

BIODEGRADATION OF XENOBIOTICS

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Summary

Xenobiotic compounds are chemicals which are foreign to the biosphere. Depending on their fate in air, water, soil, or sediment, xenobiotic pollutants may become available to microorganisms in different environmental compartments. Actually, the dominant means of transformation and degradation of xenobiotic compounds on Earth resides in microorganisms. In natural habitats, the physicochemical properties of the environment may affect and even control biodegradation performance. Sorption to soil and sediment as well as micropore entrapment are major causes for the persistence of many xenobiotics.

Polycyclic aromatic hydrocarbons, halogenated aliphatic as well as aromatic hydrocarbons, nitroaromatic compounds, azo compounds, s-triazines, organic sulfonic acids, and synthetic polymers are important classes of pollutants with xenobiotic structural features. This article is focused on the mechanisms and pathways of microbial

degradation of these compounds. Fungi, and aerobic as well as anaerobic bacteria are involved in the degradation of xenobiotics. Sometimes these microbial transformation processes are fortuitous, a phenomenon that is not uncommon in microbiology. On the other hand, microorganisms may use xenobiotic compounds as a source of energy, carbon, nitrogen, or sulfur. Degradation of many xenobiotic chemicals requires microbial communities. Some xenobiotics, however, appear to resist microbial attack. In the near future, the collective knowledge in the field of microbial degradation may enable scientists to establish rules to predict the biodegradability and the biodegradation pathways of xenobiotic compounds.

1. Introduction: General Features of the Microbial Degradation of Xenobiotics

1.1. Biodegradation, Biotransformation, and Co-metabolism

More than ten million organic compounds are generated by biosynthetic pathways in animals, plants, and microorganisms, by other natural processes, and by industrial synthesis. Whilst the organic structures found in nature are created by many organisms and processes, microorganisms (bacteria and fungi) perform most of the biodegradation of both natural products and industrial chemicals. Collectively, microorganisms play a key role in the biogeochemical cycles of the Earth.

The substances transformed or degraded by microorganisms are used as a source of energy, carbon, nitrogen, or other nutrient, or as final electron acceptor of a respiratory process [see also - Cell thermodynamics and energy metabolism]. 'Biodegradation' involves the breakdown of organic compounds, usually by microorganisms, into biomass and less complex compounds, and ultimately to water, carbon dioxide, and the oxides or mineral salts of other elements present. The complete breakdown of an organic compound into inorganic components is termed 'mineralization', but '(ultimate/complete) biodegradation' and '(complete) mineralization' are often used interchangeably, although 'biodegradation' involves the formation of biomass as well as inorganic compounds. Of course, biomass finally will also undergo mineralization. Degradation of an organic compound to a less complex organic compound is referred to as 'incomplete (partial) biodegradation'.

'Biotransformation' is the metabolic modification of the molecular structure of a compound, resulting in the loss or alteration of some characteristic properties of the original compound, with no (or only minor) loss of molecular complexity. Biotransformation may effect the solubility, mobility in the environment, or toxicity of the organic compound.

A microbial population growing on one compound may fortuitously transform a contaminating chemical that cannot be used as carbon and energy source, a process referred to as 'co-metabolism'. The phenomenon has also been called 'co-oxidation' and 'gratuitous' or 'fortuitous' metabolism. Usually, the primary substrate induces production of (an) enzyme(s) that fortuitously alter(s) the molecular structure of another compound. The organisms do not benefit from the co-metabolic process. Co-metabolic transformation may result in a minor modification of the molecule, or it may lead to incomplete or even complete degradation.

The products of partial biodegradation, or biotransformation, or co-metabolic conversion of a xenobiotic may be less harmful as the original compound, or they may be as hazardous or even more hazardous as the original compound. For example, tetrachloroethene and trichloroethene can be microbially reduced to vinyl chloride, a known carcinogen, in anoxic habitats. In natural environments, the products of bioconversion processes may be further transformed or degraded by other microorganisms, maybe eventually leading to complete degradation by the microbial consortium. Co-metabolic processes, and biodegradation by microbial consortia are thought to be of enormous ecological importance. However, persistent xenobiotics and metabolic dead-end products will accumulate in the environment, become part of the soil humus, or enter the food chain leading to biomagnification. Figure 1 summarizes the possible fate of xenobiotic compounds.

