygen	O_2	H_2O	$\frac{1}{8}O_2 + H^+ + e^- = \frac{1}{2}H_2O$
Nitrate	NO_3^-	N_2	$\frac{1}{5}NO_3^- + \frac{6}{5}H^+ + e^- = \frac{1}{10}N_2 + \frac{3}{5}H_2O$
Manganate	MnO_2	Mn^{2+}	$\frac{1}{2}MnO_2 + 2H^+ + e^- = \frac{1}{2}Mn^{2+} + H_2O$
Ferric iron	Fe_2O_3	Fe^{2+}	$\frac{1}{2}Fe_2O_3 + 3H^+ + e^- = Fe^{2+} + \frac{3}{2}H_2O$
Sulfate	SO_4^{2-}	$H_2S + HS^-$	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^- = \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$
Carbon dioxide	CO_2	CH_4	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4$
Perchlorate	ClO_4^-	Cl^-	$\frac{1}{8}ClO_4^- + H^+ + e^- = \frac{1}{8}Cl^- + \frac{1}{2}H_2O$
PCE	$CCl_2{=}CCl_2$	$CH_2{=}CH_2$	$\frac{1}{8}CCl_2{=}CCl_2 + \frac{1}{2}H^+ + e^- = \frac{1}{8}CH_2{=}CH_2 + \frac{1}{2}Cl^-$
TCE	$CHCl{=}CCl_2$	$CH_2{=}CH_2$	$\frac{1}{6}CHCl{=}CCl_2 + \frac{1}{2}H^+ + e^- = \frac{1}{6}CH_2{=}CH_2 + \frac{1}{2}Cl^-$
Chromate	CrO_4^{2-}	$Cr(OH)_2$ (*s*)	$\frac{1}{3}CrO_4^{2-} + \frac{5}{3}H^+ + e^- = \frac{1}{3}Cr(OH)_3 + \frac{1}{3}H_2O$
Permanganate	MnO_4^-	MnO_2 (*s*)	$\frac{1}{3}MnO_4^- + \frac{4}{3}H^+ + e^- = \frac{1}{3}MnO_2 + \frac{2}{3}H_2O$
Peroxide	H_2O_2	H_2O	$\frac{1}{2}H_2O_2 + H^+ + e^- = H_2O$

Multiplying by the least common denominator of 24 yields the typical reaction:

$$2CH_3CH_2OH = 3CH_4 + CO_2 \qquad \text{(Eq. 2.2)}$$

This balanced equation indicates that in this anaerobic reaction, 2 moles (mol) (92 g) ethanol is converted to 3 moles methane and 1 mole carbon dioxide.

Oxidation-reduction reactions of interest may be purely chemical (abiotic) or biological. An abiotic example is permanganate oxidation of an organic contaminant to carbon dioxide and water. A biological example is microbial oxidation of an organic contaminant to carbon dioxide and water when oxygen is available. Permanganate and oxygen are just two of the many different oxidants or electron acceptors that are used to enhance oxidations of interest. At times, rather than adding an oxidant to transform a contaminant, a reductant might be added. Hexavalent chromium (CrO_4^{2-}) is very soluble, but it can be reduced chemically or biologically by adding a suitable electron donor to form the insoluble trivalent chromium form ($Cr(OH)_3(s)$) which precipitates and is thus removed from the aqueous phase. The trivalent form is also less toxic than the hexavalent form, so reduction reduces both the solution concentration and the toxicity. For chemical reduction, sulfur dioxide might be added, or for biological reduction, hydrogen (H_2) or an organic electron donor might be added. Biological reduction is also commonly used for bioremediation of chlorinated solvents. Here, H_2 or an organic electron donor is added for the reduction of chlorinated solvents, a process in which the chlorines on the compound are biologically replaced with hydrogen atoms. Thus, tetrachloroethene ($CCl_2{=}CCl_2$) might be converted to the less harmful ethene ($CH_2{=}CH_2$). In this case,

the chlorine removed enters solution as hydrochloric acid, thus tending to lower pH. Thus, pH control may be necessary in order to maintain the near neutral range generally desired for biological reactions.

The first eight electron donors in Table 2.6 (hydrogen through carbohydrates) are often added to groundwater for chemical or biological remediation of some of the hazardous electron acceptors such as nitrate and perchlorate through chromate listed in Table 2.7. Fatty acids are often added in the form of emulsified vegetable oil and carbohydrates in the form of compounds such as sugar or molasses. Also listed as electron donors in Table 2.6 are several organic compounds from benzene through dichlorobenzene. These are at times oxidized by the addition of electron acceptors listed in Table 2.7 such as oxygen, nitrate, or through the action of an electron acceptor commonly present in groundwater or formed in the reaction itself, carbon dioxide. Sulfate and ferric iron are also often present naturally in groundwater and may serve as electron acceptors for oxidation. When the electron acceptors required for oxidation of an electron donor are already present in the aquifer, then natural attenuation is possible, but may require a mixing process to bring the reactants together.

Precipitation reactions (the precipitate is indicated by (*s*) following the chemical) are of importance when stabilization of a chemical is desired, such as by its removal from the water phase and formation of a solid phase that does not contaminate or move with groundwater. For example, formation of the precipitate $Cr(OH)_3(s)$ removes chromium from water. Some other important low-solubility metal complexes are listed in Table 2.4. The low solubility product of many sulfide species suggests that they would be good candidates for removal from groundwater. Sulfides for this purpose might be formed from sulfate reduction under anaerobic conditions.

Precipitation, while often beneficial, can also cause serious problems, such as clogging by calcium carbonate ($CaCO_3(s)$) which is often encountered in groundwater remediation. Clogging may be undesirable because it can re-route the direction of groundwater flow leading to migration of contaminated water into previously uncontaminated regions and/or delivery of added chemicals to regions that are uncontaminated. The outcome may be an inefficient and wasteful use of added chemicals and the creation of regions left untreated or poorly treated.

Solubilization is a mass-transfer process related to the movement of a chemical between a solid phase and the aqueous phase. Solubilization may also occur through the dissolution of a non-miscible liquid into water, such as benzene or trichloroethene. Mixing often enhances solubilization by enhancing mass transfer. Additionally, chemicals can be added that enhance solubilization. For example, detergents may be used to increase the solubility of liquid-phase chlorinated solvents so that they can be extracted more readily from groundwater. Solutions containing high concentrations of water-soluble solvents such as ethanol may be used for this purpose as well. Detergent and solvent enhanced solubilization are major remediation processes that require the introduction and mixing of chemicals for groundwater remediation.

Sorption is another mass-transfer process that results in the movement of a chemical species from one phase to another, i.e. from an aqueous phase to a solid phase. At times this process also may not involve addition of a different chemical species, but instead may be aided by mixing to enhance mass transfer rates. However, it should be noted that different forms of a chemical differ in their susceptibility to volatilization or sorption. For example, CO_2 is a volatile gas, while HCO_3^- (bicarbonate) is not, just as H_2S (hydrogen sulfide) is a volatile gas, while HS^- (bisulfide) is not. The sorption characteristics of Zn^{2+} are different from those of $ZnOH^+$. The pH affects the relative proportions of these different species, and thus by implementing pH control, the potential for volatilization or sorption can be made to vary considerably. This again illustrates the importance that pH control can have on the movement and fate of chemicals in groundwater.

Advection and diffusion or dispersion are transport processes associated with the fluid in which the chemicals are contained. For example a chemical discharged into a flowing river is carried downstream with the flowing water by advection. As it moves downstream, the chemical spreads out and becomes more dilute through mixing caused by the turbulent action of water, a process called dispersion. In very still waters or in water moving by laminar flow, mixing may be more limited. Advection, dispersion, and diffusion are major processes of importance in bringing chemicals together for reaction in groundwater, and are addressed in more detail in Chapter 3, as well as later in this chapter and elsewhere in this volume.

2.3.4 Reaction Kinetics

Reaction rate processes are discussed in detail in general textbooks (Bailey and Ollis, 1986; Levenspiel, 1999; Weber and DiGiano, 1996) and will only briefly be summarized here. There are two basic classifications of reactions, *homogeneous* and *heterogeneous*. A homogeneous reaction is one that takes place in one phase only, such as in water. A heterogeneous reaction occurs in two phases, or at an interphase, such as between groundwater and aquifer solids, or between groundwater and microorganisms. Thus, in groundwater systems both homogeneous and heterogeneous reactions are likely to occur. Many variables may affect reaction rates such as temperature and pressure. Heterogeneous reactions are much more complex; here mass transfer effects are likely to play a key role in overall observed reaction rates. Mass transfer effects such as diffusion of a chemical to and into aquifer solids are likely to be involved. When a reaction consists of a number of steps in series, it is the slowest step in that series that controls the overall rate of the reaction. If one knows what step that is, whether mass transfer or reaction rate, then the rate can be modeled by consideration of that step alone. The transformation of a contaminant in a biofilm is just one case where both mass transfer rate and reaction rate are involved, an example of such a case is discussed in Section 2.4.4, while mass transfer effects overall are discussed in detail in Chapter 3. The following discussion concentrates only on the reaction term portion of a reaction rate series.

Let us first consider the rate of change, r_i, in one component i in a reaction, we indicate this rate by the change with time in its molar concentration N_i to be dN_i/dt. The reaction rate may be expressed in different ways, depending upon the basis of the reaction:

Basis for reaction	Reaction form	
Unit volume of reacting fluid	$r_i^0 = \frac{1}{V}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{volume of fluid})(\text{time})}$	(Eq. 2.3)
Unit mass of solid in fluid	$r_i^1 = \frac{1}{W}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{mass of solid})(\text{time})}$	(Eq. 2.4)
Unit interfacial surface of solid in fluid	$r_i^2 = \frac{1}{S}\frac{dN_i}{dt} = \frac{\text{moles } i \text{ formed}}{(\text{unit suface})(\text{time})}$	(Eq. 2.5)

Equation 2.3 is generally the form used in homogenous groundwater reactions when all the reactants are in the aqueous phase. Equations 2.4 and 2.5 are used primarily with heterogeneous reactions. Equation 2.5 may be the more accurate equation of the two, but frequently surface area is not readily determined because of the greatly differing characteristics and sizes of aquifer solid particles, so Equation 2.4 is frequently used as a more convenient substitute.

Beginning with a homogeneous reaction and Equation 2.3, let us first consider a simple reaction involving two reactants in aqueous phase that form two aqueous phase products:

$$\mathrm{aA} + \mathrm{bB} = \mathrm{cC} + \mathrm{dD}$$

We may then become interested in the rate of loss of component A, having a molar concentration C_A. This may be expressed in many different ways depending upon the factors affecting the reaction. Some example reaction expressions are:

Reaction type	Reaction equation	Units for *k*	
First-order	$-r_A = kC_A$	T^{-1}	(Eq. 2.6)
Second-order	$-r_A = kC_A^2$	$L^3M^{-1}\,T^{-1}$	(Eq. 2.7)
Second-order	$-r_A = kC_AC_B$	$L^3M^{-1}\,T^{-1}$	(Eq. 2.8)
Zero-order	$-r_A = k$	$ML^{-3}\,T^{-1}$	(Eq. 2.9)
Complex reaction	$-r_A = \frac{kC_AC_X}{K+C_A}$	T^{-1}	(Eq. 2.10)
Complex reaction	$-r_A = \frac{kC_AC_X}{K_A+C_A}\,\frac{C_B}{K_B+C_B}$	T^{-1}	(Eq. 2.11)

Where the symbols M, L and T refer to standard units – M is mass (generally expressed in milligrams [mg] or micrograms [μg]), L is length (usually expressed in meters [m] or centimeters [cm]), and T is time (generally expressed in days [d] or seconds [s]).

The order of the reaction is generally given by the sum of the exponents on the concentration terms in the reaction. Thus, Equations 2.7 and 2.8 are both second order reactions, the first depending upon the square of component A's concentration and the second on the product of the concentration of two different components. In the zero-order reaction, the rate is independent of the concentration of any of the reactants.

Complex reactions, however, cannot be described by the order concept. The complex reactions shown are just two of many possibilities. These are non-linear equations that are difficult to use when an analytical solution for a groundwater model is sought, their use generally requires some form of numerical solution. These two particular equations are similar to variations used in the Monod expression for biological processes. Here, C_X would represent the concentration of the acting microorganisms. Equation 2.10 is the form generally used when component A is in limiting supply and controls the overall reaction. Equation 2.11 is used when either reactant component, A or B, may be limiting at times, so both need consideration in a numerical model. An example where Equation 2.11 might be useful is in modeling the biological oxidation of toluene by organisms using nitrate as an electron acceptor. At the point where toluene first comes in contact with an aquifer that contains nitrate, the nitrate concentration may be high and non-limiting compared with toluene. But as the groundwater moves through the toluene spill, nitrate concentration decreases – the nitrate concentration then may become rate limiting. If nitrate is taken to be component B in Equation 2.11, we see that in the first case of high nitrate (this means high with respect to the constant K_B), then the expression $C_B/(K_B + C_B)$ approaches 1. When C_B decreases to the point where it equals K_B, then the expression equals one-half, meaning the overall rate is halved. This is the reason K_B is often called the half-velocity coefficient.

In selecting the most appropriate rate expression, the modeler should chose one that is complex enough to describe the situation adequately for the purpose intended, but not so complex that the model solution becomes overly difficult. At times, one may wish to use a more appropriate rate expression, but the information required for input to the model is not available. Perhaps too often, simple models that are inadequate for predictive purposes are used simply because they are simpler to use, often leading to grossly erroneous predictions. However, simpler models are sometimes justified for use when the field situation deems it appropriate. For example, which model might be most appropriate for conversion of acetate to methane (methanogenesis)?

