

November 9, 2005

Lecture 22 Kinetics (Abiotic/Biotic) & Reactivity (a.k.a. Environmental Attenuation of Contaminants)

I. Definitions

- A. Attenuation—decrease in concentration of a chemical in one of the environmental phases or compartments.
 - 1. Attenuation could be due to a degradative mechanism (as defined below) or due to mass transport out of the compartment.
- B. Degradation--decrease in concentration of a contaminant due to nonreversible alteration of chemical structure
- C. Mineralization--biologically mediated degradation of chemical resulting in release of carbon dioxide
- D. Persistence--longevity of contaminant residues in a medium or phase
- E. Detoxification--degradation resulting in loss of toxicity or biological activity
- F. Transformation--partial change in structure of contaminant due to biological (biotic) or nonbiological (abiotic) reaction; transformation product may still retain toxicity
- G. Bound residue--the residue remaining after exhaustive extraction of a soil, water, or biological matrix; covalent incorporation of a transformation product into the natural biochemical matrix; for ex., a pesticide transformation product might be covalently bound with natural organic matter
- H. Reaction mechanism--process by which a contaminant is degraded; normally divided into phase I and phase II reactions. Phase I reactions may be biologically or nonbiologically mediated.
 - 1. Phase I
 - a. Hydrolysis
 - b. Oxidation
 - c. Reduction
 - 2. Phase II
 - a. Conjugation

II. Important Considerations:

- A. Reactions in the environment occur much slower than proton transfers (i.e., loss of H^+ from an acid [i.e., $-COOH$ functional group] or base [$-NH_3^+$ functional group] in aqueous solution; i.e., proton transfers come to equilibrium nearly instantaneously, but breaking and reforming of bonds are much slower, especially in a complex matrix.
 - 1. Therefore with chemical reactions (breaking and reforming of bonds), we become interested in the mechanisms and kinetics of reactions, rather than just the thermodynamics (i.e., equilibrium state).
- B. Questions to ask:
 - 1. Is there only one or are there several different reactions by which a given compound may be transformed under given environmental conditions; what are the reaction products?
 - 2. What are the kinetics of the different reactions? What is the overall rate of the reaction by which a compound is eliminated from the system?

3. What is the influence of important environmental variables like temperature, pH, redox condition, ionic strength, and presence of other solutes, or concentration and type of solids?

III. Degradation in Soil & Water vs. Plant & Animal

A. Abiotic vs. Biotic

1. Both abiotic and biotic reactions occur in soil and water
2. Degradation in plants or animals would involve strictly biotic reactions, which is called metabolism
3. Basic kinds of reactions--the products of the reactions are generally the same regardless of the specific phase (or medium)
 - a. Nucleophilic substitution, reduction, hydrolysis (a form of nucleophilic substitution), oxidation
 - b. Conjugation
 - c. Sequestration/storage--refers to plants or animals
4. Abiotic reactions lead to other organic compounds whereas biotic reactions could lead to mineralization, i.e., CO_2 and H_2O evolution, or to other organic compounds (i.e., biotic reactions may be complete and faster than abiotic reactions, but transformation products may be the end products).

B. Distinguishing Abiotic (Chemical and Photolytic) and Biotic Degradation

1. The sterile soil/water experiment
 - a. Soil or water can be sterilized either by autoclaving (heat and pressure, chemically (mercuric chloride, propylene oxide, sodium azide), or by irradiation (cobalt 60).
 - b. Contaminant is added to sterile soil and water or natural soil and water; experiment is facilitated by using a ^{14}C radiolabelled tracer
 - c. Degradation & transformation products are followed and compared in each treatment; rates of degradation/transformation are compared (Figure 1).
 1. If the reaction is abiotic, expect sterilization to have little effect on degradation rate, k ;
 2. If the reaction is biotic, expect some CO_2 to be evolved; however, some biological reactions may not result in mineralization.
 3. In reality, both biotic and abiotic reactions may be occurring simultaneously.

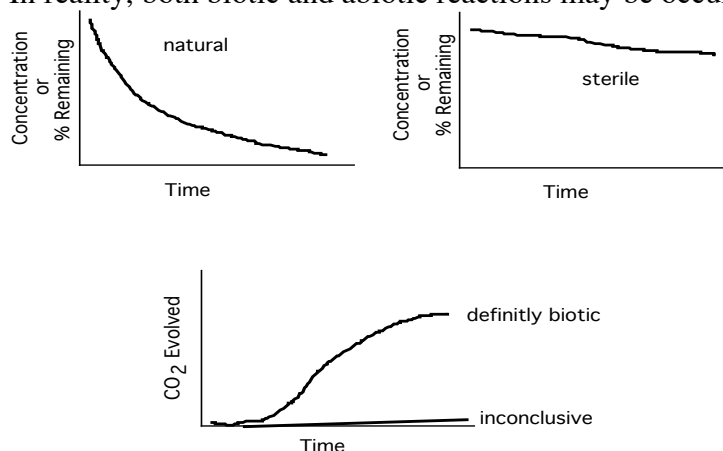


Figure 1. Model degradation curves for biotic and abiotic reactions.

IV. Studying Fate of Contaminant in Soil or Plants

- A. The most common way to study reactions involved in degradation is to add a radiolabelled contaminant to soil or water (or a plant through the soil) and monitor the appearance of degradation products or metabolites at various times after applying the compound (Figure 2).
1. Volatile products would also be trapped.
 2. At the same times that samples are taken to look for degradation products or metabolites, the soil (or plant) is exhaustively extracted and then combusted to look for bound residues.
 - a. In water, bound residues are not studied, although bound residues could be associated with sediment or biomass in water.

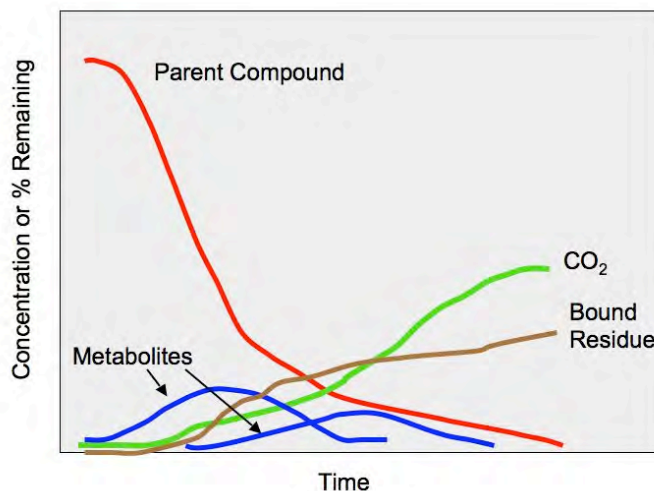


Figure 2. Degradation of a contaminant and subsequent release of CO₂ and appearance of several metabolites. The contaminant may be partially transformed and the transformation products may be incorporated into the soil organic matter matrix, forming bound residues.

3. It should be noted that degradation can result in a detoxification of a molecule, but it can also result in a product that is as toxic or sometimes more toxic than the parent.

V. Review of Reaction Kinetics

- A. Rate law=a mathematical function or differential equation describing the turnover rate of a compound as a function of the concentration (activity coefficients are assumed to be 1, and therefore concentrations are used directly).
- B. Power rate law
1.
$$\text{Rate} = \frac{-dC}{dT} = kC^n$$
 - a. where C=concentration, k=rate constant, n=order of the reaction
 1. Usually used to describe reactions in homogeneous solutions, but is also applied to soil
 2. When n=1, equation becomes the first-order rate law

3. If plot the disappearance or transformation of a parent compound relative to time, observe an exponential decrease in concentration. At any time, t , the turnover rate is proportional to the actual concentration of the parent compound and

$$\frac{d[C]_t}{dt} = -k[C]_0$$

or

$$[C]_t = [C]_0 \cdot e^{-kt}$$

- C. The integrated form of the first order rate law is a straight line described by the following integrated equation:

$$1. \ln [C]_t = -kt + \ln [C]_0$$

- a. Where \ln is the natural logarithm
 - b. $[C]_t$ is the concentration of contaminant at time t
 - c. $[C]_0$ is the initial concentration of contaminant
 - d. $-k$ is the rate coefficient or “constant” (units of $1/\text{time}$ or t^{-1}).
2. A plot of the natural logarithm of $[C]_t/[C]_0$ (proportion remaining at time t , or percent remaining) versus time should yield a straight line with the slope $-k$; thus the rate constant $-k$ can be calculated using linear regression.
1. The rate of the reaction is proportional to the concentration, but the half-life will be independent of concentration (in other words, the slope of the curve, when expressed on a logarithmic scale, will be constant regardless of concentration of the parent compound remaining);
 2. Half-life, or the time when 50% of the initial concentration has degraded or dissipated is given by the equation:
 - a. $t_{1/2} = \ln 2/k = 0.693/k$ (in other words, half-life is when the concentration of the parent compound is lowered by a factor of 2, i.e., 50%)
 - b. For first order kinetics in a homogenous liquid like water, we are assuming the water concentration to be constant but not a rate-limiting component.
- D. Given the number of data points usually taken in the course of an experiment, most environmental reactions are fit by the first order power rate law whether the reaction is biotic or abiotic.
- a. In reality, many studies have shown that immediately after application of a contaminant, the rate appears to be first order with respect to concentration;
 1. However, after a period of time, the rate slows down significantly, and further degradation occurs very slowly (Figure 3).
 - a. The reasons for the slow down could be due to the residue aging phenomenon making the older residues less available for degradation or transport processes.
 2. Thus, the rate law over the life of the chemical in soil is only pseudo first-order;
 - a. The rate is actually biphasic with two distinct lines if the data are graphed logarithmically.

