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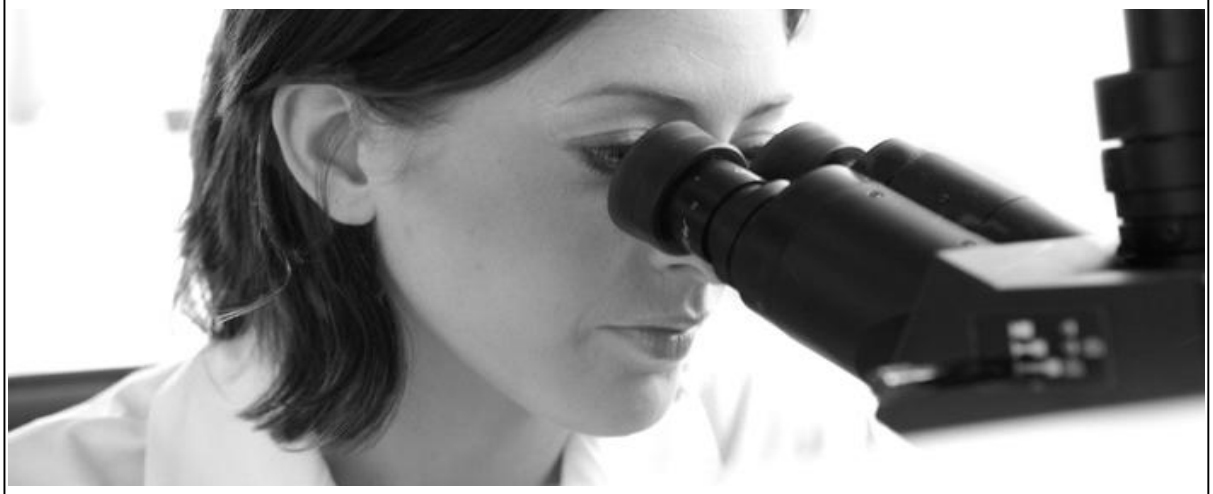

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RESEARCH ARTICLE

BIODEGRADATION OF XENOBIOTIC COMPOUNDS

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INTRODUCTION

Biodegradation

Biodegradation involves the breakdown of organic compounds, usually by microorganisms into biomass and less complex compounds, and ultimately to water, carbon-dioxides and oxides of other elements. Microorganisms act upon these compounds in following different ways:

- By using them as substrates for energy and biomass production.
- ***Co-metabolism:*** Degradation of xenobiotics in the presence of another compound (co-metabolite) which induces the necessary enzymes and metabolism of which provides both energy and reducing equivalent as well as carbon, energy etc.
- ***Gratuitous metabolism:*** Xenobiotic compounds are degraded by an existing pathway and used as source of energy and reducing equivalents by microbes.

Xenobiotic Compounds: These are man-made chemicals that are present in the environment at unnaturally high concentrations e.g. halogenated hydrocarbons, aromatics, pesticides etc. Xenobiotics present a number of potential hazards to man and environment like:

- ***Toxicity:*** Halogenated and aromatic hydrocarbons are toxic to most live forms. At low levels they may cause skin problems and reduce reproductive potential.
- ***Carcinogenecity:*** Certain halogenated hydrocarbons have been shown to be carcinogenic.
- ***Bioaccumulation:*** *These* compounds are taken up from the environment and accumulated in the lipid deposits of the body eg. 100 fold accumulation of DDT by plankton from environment.

Factors affecting xenobiotic biodegradation by microorganisms

- ***Rate of contaminant degradation:*** It depends upon the concentration of the contaminant if high requires dilution, if low requires higher density of degrading microbes and number of organisms able to metabolise the contaminant as well as the amount of enzyme(s) produced by each cell.
- ***Extent of contaminant degradation:*** It is largely a function of the specific enzyme involved and their "affinity" for the contaminant and the availability of the contaminant.
- ***C:N ratio:*** Microbial cells comprised of carbon (C), nitrogen (N) and phosphorus (P) at an average C:N:P ratio of 50:14:3. Sufficient amount of these nutrients must be available in a usable form and in proper proportions for unrestricted microbial growth to occur.
- ***Temperature:*** Temperature up to a maximum of about 65°C directly influences the rate of biodegradation by controlling the rate of enzyme catalysed reaction. ***Moisture:*** Moisture (water) influences the rate of contaminant metabolism because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems.
- ***pH:*** A pH range of 6.5 to 8.5 is considered to be optimum for biodegradation.
- ***Bioavailability:*** The fraction of contaminant actually available to microorganisms is said to be bioavailable.