High concentrations in the thousands of mg/L range of acetate often result from fermentation of organic electron donor added to aquifers for biological remediation of chlorinated solvents. The acetate emerging in resulting anaerobic plumes can be converted to methane gas by methanogens. One may wish to model this process and might first consider using Equation 2.11. Here, no electron acceptor is needed, so that C_B in Equation 2.11 is zero, thus, Equation 2.10 would be adequate instead. Also, the K_A for acetate is on the order of 100 mg/L, so if acetate concentration is 1,000 mg/L or above, the term $C_A/(K_A + C_A)$ essentially equals 1. Eliminating that element means that the first order Equation 2.6 is adequate with C_X being substituted for C_A. However, measuring C_X is very difficult as the organisms it represents are mostly attached to aquifer solids and not adequately determined from analysis of extracted groundwater. The organism concentration also changes with growth through acetate utilization. Because of this difficulty, modelers often then tend to assume C_X is constant, essentially meaning that the zero-order Equation 2.9 is sufficient. Others just assume Equation 2.6 is adequate. Neither really fits the case. It would be better here to develop a model that includes changes in C_X with time and acetate utilization. A typical model for change in C_X through normal biological growth and decay is as follows:

$$\frac{dC_X}{dt} = Yr_A - bC_X \qquad \text{(Eq. 2.12)}$$

Here, Y equals the yield of organisms per mole of acetate consumed, r_A is the rate of acetate utilization, and b is a first-order decay rate coefficient (T^{-1}) for the microorganisms.

We see here that one could obtain appropriate results using the more complex Equation 2.11 or the simplified Equation 2.9 as long as an appropriate value as derived from Equation 2.12 were included in the overall model. Modeling thus sometimes becomes as much of an art as it is a science.

In the above example for biological transformation, it is seen that microorganisms were considered to be part of a homogeneous reaction. Microorganisms actually act as a catalyst to bring about the reaction, extracting energy for growth from the process. Thus, Equations 2.10 and 2.11 may be used as well to describe rates resulting from catalyst addition to an aquifer for chemically enhancing a reaction rate. Current interest is in using nanoparticles for this purpose. However, like microorganisms, catalyst or reactants may be attached to aquifer material, so treatment as if it were a homogeneous reaction may not be appropriate. Reaction rate instead may be a function of surface area exposed rather than solution concentration. A good example here is a permeable reactive barrier wall, such as one containing zero-valent iron, as described in Chapter 7. Chemicals, such as a chlorinated solvent contained in groundwater passing through the barrier wall must then be mass transported such as by diffusion from the water to the iron surface, where the dechlorination reaction takes place, oxidizing the iron in the process. Equation 2.5 then becomes the appropriate reaction term for use, and the reaction rate for solutes is then expressed in mass per time per unit surface area. The difficulty here is that diffusive mass transport to the reacting surface becomes of importance as does knowledge of the surface area of the material with which it is reacting. These may be difficult to determine. Simplifications such as use of Equation 2.4 are then often resorted to in zero-valent barrier walls as the mass quantity of iron added is generally known, if not its surface area. In other cases, modelers simply resort to first- or zero-order reaction rates as determined from empirical field measurements. Such models generally do not involve sufficient knowledge of system characteristics to be useful for sound predictions. Great care thus needs to be taken in their use.

Temperature is an important factor affecting reaction rates as is pH and reaction inhibitors. There are several different theoretical models that indicate how reaction rate varies with

temperature. In general, most result in a logarithmic expression that modifies the rate coefficient as in the following:

$$k_T = k_{T^0} e^{K_T(T-T^0)} \quad \text{(Eq. 2.13)}$$

where k_T is the rate constant at temperature T, k_{T^0} is the rate at some standard temperature T^0 such as 20°C, and K_T is a temperature constant. In the normal groundwater temperature range between 10°C and 30°C, rate is commonly considered to double with each 10°C rise in temperature. This corresponds with a value for K_T of 0.069/°C.

Inhibiting the reaction rate are such things as high concentration of the substrate being consumed, high concentration of a reaction product, or competition for key enzymes by different substrates. Typical models for each are listed below, illustrating how they might be incorporated to modify Equation 2.10.

Inhibition factor	Example incorporation into Equation 2.10	
Substrate inhibition	$-r_A = \frac{kC_AC_X}{K+C_A\left(1+\frac{C_A}{K_I}\right)}$	(Eq. 2.14)
Product inhibition	$-r_A = \frac{kC_AC_X}{K+C_A}\frac{K_I}{K_I+C_P}$	(Eq. 2.15)
Competitive inhibition	$-r_A = \frac{kC_AC_X}{K\left(1+\frac{C_c}{K_I}\right)+C_A}$	(Eq. 2.16)
Non-competitive inhibition	$-r_A = \frac{kC_AC_X}{\left(1+\frac{C_c}{K_I}\right)(K+C_A)}$	(Eq. 2.17)

Here, K_I is the relevant inhibition constant, C_P is concentration of a product of a reaction, and C_c is the concentration of a reactant C that is competing for a key enzyme involved in transforming reactant A. Substrate inhibition may be experienced in the reductive dehalogenation of a chlorinated solvent such as TCE by high TCE concentrations that exist near a dense nonaqueous phase liquid (DNAPL) or of benzene near a gasoline-spill produced light nonaqueous phase liquid (LNAPL). Product inhibition may result during TCE reductive dehalogenation from a large increase in the concentration of *cis*-DCE, the product of TCE reduction. Competitive inhibition in chlorinated solvent biodegradation can occur during the reductive dehalogenation of *cis*-DCE and VC when these two electron acceptors compete for the same electron transfer train in a single organism. Generally the organism, which can use either, will select to use that electron acceptor in highest relative concentration. Non-competitive inhibition represents the adverse impact of one compound on the transformation of another. The similarity between Equations 2.17 and 2.15 should be readily apparent, they are mathematically the equivalent of each other.

2.3.5 Summary

In summary, there are many different reactions and phase changes that might be brought about through the delivery and mixing of chemicals for *in situ* remediation of groundwater. Selecting the correct chemical and correct amount is one part of the challenge. Reaction stoichiometry helps in this selection. The other is in the delivery and mixing of the chemical where needed in order to bring about the desired change. These are rate processes that also need to be understood. Both are challenges, but the latter is perhaps the bigger of the two, and the major emphasis given in this volume. This chapter, however, emphasizes the first challenge, the

selection of the right chemicals and amounts for *in situ* remediation, although some brief discussion of mass transfer and reaction rates is also provided.

2.4 BIOLOGICAL PROCESSES

Most naturally occurring organics that percolate down through the soil are degraded by naturally occurring bacteria, thus rendering them harmless so that they pose no serious threat to groundwater quality. Many anthropogenic chemicals can be destroyed readily by microorganisms. It is only some of the anthropogenic organic chemicals that pose a significant threat, and these, for the most part are the ones that are difficult to biodegrade, compounds that are termed "persistent organic pollutants," or POPs. Included here are many halogenated compounds such as pesticides, chlorinated solvents, chlorinated benzenes and phenols, and dioxin, many of which are listed as frequently detected contaminants in Table 2.1. Also included are some with difficult to degrade structures such as complex ethers (e.g., MTBE). There are many inorganic chemicals of concern in groundwater as well that can be transformed biologically to less harmful forms, such as nitrate, perchlorate, chromate, and uraninite. Most biological reactions of interest in remediation are oxidation-reduction reactions, and in these reactions, the target contaminant may be rendered less harmful either through its oxidation or its reduction as already indicated. More detailed information about biological processes can be obtained from textbooks (Rittmann and McCarty, 2001).

2.4.1 Biological Processes

Microorganisms bring about oxidation-reduction reactions in order to obtain energy for growth, thus organism growth must be considered as part of the reaction. In order to grow, microorganisms also need certain mineral nutrients to form necessary cellular components such as nucleic acids, enzymes, proteins, carbohydrates, and fats. Of major importance here are the elements carbon, nitrogen, phosphorus, sulfur, and iron. Certain trace chemicals such as nickel and manganese may also be required for enzyme activity. These may or may not be present in excess in the aquifer solids surrounding groundwater—often they are, and so such nutrient additions may not be needed. As an example, a balanced stoichiometric equation of the overall reaction for transformation of an organic contaminant (benzoate) through reduction of an inorganic contaminant (nitrate) is as follows:

$$C_6H_5COO^- + 3.29NO_3^- + 3.29H^+ = 0.588C_5H_7O_2N + 1.35N_2 + HCO_3^- + 3.05CO_2 + 1.58H_2O \quad \text{(Eq. 2.18)}$$

Here, $C_5H_7O_2N$ is used as an empirical formula for cells and indicates the relative proportion of various elements in the cells. Nitrogen represents about 12% of the weight of the cell. Phosphorus, another major element required is not shown in this formulation, but represents about 2% of the cell weight.

Equation 2.18 indicates that for oxidation of 1 mole of benzoate (121 g) 3.29 moles of nitrate (46 g nitrate-N) would be reduced, with most being converted to N_2 gas. In this process, 0.588 mole of cells (66 g) would be formed. The reaction is a basic one as indicated by consumption of 3.29 moles H+ on the left side and formation of 1.0 mole of the basic bicarbonate anion on the right side. This balanced equation is thus useful for indicating how much of one chemical is required in order to bring about the destruction of the other. This is the kind of information needed in order to properly design a chemical feed system. Interesting here is that according to this reaction, benzoate could be added to destroy nitrate contamination, or nitrate could be added to treat benzoate contamination. However, benzoate itself can be toxic, so if the goal is

to remove nitrate, a different electron donor would generally be added, such as acetate, ethanol, or lactate.

Reaction 2.18 can be divided into two components (Rittmann and McCarty, 2001), the energy component and the synthesis component:

Energy component:

$$0.45C_6H_5COO^- + 2.7NO_3^- + 2.7H^+ = 1.35N_2 + 0.45HCO_3^- + 2.7CO_2 + 2.25H_2O \quad \text{(Eq. 2.19)}$$

Synthesis component:

$$0.55C_6H_5COO^- + 0.59NO_3^- + 0.59H^+ + 0.67H_2O = 0.588C_5H_7O_2N + 0.55HCO_3^- + 0.35CO_2 \quad \text{(Eq. 2.20)}$$

Adding Equation 2.19 to Equation 2.20 results in Equation 2.18. From this it can be seen that here 45% of the benzoate is consumed in denitrification, or the conversion of nitrate into N_2, while 55% is used for synthesis of cells. Most of the nitrate is destroyed by denitrification, but about 18% is used in cell synthesis. In considering demand for electron donor, that portion associated with both energy production and synthesis needs evaluation.

The synthesis component of the biological reaction can be obtained by adding the synthesis half reaction to the electron donor half reaction. The synthesis half reaction is:

$$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O \quad \text{(Eq. 2.21)}$$

While the stoichiometry of a biological reaction is given by a balanced overall reaction, such as Equation 2.18, the quantity of electron donor required for the reaction can also be estimated by considering just the energy portion of the reaction and then including in the calculations sufficient excess donor to satisfy the need for biological synthesis. The fraction of donor used for synthesis is highest for aerobic reactions and denitrification, with as much as 50% then being used for synthesis during active bacterial growth. Thus, about twice the electron donor required for the energy reaction would need to be present to also satisfy the need for biological growth. In groundwater remediation, growth rate is usually not maximal, and perhaps only about 50% excess donor is needed to satisfy the synthesis demand in the above cases. However, with anaerobic reactions (those not involving O_2), the amount of donor associated with synthesis is generally much less. When methane production or sulfate reduction are the dominant reactions, the excess amount of donor needed for synthesis varies between about 5% when fatty acids are used as donors up to about 20% with carbohydrates. In reductive dehalogenation, the additional amount needed for synthesis may be closer to 10–15%.

Some discussion is justified concerning the energy reactions involved in anaerobic processes. For this example, acetate will be used. Several possible energy reactions with acetate are listed in Table 2.8. The first four reactions represent the typical ones for which microorganisms are common and ubiquitous in the environment. The first is the aerobic reaction with oxygen as electron acceptor. The next three are anoxic reactions, the first, denitrification with nitrate, next, sulfate reduction or sulfidogenesis, and the fourth, methanogenesis. The energy derived from each reaction is noted on the right side of Table 2.8. Aerobic oxidation of organic substances yields the highest energy and so growth on a given amount of acetate is higher here, that is the portion of electron donor used for synthesis is higher as already noted. Nitrate energy yield is not far behind. However, the energy from sulfate reduction and methanogenesis are much less, with that from methanogenesis the smallest. Methanogens and sulfate-reducing

Table 2.8. Energy Reactions Involving Acetate

Electron acceptor	Energy reaction	$\Delta G^{0\prime}$ (kJ)
O_2	$CH_3COO^- + 2O_2 \rightarrow CO_2 + HCO_3^- + H_2O$	−849
NO_3^-	$CH_3COO^- + 1.6NO_3^- + 1.6H^+ \rightarrow CO_2 + HCO_3^- + 0.8N_2 + 1.8H_2O$	−797
SO_4^{2-}	$CH_3COO^- + SO_4^{2-} + 1.5H^+ \rightarrow CO_2 + HCO_3^- + 0.5H_2S + 0.5HS^- + H_2O$	−52
CO_2	$CH_3COO^- + H^+ \rightarrow CO_2 + CH_4$	−36
Fe(III)	$CH_3COO^- + 4Fe_2O_3 + 16H^+ \rightarrow CO_2 + HCO_3^- + 8Fe^{2+} + 9H_2O$	
ClO_4^-	$CH_3COO^- + ClO_4^- \rightarrow CO_2 + HCO_3^- + Cl^- + H_2O$	−972
PCE	$CH_3COO^- + 2CCl_2{=}CCl_2 + 3H_2O \rightarrow CO_2 + HCO_3^- + 2CHCl{=}CHCl + 4Cl^- + 4H^+$	−463

Note: *kJ* kilojoules

bacteria (SRB) must therefore oxidize a larger fraction of the electron donor so as to have sufficient energy for cell synthesis. This is why the cell yield from these reactions is so low, and why the organisms grow so slowly under sulfidogenic and methanogenic conditions. Doubling times for aerobic organisms are on the order of hours, while that for sulfidogenic and methanogenic conditions are on the order of days.

Significant rates of conversion of substrates by microorganisms require an organism concentration on the order of one million per milliliter (mL) of water. When the doubling time for the organism is 1 h – as in the case of aerobic growth on organics – the concentration of organisms can increase from one to one million per milliliter in less than 1 day. The same job requires 60 days when the doubling time is 3 days, as is the case for methanogens. The slow doubling time of anaerobic microorganisms is why it often takes months to begin to see significant degradation of hazardous compounds once the remediation process is initiated, even if the needed microorganisms may already be present in small concentrations.