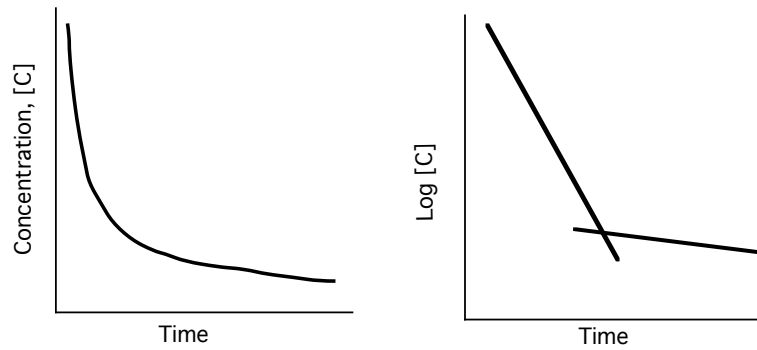
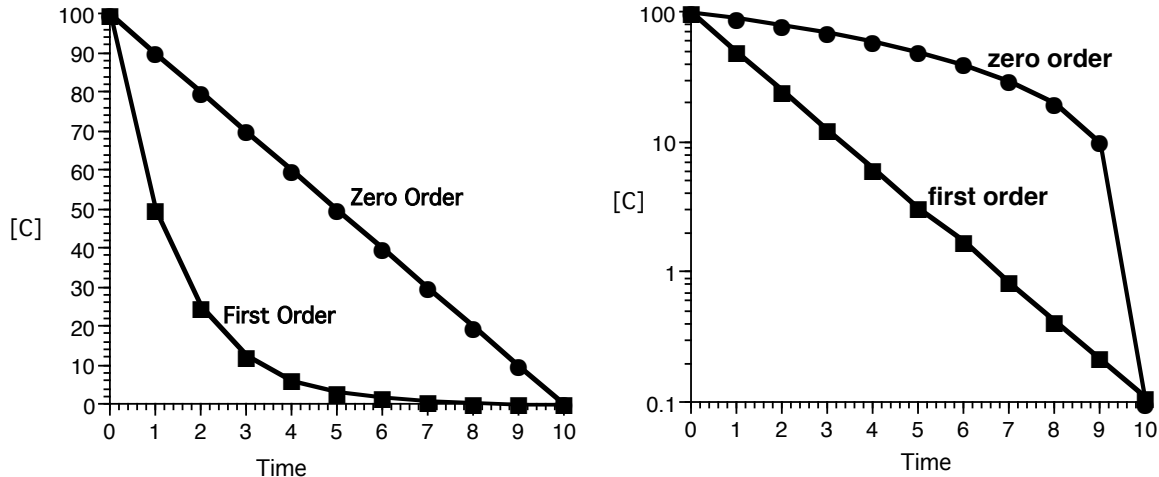


Figure 3. Pseudo first order degradation curve for a contaminant

3. Sometimes, the disappearance of a compound is independent of the concentration; an arithmetic plot of this type of kinetics, known as **zero order** (when $n = 0$), yields a straight line and the logarithmic plot shows a downward sloping (concave) curve



- a. Zero order kinetics are not often invoked in studying dissipation of contaminants in the environment.
 1. However, zero order kinetic behavior may be desirable if one wishes a constant release of a substance from a matrix such as a an implant for delivery of a drug (thus release should be independent of how much is left in the matrix and deliver the same concentration over all time intervals).
4. Case Study:
 - a. Nash and Woolson (1967) conducted an experiment at the USDA in Beltsville, MD. Small plots were treated with DDT and monitored over a 17 year period (Figure 4).
 - b. Note that persistence, as measured by percentage of initially applied DDT remaining (measuring all metabolites and parent) differed depending on the initial application rate.
 - c. Also, the data for the 50 ppm level of application suggest that the kinetics of degradation (or disappearance) are not strictly first-order

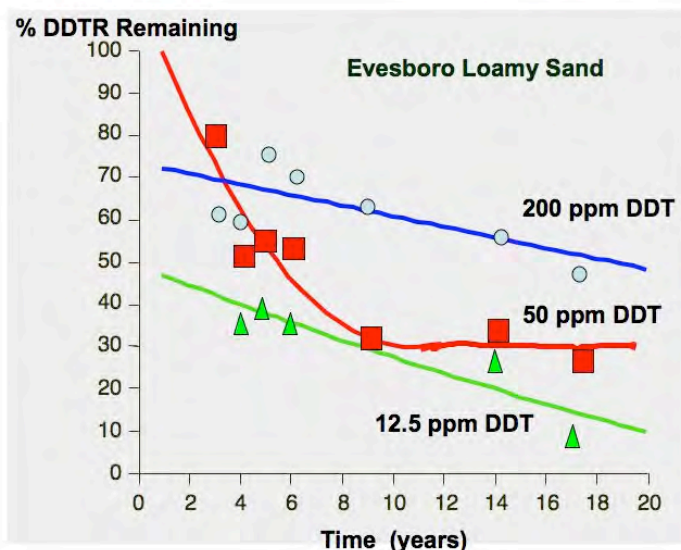


Figure 4. Degradation of DDT and residues in soil during a long term experiment

- d. Indeed, studies by Wheatley (1964) in the U.K. noted that the half-life of DDT changed from 2.5 years to 25 years by the ninth year after application, suggesting the classical biphasic degradation kinetics pattern

E. Second Order Kinetics

1. If water molecules or other components in the reaction are rate limiting (i.e., a reactant involved in the slowest part of the reaction sequence), then the rate law becomes second order and the rate is dependent on the concentration of both the reacting parent compound and some other species.

$$\frac{d[C]}{dt} = -k^- [C][B]$$

2. However, in the open system of the environment, reactants like water are not likely rate limiting. Indeed, if we assume the concentration of one of the reacting species is constant during a second order rate mechanism, the equation can simplify to a first order rate law. This rate law is called pseudo-first order.
 - a. This exercise is used to describe and simplify many environmental reactions; it should only be applied when one of the reacting species is in large excess and its concentration is not altered significantly during the course of the reaction.

F. Hyperbolic kinetics or rate law

1. Usually used to describe reactions catalyzed by adsorption to surfaces or complexing with catalyst molecules, like enzymes (Michaelis-Menton equation--see (3) below)

$$Rate = \frac{-dC}{dT} = \frac{k_1 C}{k_2 + C}$$

- a. Where k_1 is the maximum rate approached with increasing concentration and k_2 is a pseudoequilibrium constant (pseudo because as the reaction occurs it is constantly unbalancing the equilibrium represented by the constant)

- The kinetics are considered hyperbolic because the rate slows down as the concentration of the substrate increases (this rate law considers that the concentration of the enzyme or catalyst is constant or limiting and the substrate is in greater concentration) (Figure 5).