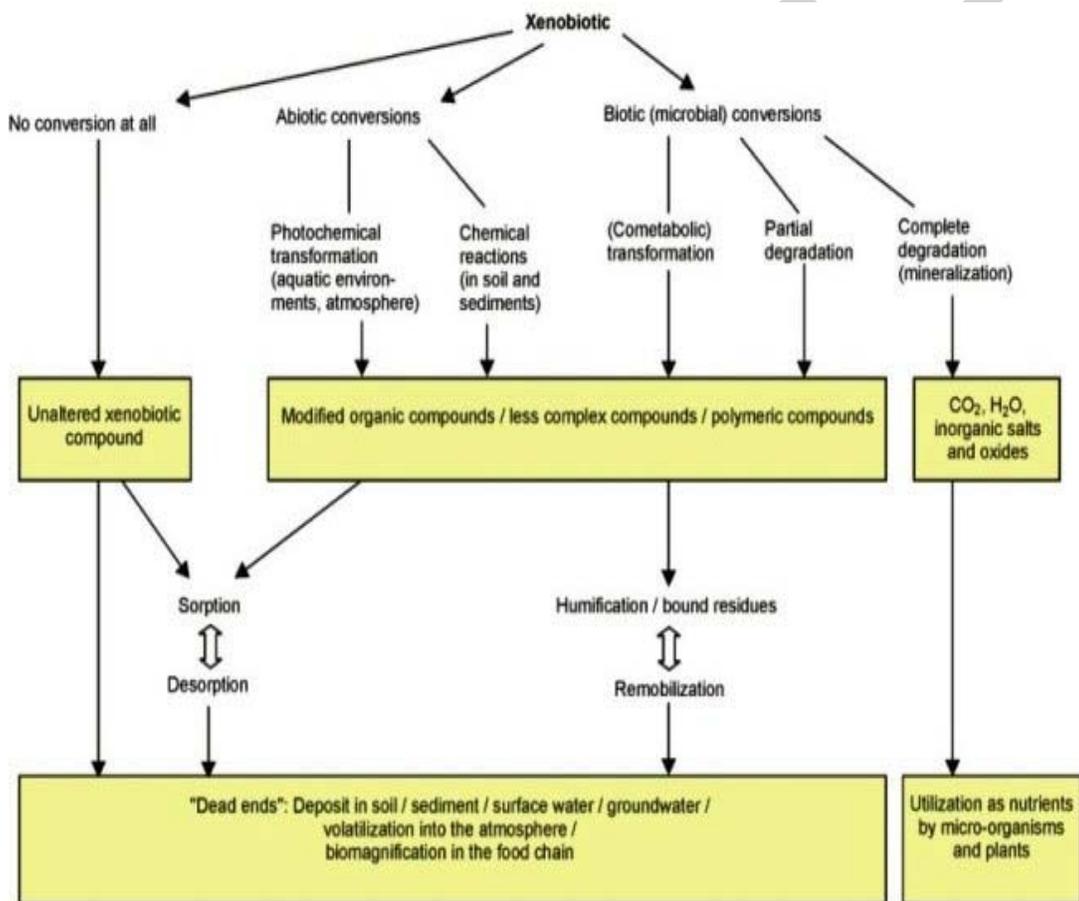


Figure 1. Possible environmental fate of a xenobiotic compound.

1.2. What are Xenobiotics?

Xenobiotics (greek *xenos* = strange, foreign, foreigner) are chemically synthesized compounds that do not occur in nature and thus are 'foreign to the biosphere'. They have 'unnatural' structural features to which microorganisms have not been exposed to during evolution. Xenobiotics may resist biodegradation, or they undergo incomplete

biodegradation or just biotransformation. The definition of xenobiotics as compounds 'foreign to life' exhibiting 'unnatural' structural features does not necessarily imply that xenobiotics are toxic compounds, but many xenobiotics indeed are harmful to living organisms.

Whereas xenobiotics may persist in the environment for months and years, most biogenic compounds are biodegraded rapidly. Exceptions are lignin, the structural polymer of woody plants, and, above all, the melanin polymers which are constituents of the cell wall of the spores of a number of fungi. Recalcitrance (i.e., the structure-immanent stability) of a xenobiotic molecule is mainly due to 'unphysiological' chemical bonds and/or substituents, which block the attack by microbial catabolic enzymes (see Table 1 and Figure 2). Type, number and position of bonds and substituents affect the xenobiotic character. However, it is not always easy to determine which structural moieties indeed are xenobiotic in the sense of 'foreign to life'. Some natural compounds show principally the same unusual structural features as xenobiotics, such as halogen substituents or nitro groups found in some antibiotics, or they contain stable chemical bonds like the ether and carbon-carbon bonds stabilizing lignin. Moreover, microorganisms throughout geological time have also been exposed to a variety of chemicals produced by abiotic natural processes:

"Many of these compounds bear little relationship to the biological products from which they were originally derived. For example, soils and young sediments contain thousands of substituted polycyclic aromatic hydrocarbons. These molecules, formed by the thermal alteration of cellular material, have been in contact with living organisms throughout evolutionary periods of time. Consequently, one would predict the existence of microorganisms that will degrade them, and organisms that metabolize aromatic hydrocarbons ranging in size from benzene to benzo[a]pyrene have been described." (D. T. Gibson, 1980).

•	High molecular mass
•	Low solubility in water
•	Condensed benzene and pyridine rings, especially: polycyclic structures
•	Three-fold substituted N atoms
•	Quarternary C atoms
•	Unphysiological bonds and substituents R-X (especially, polysubstitution): -X = -O-R -N=N- -F, -Cl, -Br -NO ₂ -CF ₃ -SO ₃ H

Table 1. Typical features of recalcitrant organic compounds. Type, number, and position of 'unphysiological' substituents influence recalcitrance.

It should be noted that organic chemicals of anthropogenic origin are not necessarily recalcitrant. There are a number of industrial products that are degraded by microorganisms. These compounds obviously are readily recognized by microbial catabolic enzymes. Besides, research in biodegradation has demonstrated that a number of xenobiotic compounds such as polychlorinated biphenyls (PCBs) and nitroaromatics

which once were thought to be recalcitrant are subject to microbial attack (see the following sections).

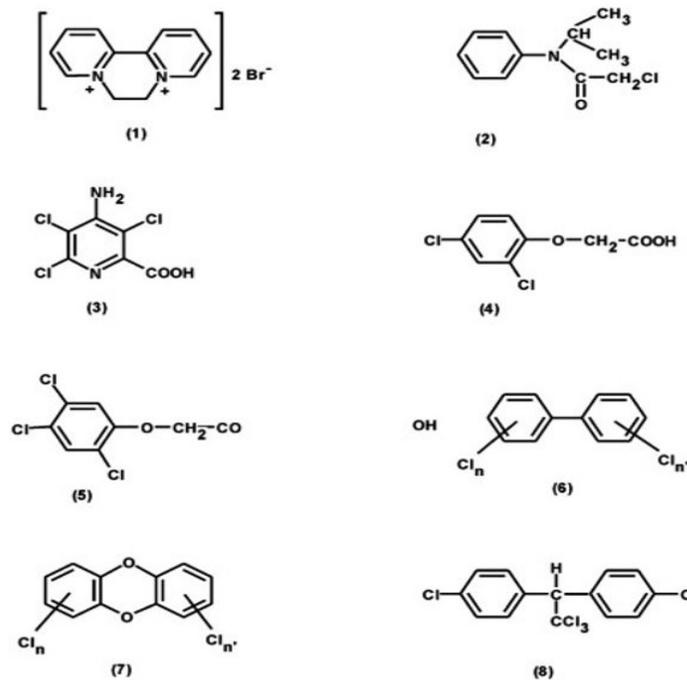


Figure 2. Examples of relatively persistent xenobiotics showing 'typical' features of recalcitrant compounds.

- (1) The herbicide diquat-dibromide, DT90 (degradation time for 90% removal) is up to 12 months; (2) the herbicide propachlor, DT90 is 2 to 12 months; (3) the herbicide picloram, DT90 is 2 to 18 months; (4) the herbicide 2,4-D, DT90 is 0.5 to 1.5 months; (5) the herbicide 2,4,5-T, DT90 is 6 to 12 months; (6) PCBs, used e.g. as insulating and cooling fluids, hydraulic fluids, and additives for lubricants; DT90 depends on the position and extent of substitution, but is generally much higher than 12 months; (7) PCDDs, by-products in the synthesis of herbicides such as 2,4-D and 2,4,5-T, and by-products of combustion processes; DT90 is much higher than 12 months; (8) the insecticide DDT, DT90 is many years.