Another factor of importance when considering the first four reactions in Table 2.8 is that the fourth reaction, methanogenesis, occurs in the absence of an external electron acceptor. In other words, if a compound is amenable to decomposition under methanogenic conditions, it can be degraded in groundwater without an added electron acceptor. All that is needed are sufficient microorganisms capable of degrading the target contaminants and the trace nutrients necessary for their growth. Necessary trace nutrients are commonly present in aquifer minerals, so they may not need to be added either. Most commonly, natural attenuation of hazardous organic compounds occurs because the compounds are amenable to methanogenesis, which generally requires a consortium of different species working together to process the organic through the steps of fermentation, acidogenesis, and then methanogenesis. Potential for conversion through methanogenesis is the case with most naturally occurring organic compounds. Included are many hazardous compounds, such as phenol, styrene, and the BETX compounds. Some numerical models of natural attenuation assume that external electron acceptors are required for anaerobic degradation of these compounds in groundwater, but this is not actually necessary through methanogenesis as well demonstrated in the landmark publication by Gribić-Galić and Vogel (1987) and numerous subsequent articles. While the consortia of anaerobic microorganisms required for the conversion of these compounds to methane are not always present in groundwaters, they are sufficiently common that natural attenuation often can be counted upon to rid groundwater of such chemicals. When the required organisms are not present, then bioaugmentation with suitable microorganisms might be considered. The process used for introduction and mixing of the microorganisms then becomes an issue.

2.4.2 Chlorinated Solvents

Because of their importance as major groundwater contaminants and the variety of ways by which they may be transformed in groundwater (Vogel et al., 1987), some specific comments about them are included here. Methylene chloride can be biodegraded under either aerobic or anaerobic conditions while supplying energy to the microorganisms and using typical electron acceptors as listed in Table 2.7 just as is the case with many other common organic non-halogenated compounds. However, this is not the case with the other four main chlorinated solvents, PCE, TCE, TCA, and CT. There is little evidence that any of them can be degraded aerobically or through denitrification in a manner that is beneficial to microorganisms. TCE and TCA, however, can be aerobically transformed through cometabolism, primarily by organisms that contain an oxygenase used for initiating oxidation of hydrocarbons or ammonia. Anaerobically, when neither oxygen nor nitrate is present, PCE, TCE, and TCA, but not CT, can be used by certain microorganisms as electron acceptors in energy metabolism. Here, the reaction is stepwise, one chlorine at a time is removed and replaced with hydrogen, a process termed reductive dehalogenation. In this process, several intermediate chlorinated species result as illustrated in Figure 2.1. Generally, compounds with more chlorine atoms tend to be transformed faster than those with fewer chlorine atoms, often resulting in the buildup of the intermediate compounds. Frequently, specific dechlorinating microorganisms can remove only some of the chlorine atoms from some of the compounds of concern so that complete removal of all chlorine atoms from a chlorinated compound may require the action of more than one dehalogenating organism. The electron donor that appears to be most generally preferred by dehalogenating organisms is H_2, and this is the only electron donor found so far to be acceptable by organisms that reductively dehalogenate *cis*-DCE and VC. Some organisms can use other electron donors, such as acetate or lactate, for at least partial dehalogenation of some compounds, such as TCE and PCE. Additionally, TCA can be transformed partially abiotically to form other chemicals of concern.

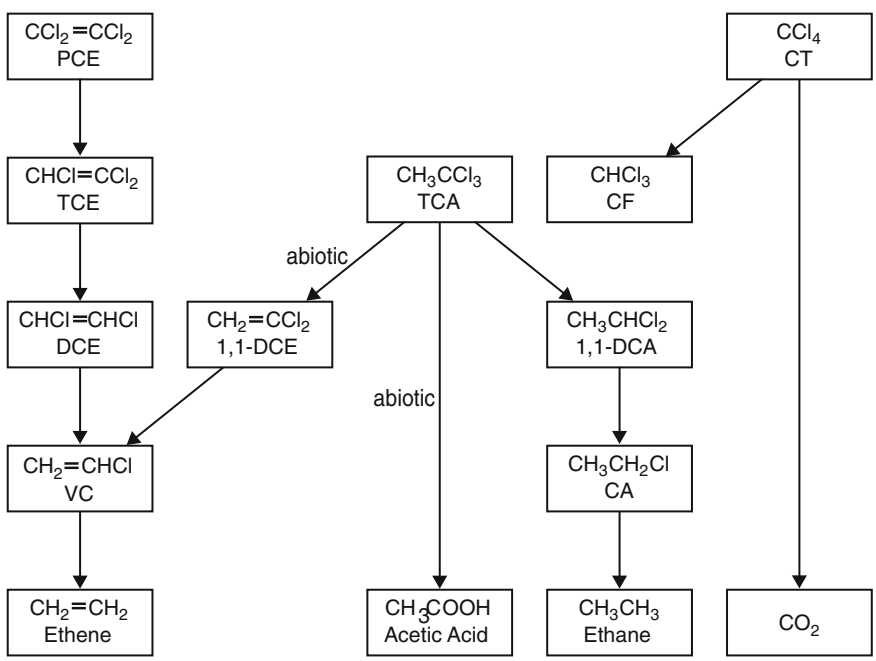

Figure 2.1. General scheme for anaerobic biological transformations of chlorinated aliphatic compounds (some spontaneous abiotic steps also indicated).

Table 2.9. Abiotic and Biotic Reactions for PCE, TCE, TCA, and CT

Reaction	Reactant	Product	Other electron donors possible?
ANAEROBIC – METABOLIC ENERGY YIELDING			
Tetrachloroethene (PCE)			
$CCl_2{=}CCl_2 + H_2 \rightarrow CHCl{=}CCl_2 + H^+ + Cl^-$	PCE	TCE	Yes
Trichloroethene (TCE)			
$CHCl{=}CCl_2 + H_2 \rightarrow CHCl{=}HCl + H^+ + Cl^-$	TCE	*cis*-DCE	Yes
$CHCl{=}CHCl + H_2 \rightarrow CH_2{=}CHCl + H^+ + Cl^-$	*cis*-DCE	VC	–
$CH_2{=}CHCl + H_2 \rightarrow CH_2{=}CH_2 + H^+ + Cl^-$	VC	Ethene	–
1,1,1-Trichloroethene (TCA)			
$CH_3CCl_3 + H_2 \rightarrow CH_3CHCl_2 + H^+ + Cl^-$	TCA	1,1-DCA	–
$CH_3CHCl_2 + H_2 \rightarrow CH_3CH_2Cl + H^+ + Cl^-$	1,1-DCA	CA	–
ABIOTIC			
1,1,1-Trichloroethene (TCA)			
$CH_3CCl_3 \rightarrow CH_2{=}CCl_2 + H^+ + Cl^-$	TCA	1,1-DCE	–
$CH_3CCl_3{+}2H_2O \rightarrow CH_3COOH + 3H^+ + 3Cl^-$	TCA	Acetic acid	–
COMETABOLIC			
Trichloroethene (TCE)			
$CHCl{=}CCl_2 + NADH + H^+ + O_2 \rightarrow CHClOCHCl + NAD^+ + H_2O$	TCE	TCE Epoxide	–
Carbon Tetrachloride (CT)			
$aCCl_4 + \text{cofactors} \rightarrow bCHCl_3 + cCO_2 + d\text{Other}$	CT	$CHCl_3$[a]	–

[a]Chloroform generally is one of the products formed from CT transformation, but not always depending upon the organism involved

Table 2.9 provides a listing of chemical (abiotic) and biological (biotic) transformations commonly observed in groundwater. In the examples provided where oxidation-reduction is involved, H_2 is indicated as the electron donor for simplicity with a note indicating when other electron donors might also be used.

The anaerobic transformation of organic compounds is fairly complex and often relies on a variety of microorganisms to complete the transformation. A general scheme for anaerobic transformation is illustrated in Figure 2.2. Here, complex organics such as carbohydrates, proteins, and fats, are first hydrolyzed to form simple sugars, amino acids, and fatty acids, which are then fermented and partially oxidized by a variety of microorganisms to produce hydrogen and acetic acid. Generally, about 2 moles H_2 will be produced per mole of acetate that is formed, but this ratio varies somewhat depending upon the starting electron donor. The hydrogen and acetic acid formed can then be used by methanogens and converted into methane, or by other organisms that compete for hydrogen, such as sulfate reducers, iron reducers, or dehalogenators (Table 2.10). In order to supply hydrogen as needed by *cis*-DCE and VC dehalogenators, any of a variety of organic donors might be used, as the anaerobic degradation of most will produce the needed hydrogen. Elemental hydrogen itself might be added to satisfy

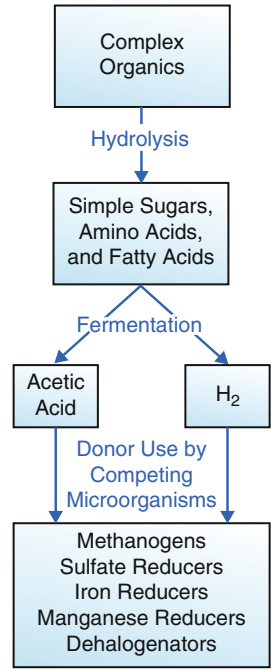

Figure 2.2. The mixed-culture anaerobic transformation of organic compounds.

Table 2.10. Energy Reactions Using H_2 as Electron Donor with Various Electron Acceptors

Electron acceptor	Energy reaction	Effect on pH
O_2	$H_2 + \frac{1}{2}O_2 \rightarrow H_2O$	Neutral
NO_3^-	$H_2 + \frac{1}{5}NO_3^- + \frac{1}{5}H^+ \rightarrow \frac{1}{10}N_2 + \frac{3}{10}H_2O$	Basic
SO_4^{2-}	$H_2 + \frac{1}{4}SO_4^{2-} + \frac{3}{8}H^+ \rightarrow \frac{1}{8}H_2S + \frac{1}{8}HS^- + \frac{1}{2}H_2O$	Basic
CO_2	$H_2 + \frac{1}{4}HCO_3^- + \frac{1}{4}H^+ \rightarrow \frac{1}{4}CH_4 + \frac{3}{4}H_2O$	Basic
Fe(III)	$H_2 + Fe_2O_3 + 4H^+ \rightarrow 2Fe^{2+} + 3H_2O$	Basic
ClO_4^-	$H_2 + \frac{1}{4}ClO_4^- \rightarrow \frac{1}{4}Cl^- + H_2O$	Neutral
PCE	$H_2 + \frac{1}{4}CCl_2{=}CCl_2 \rightarrow \frac{1}{4}CH_2{=}CH_2 + Cl^- + H^+$	Acidic

this need, but at the higher concentrations that result, homoacetogenic microorganisms can grow from the energy produced by reducing carbon dioxide with hydrogen to produce acetic acid. This is generally not considered a desirable outcome, because it results in some unwanted loss of the hydrogen and produces an acid that may adversely impact solution pH. Generally for reductive dehalogenation, organic electron donors that release hydrogen slowly and only when the concentration is brought below a threshold for the homoacetogens of about 300 nanomolar (nM) are desired. This is generally the case with fatty acids containing three or more carbon atoms such as propionic or butyric acids. Another example is the vegetable oils commonly added as electron donors and consisting primarily of 16- to 18-carbon fatty acids such as palmitic, oleic and linoleic acids.

2.4.3 Biological Reaction Kinetics

The reaction rate for a biological reaction is often characterized by Monod kinetics, which can be formulated as follows (Rittmann and McCarty, 2001):

Rate of substrate utilization:

$$-\frac{dS}{dt} = qX\frac{S}{K+S} \qquad \text{(Eq. 2.22)}$$

where,

S = rate-limiting substrate concentration, mg/L
t = time, days
q = maximum substrate utilization rate, mg substrate per mg cells per day (d)
X = cell concentration, mg/L
K = half-velocity coefficient, mg/L

This equation assumes that only a single substrate is rate limiting, all other nutrients needed by the organisms for growth are in excess concentration and so do not affect the rate of the reaction. The relationship between substrate utilization rate and substrate concentration is illustrated in Figure 2.3. At low substrate concentration, the rate is directly proportional to substrate concentration, but at high substrate concentration, the rate reaches a maximum with a value of q.

Rate of organism growth:

$$\frac{dX}{dt} = Y\left(-\frac{dS}{dt}\right) - bX \qquad \text{(Eq. 2.23)}$$

where,

Y = organism yield, mg organism produced per mg substrate consumed
b = organism decay rate, day^{-1}

Combining Equations 2.22 and 2.23 yields:

$$\frac{dX/X}{dt} = Yq\frac{S}{K+S} - b \qquad \text{(Eq. 2.24)}$$

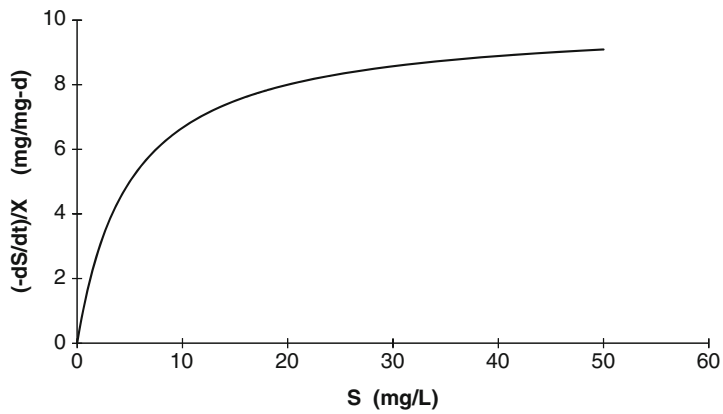

Figure 2.3. Relationship between the concentration of a rate-limiting substrate and biological reaction rate (Y = 0.6 mg cells/mg substrate, K = 5 mg/L, q = 10 g substrate/g cells/day, b = 0.2 day^{-1}).