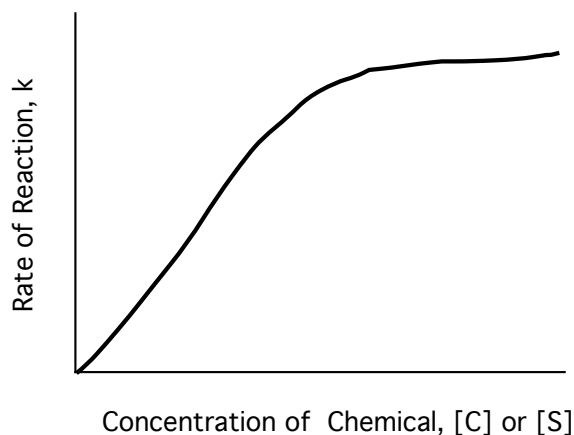
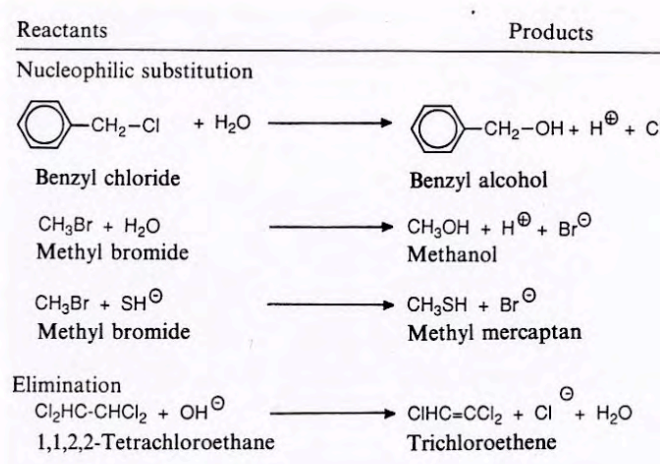


Figure 5. For reactions that conform to hyperbolic functions, the rate slows down because the catalyst (enzyme or clay surface) is rate-limiting. Once all of the sites to which the contaminant would bind in the reaction are filled, then no more can bind.

VI. Overview of Environmentally Important Reaction Types (Figure 6)

A. Hydrolysis (Phase I reaction)--these can also be described as a special case of nucleophilic substitutions

- These reactions are abiotic and normally studied in water, although they can be catalyzed by clay surfaces in soils and sediments. Reactions are pH-dependent.
- Biologically produced metabolites can be the same as hydrolysis degradation products, but these are formed during enzymatic reactions and the kinetics will be very different and not influenced by pH of the environment (as long as the pH is optimal for the cell).



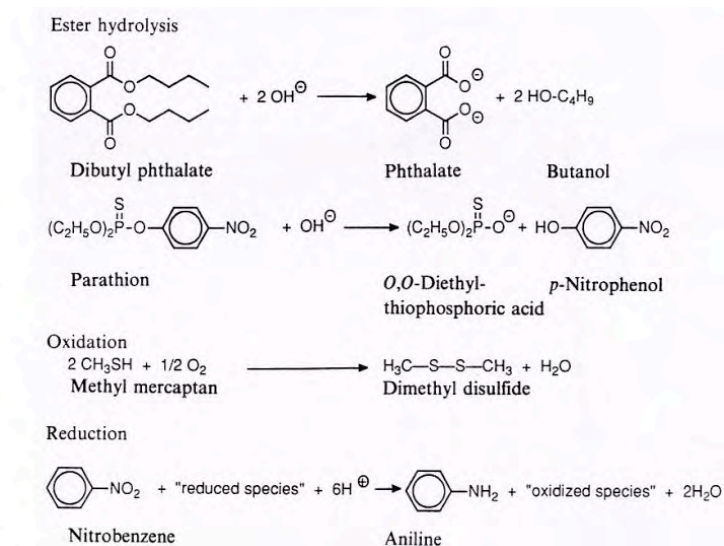
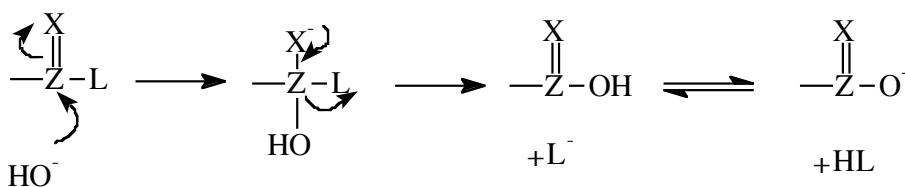
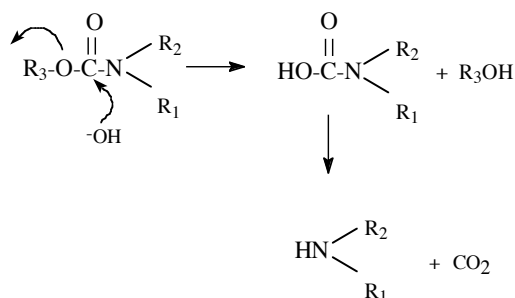


Figure 6. Environmentally important reaction mechanisms (from Schwarzenbach et al. 1993)

3. These reactions are known as nucleophilic substitutions and involve either a proton (H^+), water (H_2O), or hydroxyl (OH^-) as the nucleophile (a functional group "attracted" to an electron deficient atom; for example, a carbonyl group, i.e., $\text{C}=\text{O}$, has an electron deficient carbon because oxygen is more electronegative and pulls the electrons in the bonding orbital toward its nucleus).
 - a. Acid derivatives (shown below) are very susceptible to hydrolysis.
4. Basic reaction: electrophilic (or electron deficient atom) is attacked by nucleophilic functional group (like OH^-); the leaving group is then displaced.
 - a. Rate of reaction will be controlled by electronegativity of leaving group (the more electronegative the easier the nucleophilic substitution because of the pull of electrons away from the $\text{P}=\text{X}$ or $\text{C}=\text{O}$).



1. The above generalized equation represents the reaction of an acid derivative with hydroxyl anion (OH^-).
 - a. Z is commonly C, P, or S.
 - b. X is O, S or NR, where R is an alkyl group.
 - c. L is a leaving group, commonly RO^- , $\text{R}_1\text{R}_2\text{N}^-$, RS^- , and Cl^- .
- b. Example reaction: base hydrolysis of a carbamate ester (typical structure in some insecticides)



- The rate of the hydrolysis depends on whether the carbamate is a primary or secondary carbamate.
 - primary: $\text{R}_1 = \text{H}$; $\text{R}_2 = \text{alkyl}$
 - secondary: $\text{R}_1 = \text{alkyl}$; $\text{R}_2 = \text{alkyl or phenyl group}$
 - primary hydrolysis much faster than secondary hydrolysis (half-life of days vs. years)
- Thus, the substituent functional groups in a structure greatly influence the rate of degradation, as shown in the next table (Table 1). The table shows how the substituent groups on a carbamate ester affect hydrolysis half-life.

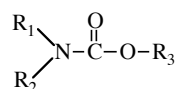
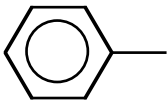
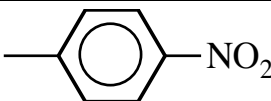
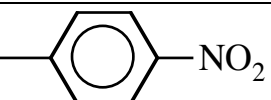
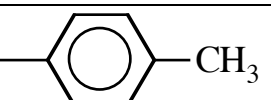
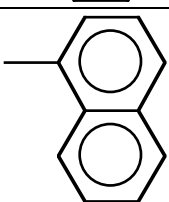


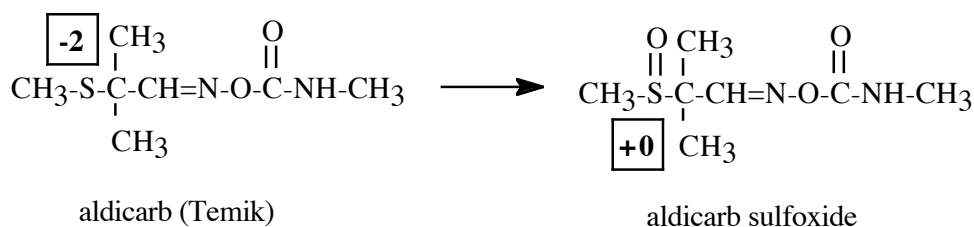
Table 1. Influence of structure on hydrolysis rate constant and half-life for some carbamate ester structures (based on Schwarzenbach 1993).

R1	R2	R3	k_B (M^{-1}s)	Half-Life @ pH 7
$\text{CH}_3\text{—}$	$\text{CH}_3\text{—}$	$\text{—CH}_2\text{CH}_3$	4.5E-06	50,000 y
$\text{CH}_3\text{—}$		$\text{—CH}_2\text{CH}_3$	4.0E-06	55,000 y
$\text{CH}_3\text{—}$	$\text{CH}_3\text{—}$		4.0E-04	550 y
H—	$\text{CH}_3\text{—}$		6.0E02	3 h
H—	$\text{CH}_3\text{—}$		5.6E-01	70 d
H—	$\text{CH}_3\text{—}$		5.0E01	33 h

5. Whether the nucleophile is H^+ , H_2O , or OH^- depends on the pH of the reaction medium and whether the attacked molecule is susceptible to acid, neutral, or base hydrolysis.

