

CHEMISTRY OF ORGANIC POLLUTANTS

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Contents

1. Introduction
 2. Aliphatic compounds
 - 2.1. Hydrocarbons
 - 2.2. Halogenated Alkanes, Alkenes and Alkanooates
 - 2.3. Ethers, Amines, Sulfides, Nitriles and Phosphonates
 - 2.4. Carboxylate, Nitrate, Sulfate, Sulfonate and Phosphate Esters, Amides and Derivatives
 - 2.5. Siloxanes and Related Compounds
 - 2.6. Perfluoroalkyl Compounds
 3. Cyanide and carbon monoxide
 4. Aromatic carbocyclic compounds
 - 4.1. Hydrocarbons
 - 4.2. Arenes with Carboxyl, Halogen, Sulfonate and Trifluoromethyl Substituents
 - 4.3. Phenols
 - 4.4. Nitroarenes without Carboxyl Groups
 5. Aromatic rings containing nitrogen, oxygen or sulfur
 - 5.1. Furan, Thiophene and Pyrrole
 - 5.2. Carbazole, Dibenzofuran and Dibenzo-*p*-dioxin
 - 5.3. Benzothiophenes and Dibenzothiophenes
 - 5.4. Pyridine, Quinoline, and Pyrimidine
 - 5.5. Triazines
 6. Abiotic reactions
 - 6.1. Tropospheric Reactions
 - 6.2. Reactions in the Aquatic and Terrestrial Environment
 7. The natural environment
- Glossary
Bibliography
Biographical Sketches

Summary

The major groups of organic pollutants, the principal reactions for their degradation or

transformation, and the responsible enzymes are discussed. The design of the relevant experiments and factors that determine their environmental significance are given. Although emphasis is placed on biotic reactions carried out by bacteria, important reactions mediated by fungi primarily in the terrestrial environment are provided and contrasted with the analogous reactions mediated by bacteria. Reaction pathways used by bacteria under both aerobic and anaerobic conditions are provided to illustrate their essential differences and the relevant enzymes are noted. Emphasis is placed on the occurrence of partial degradation of pollutants and the role of intermediates that are toxic to the organisms, inhibit further degradation or have adverse effects on other biota. Attention is drawn to the occurrence of reactions carried out by several organisms, and to the importance of sequential biotic and abiotic reactions. The limitations to biodegradation imposed by specific structures are noted: branching in alkanes, PAHs with five or more rings, aromatic rings with more than three substituents, or trifluoromethyl groups may be recalcitrant. A brief summary of abiotic reactions is given together with examples of transformations in the troposphere. The chemical and photochemical degradation of a selection of agrochemicals in the aquatic and terrestrial environment is given.

1. Introduction

This chapter discusses the biodegradation and biotransformation of major groups of organic pollutants, the principal reactions and the enzymes responsible, the design of experiments, and factors that determine their environmental significance.

Major classes of organic pollutants

Important groups of potential organic pollutants are summarized.

- Refined petrochemicals (gasoline, monocyclic aromatic hydrocarbons)
- Bulk chemicals (a range of compounds including nitrobenzene, aniline, plastic monomers e.g. phthalate and styrene, solvents)
- Agrochemicals including herbicides and pesticides
- Chemicals used in plastics, mining, metal-working, wood preservation, paints, textiles, paints and pigments, flame retardants, household products, and pharmaceuticals.

The diverse groups to which these belong include the following.