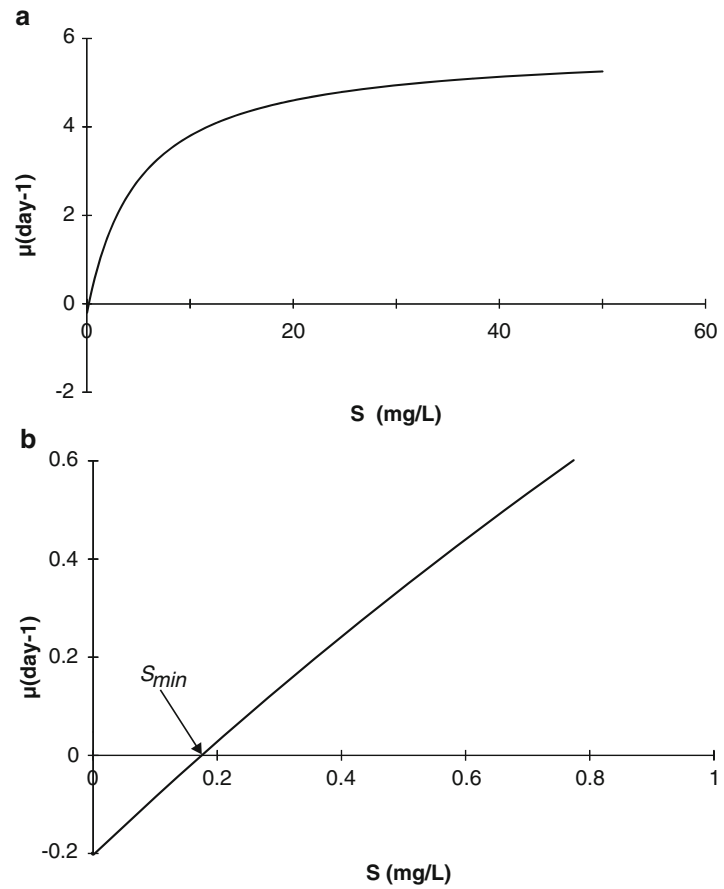

Figure 2.4. Relationship between the concentration of a rate-limiting substrate and microorganism growth rate (same conditions as in Figure 2.3). Expanded Figure 2.4b illustrates negative growth rate when *S* is below S_{min} of 0.17 mg/L).

The net specific growth rate of microorganisms (dX/Xdt) is generally represented by the symbol μ with units of day^{-1}, and the product Yq equals the maximum growth rate μ_m so that,

$$\mu = \mu_m \frac{S}{K + S} - b \qquad \text{(Eq. 2.25)}$$

The relationship between organism growth rate and substrate concentration is illustrated in Figure 2.4. Of interest to note is that there is a substrate concentration S_m, below which the net growth rate of organisms is less than zero, in other words, the organisms are in net decay because they decay away faster than they grow. The relationship between S_m and other variables of interest can be found by setting the net growth rate to zero in Equations 2.24 and 2.25. This results in the following:

$$S_m = K \frac{b}{Yk - b} = K \frac{b}{\mu_m - b} \qquad \text{(Eq. 2.26)}$$

Equation 2.26 indicates that S_m is a function of K. The relationship between the two is given by the ratio $(\mu_m - b)/b$. Typical values for this ratio or maximum growth rate to decay rate are 20–100, suggesting that S_m typically is in the range of perhaps 10–500 micrograms per liter

(μg/L) when K is in the range of 1–10 mg/L. S_m represents the lowest concentration for a substrate under steady-state conditions when that substrate is the only substrate for organism growth and all other growth requirements are in excess supply. At times then, the minimum concentration to which a contaminant can be biodegraded in an aquifer can be limited by S_m.

Often it is observed that compounds in groundwater are being degraded to concentrations below S_m. This can occur when they are used as secondary substrates or through cometabolism. Degradation as a secondary substrate occurs when an organism is provided with a sufficient amount of a primary substrate in order to maintain itself and produce the enzymes necessary for the simultaneous consumption of the secondary substrate. For example, an organism might be able to aerobically consume and grow on either acetate or benzene. Benzene by itself at a concentration of 10 μg/L might not be able to support net biological growth, but if the organism were at the same time given 1,000 μg/L of acetate, which is above its S_m level, it could grow on the acetate and simultaneously degrade the benzene down to 1 μg/L of benzene or less.

Cometabolism is the degradation of a compound by an organism using enzymes that serve some purpose for the cell other than degradation of that compound. The organism obtains no benefit from the transformation, indeed it may harm them. For example, some organisms that aerobically oxidize toluene initiate the oxidation using an enzyme called an oxygenase that adds elemental oxygen to toluene forming cresol. Commonly, the oxygenase also fortuitously adds elemental oxygen across the double bond in TCE to form TCE epoxide, which is chemically unstable and degrades to a series of simpler compounds that are used by other organisms for food. In this manner, TCE is destroyed by an organism that obtains no benefit from the transformation, indeed the epoxide formed may not only sap some energy away from the organism, but the epoxide itself can also be quite lethal to them. Nevertheless, cometabolism has been demonstrated to be useful as a method for aerobic destruction of TCE in groundwater (McCarty et al., 1998a). Another example is the cometabolic transformation of CT by the denitrifying bacterium *Pseudomonas stutzeri* KC. Strain KC secretes a biomolecule – pyridine-2,6-bis-thiocarboxylate (PDTC) – that has a primary role in trace metal acquisition, but also fortuitously degrades CT to harmless end products when it is chelated to copper (Dybas et al., 1995b; Lee et al., 1999).

S_m as a concept in groundwater is important in setting a lower substrate bound below which organisms cannot be in net growth. However, when the concentration is above S_m, as it usually is at the point of injection when substrates are added to aquifers to stimulate microbial growth, growth rate will be positive. Indeed, it will remain positive as long as a rate limiting substrate is above S_m. When this occurs at the point of chemical injection into an aquifer, organisms can continue to grow until the pore spaces between aquifer minerals are filled with them, clogging the aquifer. This is a problem that needs to be prevented at points of continuous substrate injection into aquifers, such as in wells. Methods to address this potential problem are outlined in detail in ESTCP (2005). These include pulsing of substrates instead of continuous injection so that periods of organism starvation and population decrease will occur, or periodic or continuous injection of a bacterial toxicant such as hydrogen peroxide to reduce clogging by organism growth near the injection well.

2.4.4 Mass Transfer Limitations

Frequently in groundwater, reaction rates are limited by the rate of transport of a needed substance to the point of reaction. Transport processes include advection, dispersion, sorption, and diffusion. Advection, dispersion, and sorption are covered adequately in other parts of this volume. Diffusion controlled reactions are as well, but will be mentioned briefly here to compliment the discussion of biological kinetics. The rate of a chemical or biological reaction

Table 2.11. Spreading Time for Chemicals as Function of Distance. Chemicals must move by diffusion to site of reaction. Assumed coefficient of molecular diffusion $D = 10^{-9}$ m^2/s. Spreading in time t is $t = l^2/2D$, where l is the diffusion distance.

Diffusion distance	Time required
1 μm (scale of a bacterium)	10^{-3} s
1 mm (scale of a grain of sand)	8 min
1 cm	1 day
10 cm	5 months
1 m	16 years
10 m	4,000 years

at some specific location may be controlled mainly by the intrinsic rate of a reaction or by the rate of diffusion of a needed substance to that location. The two rate processes involved are diffusion and biotransformation. At times one may be more limiting than the other. Which is limiting in a given case affects how best to operate a chemical delivery system.

As shown in Table 2.11, spreading by molecular diffusion is fast over the distance scale of a bacterium or a grain of sand, occurring in seconds to minutes. But the time required for spreading is proportional to the distance squared. So over longer distances, much more time is needed. If reactants can only be delivered to a location within 10 cm of a target contaminant, 5 months are required. Clearly, patience is needed when contaminants and/or other reactants must diffuse through micro-fractures or small channels before becoming accessible for degradation.

Even when chemicals can be effectively distributed or delivered close to the contaminants, diffusion remains important. Microorganisms in aquifers for the most part are attached to aquifer material or exist as large immobile bundles of organisms living in the interstitial spaces between aquifer mineral particles. As such, they act as biofilms. Here as groundwater moves past, substrates must be conveyed from the water to and into the biofilm for biodegradation. Mass transfer from the water to the biofilm, and diffusion within the biofilm is required to bring substrate to the microorganisms. Mass transfer rather than intrinsic biodegradation rate may limit the rate of the biological reaction. This is often the case with natural attenuation.

Consider a simple case of steady-state diffusion of a rate-limiting substrate from the aqueous phase to a biofilm attached to the surface of some aquifer minerals. This is illustrated in Figure 2.5. The rate of mass diffusion (dM/dt) across a unit area of the boundary layer to the biofilm is proportional to the concentration gradient (Rittmann and McCarty, 2001):

$$-\frac{dM}{dt} = k_d(S_x - S_s)A \qquad \text{(Eq. 2.27)}$$

where dM/dt represents the mass of substance moving across the boundary layer into an area A of biofilm per unit time, k_d is the rate of mass transport (length over time), and S_x and S_s are the concentration of the substance in the bulk water and at the biofilm surface at the given location within the aquifer. Biodegradation of the limiting substrate within the biofilm itself is a function of the concentration as given by Equation 2.27, but the substrate concentration decreases with distance within the biofilm, making the relationship somewhat complicated. A general solution for this case is (Rittmann and McCarty, 2001):

$$-\frac{dM}{dt} = \left[2qXD\left((S_s - S_w) + K\ln\left(\frac{K + S_w}{K + S_s}\right)\right)\right]^{1/2} \qquad \text{(Eq. 2.28)}$$

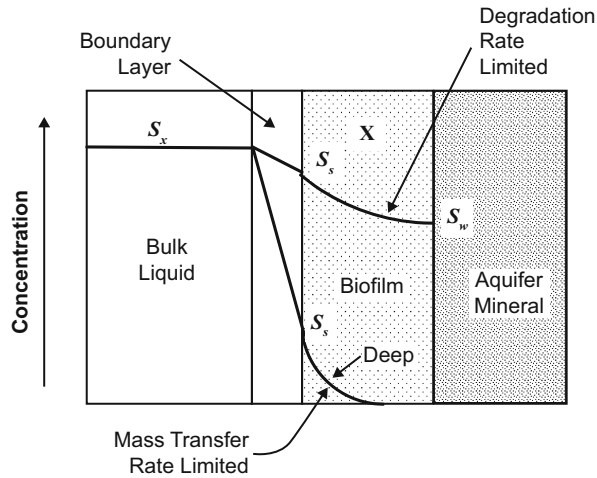

Figure 2.5. Substrate diffusion across a boundary layer and into a biofilm, illustrating cases of reaction rate limited by mass transfer and by biological reaction kinetics.

where D is the rate of molecular diffusion of the substrate through the biofilm and S_w is the substrate concentration at the point of biofilm attachment to the aquifer minerals. Under near steady-state conditions, which are typical in aquifers, the rate of diffusion of substrate into the biofilm just equals the rate of biological transformation, thus,

$$k_d(S_x - S_s) = \left[2qXD\left((S_s - S_w) + K\ln\left(\frac{K + S_w}{K + S_s}\right)\right)\right]^{1/2} \quad \text{(Eq. 2.29)}$$

Figure 2.5 illustrates various likely outcomes from the solution of this equation, depending upon the relative values of the various rate constants involved. If the process is diffusion limited (relatively low k_d) then S_s will be much less than S_x, but if biodegradation is rate-limiting (relatively high k_d), then S_s will be similar in value to S_x. With a small starting seed of microorganisms, the depth of the biofilm may be very small so that S_w is almost equal to S_s. The rate of biotransformation then is very low and thus rate limiting. Changing the rate of mass transfer to the biofilm will make little difference. However, as microorganisms grow and the biofilm thickens, S_w decreases and may eventually approach zero, in which case, biotransformation becomes maximum. The limiting factor may then be the rate of mass transfer to the biofilm surface. In this case, changing the rate of mass transfer may increase reaction speed. This might be accomplished by increasing the fluid velocity passed the biofilm, such as by artificially increasing mixing speed through groundwater recirculation. This indicates the importance of understanding what factors are affecting reaction rate within an aquifer.

2.4.5 Bioaugmentation

Frequently, the microorganisms required for biodegradation of contaminants are naturally present in an aquifer and to bring about contaminant destruction only requires a non-toxic environment and that the microorganisms be brought into contact with an adequate mixture of electron donor and electron acceptor for energy production, and necessary nutrients for growth. In some cases, however, the needed organisms may not be present so that bioaugmentation may be desirable. External production of the degrading organisms and introduction into

the aquifer with adequate mixing is then required. Bioaugmentation at times has not been successful, but if conditions are correct, bioaugmentation can be very successful. For bioaugmentation to succeed well, the bioaugmented microorganisms must be filling a niche not already being filled by other microbes. Examples of unsuccessful, partially successful, and highly successful bioaugmentation are provided in the following.

Bioaugmentation was attempted to enhance the aerobic cometabolism of chlorinated solvents in two separate studies at the Moffett Field experimental field site in Mountain View, California. The first was unsuccessful and the second was partially successful. In previous studies at Moffett Field, the use of toluene as an electron donor was found to be quite successful for stimulating microorganisms that cometabolize TCE. However, use of toluene for this purpose is sometimes of concern because it is a regulated compound, and so a more generally acceptable donor is desired. Toward this end, an organism that produces the toluene ortho monooxygenase enzyme that cometabolizes TCE efficiently was genetically modified to grow on lactate, a generally acceptable donor, while still maintaining a high concentration of toluene ortho monooxygenase. Laboratory studies indicated that the organism, *Burkholderia* G4, performed well in pure culture, but column studies with natural aquifer materials indicated that maintaining good activity over time might be difficult (Munakata-Marr et al., 1996). This was demonstrated in subsequent field studies in which high concentrations of the microorganisms were continually injected into the aquifer in hopes of allowing it to compete well with native lactate using microorganisms (McCarty et al., 1998b). Good TCE cometabolism was achieved for about 10 days, and then it declined as competing lactate users that did not cometabolise toluene came into dominance. It was also noted that continuous addition of high organism concentrations resulted in growth of a predatory population of protozoa that consumed the bioaugmented organisms. This study indicated the difficulty of trying to out compete native organisms with the same or better ability at electron donor and acceptor utilization.