B. Oxidations (Phase I reaction)

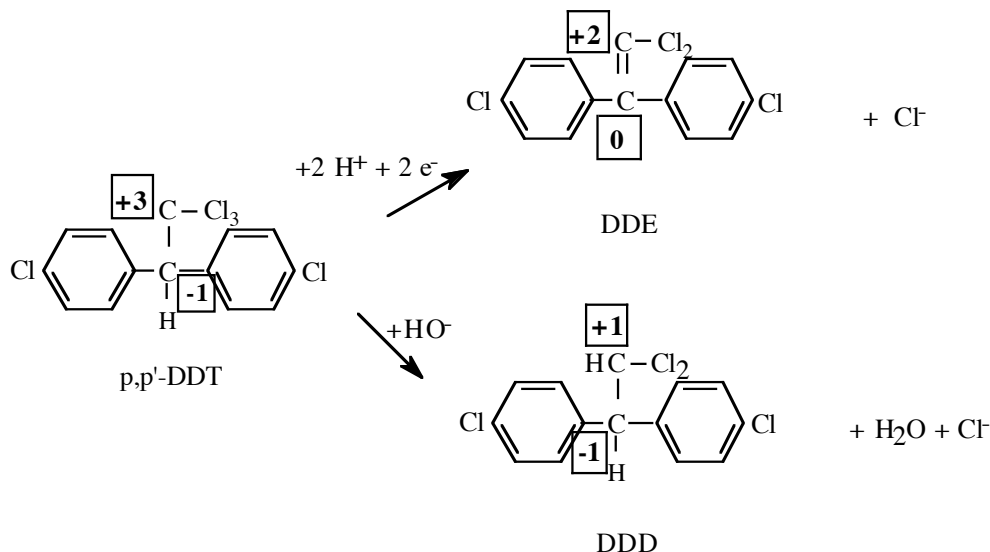
- Involve the removal of electrons from carbon or a heteroatom;
 - Often characterized by the addition of oxygen to heteroatoms or carbon, for ex.,



- These reactions are catalyzed by mineral surfaces or they may be enzymatic; the abiotic reactions do involve the transfer of electrons and are therefore types of redox reactions

C. Reductions (Phase I reaction)

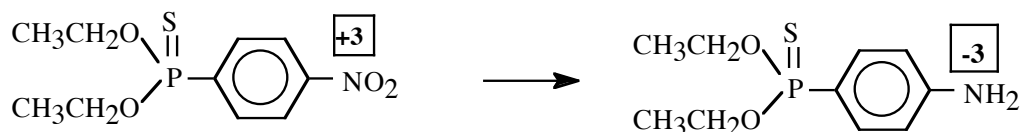
- Actually, these reactions are also redox reactions because they involve the transfer of electrons to an acceptor molecule.
- The most well known reaction is the mineral catalyzed (perhaps by Fe) reduction of DDT to DDD in low redox environments like anaerobic sediments



- Formation of DDE can be catalyzed also by Fe, but usually DDE is formed under aerobic conditions as the primary metabolite by a reaction known as an elimination (or dehydrodehalogenation), which is a type of nucleophilic substitution (there is not change in oxidation state of carbon, i.e., it did not accept electrons as the carbon in DDD); it could be formed by microbial metabolism, but it is then recalcitrant to further metabolism in the soil (it does dissipate but very

slowly; volatilization may be a major mechanism for further dissipation; it can be further metabolized but slowly in animals)

- Reduction of nitro groups to amino groups can increase the adsorption of compounds in soil; for ex., parathion may be transformed to amino-parathion under flooded soil conditions:



D. Environmentally Important Oxidation-Reduction (Redox) Reactions (Figure 7)

Oxidized Species	Reduction ⇌ Oxidation	Reduced Species	Equation Number
<i>Change in oxidation state of carbon atom(s)</i>			
$\text{R}-\text{COOH} + 2\text{H}^{\oplus} + 2\text{e}^-$	←	$\text{R}-\text{CHO} + \text{H}_2\text{O}$	(12-72)
$\text{O}=\text{C}_6\text{H}_4=\text{O} + 2\text{H}^{\oplus} + 2\text{e}^-$	⇌	$\text{HO}-\text{C}_6\text{H}_4-\text{OH}$	(12-73)
$-\text{C}-\text{X} \text{ (X=Cl, Br, I)} + \text{H}^{\oplus} + 2\text{e}^-$	→	$-\text{C}-\text{H} + \text{X}^{\ominus}$	(12-74)
$-\text{C}-\text{C}-\text{X} \text{ (X=Cl, Br, I)} + 2\text{e}^-$	→	$>\text{C}=\text{C}< + 2\text{X}^{\ominus}$	(12-75)
$2-\text{C}-\text{X} \text{ (X=Cl, Br, I)} + 2\text{e}^-$	→	$-\text{C}-\text{C}- + 2\text{X}^{\ominus}$	(12-76)
<i>Change in oxidation state of nitrogen atom(s)</i>			
$\text{R}-\text{C}_6\text{H}_4-\text{NO}_2 + 6\text{H}^{\oplus} + 6\text{e}^-$	⇌	$\text{R}-\text{C}_6\text{H}_4-\text{NH}_2 + 2\text{H}_2\text{O}$	(12-77)
$\text{R}-\text{C}_6\text{H}_4-\text{N}=\text{N}-\text{C}_6\text{H}_4-\text{R} + 2\text{H}^{\oplus} + 2\text{e}^-$	⇌	$\text{R}-\text{C}_6\text{H}_4-\text{NH}-\text{NH}-\text{C}_6\text{H}_4-\text{R}$	(12-78)
$\text{R}-\text{C}_6\text{H}_4-\text{NH}-\text{NH}-\text{C}_6\text{H}_4-\text{R} + 2\text{H}^{\oplus} + 2\text{e}^-$	⇌	$2 \text{ R}-\text{C}_6\text{H}_4-\text{NH}_2$	(12-79)
<i>Change in oxidation state of sulfur atom(s)</i>			
$\text{R}-\text{S}-\text{S}-\text{R} + 2\text{H}^{\oplus} + 2\text{e}^-$	⇌	$2\text{R}-\text{SH}$	(12-80)
$\text{R}-\text{S}(=\text{O})-\text{R} + 2\text{H}^{\oplus} + 2\text{e}^-$	⇌	$\text{R}-\text{S}-\text{R} + \text{H}_2\text{O}$	(12-81)

Figure 7. Examples of redox reactions (from Schwarzenbach et al. 1993)

- Conjugations (these are called phase II reactions to distinguish them from phase I reactions, which are collectively the hydrolysis, oxidation, and reduction reactions)

- Biological reaction; not as well known from soil degradation experiments as from plant and animal metabolism

2. A pesticide will be transformed, perhaps by an oxidative reaction resulting in a hydroxyl group; then through another enzymatic reaction, the metabolite is linked covalently with a sugar molecule or perhaps the tripeptide glutathione.
3. The end result is a much more water soluble metabolite that is excreted more easily
4. Conjugation products can be determined by hydrolyzing (with acid) the water soluble fraction of an extract; the unconjugated metabolite, which will contain the radiolabel will be obtained

VII. Abiotic Degradation Reactions

A. Chemical Degradation

1. These processes can occur in water if pesticide molecular structure is susceptible to acid, water, or base hydrolysis (nucleophilic substitutions); they may occur in soil at extreme acid or basic pHs; however, few studies have shown that soil pH has much effect, even for OPs.
 - a. Essentially, all non-biological transformations are chemical degradations, but specifically this term is used to describe nucleophilic additions (which includes hydrolytic reactions), elimination reactions (loss of hydrogen and chlorine), and redox (oxidation & reduction) reactions that occur without the benefit of microbial metabolism.
2. Mineral surfaces, whether clays or metals, can catalyze oxidations of certain types of pesticides in the presence of water
 - a. For ex. parathion has been shown to be hydrolyzed by catalysis on clay surfaces;
 - b. Cu ions in solution have been shown to catalyze the hydrolysis of chlorpyrifos
3. In general, chemical degradations are slower than microbial metabolism and the products are only partial transformations of a molecule rather than mineralization; however, the partially transformed molecule can be then subjected to microbial degradation.