- *Hydrocarbons*: Products of degradation of higher plants including the simplest (methane), aliphatic (alkanes and alkenes), alicyclic and aromatic structures with one or more rings
- *Organohalogen compounds*: Halogen (fluorine, chlorine, bromine or iodine) bound to aliphatic, alicyclic and aromatic structures including halogenated alkanes and alkenes, chlorinated alkanes, halogenated arenes, halogenated anilines, halogenated phenols, halogenated phenoxyacetates, polybrominated phenols and polybrominated diphenyl ethers
- *Substituted aromatic hydrocarbons*: Phenols, anilines, benzoates and phthalates, nitro- and sulfonated arenes

- *Nitrogen compounds*: Aliphatic and aromatic amines, amides, carbamates and nitriles, nitro- and azaarenes, nitrophenols, heteroarenes, nitrate esters, nitramines
- *Oxygenated compounds*: Aliphatic diols, aliphatic and aromatic esters and ethers, dibenzofurans, dibenzo-*p*-dioxins
- *Sulfur compounds*: Aliphatic and aromatic sulfate esters and sulfonates, dibenzothiophenes
- *Phosphorus compounds*: Organophosphates and organothiophosphates, aliphatic phosphonates, phosphorofluoridates

Their occurrence is given for each of the classes of pollutants that are discussed in Sections 2 to 6.

In addition, important plastics are prepared from monomeric intermediates. Examples include those produced by polymerization of substituted ethylenes (e.g. polyethylene, polystyrene, poly-tetrafluoroethylene, polyvinyl chloride, polyvinylacetate, and polyacrylonitrile) and condensation polymers (e.g. polyurethane from aliphatic diols and diisocyanates, nylon from diaminohexanoate and aliphatic dicarboxylic acids, and polyethylene terephthalate from terephthalic acid and ethylene glycol).

Major reactions and enzymes

For biodegradation of organic compounds to occur it is necessary that biochemical reactions produce metabolites that are incorporated into cell material and used for production of energy. Reactions catalyzed by enzymes follow broadly those encountered in organic chemistry: oxidation, dehydrogenation, reduction, hydrolysis, nucleophilic substitution, elimination, hydration, decarboxylation, rearrangement or carboxylation. The end products of degradative sequences are low molecular weight compounds including CO₂ and lower fatty acids. The primary reactions in both biodegradation and biotransformation involve the introduction of oxygen atoms (from di-oxygen or water), the cleavage of carbon-carbon, carbon-oxygen, carbon-halogen, carbon-nitrogen, carbon-sulfur and carbon-phosphorus bonds, and the reduction of compounds at elevated oxidation levels. The major groups of enzymes include the following.

- *Carboxylases*: Addition of the elements of carbon dioxide during aerobic degradation of branched alkanes and anaerobic degradation of alkanes and phenols
- *Dehydrogenases*: Loss of hydrogen from e.g. alkanols to produce aldehydes
- *Epoxidases*: Addition of a single atom of dioxygen to epoxides
- *Halohydrolases*: Breakage of carbon-halogen bonds in organohalogen compounds
- *Hydratases*: Addition of the elements of water to carbon-carbon triple bonds or carbon-nitrogen triple bonds
- *Hydrolases*: Hydrolysis of esters, amides and related compounds, sulfates, sulfonates, phosphates and thiophosphates
- *Hydroxylases*: Introduction of oxygen from water into chlorinated arenes and azaarenes
- *Oxygenases*: Introduction of oxygen from dioxygen (monoxygenases and dioxygenases)
- *Reductases*: Cleavage of carbon-halogen bonds in aliphatic and aromatic compounds, cleavage of carbon-oxygen bonds in nitrates, carbon-phosphorus bonds

in organophosphonates and carbon-mercury bonds in organomercurials, reduction of aromatic nitro compounds

Significance of metabolites

Metabolites may critically affect the degradation pathway or have undesirable environmental consequences: they may (a) inhibit further degradation of the initial substrate and represent terminal products (b) have properties different from those of their precursors e.g. be lipophilic and accumulate in biota or (c) have adverse effects on other organisms in the ecosystem. Metabolites should therefore be taken into account in assessing the biodegradability and the impact of pollutants, the effectiveness of remediation strategies for polluted sites, and in environmental monitoring that generally takes into account only the original pollutant.

On the other hand, metabolites have proved valuable: (a) as indicators of degradation or transformation in the environment e.g. arene dihydrodiols produced from arenes by bacterial dioxygenation, chlorobenzoates from the aerobic degradation of PCB congeners, or succinate derivatives from the anaerobic degradation of hydrocarbons, (b) as commercial products or as the basis for further chemical elaboration. In addition the organisms may be the source of biocatalysts.