In another study at Moffett Field, an organism that cometabolises TCA well while growing on butane was used for bioaugmentation (Semprini et al., 2007). Here, bioaugmentation resulted in a rapid increase in the ability to grow on injected butane and to cometabolize TCA, but after about 1 month, TCA degradation decreased. In a control system without bioaugmentation, a native population of butane-oxidizing bacteria that lacked the ability to cometabolize TCA eventually became established. Cometabolism could be continued if the native population was controlled by periodic additions of high hydrogen peroxide concentration. In this case, bioaugmentation was partially successful, but its maintenance was difficult in the presence of non-TCA utilizing organisms that could compete effectively for the added butane.

Bioaugmentation has been successful when competition by other organisms for electron donor or electron acceptor is either not an issue or is suppressed. This has been the case with bioaugmentation for anaerobic PCE, TCE, DCE, and VC reductive dehalogenation. Since these electron acceptors are not used under anaerobic conditions for processes other than reductive dehalogenation, the organisms carrying out these reactions have no competition for their respective electron acceptors, even though competition is quite strong for the electron donors that they use. As long as the dehalogenators can compete successfully with other hydrogen-using microorganisms, they survive well in anaerobic groundwater environments. This has been demonstrated in field-scale demonstrations at Dover Air Force Base (AFB), Deleware (Ellis et al., 2000) and Kelly AFB, Texas (Major et al., 2002).

Suppression of competition was demonstrated in pilot- and demonstration-scale bioaugmentation studies conducted at Schoolcraft, Michigan, in an aquifer contaminated with CT and nitrate. The added organism was *Pseudomonas stutzeri* KC, the denitrifying, CT-degrading

bacterium described earlier (Dybas et al., 1998, 2002; Hyndman et al., 2000). The concept was to introduce strain KC into the aquifer ahead of the CT plume and to maintain it as a biofilm through weekly additions of acetate. A challenge was how to prevent indigenous denitrifying bacteria from outcompeting strain KC for the added acetate. When stimulated by acetate addition, the other denitrifying organisms at the site could also convert CT to chloroform, an unwanted and persistent product. Failure to selectively stimulate strain KC would result in the formation of chloroform and failure of the bioaugmentation effort.

A laboratory comparison of the specific growth rates of strain KC and the indigenous microflora at different pH levels revealed a solution to the problem of competition (Dybas et al., 1995a). At Schoolcraft, the native denitrifying bacteria had adapted to the background groundwater at a pH of 7.2, and as expected, their maximum specific growth rate was highest at that pH level. Increasing the pH to 8.0–8.2 caused precipitation of Fe(III) and created conditions unfavorable for the indigenous microflora, but favorable for the growth of strain KC, an effective iron scavenger. Thus, adjusting the aquifer pH to 8 prior to introduction of strain KC conferred a colonization advantage on the strain KC and enabled long-term control of the CT degradation pathway.

Other challenges at Schoolcraft included: how to introduce alkalinity, strain KC, and acetate across a large and deep aquifer in a uniform fashion, and how to maintain sufficient concentrations of strain KC within the biocurtain to insure reliable CT degradation to levels below the U.S. Environmental Protection Agency (USEPA) drinking water standard (five parts per billion [ppb]) over a period of years, as the CT plume slowly passed through. These challenges were overcome by weekly 6-h chemical delivery periods in which groundwater amended with acetate and adjusted to pH 8 was recirculated through a "picket fence" of closely spaced (1 m apart) extraction/injection wells screened over the entire depth of contamination and positioned normal to the direction of groundwater flow. The resulting recirculation patterns between these wells allowed for pH adjustment, introduction of strain KC, formation of a well-colonized biocurtain, and maintenance of the biocurtain for a period of years.

Delivery of strain KC into the subsurface was not problematic. This was because wells for chemical and organism delivery were spaced close together (1 m apart) and because delivery of chemicals occurred in the same wells used to deliver the organism. In general, organism delivery has not been a problem for bioaugmented systems when the added organisms are introduced at the same wells or near wells where donor or acceptor are later added. Growth near the well is rapid, and the added organisms tend to spread rapidly through the aquifer as they multiply in response to the presence of growth factors. Only a small fraction of the organisms need to be carried through the aquifer to act as seed throughout the system. This was clearly shown at the Dover AFB demonstration (Ellis et al., 2000).

The general strategy used at Schoolcraft to control competition – chemical conditioning a region of the subsurface to prepare for the introduction of a new organism – is useful when specific organisms or groups of organisms need to be encouraged or discouraged. For bioaugmentation, the native microflora will typically be adapted to the pH of their environment, and that pH is likely to be different from the optimum for the added organism. A pH shift can thus encourage survival and growth of the introduced organism while selecting against the indigenous competitors. Table 2.12 lists strain KC along with other major microbial groups and some optimal pH ranges for each. It is important to keep in mind, however, that most of the listed groups in Table 2.12 also contain highly specialized representatives capable of growth under extreme acidic conditions (acidophiles) and extreme alkaline conditions (alkalophiles), so the ranges indicated represent "non-extreme" values for each group.

Table 2.12. Optimum pH Ranges for Different Microorganisms and Functions

Organism type	Function	Optimal pH range
Heterotrophs	Oxidize ammonia	6–9
Nitrifiers	Oxidize ammonia	6–9
Denitrifiers	Reduce nitrate to N_2	6–9
Acidogens	Convert complex organic matter to weak acids	3–6
Acetogens	Convert propionic acid and butyric acid to acetic acid	6–7
H_2-utilizing methanogens	Convert H_2 + CO_2 to methane	6.2–7.2
Acetoclastic methanogens	Convert acetic acid to methane	6.6–7.2
Sulfate-reducing bacteria (SRB)	Reduce sulfate to hydrogen sulfide, remove metals as sulfide precipitates	4–10
Specialized cultures, example: *Pseudomonas stutzeri* KC	Function depends on the organism. In the case of strain KC, denitrification and dechlorination of CT are important	>7 (8.2 optimal)

2.4.6 Organic Bioremediation Example: Edwards AFB, California

A full-scale evaluation for *in situ* aerobic cometabolic biodegradation of TCE at Edwards AFB in southern California serves as an example to illustrate how chemicals needed for biodegradation can be successfully introduced and mixed to enhance biodegradation (McCarty et al., 1998a). Cometabolism is the fortuitous biodegradation of a compound by enzymes that are used by organisms to carry out some other essential function in the organism. There have been several field demonstrations of successful use of cometabolism for biodegradation of TCE and other halogenated aliphatic compounds.

At Edwards AFB a TCE contaminated plume emanated from a location where TCE contaminated wastewater was discharged onto the ground surface in the 1950s and 1960s. At the downgradient location where *in situ* cometabolism was applied, the groundwater was divided between two aquifers separated by a 2 m thick clay aquitard (Figure 2.6). The upper unconfined aquifer was 9 m below ground surface (bgs) and was 8 m in depth. The lower aquifer was 5 m deep. The substrate selected here for cometabolism was toluene, which was shown from earlier pilot studies at Moffet Federal Air Field to be a good substrate for efficient cometabolism of TCE (Hopkins and McCarty, 1995). Studies with aquifer material from Edwards AFB indicated that the necessary microorganisms for toluene consumption and efficient TCE cometabolism were naturally present throughout the aquifer (Jenal-Wanner and McCarty, 1997). Bioaugmentation was not necessary. In order to achieve cometabolism, both toluene and oxygen for its oxidation were added to the aquifers, and both were mixed with the TCE contaminated water and brought together for consumption by toluene-using microorganisms in order to enhance biodegradation of TCE. Two potential problems had to be considered in designing the delivery system.

The first potential problem was how to bring toluene, oxygen, TCE, and the toluene-consuming microorganisms together at the same location within the aquifer. Oxygen must be present in order for microorganisms to oxidize the toluene and grow, producing the toluene monooxygenase enzyme needed for TCE cometabolism. TCE had to be present when the enzyme was induced so that it would be biodegraded. However, toluene and TCE compete

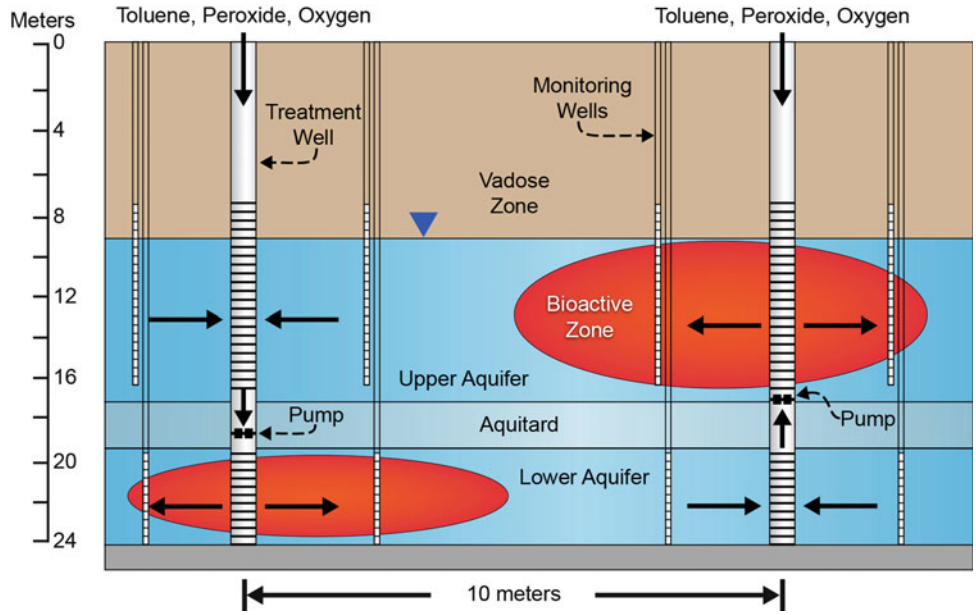

Figure 2.6. Treatment scheme used for adding and mixing chemicals for *in situ* aerobic cometabolic biodegradation of TCE at Edwards AFB, California. Reprinted from McCarty et al., 1998a with permission by American Chemical Society.

for the same enzyme, and so it was desirable to have the toluene present in low concentration, at least occasionally, so that TCE could be biodegraded efficiently. Also, TCE cometabolism as well as toluene oxidation could take place only under aerobic conditions. Thus, aerobic conditions in the aquifer had to be maintained.

The second major potential problem was how to prevent clogging of well screens in wells where toluene and oxygen were to be added to the aquifer. The two potential problems were addressed at Edwards AFB through use of tandem recirculating wells as illustrated in Figure 2.6. The two tandem wells were established for mixing and for adding toluene and oxygen. Each well had two screens, one in the upper aquifer and one in the lower aquifer. Each well contained a pump for water circulation, water in one was pumped downward from the upper aquifer to the lower aquifer, and in the other water was pumped upward from the lower aquifer to the upper aquifer. This created a circular pattern of water movement in the aquifer. The circulating water contained the TCE contaminant. A static mixer was placed in the exit line from the pump so that toluene and oxygen could be added to the circulating water as it passed through the well. Neat toluene was pumped and gaseous oxygen was allowed to flow from a pressure cylinder into the static mixer for mixing with the TCE contaminated water. In this manner, mixing between the toluene and oxygen and the TCE could be achieved within the well. The mixture then flowed into the aquifer to come in contact with the biodegrading microorganisms. Thus, all four necessary ingredients for organism growth, enzyme induction, and TCE cometabolism were brought together as needed.

The other problem to solve was that of well clogging through excessive biological growth near the well screens, a problem that was likely to exist as the injection concentrations of toluene and oxygen were well above their respective S_m values so that microorganism growth would continue until the pores between aquifer minerals were completely filled. Three strategies were used here to reduce this problem. The first was toluene pulsing. The needed oxygen was added continuously, but toluene was added in high-concentration pulses only three times

per day. During the toluene pulse, the oxygen concentration at that time was insufficient for oxidizing most of the toluene and became depleted rapidly near the well, thus minimizing growth there. However, as the toluene moved radially from the injection well and into the aquifer it mixed with the oxygen further away from the injection well through lateral and longitudinal dispersion, which helped greatly to spread biological growth out into the aquifer system. This is a great benefit derived from pulsing substrates. The second strategy was to continuously add hydrogen peroxide, a biocide that is hydrolyzed by bacterial enzymes in the aquifer to form oxygen and water:

$$2H_2O_2 \rightarrow O_2 + 2H_2O \qquad \text{(Eq. 2.30)}$$

A mass balance indicates that two moles H_2O_2 or 68 g produces 32 g O_2. Thus, hydrogen peroxide addition was beneficial in two ways. First, it killed biological growth within the well itself and for some distance beyond it. Its hydrolysis then resulted in the production of oxygen away from the well as needed there by microorganisms for toluene oxidation. The third strategy to mitigate well clogging was to use well development, which was required infrequently because of the relative success of the first two approaches.

The quantity of chemicals to add was based upon reaction stoichiometry. The energy reaction for toluene oxidation is:

$$C_6H_5CH_3 + 9O_2 \rightarrow 7CO_2 + 4H_2O \qquad \text{(Eq. 2.31)}$$

This equation indicates that 9 moles oxygen is required for each mole of toluene. However, some of the toluene is converted to cells and so the actual oxygen requirement is less than this. A laboratory study to simulate field conditions was conducted in order to determine reaction stoichiometry for aerobic oxidation of toluene when organism growth was included, showing that the actual need was for only 6 moles oxygen (192 g) per mole toluene (92 g), or 2.1 g oxygen per g toluene (Jenal-Wanner and McCarty, 1997).