B. Photolysis

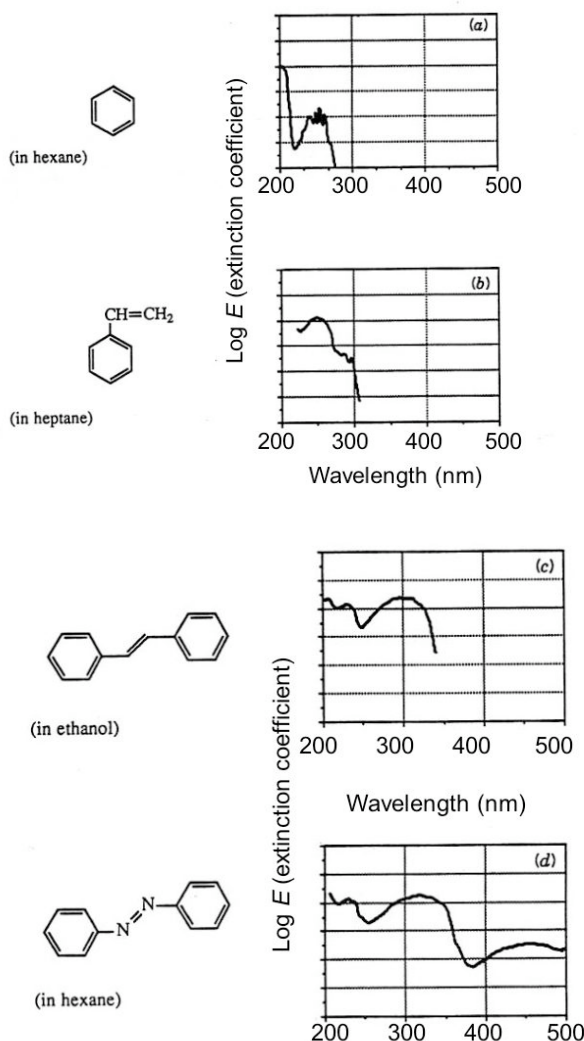
1. Light has a wave-like and particle-light character; its energy is proportional to its frequency and inversely proportional to its wavelength; thus, longer wavelengths have lower energy and shorter wavelengths have higher energy
2. Whether or not a reaction will take place depends on the probability that a given compound absorbs a specific wavelength of light, and on the probability that the excited molecular species undergoes a particular reaction
 - a. Energies associated with UV light are about the same as energies associated with molecular bonds.

Table 2. Bond energies and absorption maxima of some types of functional groups.

Bond	Bond Energy (kJ mol ⁻¹)	Wavelength (nm)
O-H	465	257
H-H	436	274
C-H	415	288
N-H	390	307
C-O	360	332
C-C	348	344

C-Cl	339	353
Cl-Cl	243	492
Br-Br	193	630
O-O	146	820

3. Note the UV absorption spectra for the following compounds (Figure 8); those that absorb strongly in the UV region (<360 nm) are more susceptible to direct photodegradation.
- Also note that the compounds absorbing strongly in UV light (as indicated by the magnitude of $\log E$, the molar extinction coefficient) have conjugated double bonds (i.e., alternating double and single bonds).
 - Note also how the absorption maxima shift as the functional groups or structure of the contaminant changes.
 - This shift suggests that contaminants have different susceptibilities to photodegradation because UV absorption spectra are different.



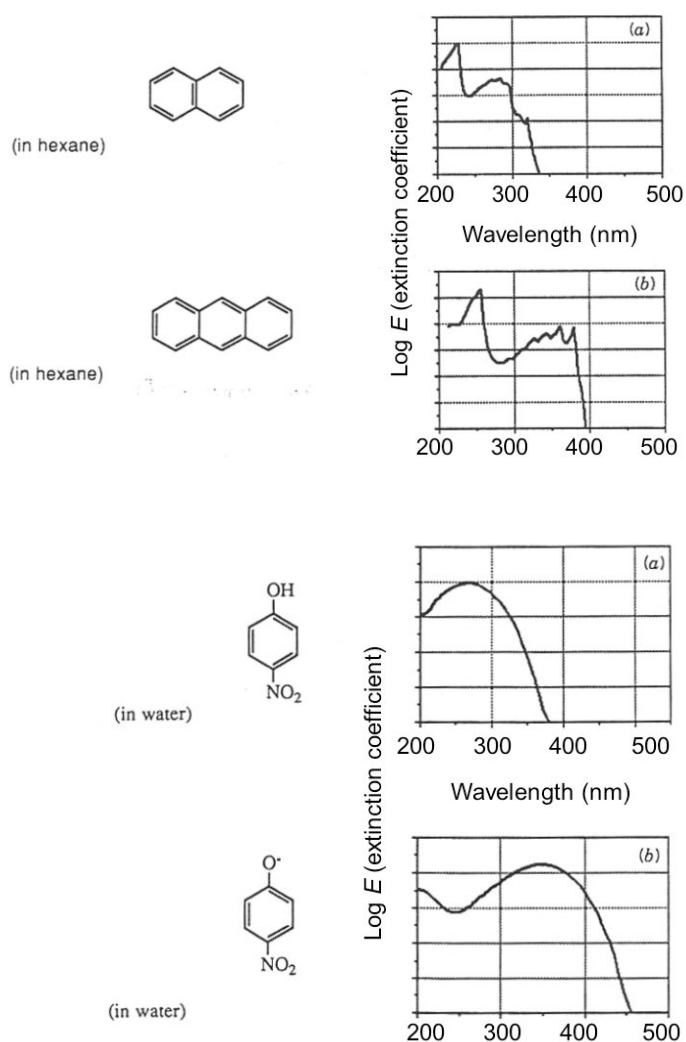


Figure 8. Absorption maxim of selected environmental contaminants (Schwarzenbach et al. 1993).

4. When a molecule absorbs light, electrons in its bonding orbitals (i.e., electrons are said to be in their ground state) are promoted to antibonding orbitals, placing the electrons in an excited state.
5. In the excited state, the bonds may break or the electrons can return to their ground state giving off heat to the environment; alternatively, energy can be given off as light (fluorescence and phosphorescence) or transferred to another molecule in the environment (photosensitization).
6. Contaminant in water and/or air will be more susceptible to photolysis (or photodegradation) than pesticides in soil; in soil UV light is very rapidly attenuated or absorbed by the soil.
7. Reactions occurring by direct photolysis (i.e. a chemical absorbs light directly and chemical reaction occurs) have not been seen very often. Photolytic reactions of contaminants under environmental conditions are more likely to occur when a

photosensitizer molecule (like dissolved organic matter in water) absorbs a photon and then transfers energy to an acceptor molecule.

- a. Oxygen is usually the acceptor molecule; forms either excited oxygen species (known as superoxide or singlet oxygen) or hydroxyl and peroxy radicals;
 1. These “excited” or reactive species can then attack the contaminant directly.
 2. The resulting degradation products may look a lot like the degradation or metabolic products from typical chemical or enzymatic reactions.

VIII. Biodegradation

A. Kinetics of Biodegradation (see Lecture 4/5 Handout)

1. In the real world, microbial populations will be changing, for example growing on organic substrates, or perhaps growing as a result of metabolism of the substrate itself; in this case Monod kinetics (which is derived from Michaelis-Menton kinetics) can be used to help analyze the rate of biodegradation:

$$\mu = \frac{\mu_{\max} S}{K_s + S} \quad \text{and} \quad v \text{ (rate of reaction)} = \frac{d[S]}{dt} = \frac{\mu_{\max} [B] \cdot Y^{-1} [S]}{K_s + S}$$

- a. Note that $K_s = K_m$
- b. $B = \text{cells/L}$
- c. $Y = [\text{cells grown/moles of substrate used}] = \text{Yield}$
2. Using nonlinear curve fitting techniques, the equations for growth kinetics of reaction can be determined and the various constants (V_{\max} and K_m) can be determined so that rate of biodegradation of different substrates can be compared (Figure 9).

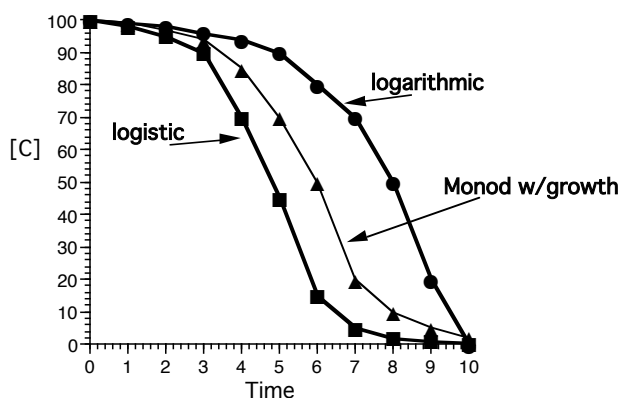


Figure 9. Degradation curves for various biodegradation models accounting for growth of microorganisms.