Design of experiments

Experiments on the metabolism of organic compounds have been carried out under different conditions: (a) degradation during growth at the expense of the compound which supplies energy and carbon (or nitrogen, or sulfur) for biosynthesis, (b) metabolism in the presence of an auxiliary substrate that is provided for growth of the cells (cometabolism), (c) growth of cells with an alternative substrate that is capable of inducing the appropriate degradative enzymes and incubation of cell suspensions under conditions in which cell growth does not take place. Although probably few environments are contaminated with only a single contaminant, environmental relevance can be incorporated by simulating the natural environment, for example, by using low concentrations of the test compound, and determining its degradation or transformation during growth with plausible naturally occurring growth substrates. When appropriate, both aerobic and anaerobic conditions should be examined.

Limitations in this study

This review is focused on the degradation and transformation of major groups of pollutants. It makes no attempt to provide taxonomic definition and description of the microorganisms used for degradation of organic pollutants, or to discuss the enzymology or the genetics of the degradation pathways. Although most examples are drawn from reactions carried out by bacteria, the important role of yeasts in the aquatic environment and fungi in the terrestrial environment should be appreciated.

2. Aliphatic Compounds

2.1. Hydrocarbons

Hydrocarbons are major components of crude oil and of refined products such as petroleum and lubricating oils. They may enter the environment as a result of accident and bioremediation has been attempted in the aquatic and the terrestrial environments. They may enter groundwater in which anaerobic degradation is significant.

The degradation of methane is initiated by monooxygenation to formaldehyde that is degraded by either of two pathways. In addition, methane monooxygenase is able to accept a range of other substrates including methyl fluoride, while other monooxygenases carry out the first stage in the degradation of other substrates containing one or two carbon atoms: these include secondary and tertiary methylamines, methane sulfonate, dimethyl sulfide and diethyl ether.

The degradations of hydrocarbons exemplify reactions of metabolites that are formed by cleavage of the rings in aromatic compounds.

Bacterial degradation under aerobic conditions is initiated by either of two reactions: (1) terminal hydroxylation followed by successive dehydrogenation. The resulting carboxylates are further degraded by β -oxidation to yield ultimately acetate from even-membered alkanes or propionate from odd-membered alkanes (Figure 1) or (2) sub-terminal hydroxylation followed by oxidation to ketones that are degraded by oxygenation to lactones, and hydrolysis followed by the sequences as for terminal hydroxylation (Figure 2). These reactions are also used for the degradation of intermediates formed after cleavage of aromatic rings.

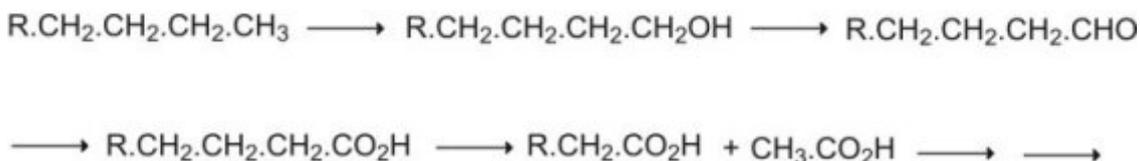


Figure 1. Terminal oxidation of alkanes

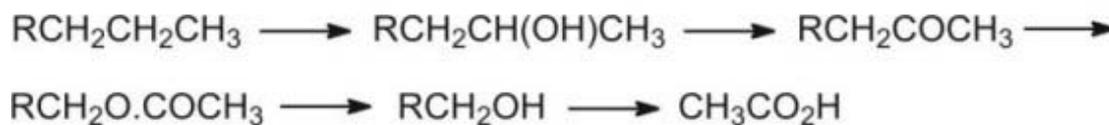


Figure 2. Sub-terminal oxidation of alkanes

For long-chain alkanes, hydroxylation may occur at both ends of the chain with production of methylmalonate. When chain-branching prevents β -oxidation, carboxylation is able to overcome this obstacle (Figure 3).