The pulsing of toluene to give a time-averaged concentration of 9.0 mg/L, and continuous addition of pure oxygen (44 mg/L) and hydrogen peroxide (47 mg/L) resulted in good steady-state operation with little problems from aquifer clogging. With 9 mg/L toluene, only about 20 mg/L of oxygen was actually needed based upon reaction stoichiometry, but the excess added insured that aerobic conditions remained throughout the aquifer, a desirable condition. TCE removal during this period varied between 83% and 87% of the TCE passing through the treatment well. However, toluene removal was much higher. Within 2.5 m of the injection wells, toluene concentration was mostly consumed, and by the time the water reached the four 15-m sampling locations surrounding the treatment system, the concentration of toluene had been reduced to an average of 1.3 μg/L, well below the taste and odor threshold of 20 μg/L and the drinking water maximum contaminant level (MCL) of 1,000 μg/L.

2.5 CHEMICAL PROCESSES

An advantage of biological processes is that they can result in the destruction as well as removal of chlorinated solvents. The same can be said of some chemical processes. As with biological processes, the chemical processes can be divided into oxidative and reductive processes. Oxidative processes would logically result in the oxidation of organic carbon in the chlorinated solvents to carbon dioxide, while releasing organic chlorine as chloride. Reductive processes on the other hand, reduce the organic carbon in chlorinated solvents to a lower oxidation state such as ethane, while again releasing the organic chlorine as chloride. Some chemical processes for chlorinated solvent transformation occur under the ambient environmental conditions associated with aquifers (Rheinhard et al., 1997). Often natural chemical

transformations do not result in complete conversion to harmless end products. Nevertheless, an understanding of these natural processes is important for assessing the source of contaminants that may be found at a site and in selecting processes and strategies for remediation. Engineered remediation using chemical processes for *in situ* contaminant destruction have been broadly studied, and some have been frequently applied.

2.5.1 Oxidative Chemical Processes

Chemical oxidants have been used in the water treatment industry for decades for the destruction of unwanted organic chemicals. Most frequently used have been ozone, permanganate, and Fenton's reagent. However, possible use of chemical oxidants for *in situ* destruction of chlorinated solvents has been explored in detail only in recent years, and that has been for addressing the difficult problem of DNAPL destruction. This has become known as ISCO or *in situ* chemical oxidation. Perhaps the first to explore the use of permanganate for this purpose was Schnarr et al. (1998). They reported on both laboratory and field experiments for PCE and TCE destruction in which 10 g/L permanganate was found to completely oxidize the compounds to carbon dioxide and chloride. Two field experiments were conducted. In the first, 1 L PCE that was added to a confined area was completely removed within 120 days by flushing through 100 L/day of the 10 g/L $KMnO_4$ solution. For the second, 8 L of a mixed PCE/TCE DNAPL was added to a test cell, and after 290 days of flushing with 10 g/L permanganate, 62% of the initial source had been oxidized. In this oxidation process, the MnO_4^- oxidant is reduced to form the insoluble MnO_2. Subsequently, many studies by a wide range of researchers have been conducted to further evaluate the use of permanganate.

Fenton's reagent is a mixture of hydrogen peroxide and ferrous iron, which serves as a catalyst, forming hydroxyl radicals, the main oxidizing species in Fenton's reagent. An earlier experiment using Fenton's reagent for oxidation of PCE was conducted by Leung et al. (1992), who reported mineralization of 1 g PCE per kilogram (kg) aquifer solids within 3 h with a solution containing 2.1 molar (M) H_2O_2 and 5 millimolar (mM) $FeSO_4$. TCE appears to be oxidized somewhat more slowly than PCE (Teel et al., 2001). Fenton's reagent also degrades CT even though its carbon is already in the fully-oxidized state (Teel and Watts, 2002). This apparently occurs by a reduction mechanism in which a superoxide radical anion is involved (Smith et al., 2006). Many studies using Fenton's reagent for destruction of chlorinated solvents have now been conducted.

2.5.2 Reductive Chemical Processes

Perhaps the first to recognize the potential for abiotic reduction of chlorinated solvents for *in situ* destruction was Gillham and O'Hannesin (1994), who found that 100-mesh zero-valent iron was capable of removing chloride from 14 different chlorinated methanes, ethenes, and ethanes, and replacing the chlorides with hydrogen. In the process Fe(0) is converted to Fe(II). The rates of transformation were sufficiently fast for field application, except perhaps for dichloromethane. Gillham and O'Hannesin proposed that zero-valent iron might be used for either *in situ* or aboveground applications for remediation of contaminated groundwater. A field demonstration of the technology was initiated in 1991 at Canadian Forces Base, Borden, Ontario, to treat a plume containing 268 mg/L TCE and 58 mg/L PCE (O'Hannesin and Gillham, 1998). Here, a mixture of 22% granular iron and 78% sand installed as a permeable "wall" across the path of the plume removed approximately 90% of the TCE and 86% of the PCE. The first full-scale application of granular zero-valent iron was a reactive wall installed in 1996 in North Carolina to treat overlapping plumes of chromate and chlorinated solvents (Puls et al., 1998).

This passive approach to the control of plume migration, while involving a relatively high capital expenditure, has been an attractive alternative to those wishing to avoid an active program of control, which has lower capital but higher maintenance costs.

Experiments with zero-valent iron have been conducted for other than plume-migration control. For example, a demonstration was conducted in which zero-valent iron was mixed with aquifer material contaminated with TCE DNAPL using a large-diameter mixing blade (Wadley and Gillham, 2003). Here, bentonite was added as well to serve as a lubricant to facilitate injection of the iron and to isolate the contaminated zone. PCE was reported to decrease to non-detectable levels within the 13-month monitoring period. Alternatively, Cantrell and Kaplan (1997) proposed using colloidal sized suspensions of zero-valent iron that could be injected directly into an aquifer without the need to build a reactive wall. This has been carried further by Zhang et al. (1998), who have suggested use of nanoscale bimetallic particles in which one metal (Fe or Zn) serves as the reductant, while palladium or platinum serves as a catalyst to speed up the reaction. Much research and field studies have been conducted on this alterative approach. In a further alternative that also uses a palladium catalyst, Schreier and Reinhard (1995) demonstrated that molecular hydrogen could be used instead of iron as the reductant. Here, the reaction is sufficiently faster so that the system lends itself to a down-well or surface reactor. Thus, many alternatives for treatment of chlorinated solvent contaminated plumes as well as DNAPL source areas using reductive chemical processes have emerged in recent years.

2.5.3 Precipitation

Precipitation may be desired for stabilization of hazardous chemicals within an aquifer so that they do not contaminant water passing by. Chemical species for which this may be an option are generally metal cations that have very low solubility in water under given aquifer chemical, redox and pH conditions.

Metals as such cannot be destroyed, and so either stabilization in some manner within the subsurface where contamination exists or removal may be the only viable remediation options. Possible metals for the stabilization option are chromium, cadmium, zinc, lead, mercury, uranium, and plutonium. If stabilization by precipitation is to be an option for remediation, then the aquifer conditions that promote precipitation should not change over time, otherwise the metals may become soluble to contaminate groundwater. Metals most frequently occur as cations, which is the form most susceptible to precipitation. Some metals, such as hexavalent chromium (CrO_4^{2-}), may also exist as oxyanions that generally do not precipitate well. Some metal cations precipitate well in one oxidation state, but not in another. For example, Fe(III) hydroxide is quite insoluble, while Fe(II) hydroxide is not.

Factors involved in precipitation can be quite complex and are not discussed in detail here. Further information can be found in general textbooks on environmental chemistry (Benjamin, 2002; Morel and Hering, 1993; Sawyer et al., 2003; Stumm and Morgan, 1996). The general principle involved, however, is the solubility product (K_{sp}) of the metal with an anion:

$$Cd^{2+} + 2OH^- = Cd(OH)_2(s) \qquad [Cd^{2+}][OH^-]^2 = K_{sp} = 2 \times 10^{-14} \qquad \text{(Eq. 2.32)}$$

$$Fe^{3+} + 3OH^- = Fe(OH)_3(s) \qquad [Fe^{3+}][OH^-]^3 = K_{sp} = 6 \times 10^{-38} \qquad \text{(Eq. 2.33)}$$

$$Pb^{2+} + S^{2-} = PbS(s) \qquad [Pb^{2+}][S^{2-}] = K_{sp} = 1 \times 10^{-28} \qquad \text{(Eq. 2.34)}$$

From the ionization product of water, ($[H^+][OH^-] = 10^{-14}$), the hydroxide concentration at pH 7.0 is found to equal $10^{-14}/10^{-7} = 10^{-7}$. At pH 7, cadmium(II) in the form of its hydroxide is quite soluble while iron (III) is not. Based upon Equations 2.32 and 2.33, the concentrations of

Cd^{2+} and Fe^{3+} are thus 2 M and $6(10^{-14})$M, respectively. Thus, we would not expect Cd^{2+} to be stabilized in groundwater at pH 7 while we would with iron. But these simple calculations are not sufficient. An important regulatory consideration is the total solubility of a metal, including all soluble forms in equilibrium with the solid phase. An estimate for this value requires a knowledge of the solubility product data in Table 2.4 along with equilibrium coefficients for other equilibria that involve the metal of interest.

As discussed previously, chromium can be removed by reduction to Cr(III) hydroxide, $Cr(OH)_3$. This solid will be in equilibrium with Cr^{3+}. At pH 7.5, the concentration of Cr^{3+} can be estimated from the solubility product:

$$\begin{aligned} Cr(OH)_3(s) &= Cr^{3+} + 3OH^- \\ K_{sp} &= 10^{-30.2} \\ K_{sp} &= [Cr^{3+}][OH^-]^3 \\ 10^{-30.22} &= [Cr^{3+}](10^{-6.5})^3 \\ [Cr^{3+}] &= 1.9 \times 10^{-11}\text{M} = 9.9 \times 10^{-4}\mu g/L \end{aligned} \qquad \text{(Eq. 2.35)}$$

The above concentration is much less than the USEPA regulatory standard of 100 μg/L Cr, but it only represents the soluble Cr(III) that is chelated to H_2O ligands. Other dissolved Cr(III) species are present and must be accounted for. The nature of these species will depend on whatever additional ligands are present and their equilibrium binding constants. If the only other ligands are hydroxyl groups from water, the total soluble Cr(III) at pH 7.5 can be estimated from K_{sp} and the relevant equilibrium constants (K_1 through K_4), where:

$$K_1 = 10^{10.0} = [Cr(OH)^{2+}]/\{[OH^-][Cr^{3+}]\}$$

Thus, $[Cr(OH)^{2+}] = 10^{10.0}[10^{-6.5}][1.9 \times 10^{-11}] = 6.0 \times 10^{-8}\text{M} = 3\ \ \mu\text{g/L Cr}$

$$K_2 = 10^{8.3} = [Cr(OH)_2{}^+]/\{[OH^-][Cr(OH)^{2+}]\}$$

So that, $[Cr(OH)_2{}^+] = 10^{8.3}[10^{-6.5}][6.0 \times 10^{-7}] = 3.8 \times 10^{-6}\text{M} = 198\ \ \mu\text{g/L Cr}$

$$K_3 = 10^{5.7} = [Cr(OH)_3(aq)]/\{[OH^-][Cr(OH)_2{}^+]\}$$

Solving gives, $[Cr(OH)_3(aq)] = 10^{5.7}[10^{-6.5}][1.2 \times 10^{-5}] = 6.0 \times 10^{-7} = 31\ \ \mu\text{g/L Cr}$

$$K_4 = 10^{4.6} = [Cr(OH)_4{}^-]/\{[OH^-][Cr(OH)_3(aq)]$$

This means $[Cr(OH)_4{}^-] = 10^{4.6}[10^{-7}][6.0 \times 10^{-6.5}] = 7.6 \times 10^{-9}\text{M} = 0.4\ \ \mu\text{g/L Cr}$

The above calculations show that for this pH, most of the dissolved Cr(III) is present as $Cr(OH)_2{}^+$. The total mass concentration in solution is the sum of the concentrations of all dissolved species: $9.9 \times 10^{-4} + 3 + 198 + 31 + 0.4 = 232\ \mu g/L$. This value for total Cr exceeds the regulatory standard. Soluble chromium concentration from operation at a slightly higher pH (~8) would meet the standard.

Anions often considered for stabilization of metals in water are hydroxide, carbonate, phosphate, and sulfide. These anions are all commonly found associated with groundwater and aquifer minerals. Figure 2.7 indicates the relative solubility of various metal salts of these anions. Several general conclusions might be drawn from this figure. Phosphate and sulfide salts are in general less soluble than hydroxide salts. Of the four, carbonate salts are the most soluble. The graph for hydroxide salts indicates one of the impacts of pH. At pH of 7 (log hydroxide concentration of −7), Fe(III) as well as Cr(III) are quite insoluble, but most of the other metals are not. Precipitation of hydroxides is better at higher pH (higher hydroxide