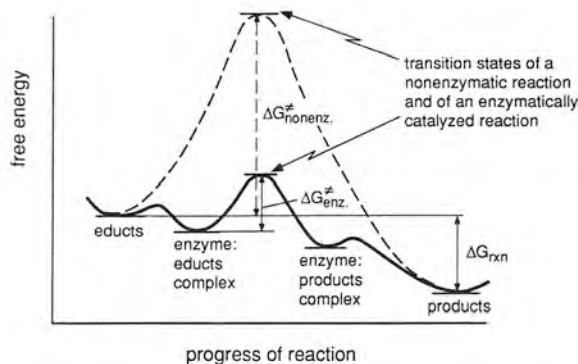
- a. Logarithmic kinetics: initially little loss of chemical occurs because cell numbers are limited, but as mass of cells becomes larger and double logarithmically, a rapid loss of chemical occurs. In this situation, pesticide concentration would be much greater than the concentration equivalent to the affinity constant
- b. Logistic kinetics: when the pesticide (or nutrient) concentration is present at a concentration less than K_m , degradation is slow at first until cell numbers begin to

increase; rate of loss increases as size of the microbial population increases, but as the pesticide (or nutrient substrate) decreases, the rate of population doublings (i.e., growth) also decreases, and the rate of substrate loss decreases.

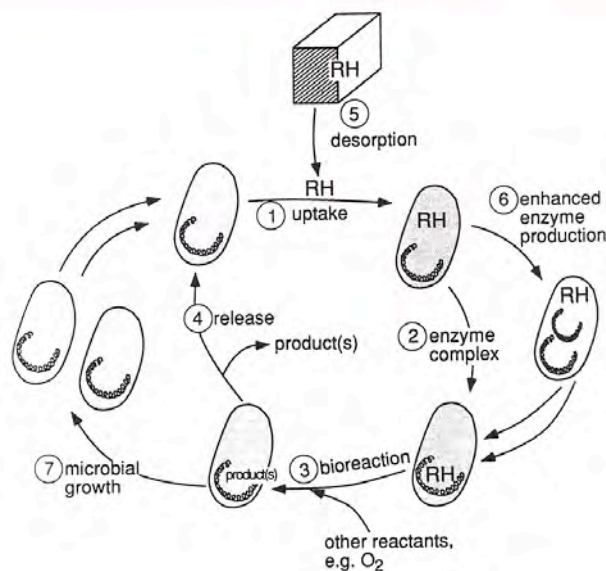
- c. **Monod-with-Growth kinetics:** when pesticide (or nutrient) concentration is about equal to the concentration equivalent to the affinity constant, then degradation rate is neither logistic nor logarithmic; it is somewhat in between; the substrate concentration is neither high enough to allow sustained microbial population growth, nor low enough to cause rate of degradation to slow too quickly as in the logistic kinetic model.
3. Which kinetic model is appropriate?
 - a. In many experiments, especially those conducted in the field, too few data points are collected to accurately predict the appropriate kinetic model, yet the importance of the correct model can be illustrated by considering the amount of time it takes for a contaminant to reach its regulatory safety level; for example (example taken from Alexander, 1994, p. 87):
 1. Consider a polluted site with 10 mg/L initially and 9 mg/L after 30 days. If predictions were made of the time for the concentration to fall to a safe level of $10 \mu\text{g/L}$, the following models would predict vastly different times:
 - a. logarithmic model--33 days
 - b. first order model--5 years

B. Biochemical Ecology of Biodegradation

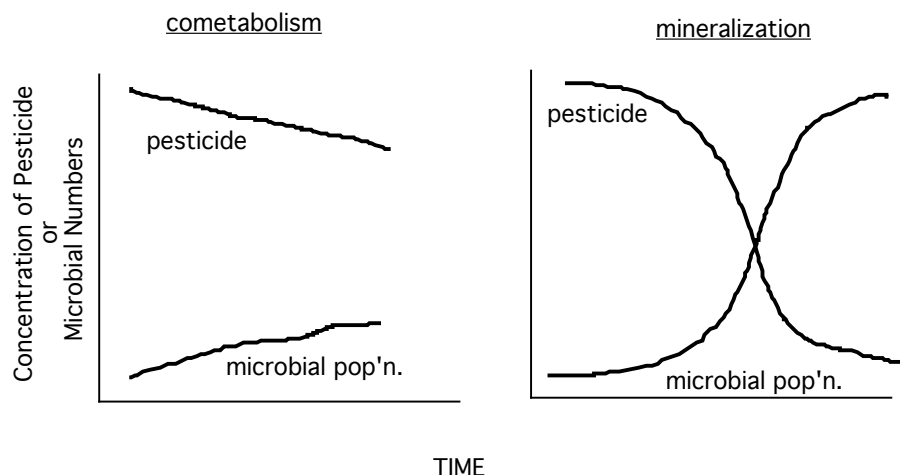
1. End products either represent
 - a. **Mineralizations** (i.e., the molecule of interest is completely degraded to inorganic forms; usually this would be CO_2 and H_2O)
 - b. or **Transformations** (i.e., the structure is altered; only part of the molecule is actually mineralized to CO_2 if at all; the biotransformed product can be bound to soil constituents or plant/animal cellular components; the biotransformed product will usually be more water soluble than the parent, and usually less toxic, but not always.
2. Biochemical reactions involve catalysis by enzymes
 - a. Biochemical reactions tend to be faster than abiotic reactions (although abiotic reactions are thermodynamically favorable they may occur very slowly) because enzymes significantly lower the activation energy by several tens of kJ per mole, speeding transformations by 10^9 or more



- b. Enzymes hold the substrates (reacting compounds) in an advantageous orientation relative to one another, thereby reducing entropy limitations.
3. Rate of biodegradation is controlled by factors beyond just interaction of the substrate with the enzyme.
 - a. Rate of delivery of substrate molecules to the microbial cells
 1. Consideration of microbial ecology:
 - a. Where are cells in soil relative to distribution of chemical; clumpiness of microbial communities; biofilms (cells layered on top of one another)
 - b. Availability of energy substrates
 - b. Rate of diffusion of substrates across intervening media
 1. Sorption effects
 2. Moisture content; vapor phase transport
 - c. Rate of uptake by microbial cells
 1. Active transport or uptake
 2. Passive uptake
 - d. Biochemical effects
 1. Induction--presence of substrate causes genes to be turned on for manufacturing more enzyme--can increase rate
 2. De-repression--causes existing enzymes to be enabled (may not be in a functional state until the substrate "de-represses" them, perhaps by interacting with the part of a gene that is producing a repressor molecule)
 3. Mutation--new genetic codes resulting in operable enzyme (or more efficient enzyme)
 4. Constitutive enzymes--enzyme already present
 5. Adaptation--increase in cell numbers owing to metabolism of substrate that yields substantial energy or cell-building materials;
 - a. Overall rate of degradation in environment will be dictated by the rate of microbial population increase.
4. Conceptualization of the biotransformation process (Fig. 14.2 from Schwarzenbach et al. 1993)



- a. Bacterial cell containing enzymes takes up organic chemical RH;
 - b. RH binds to suitable enzyme;
 - c. Enzyme-RH complex reacts producing the transformation products of RH;
 - d. The products are released from the enzyme; several additional processes may influence the overall biotransformation rate, including—
 - e. Desorption of RH from solids making it available to the microorganisms;
 - f. Production of new or additional enzyme capacity (e.g., due to turning on genes [i.e., induction]; due to removing materials which prevent enzyme operation [activation]; or due to acquisition of new genetic capabilities via mutation or plasmid transfer;
 - g. Growth of the total microbial population carrying out the biotransformation of RH.
- C. Biodegradation rates are the least well understood inputs to chemical fate modeling
- D. Soil Microbial Biochemical Strategies
1. Mineralization
 - a. Usable energy from metabolism of compound
 - b. Characterized by lag period--period of time where disappearance of chemical is too small or undetected; probably corresponds to either enzyme induction and/or proliferation of microbial numbers
 - c. Log phase--rapid decline in substrate concentration, accompanied by very rapid proliferation of microbial cells
 - d. Stationary phase--growth of cells ceases; most of chemical has been metabolized
 2. Cometabolism
 - a. Compounds other than the substrate of interest serve as primary energy sources; substrate itself is not an energy source
 - b. No growth of community or proliferation of microbial cells
 - c. Compound transforms at a comparatively steady rate; i.e., the rate of transformation is constant relative to the concentration



- d. Explanations for cometabolism (i.e., why does an organic chemical that is a substrate for an enzyme not support growth but is converted to products that accumulate) (from Alexander 1994)