1.3. Parameters Influencing Bioavailability and the Rate of Biodegradation

The extent of biodegradation and the rate at which it occurs depend on the chemical structure and concentration of the compound being degraded, the type and number of microorganisms present, and the physicochemical properties of the environment. In laboratory as well as environmental systems, only the fraction of the xenobiotic pollutant that is dissolved in the aqueous phase is generally assumed to be available to the microorganisms for degradation. Bioavailability is controlled by parameters such as the physical state of the pollutant compound (solid, liquid, gaseous), its solubility in water, and its tendency to adsorb or bind to soil or sediment particles. In soil aggregates or other solids, microbes may be excluded from entering the smaller micropores. Xenobiotics present in the micropores are thus unavailable to the microorganisms and must diffuse through pore water to the grain surface in order to be degraded. However,

diffusion in soil systems may well be sorption-limited. Actually, sorption, immobilization and micropore entrapment are major causes for the persistence of many xenobiotics. 'Aging', i.e. the length of time a soil or sediment has been exposed to contamination, also affects bioavailability: Pollutants may undergo reactions that lead to strong binding to soil and sediment material, becoming increasingly unavailable to microorganisms with the progress of time. Many xenobiotics, for example the polycyclic aromatic hydrocarbons and the polychlorinated biphenyls, are poorly soluble in water, and tend to adsorb to and be immobilized by the soil matrix and sediment material.

As mentioned above, the structure of xenobiotic molecules is characterized by 'unphysiological' substituents and stable chemical bonds, which impede or even prevent biodegradation. Unfavorable concentrations of the xenobiotic compound also affect biodegradation. In high concentrations, many xenobiotics are toxic to organisms, including the degradative bacteria. On the other hand, there may be a minimum concentration below which a compound is not degraded any more. Synthesis of catabolic enzymes may not occur when the concentration of a chemical is below a level that is effective for induction of the corresponding catabolic genes. Besides, the minimal threshold concentration depends mainly on the kinetic parameters of growth and metabolism, but also on the thermodynamics of the overall transformation reaction. Actually, the substrate affinity constant is the most important parameter with respect to the biodegradation of contaminants to very low concentrations. Typical minimal substrate concentrations for aerobic systems may be in the range of 0.1 to 1.0 mg L⁻¹, but the desired end concentrations in environmental systems often are 1 µg L⁻¹ or less. Other factors that influence biodegradation involve environmental conditions such as temperature, pH, water content and salinity, presence of inhibitory chemicals, availability of electron donors and nutrients, and availability of oxygen or other electron acceptors. In soil, for example, oxygen availability is very often the limiting factor of aerobic biodegradation processes. Moreover, the presence of competing microorganisms, or of predators grazing on the microbial consortium, also affect biodegradation.

When determining biodegradation rates, it is important to keep in mind that an observed 'disappearance' of a xenobiotic from an ecosystem does not necessarily mean that it was biodegraded, since loss can also occur by partial degradation, biotransformation, or by volatilization, leaching, or chemical conversion (polymerization, modification, breakdown). In monitoring the environmental fate of a chemical, one must also monitor the products formed, not simply the disappearance of the parent compound.

The rates of xenobiotic biodegradation in the environment may range from days and weeks to years and decades. The organophosphate insecticide malathion disappears from soil within approximately one week, and the herbicide 2,4-D (2,4-dichlorophenoxyacetic acid) is degraded within four to six weeks in soil. Modern herbicides are designed to undergo biodegradation within one cropping season. On the other hand, there are recalcitrant xenobiotics that persist in the environment for many years. Simple structural changes of a molecule, such as the addition of a chlorine substituent, can convert a readily biodegradable compound such as 2,4-D into a more persistent substance such as 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) which is

degraded in soil within approximately six to twelve months (see Figure 2). A prominent example of a very persistent xenobiotic is the insecticide DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane, see Figure 2), which was used extensively from the 1930s until its ban in 1979. DDT was found to persist with an average half-life of 4.5 years in field soils, and a half-life in anoxic soils of about 700 days. Stable metabolites of DDT have been detected in soil, groundwater, and in the tissue of organisms.

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Biographical Sketch

Susanne Fetzner, born in 1963, studied biology at the University of Hohenheim (1982-1988). Her graduate work involved studies of the bacterial degradation of haloaromatic compounds. After receiving her doctoral degree from the University of Hohenheim in 1990, her postdoctoral work focused on bacterial enzymes catalyzing hydroxylation and heterocyclic-ring cleavage reactions. Since 1996, she has been deputizing for the Professor of Microbiology at the University of Oldenburg. The research interests of her group include enzymology, mechanisms of biocatalysis, and the genetic organization of catabolic enzymes and pathways.