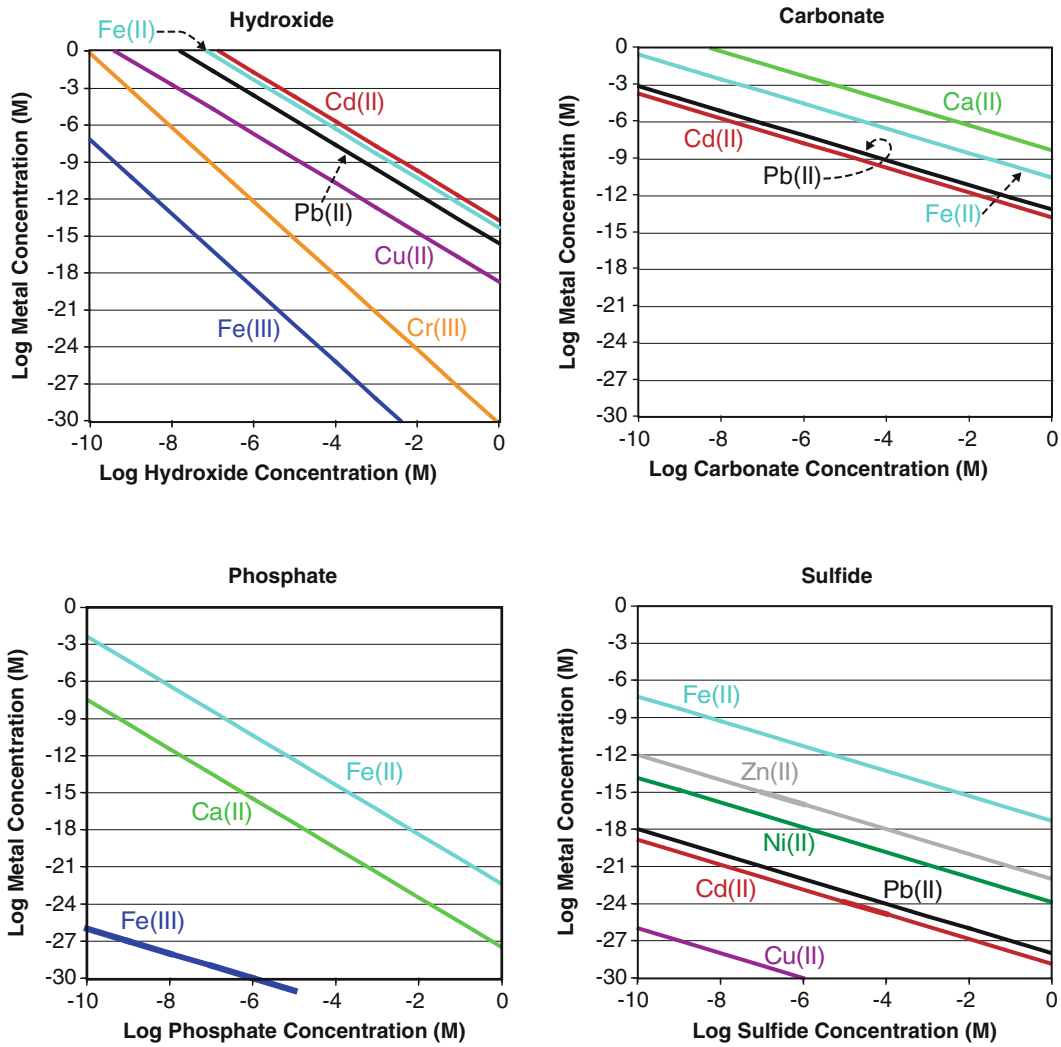

Figure 2.7. Solubility of various metal salt hydroxides, carbonates, phosphates, and sulfides based upon solubility product data. From Sawyer et al., 2003.

concentration). Fe(II) is quite soluble at pH 7, indicating why the anaerobic reduction of Fe(III) in Fe(III)-containing minerals results in the formation of soluble Fe(II).

On the other hand, the solubility of FeS, indicated in the last graph, is quite low. Thus, while anaerobic conditions may result in the reduction of Fe(III) to Fe(II), they may also result in the reduction of sulfate to sulfide so that some or all of the Fe(II) becomes stabilized in a sulfide precipitate. This illustrates the important role that microbial processes often play in the movement and fate of chemicals in groundwater. Chemicals added to an aquifer for metal stabilization may be those that react directly with the metal such as the four anions illustrated in Figure 2.7, or they may be chemicals that promote biological and redox conditions that bring about stabilization, such as reduction of soluble Cr(VI) to form the insoluble Cr(III), or the production of sulfide. Once stabilization is achieved, then chemical and biological conditions that may result in solubilization must be prevented from occurring. The many factors involved in chemical stabilization should be well understood before this is deemed an acceptable method of control.

2.5.4 pH Control

Many of the reactions listed in Table 2.5 involve hydrogen (H^+) or hydroxyl (OH^-) ions, indicating that the reactions can change pH, some reactions cause a decrease in pH and others cause an increase in pH. Reaction rates and the speciation of chemicals are greatly affected by pH, and thus it is often important for effective groundwater remediation to apply pH control. Thus, chemicals that control pH may need to be added to and be mixed with existing groundwater. The main pH buffer in groundwater is bicarbonate (HCO_3^-), so its equilibrium with carbonate (CO_3^{2-}) and CO_2 is of importance in the control of chemical speciation.

Equilibrium reactions of importance here are listed below:

$$H_2O = H^+ + OH^- \quad \text{(Eq. 2.36)}$$

$$CO_2 + H_2O = H_2CO_3 \quad \text{(Eq. 2.37)}$$

$$H_2CO_3 = H^+ + HCO_3^- \quad \text{(Eq. 2.38)}$$

$$HCO_3^- = H^+ + CO_3^{2-} \quad \text{(Eq. 2.39)}$$

Equilibrium equations of importance as derived from the above and their respective equilibrium constants at 20°C are:

$$[H+][OH^-] = K_w = 10^{-14} \quad \text{(Eq. 2.40)}$$

$$\frac{[CO_2(g)]}{[H_2CO_3]} = K_H = 36 \text{ atm/mol} \quad \text{(Eq. 2.41)}$$

$$\frac{[H^+][HCO_3^-]}{H_2CO_3} = K_1 = 4.8 \times 10^{-7} \quad \text{(Eq. 2.42)}$$

$$\frac{[H^+][CO_3^{2-}]}{[HCO_3^-]} = K_2 = 4.9 \times 10^{-11} \quad \text{(Eq. 2.43)}$$

Equation 2.41 indicates the relationship between the partial pressure of carbon dioxide and its concentration in solution. If the partial pressure of CO_2 plus that of the other gases in solution (N_2, O_2, H_2S, CH_4) exceeds 1 atm (at sea level) plus the hydrostatic pressure at a given point in an aquifer (total pressure = atm pressure + D/10.3, where D equals hydrostatic pressure in m), then the gases would exceed saturation and bubble formation is likely. This could lead to clogging of the aquifer at that point.

Concerning pH, Equation 2.42 is of importance at near neutral pH. Taking the $\log_{10}$ of both sides of Equation 2.42, and remembering that pH = −log[H+], the pH can be found as follows:

$$pH = 6.3 + \log \frac{[HCO_3^-]}{[H_2CO_3]} \quad \text{(Eq. 2.44)}$$

Here, $[HCO_3^-]$ equals the molar concentration of bicarbonate (mg HCO_3^-/L/61,000) and $[H_2CO_3]$ is the molar solution concentration of CO_2 (mg CO_2/L/44,000). The water saturation concentration in equilibrium with 1 atm of CO_2 based upon Equation 2.41 is 0.028 M. In order to maintain a pH of 7, then according to Equation 2.42, the bicarbonate concentration would need to be 4.8 times higher or 0.134 M, which corresponds to an alkalinity of 6,700 mg/L as $CaCO_3$. If a lower pH, say 6.5 were acceptable, then the bicarbonate alkalinity would only need to be 1.58 times higher or 2,200 mg/L as $CaCO_3$. Generally, the CO_2 concentration is much less than 1 atm partial pressure, and so the need for bicarbonate alkalinity to maintain the desired pH would be proportionally less.

Some biological reactions are basic, causing pH to rise, while others are acidic, causing it to fall. The impact of various electron acceptors on bicarbonate and CO_2 concentration are illustrated by the listing in Table 2.10 using H_2 as a pH neutral electron donor. It can be seen that when oxygen or perchlorate is the electron acceptor, there is no impact of electron acceptor itself on pH. When nitrate, sulfate, bicarbonate, or ferric oxide is the electron acceptor, H^+ is consumed, and so pH tends to rise. Ferric oxidation consumes more H^+ per mole of H_2 oxidized than the other three electron donors and thus is a more basic reaction. By contrast, reductive dehalogenation of a chlorinated solvent such as PCE produces H^+ and thus is an acidic reaction, causing pH to decrease. Oxidations of many organic electron donors result in H^+ production, and thus are generally acidic as well.

2.5.4.1 Example

Problem. Lactic acid is sometimes added to accomplish reductive dehalogenation of TCE to ethene. How much lactic acid would be required to reductive dehalogenate a solution containing 1 mM TCE, and what would be the resulting pH of the solution? Assume that the initial HCO_3^- concentration is 6 mM ($6 \times 50 = 300$ mg alkalinity as $CaCO_3$/L), the initial pH is 7.0, and no other oxidation-reduction reaction occurs.

Solution. The initial aqueous CO_2 concentration (which equals the carbonic acid concentration) can be estimated using Equation 2.42:

$$[H_2CO_3] = [H^+][HCO_3^-]/4.8 \times 10^{-7} = 10^{-7}(0.006)/4.8(10^{-7}) = 0.00125 \text{ M} \quad \text{(Eq. 2.45)}$$

Lactic acid is fermented to produce acetic acid and hydrogen:

$$CH_3CHOHCOOH + H_2O = CH_3COOH + CO_2 + 2H_2 \quad \text{(Eq. 2.46)}$$

The hydrogen is used for reductive dehalogenation of TCE:

$$CCl_2{=}CHCl + 3H_2 = CH_2{=}CH_2 + 3\ HCl \quad \text{(Eq. 2.47)}$$

This equation indicates that 1.5 moles lactic acid are required to produce sufficient hydrogen for TCE reduction. The acetic acid and hydrochloric acids produced react with solution HCO_3^- to produce more carbonic acid, H_2CO_3.

$$CH_3COOH + HCO_3^- = CH_3COO^- + H_2CO_3$$

$$HCl + HCO_3^- = Cl^- + H_2CO_3$$

The overall reaction can thus be written (neglecting the donor associated with synthesis for simplicity):

$$1.5CH_3CHOHCOOH + CCl_2{=}CHCl + 4.5HCO_3^- + 3H_2O$$

$$= 1.5CH_3COO^- + 3Cl^- + CH_2{=}CH_2 + 7.5H_2CO_3$$

We see that for each millimole (mmol) of TCE dehalogenated, 4.5 mmoles bicarbonate are destroyed and 7.5 mmoles of carbonic acid are formed. With groundwater, there is no direct contact with the atmosphere and so the CO_2 formed as carbonic acid remains in solution as long as its partial pressure remains below atmospheric pressure, causing bubble formation. Checking with Equation 2.41, we see that the CO_2 formed remains in solution. Thus, our final pH would be:

$$\text{pH} = 6.3 + \text{Log}\left(\frac{6.0 - 4.5}{1.25 + 7.5}\right) = 5.5 \quad \text{(Eq. 2.48)}$$

This pH is too low for effective biological activity. Thus, bicarbonate must be added to the groundwater to maintain pH in a proper range, say 6.5 or above.

While groundwater chemistry is generally more complex than considered in this simple example, it nevertheless illustrates some of the factors involved in pH maintenance and control. Good knowledge of factors affecting pH as well as how to deliver and mix buffering chemicals if needed is often required in groundwater remediation.

2.5.4.2 Chemicals for pH Control

The major natural buffer system in groundwater is the carbonate system as governed by Equations 2.36 to 2.43. From Equation 2.42 or 2.44 it can be determined that the $[HCO_3^-]/[H_2CO_3]$ ratio must be maintained between 1.6 and 16 in order to control pH in the range of 6.5–7.5. If pH tends to be low, then it can be increased by an increase in the bicarbonate concentration. The bicarbonate levels can be increased directly, by adding sodium, potassium, or ammonium bicarbonate, or indirectly by adding a base that combines with carbon dioxide or carbonic acid to form bicarbonate. A summary of different chemicals that are commonly used in soils and aquifers to provide bicarbonate for pH control and the chemicals reactions that may be involved is provided in Table 2.13. As shown, different masses of each chemical can provide the same level of alkalinity: for example, one mole of alkaline buffer might be supplied by 50 g of $CaCO_3$ or, equivalently, by 17 g of ammonia gas.

The soluble forms of bicarbonate that can be introduced directly into an aquifer through mixing are $NaHCO_3$ and $KHCO_3$. Generally, concentrated solutions of each would be mixed with recirculating groundwater above ground or in a well. The solubility of sodium bicarbonate is about 70 g/L while that of potassium bicarbonate is quite a bit higher or about 200 g/L, thus solutions for mixing that approach these levels can be prepared for addition. NH_4HCO_3 is another possibility, but unless ammonium is needed as a nutrient for biological control, sodium and potassium salts are the better choice as ammonium adsorbs readily to clays, hindering its movement, and if oxidized, would be converted to nitrate, a soluble and hazardous chemical. Other chemicals that might be used for buffering are sodium or potassium carbonate or hydroxide. However, direct addition to an aquifer of concentrated solutions would tend to drive the pH too high, causing toxicity to microorganisms or precipitation of salts such as $CaCO_3$. Possible ways to add such chemicals while minimizing high pH problems are in an aboveground mixing chamber (used at Schoolcraft) or directly within a recirculation well where groundwater containing high CO_2 concentration can react with the chemicals as indicated in Table 2.13 to reduce pH. In such cases, there would need to be sufficient soluble H_2CO_3 in order to react with the sodium bicarbonate or hydroxide so that pH would decrease to 8.0 or below before entering the groundwater in order to avoid possible high pH problems as well as precipitation as $CaCO_3$ of Ca^{2+} that may be present in the groundwater.