1. The initial enzyme or enzymes convert the substrate to an organic product that is not further transformed by other enzymes in the microorganism to yield the metabolic intermediates that ultimately are used for biosynthesis and energy production;
 2. The initial substrate is transformed to products that inhibit the activity of late enzymes in mineralization or that suppress growth of the organisms;
 3. This phenomenon has important implications for spills of chemicals that would normally be fairly rapidly degraded under low concentration conditions
 - a. Spills of acetanilide herbicides have been shown to inhibit microbial activity with resultant prolonged persistence of the chemicals
 - b. The organism needs a second substrate to bring about some particular reaction
 3. Consortia
 - a. Microbial species can be found clumped together into undefined consortia that "cooperate" with dissimilar species as members of the consortium
 - b. One member of the consortium may be capable of partially transforming a substrate and releasing a metabolite (externally) that is then absorbed by another member of the consortium and used as an energy source.
 4. Exchange of Plasmids between species
 - a. Some degradation enzymes are encoded on small pieces of extrachromosomal DNA
 - b. These pieces of DNA, known as plasmids, can be transferred to unrelated species, conferring a metabolic capability that did not exist before the transfer
- E. Transformations
1. Enzymes may or may not be specific; those enzymes that are "promiscuous", i.e., they can handle a wide variety of substrates, especially if parts of the substrates resemble the molecules "naturally" metabolized will degrade environmental contaminants. This promiscuity is known as imperfect substrate specificity. The rates of reaction may be very reduced from the rates observed with the "natural substrates"
- F. Thresholds to Biodegradation
1. Many compounds can be biodegraded at fairly rapid rates and transformed extensively leading to mineralization.
 2. However, very low concentrations of these same compounds can be quite persistent in soil and water, suggesting there is a threshold for initiation of biodegradation
 - a. Note that biodegradation may occur at low concentrations over a long period of time, but the rate is slow.
 - b. The table below (from Alexander 1994, p. 105) shows the threshold concentration for normally biodegradable compounds; at or below these concentrations, biodegradation is slower than would be predicted from higher concentrations or does not occur

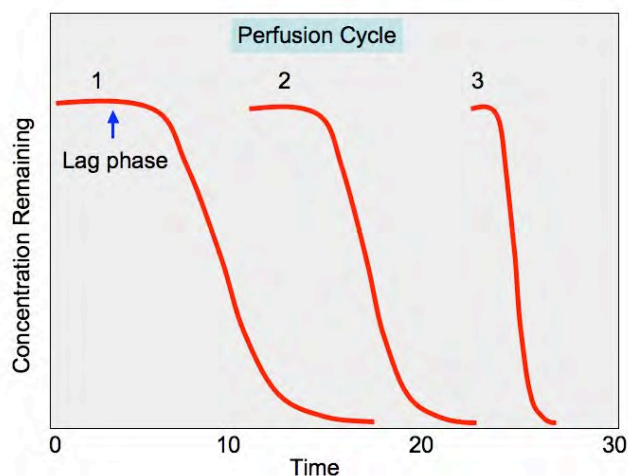
Table 3. Threshold concentrations for biodegradation.

Chemical	Environmental Source	Concentration ($\mu\text{g/L}$ water or $\mu\text{g/kg}$ soil)
2,4-D	Stream	2.2
Carbaryl	Stream	3.0

Aniline	Lake	0.1
4-nitrophenol	Lake	1.0
2,4-dichlorophenol	Lake	2.0
Styrene	Lake	2.5
Phenol	Lake	0.0015
Carbofuran	Soil	10 – 100
2,4,5-T	Soil	100
1,2-, 1,3-, & 1,4-dichloroben	Biofilm on glass	0.2 – 7.1

G. Enhanced Biodegradation

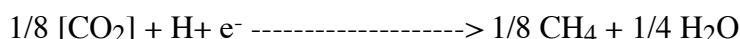
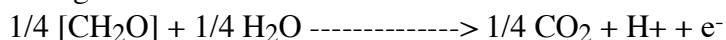
1. Contaminants that serve as energy substrates can undergo a rapid or accelerated degradation upon repeated treatment of a soil
 - a. This phenomenon has been called enhanced biodegradation and was first observed with the accelerated degradation of 2,4-D in a soil perfusion column (Audus 1949)



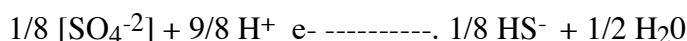
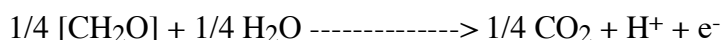
2. Characteristics of degradation curve of a compound exhibiting the potential for development of enhanced biodegradation
 - a. A lag period usually occurs in the degradation of substrates serving as energy or nutrient sources; this period may represent the growth of microbial cells--production of more enzyme
 1. Growth of microbial cells represents the production of more enzyme
 - b. Rapid degradation of a substrate upon repeated treatment
3. Idiosyncrasies of enhanced biodegradation
 - a. Development may or may not be associated with changes in population dynamics
 - b. Can develop after a single pesticide application
 - c. Capability can be transferred from one soil to another
 - d. Initial concentration may influence the development of enhanced biodegradation
 - e. Cross conditioning by related compounds and metabolites is common
 - f. Persistence of enhanced biodegradation varies among compounds and soil types
 - g. Enhanced biodegradation may be accompanied by a change in metabolic pathways

H. Anaerobic Biodegradation

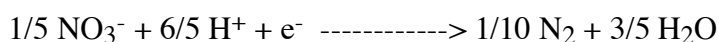
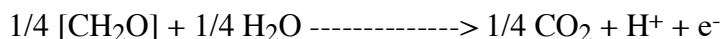
1. In the absence of oxygen, for example in anoxic sediments of low pE (redox potential very negative), microorganisms are known to exist that can dechlorinate aliphatic and aromatic compounds.
2. Anaerobic biodegradations are favored under denitrifying, sulfate reducing, and methanogenic environments.
 - a. Reactions seem to proceed fastest under methanogenic conditions
3. Nutritional Biochemistry (coupled redox reactions)
 - a. Methanogenesis



- b. Sulfate reduction



- c. Denitrification



4. The basic mechanisms include
 - a. aromatic compounds:
 1. reduction
 2. hydrolysis
 - b. nonaromatic compounds:
 1. reduction
 2. hydrolysis (substitution)
 3. vicinal reduction (dihalo elimination)
 4. dehydrohalogenation (elimination of H and X)
5. The electron source for the reactions may be a small organic substrate like pyruvate, acetate, acetone, methanol, or a sugar like glucose (and other organic compounds); these compounds are oxidized, providing the electrons that are ultimately accepted by the CO_2 , H^+ , NO_3^- , or SO_4^{2-} as the terminal electron acceptors. Oxygen will inhibit these type of reactions.
6. In some cases, the contaminant itself can be an electron acceptor; for ex. PCB have been hypothesized to be an electron acceptor during their reductive dechlorination.
 - a. Other work has shown that PCB's may be dechlorinated by a facultative anaerobic microorganism (actually can function with low O_2 tension); the dechlorinated PCB's (ortho substituted products) can then be further metabolized by aerobic organisms.
7. Compounds like chlorobenzoates are preferentially dechlorinated when the chlorines are in the meta position. Ultimately benzoate is formed which is a good bacterial growth substrate. Reaction proceeds under methanogenic conditions.
 - a. Figure 10 (Schwarzenbach et al. 1993, Fig. 12.18) shows the variation in concentration of important redox species along the flow path of a contaminant plume in ground water. The sequence results in several zones of characteristic microbial metabolism and corresponding redox conditions.

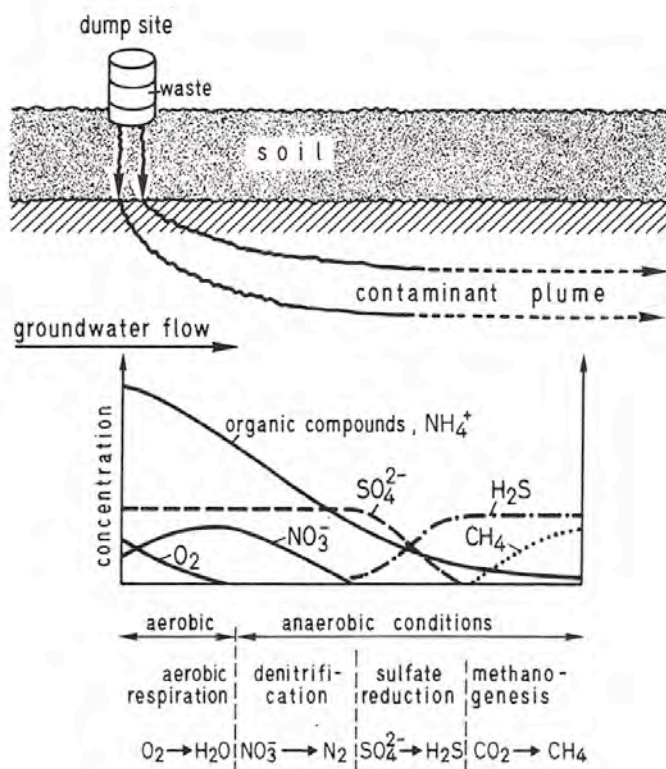


Figure 10. Differences in biodegradation mechanism as influenced by $[O_2]$ availability

IX. Influence of Environment

A. Temperature

1. Consider the Arrhenius equation:

$$k = A \cdot e^{-E_a/RT} \quad \text{or} \quad \ln k = \ln A - E_a/RT$$

where E_a is the activation energy and A is the Arrhenius constant or frequency factor, and k is the rate constant; thus, rate depends on temperature.