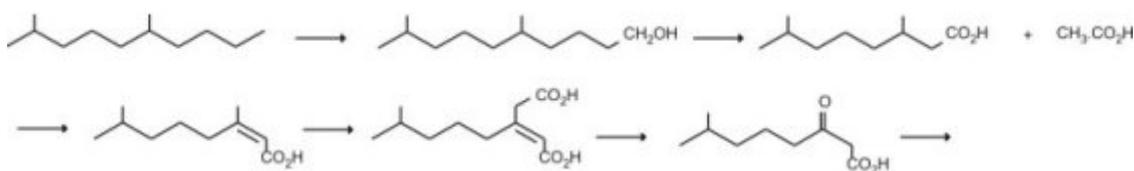


Figure 3. Degradation of branched alkanes

Alicyclic hydrocarbons are degraded by reactions involving hydroxylation followed by oxidation to the cycloalkanone followed by insertion of an oxygen atom into the ring, hydrolysis of the lactone and reactions analogous those in the degradation of alkane dicarboxylates (Figure 4).

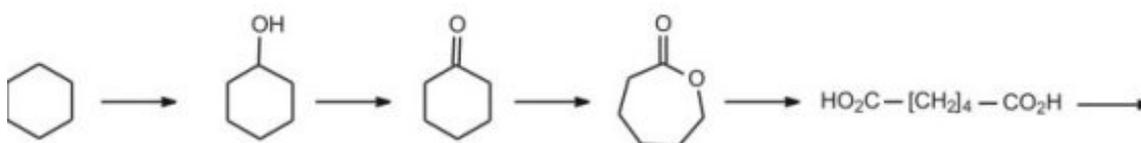


Figure 4. Degradation of cycloalkanes

Cyclohexane monooxygenase has a diverse metabolic versatility that is reminiscent of that of methane monooxygenase and, although rings with up to twelve carbon atoms have been examined, the enzymology is different for those with more than seven carbon atoms. The degradation of the monoterpene camphor has attracted attention since the initial hydroxylation in a plasmid-bearing pseudomonad is carried out by a cytochrome P-450 enzyme (designated P-450_{cam}). This enzyme displays a wide versatility that is noted later in the degradation of fluorinated alkanes, PAH degradation in a constructed bacterium and fungal metabolism of styrene.

This is the basic framework into which the degradation of other substrates may be fitted: for example, primary amines are converted into the corresponding aldehydes or ketones by oxidases or dehydrogenases that are then degraded by the pathways already described. On the other hand, the anaerobic degradation of ketones takes place by carboxylation followed by hydrolysis (Figure 5).

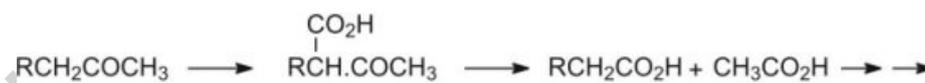


Figure 5. Anaerobic degradation of ketones

The initial step in the degradation of alkenes is epoxidation that is followed either by hydrolysis to a diol, carboxylation or a reductive glutathione-mediated reaction (Figure 6). The alkene mono-oxygenase is closely related to the aromatic monooxygenases, and is able to hydroxylate benzene, toluene and phenol. Degradation of terminal alkynes involves hydration to aldehydes followed by sequences already given (Figure 7).