In adding a chemical to prevent low pH it is also important to check for the possibility of calcium carbonate precipitation. Addition of calcium (Ca)-containing solutions such as lime is not a good idea in general as this would amplify the calcium carbonate precipitation problem near the point of injection into the aquifer. Many aquifer systems contain calcium carbonate minerals such as calcite, and the question that may then arise is whether the mineral can serve as a pH buffer by coming into solution to neutralize high acid concentrations:

$$H^+ + CaCO_3(s) = Ca^{2+} + HCO_3^- \qquad \text{(Eq. 2.49)}$$

It can to a degree, but generally at typical mineral concentrations in groundwater and at pH of 6.0 and above, the degree to which it can act as a buffer is very limited. Indeed, it is as likely to

Table 2.13. Chemicals That Might be Used to Form Bicarbonate Alkalinity for pH Control

Chemical	Reaction in formation of bicarbonate	Mass of chemical equivalent to 1 kmol (50 kg) of bicarbonate alkalinity	Comments
$CaCO_3$	$CaCO_3 + H_2CO_3 = Ca^{2+} + 2HCO_3^-$	100 g of $CaCO_3$ forms 2 moles of HCO_3^-. Thus, $100 \div 2 = 50$ kg $CaCO_3$ = 1 kmol bicarbonate	Low solubility of $CaCO_3$ limits alkalinity to 1,400–1,500 mg/L, and more so if Ca^{2+} present in groundwater
Na_2CO_3	$Na_2CO_3 + H_2CO_3 = 2Na^+ + 2HCO_3^-$	$106 \div 2 = 53$ kg	Overshooting can occur. Na deflocculates clay materials
K_2CO_3	Like Na_2CO_3	$136 \div 2 = 68$ kg	Overshooting can occur
CaO (lime)	$CaO + 2H_2CO_3 = Ca^{2+} + 2HCO_3^-$	$56 \div 2 = 28$ kg	Can result in severe precipitation of $CaCO_3$ at pH >6.8
MgO	Like CaO	$40 \div 2 = 20$ kg	Low solubility of MgO reduces chance of pH overshoot
$NaHCO_3$	$NaHCO_3 = Na^+ + HCO_3^-$	84 kg	Good but expensive. Na deflocculates clay particles
$KHCO_3$	Like $NaHCO_3$	100 kg	Good but expensive
$(NH_4)HCO_3$	Like $NaHCO_3$	79 kg	Ammonium adsorbs to clays, and if oxidized, is converted to nitrate
NaOH	$NaOH + H_2CO_3 = Na^+ + HCO_3^- + H_2O$	40 kg	Overshooting can occur, Na deflocculates clay soils
KOH	Like NaOH	56 kg	Overshooting can occur
NH_3	$NH_3 + H_2CO_3 = NH_4^+ + HCO_3^-$	17 kg	Ammonia can be toxic. Ammonium adsorbs to clays, and if oxidized, is converted to nitrate. NH_3 is naturally released when protein degrades

be removed from solution by precipitation as it is to enter solution as a buffer. One should not count on aquifer minerals or use of $CaCO_3$ itself to be good buffering solutions for pH control.

Exceptionally high pH can also be observed in groundwater, but is most likely to occur from contamination of aquifers by basic substances from industry, rather than from reactions occurring in the ground. High groundwater pH might be controlled by the addition of carbon dioxide (Equations 2.42, 2.44), or by the addition of inorganic acids such as hydrochloric acid:

$$HCl + OH^- = Cl^- + H_2O \qquad \text{(Eq. 2.50)}$$

$$HCl + CO_3^{2-} = Cl^- + HCO_3^- \qquad \text{(Eq. 2.51)}$$

In laboratory cultures, phosphate salts are often used for pH control, providing an excellent buffer near pH 7.0. However, such salts are not useful for the field because of high cost, the increased potential for causing precipitation of calcium phosphate, causing clogging problems, and the great likelihood of partitioning onto aquifer clays, preventing movement through an aquifer.

2.6 COSOLVENT AND SURFACTANT FLUSHING

Groundwater is often contaminated with a mixture of chemicals, and an early question was what effect one chemical would have on the solubility and sorption characteristics of another. Among the findings was that the presence in groundwater of highly soluble water miscible solvents such as ethanol resulted in an increased solubility and decreased sorption for another but hydrophobic chemical (Nkedikizza et al., 1985). With the growing concern about the longevity of DNAPL sources, this finding suggested one possibility in the search for new technologies with potential for DNAPL removal, and led to what is termed cosolvent flushing (Augustijn et al., 1994). Cosolvents such as methanol, ethanol, and acetone are highly soluble in water, and chlorinated solvents are much more soluble in such cosolvent mixtures than in water itself. Thus, when a cosolvent mixture containing perhaps 20% or more of the cosolvent is passed through an aquifer or injected into the groundwater near a source area, the DNAPL dissolves much more readily and can be rapidly cleansed by this process if the cosolvent solution can find its way to come into contact with DNAPL. The cosolvent/DNAPL mixture is then pumped to the surface for reuse or disposal.

2.6.1 Cosolvent Flushing

Perhaps the first application of cosolvent flushing was a field demonstration on a mixed petroleum/chlorinated solvent source area at Hill AFB, Utah in a hydraulically isolated test cell (Rao et al., 1997). Here, a cosolvent mixture consisting of 70% ethanol, 12% n-pentanol, and 18% water was pumped through the test cell over a period of 10 days, and was followed by flushing with water for 20 days. Greater than 85% mass removal of several target contaminants was observed. A pilot-scale field test of the process was later conducted for PCE removal from a site contaminated by a dry cleaner (Jawitz et al., 2000). Here, an 85% ethanol 5% water solution was pumped into the aquifer over a 3-day period, with an estimated removal of 65% of the PCE. One concern has been with the impact of the ethanol left behind with this approach, as well as with the cost of treating or disposing of the contaminated solvent removed from the aquifer. However, studies conducted 3 years after the cosolvent flushing was completed, found that the residual ethanol left behind had served as an effective electron donor for reductive dehalogenation with a significant conversion of residual PCE primarily to *cis*-DCE, but VC and ethene formation were also taking place (Mravik et al., 2003). Thus, cosolvent flushing combined with use of residual cosolvent for bioremediation emerged as a combined treatment approach for DNAPL removal.

2.6.2 Surfactant Flushing

Surfactant flushing emerged as another possible method for increasing the solubility of DNAPLs so that they could more readily be extracted from groundwater (Abdul et al., 1990; Fountain et al., 1991; Vigon and Rubin, 1989). Surfactants are organic molecules that contain a hydrophilic end with affinity for water and a hydrophobic end with an affinity for organic materials, such as chlorinated solvents. At a sufficiently high concentration of a surfactant (the critical micelle concentration), several surfactant molecules can come together to form a micelle, with the hydrophobic ends gathered together in the center and the hydrophilic ends facing out into water. Hydrophobic compounds such as the chlorinated solvents then can migrate into the hydrophobic center and hence become “solubilized.” Surfactants can also lower the interfacial tension of DNAPLs, causing them to migrate downward more readily, a problem that was early recognized (Fountain et al., 1991) and one that must be prevented from occurring (Pennell et al., 1996).

One of the first field demonstrations of surfactant flushing was in a controlled test cell at the Hill AFB, where two aquifer floods were made of the petroleum/chlorinated solvent source area (Londergan et al., 2001). The reported removal of the estimated 1,300 L of residual DNAPL in this manner was 98.5%. In a subsequent demonstration of surfactant flushing for removal of a defined release of PCE DNAPL into a confined cell at Dover AFB a smaller 68% removal was obtained (Childs et al., 2006) through ten pore volumes of flushing. Here, a surfactant formulation consisting of sodium dihexyl sulfosuccinate, isopropanol and calcium chloride was used. In a pilot field-scale demonstration of surfactant flushing of PCE DNAPL under a dry cleaning facility (Abriola et al., 2005), removal of 19 L of PCE was obtained with PCE solution concentrations decreasing by two orders of magnitude at some locations (Ramsburg et al., 2005). Here, 68 cubic meters (m^3) of an aqueous solution containing 6% by weight of Tween 80, a non-ionic food grade surfactant, were injected, with 95% recovery of the injected surfactant during extraction. An interesting observation here, as in the case with solvent flushing with ethanol, was that the residual surfactant in the aquifer stimulated the growth of PCE reducing microorganisms, leading to the formation of TCE and *cis*-DCE (Ramsburg et al., 2004). This once again demonstrated the potential for combining a chemical process for removal with a biological process for transformation of residual chlorinated solvent.

2.7 INORGANIC BIOREMEDIATION EXAMPLE: OAK RIDGE FIELD RESEARCH CENTER

A pilot-scale demonstration of uranium stabilization illustrates how both physical-chemical and biological processes can be staged and integrated to enable remediation of severely contaminated sites (Wu et al., 2006a, b). From 1951 until 1984 wastes from atomic-weapon production were stored in large unlined ponds. The ponds were drained then covered with a parking lot, but groundwater continued to percolate through the contaminated soil beneath the parking lot, resulting in three separate plumes, including one that discharged to a nearby creek. The plume depth range from 9 to 30 m bgs, in a saprolite media that had fracture densities as high as 100–200 fractures/m. These fractures accounted for less than 5–10% of matrix porosity, but carried more than 95% of the flow. The surrounding highly porous aquifer materials had a low permeability and served as a sink (and continuing source) of contamination. Groundwater contaminants included 40 mg/L of depleted uranium, 540 mg/L aluminum (Al), 930 mg/L Ca, and 11–14 mg/L nickel. Disposal of nitric and sulfuric acids lowered the groundwater pH to 3.4–3.6, and resulted in extremely high concentrations of nitrate (8–10 g/L) and sulfate (~1 g/L).

Even though the soluble uranium concentrations were high (exceeding the federal drinking water standard by over 1,000-fold), most of the uranium was associated with the solid phase, with hot spots at 200–700 mg/kg. The solid phase was thus a long-term source of U(VI) groundwater contamination. Laboratory and field tests showed that uranium sorption and desorption were strongly pH dependent with the highest adsorption observed at a pH close to 6.0.

The remediation strategy focused upon converting U(VI) into sparingly soluble U(IV). Many microorganisms, including certain SRB and iron(III)-reducing bacteria (FeRB), mediate this conversion. Reduced compounds produced by these organisms, such as sulfide and green rusts can also convert U(VI) to U(IV). The basic concept was to stimulate these reductive pathways through periodic ethanol additions. But the presence of clogging agents and inhibitors factors prevented direct implementation of this approach:

- The initial soluble uranium levels were inhibitory to microbial growth.
- Nitrate levels were inhibitory to uranium reduction and caused oxidation of U(IV) back to U(VI).

- The low pH (3–4) was unfavorable for microbial activity. The high Al acidity buffered the system at this pH and, because $Al(OH)_3(s)$ precipitates at pH 4.5–5, made it difficult to increase pH to a final level better suited for microbial activity,
- The high Ca levels were prone to precipitation at pH levels above 7 and allowed formation of soluble U(VI) calcium uranyl complexes that are difficult to reduce.

The presence of clogging agents and inhibitors motivated fabrication and operation of a multi-step conditioning system designed to remove clogging agents and to create an environment favorable for microbial activity. Stepwise conditioning is useful whenever inhibitory or clogging agents are present, though the steps and methods used in each case will differ, depending upon the contaminants present and site-specific considerations.

Prior to startup of the system, a nested circulation well system containing an inner loop and an outer loop was installed to enable hydraulic control within the targeted treatment zone. Injection of clean water into the outer loop protected the inner loop from invasion of contaminated groundwater. A bromide tracer study was conducted to characterize the well-to-well connectivity and travel times between injection and extraction wells and breakthrough curves at multilevel sampling wells located between the injection and extraction wells. The subsurface was then flushed with clean water (tap water and nitrate-free water from an aboveground treatment facility) to achieve a pH of 4.0–4.5 and to remove clogging agents and inhibitors such as Al, Ca, nitrate, and volatile organics. The extracted water was treated aboveground by vacuum stripping to remove volatile organics, two-step precipitation to remove Al and Ca, and biological treatment in a fluidized bed bioreactor to remove nitrate. The treated water was reinjected into the outer recirculation loop.

After the concentration of Al in the extracted water had fallen sufficiently – i.e., so that Al was no longer judged a clogging threat when the pH was increased – a second clean water flush at pH 6–7 was carried out. The aim of this flush was to further decrease nitrate levels and to increase pH to 6–6.5. Because nitrate had diffused deep into the matrix, these flushing operations lasted for months, as predicted by computer simulations (Luo et al., 2005), but eventually nitrate levels fell from g/L levels to low mg/L levels, and pH increased to the desired range. A pH range of 6–6.5 was selected as optimal because sorption of U(VI) was highest over this range, alleviating the potential inhibitory effect of U(VI) on microbial growth, and because this pH range was more favorable for SRB that can reduce U(VI) than for methanogens that do not, but still compete for electron donor (Table 2.12).

Weekly ethanol injections over a 1-year period sequentially stimulated *in situ* denitrification of the residual nitrate diffusing from the pores of the matrix. This was followed by sulfate- and iron(III)-reduction and U(VI) reduction. Sediment samples from the treatment zone changed color from yellow-brown to dark green or black, providing further evidence of reduction and a gradual expansion of the zone of reduction. Uranium concentrations decreased to levels below the USEPA MCL (0.03 mg/L) within those zones that were hydrologically connected to the inner loop injection well where ethanol was added. Conversion of U(VI) to U(IV) was confirmed by X-ray absorption near-edge structure spectroscopy of sediment samples. Before biostimulation, no U(IV) was observed in sediment samples. After biostimulation, up to 80% of the uranium in the aquifer was reduced to U(IV).

Before addition of ethanol, only denitrifiers were detected in the groundwater, and only at an extremely low level (3 cells/mL). After ethanol addition, most probable number estimates for denitrifiers, SRB, and FeRB in sediments (cells/g dry weight) increased to 10^7–10^8. Post-treatment tests indicated that numerous microorganisms capable of reducing U(VI) to U (IV) (including SRB *Desulfovibrio*, *Desulfoporosinus*, and *Desulfotomaculum* spp. and FeRB

Geobacter and *Anaeromyxobacter* spp.) were present. The results also suggested that ethanol addition had promoted both microbial and secondary abiotic reduction of U(VI).

Very low aqueous-phase concentrations of uranium were achieved at the Oak Ridge site despite high solid-phase concentrations. This is due to the low solubility of U(IV) and to the low rates of desorption/dissolution of U(VI) species compared to the rate of reduction. Tests to evaluate the stability of the U(IV) (Wu et al., 2007) revealed that it was stable when ethanol injections were suspended for a 50-day period but anaerobic conditions were still maintained. However, additional studies demonstrated that oxygen and nitrate can remobilize uranium, indicating that long-term bioremediation will need to incorporate strategies for removal of dissolved oxygen and nitrate or development of methods to increase the stability of immobilized U(IV) upon exposure to oxidants.

2.8 SUMMARY

Chemicals are often added in groundwater remediation for a variety of different reasons and purposes. To be effective for their intended purpose, the chemicals generally need to be added in the appropriate amounts and concentrations, and mixed in a suitable manner to have the desired effect. Knowledge of reaction stoichiometry and kinetics is needed in order to apply the appropriate amount of a chemical so that remediation can be successful. This chapter provided an overview of the various remediation processes that might require chemical additions and how to determine the appropriate amounts. Some examples are provided on how chemicals might be mixed. Subsequent chapters will address processes for mixing chemicals in a much broader context and in greater detail.

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