2. Frequency of collisions and orientation of molecules is affected by changes in temperature

- a. If one calculated the E_a and A by doing some lab experiments, then one could predict the effect of temperature on rate constant under real world conditions.

B. Moisture--probably affects biotic reactions more than abiotic reactions. Microbes usually go dormant when the soil is very dry.

1. Be aware that clay surfaces can catalyze the hydrolysis and/or oxidation of contaminants. This phenomenon has been shown with some organophosphate insecticides (phosphate esters).
2. The reaction does not require microbial activity, but it does require at least some water.

C. Sunlight: photodegradation will occur independently of microbial degradation;

1. Be aware that photodegradation only occurs in a limited area of the top of the soil surface or on leaf surfaces, but it can occur to comparatively deeper levels in bodies of water.
 - a. Studies have shown that time of year, because of incident angle of sun, can affect photolysis rate
 2. Degradation products resulting from photodegradation can be more amenable to biodegradation processes.
- D. Soil type and organic carbon content
1. Soil properties can influence biodegradation rate through the influence of sorption potential.
 2. Note in the experiment (from Alexander 1994, p. 123), that the addition of sorptive beads to a microbial culture significantly slow mineralization of biphenyl. Similar results have been noted in soils with high sorption capacity, but there are other confounding factors that could alter degradation rate (Figure 11).

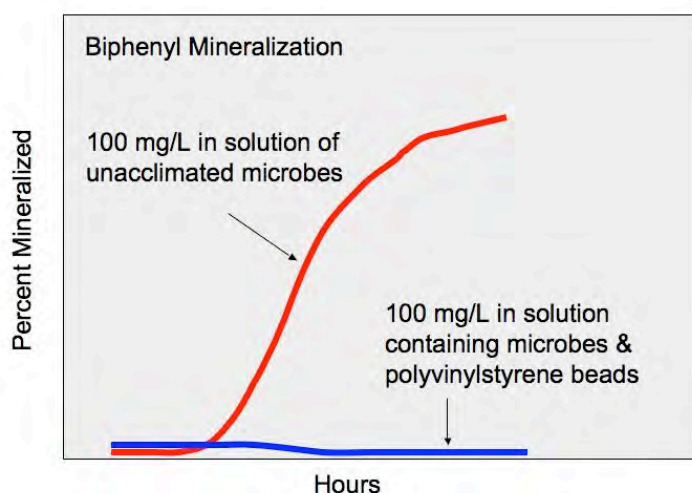


Figure 11. Influence of sorption on biodegradation.

3. Other factors include
 - a. Biochemical nature of organic matter
 - b. Microbial ecology (are organisms with requisite enzymes present?)
- E. pH
1. For ionizable compounds, pH of the bulk soil, which can be higher than the actual pH at or close to organic matter and clay surfaces, will influence the proportion of ionized (salt) and neutral (protonated) species.
 2. If sorption is favored by partitioning into organic matter, then soil pH's below the compound pKa will favor sorption, which could slow biodegradation rate.
- F. Nutrients (see figure below; effects of oxygen on degradation of naphthalene, the simplest polyaromatic hydrocarbon.)
1. Nutrient and oxygen availability can affect microbial activity and thus rate of biodegradation
 2. See anaerobic biodegradation section for effects of availability of electron acceptors

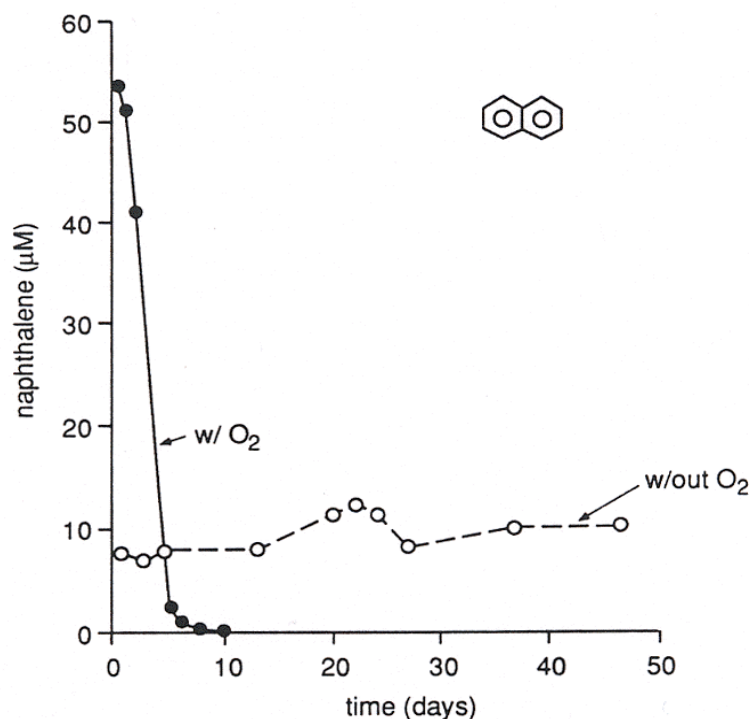


Figure 12. Effect of oxygen availability on biodegradation of naphthalene (from Schwarzenbach et al. 1993)

G. Associated formulation ingredients

1. For commercial products that are formulated in organic solvents, spills could result in microbial toxicity and a slowing of biodegradation.

H. Other chemicals and previous exposure (see below: enhanced biodegradation)

I. Aging effect

1. Hypothetically caused by non-equilibrium partitioning over a long period of time (i.e., residue aging wherein diffusion in to intra-aggregate spaces makes compounds essentially non-bioavailable)
2. The graphic below is from Steinberg et al. 1987 (Environ. Sci. & Technol. 21:1201-1208); it shows the effect of aging on biodegradation rate (Figure 13).
 - a. Two soils were sampled for EDB concentrations 19 years after the last use of the fumigant. The concentrations analyzed were noted as “native EDB”.
 - b. The soils were brought in to the lab and incubated in a ~1:1 soil:water suspension at ambient temperature (~23-28°C). Native EDB concentration changes were monitored over 25-38 days.
 - c. Meanwhile, fresh radiolabelled EDB was added and its concentration was monitored. (Note that CO₂ was trapped in addition to measuring radioactivity)

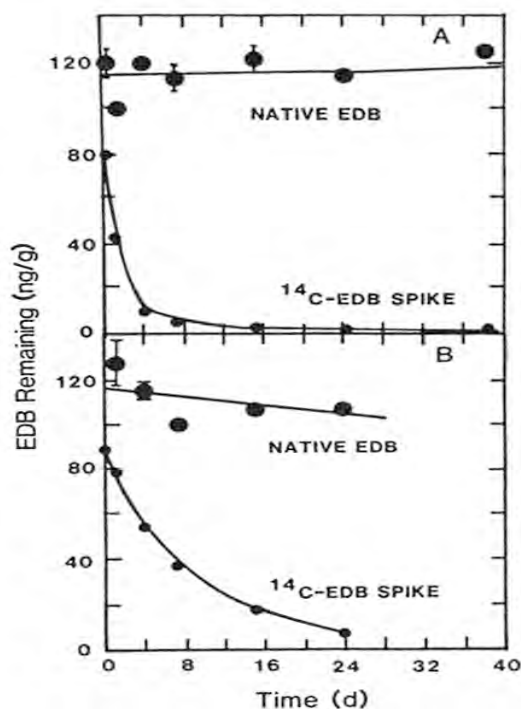


Figure 13. Effect of aging on EDB (a fumigant pesticide) degradation (Steinberg et al. 1987).

J. Degradation Rate in the Real World

1. A single half-life is given to many pesticides when they are compiled into tables or other formats that purport to describe their characteristics; however, degradation is very characteristic of the environment the pesticide ends up in
2. Below is a table of half-lives for some OP insecticides in a variety of different soil types (categorized by texture)(compiled by Racke 1992 in *Organophosphates, Chemistry, Fate, and Effects*)

Table 5. Half-life (days) of various OP insecticides in different soil types

Pesticide	Muck	Sandy loam	Silty clay loam	Loam	Clay loam	Unknown
parathion	11					
diazinon	14			22		
chlorpyrifos	18				14	19
fensulfothion	7					
isofenphos					32	
fonofos	28		35		18	46
dimethoate				11		
phorate		7	9		8	5
disulfoton		7			7	
terbufos			9		14	7
chlorfenvinphos	7	63		135		
ethoprop						11