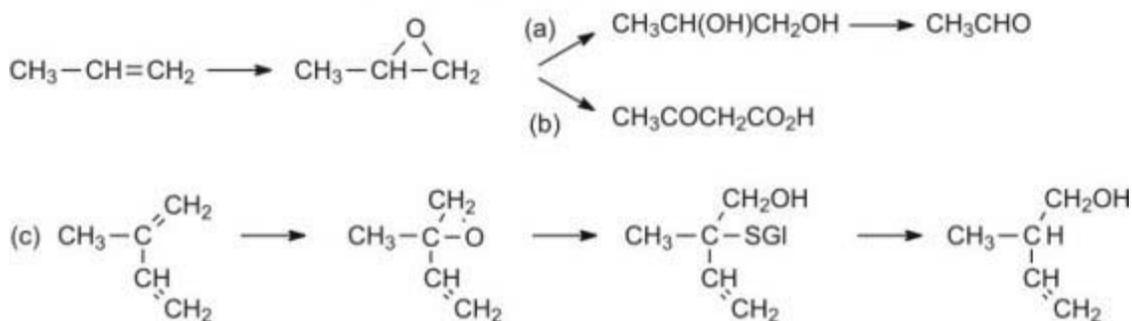


Figure 6. Alternative degradation of alkenes

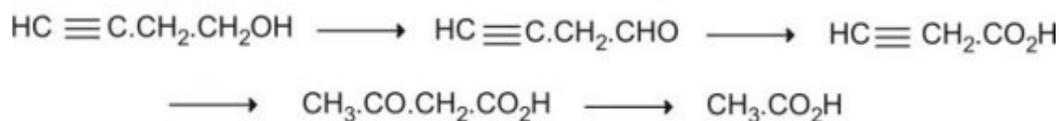


Figure 7. Degradation of alkynes

Anaerobic degradation of alkanes involves reaction at the subterminal position with fumarate followed by rearrangement and sequential loss of acetate (Figure 8). On the other hand, degradations of alkynes and ketones follow the pathways used under aerobic conditions.

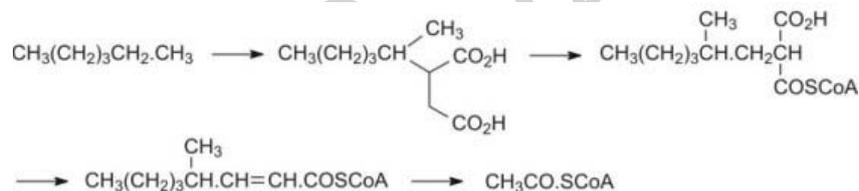


Figure 8. Anaerobic degradation of alkanes

2.2. Halogenated Alkanes, Alkenes and Alkanoates

Chlorinated alkanes have been widely used as solvents, for degreasing metal components and as feedstock while hexachlorocyclohexane and DDT are insecticides whose use has been largely discontinued. Methyl bromide that has been used as a nematicide is also a metabolite of marine algae. Attention has been given to methods for *in situ* bioremediation of groundwater contaminated with chlorinated ethylenes.

The first step in the aerobic degradation of halogenated alkanes and alkanolic acids is generally mediated by dehalogenases and the ease of replacement of the halogen is $\text{I} \approx \text{Br} > \text{Cl} \gg \text{F}$. Although the strength of the C–F bond results in the recalcitrance of many organofluorine compounds, fluoroacetate, dichlorofluoromethane and 1-chloro-1,1-difluoroethane can be degraded with loss of fluoride. Cytochrome P-450cam is capable of bringing about an elimination reaction with 1,1,1-trichloro-2,2,2-trifluoroethane (Figure 9), fluorohydrocarbons and their derivatives containing also chlorine are often recalcitrant.

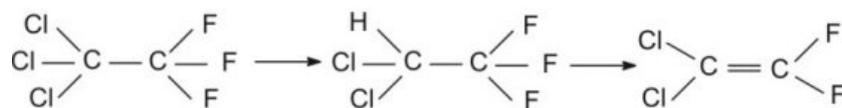


Figure 9. Cytochrome P-450 degradation of 1,1,1-trichloro-2,2,2-trifluoroethane

The degradation of both methyl chloride and methyl bromide is different from the higher analogues since alternative corrin (vitamin B₁₂)-dependent pathways for their degradation are available. For 1,2-halogenated ethanes both hydrolytic displacement and monooxygenation are involved under aerobic conditions (Figure 10), whereas elimination may take place under anaerobic conditions (Figure 11).



Figure 10. Biodegradation of 1,2-dichloroethane



Figure 11. Elimination reactions under anaerobic conditions

Under aerobic conditions epoxidation is the first reaction in the degradation of chlorinated ethylenes including vinyl chloride and cis-dichloroethylene that is a recalcitrant intermediate in the anaerobic de-chlorination of tetrachloro- and trichloroethylenes (Figure 12). Transformation of chlorinated ethylenes can also be accomplished by toluene monooxygenase, and advantage has been taken of this for bioremediation of contaminated sites when both aromatic and chlorinated aliphatic contaminants are present.

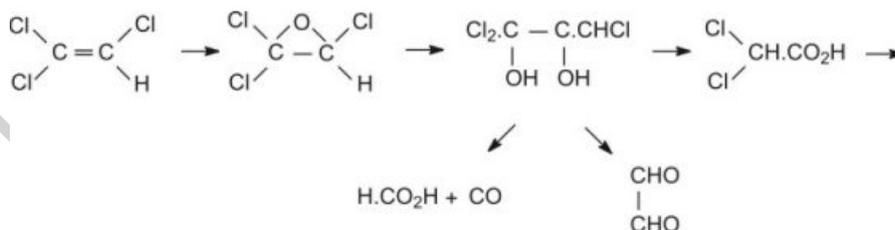


Figure 12. Epoxidation of halogenated alkenes

Under aerobic conditions, elimination is one of the early stages in the pathway for bacterial transformation of DDT (Figure 13). Although the product (DDE) is appreciably recalcitrant, it can be degraded by dioxygenation to chlorobenzoate in cells grown with biphenyl.

aromatic fluorinated compounds; atmospheric reactions; biodegradation of phosphorofluoridates]

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Biographical Sketches

Alasdair H. Neilson was Principal Scientist until retirement from the Swedish Environmental Research Institute in Stockholm. He obtained First Class Honors in Chemistry from the University of Glasgow and his Ph.D. in organic chemistry in Alexander Todd's laboratory at Cambridge. He carried out independent research in Cambridge in organic chemistry and in Oxford in theoretical chemistry with Charles Coulson. He held academic positions in the universities of Glasgow and Sussex, and obtained industrial experience in the pharmaceutical industry. He consolidated his experience by turning to research in microbiology during a prolonged stay with Roger Stanier and Mike Doudoroff in Berkeley. His interests have ranged widely and included studies on nitrogen fixation, carbon and nitrogen metabolism in algae, and various aspects of environmental science covering biodegradation and biotransformation, chemical and microbiological reactions in contaminated sediments and ecotoxicology. With his small group of collaborators, these studies have resulted in substantial publications in journals including *Applied and Environmental Microbiology*, *Journal of Chromatography*, *Environmental Science & Technology*, and *Ecotoxicology & Environmental Safety*, in chapters contributed to several volumes in the Springer Series "The Handbook of Environmental Chemistry" and "Organic Chemicals: an Environmental Perspective". He is a member of the American Chemical Society, the American Society for Microbiology, the American Crystallographic Association, and the Arctic Institute of North America.

Ann-Sofie Allard has been employed as Research Microbiologist with the Swedish Environmental Research Institute in Stockholm since 1977. She has been involved in a wide range of environmental projects covering not only microbiology but also ecotoxicology using a range of biota, and in environmental chemistry. These were initiated with extensive studies on nitrogen fixation in enteric bacteria. She has been involved in the biodegradation and biotransformation of organic compounds and developed the program on anaerobic biodegradation and transformation. She is the co-author of publications in a number of journals including *Applied and Environmental Microbiology*, *Environmental Science & Technology*, and *Ecotoxicology & Environmental Safety*, and of reviews in chapters contributed to several volumes in the Springer Series "The Handbook of Environmental Chemistry". She has most recently been concerned with aspects of the bioremediation of sites contaminated with organic compounds including the development of procedures for determining the need for them and their effectiveness. Some aspects of this have been published in *International Biodeterioration and*

Biodegradation.

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SAMPLE CHAPTERS