Enzymatic Processes in Biodegradation

Oxidation: removal of electrons or addition of oxygen

- Epoxidation: addition of an O atom bridging between two C atoms

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- β-oxidation: straight chain hydrocarbons are oxidised to two carbon atoms
- Ring cleavage: Oxygenase forms an ester in the form of a lactone which is then hydrolysed to open ring structure.

Decarboxylation: COOH is replaced with an H atom or –OH group

Hydrolysis: Addition of H_2O to a molecule accompanied by cleavage of the molecule into two species.

Subsitution: One group of atom is replaced by another

Elimination: Atoms or group of atoms are removed from adjacent carbon atoms, which remain joined by double bonds

Reduction: Addition of H atoms

Dehalogenation: Replacement of halogen group by H atom

Demethylation: Removal of methyl group

Deamination: Removal of ammonia group

Condensation: two smaller molecules are joined to produce a larger one

Conjugation: attachment of a group such as methyl group to a xenobiotic compound

The Origin of Microbial Capacity To Degrade Xenobiotics

The xenobiotic degradation ability develops due to continous exposure of microorganisms to xenobiotic compounds due to:

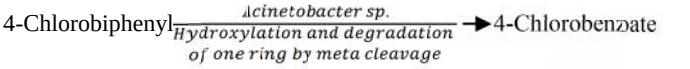
- ***Mutation:*** Mutations can be expected to either modify the active site of an enzyme for increased affinity to xenobiotics or it can eliminate regulatory control elements and enhance its production. However, they rarely generate a new enzyme function.
- ***Transfer of plasmid borne gene:*** Plasmids like TOL plasmid encodes all the enzymes for toluene degradation but the plasmids like pAC21 for p-chlorobiphenyl degradation and pAC25 for 3-chlorobenzoate degradation do not encode all the degrading enzymes, so remaining enzymes must be provided by the chromosomal genes to the cell or by another microorganism through:

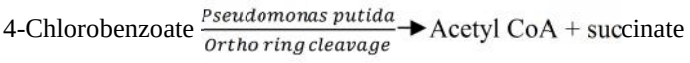
Conjugation: Allow the microorganism to acquire the genes needed to complete the xenobiotic degradation metabolic pathway or improving the rate and/or nature of degradation. For e.g. *Acaligenes* sp. degrade 4-chlorophenol to toxic product 5-chloro-2-hydroxymuconic semialdehyde (meta cleavage) preventing its further degradation. In contrast *Pseudomonas* strain B13 cleaves 4-chlorophenol by ortho pathway avoiding the production of toxic intermediates with help of plasmid encoded enzyme 1,2- dioxygenase. When a mixture of two strains is maintained in the laboratory, *Alcaligenes* sp. acquired the plasmid and the ability for ortho ring cleavage from *Pseudomonas* strain B13.

Genetic engineering: To create genetically engineered organism having recombinant pathway for xenobiotic compound degradation. For eg. Superbug with combined ability to degrade camphor, octane, xylene and naphthalene simultaneously.

Use of mixed microbial population- It is desireable as:

- The product of degradation of xenobiotic by one microorganism serves as substrate for another. Thus two microbes can together degrade a xenobiotic compound completely while either of them alone is incapable. For example:





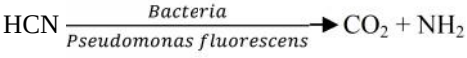
- One microorganism may produce the growth factor/nutrient provided by another.For e.g. *Nocardia* sp. breakdown cyclohexane; the breakdown products are used by *Pseudomonas* strains, which grows and releases biotin, required for growth of *Nocardia* sp., which in turn degrades cyclohexane.
- Co-culture may lead to plasmid transfer into faster growing species. For e.g. Transfer of plasmid from *Pseudomonas* sp. strain B13 into fast growing *Alcaligene* sp.
- Use of mixed inoculum increases the likelihood to degrade the mixture of xenobiotic compounds.

Some microorganisms involved in the biodegradation of xenobiotics

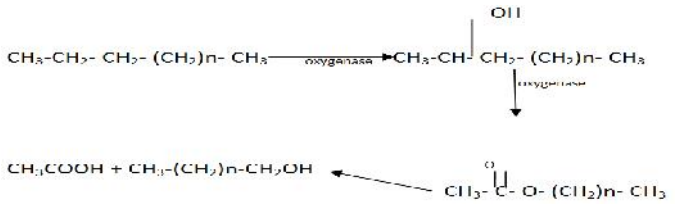
Organic pollutants	Organisms
Phenolic compound	*Achromobacter, Alcaligenes, Acinetobacter, Arthrobacter, Azotobacter, Flavobacterium, Pseudomonas putida*
Benzoate and related compound	*Arthrobacter, Bacillus* spp., *Micrococcus, P.putida*
Hydrocarbon	*E.coli, Pseudomonas putida, P. aeruginosa*
Surfactants	*Achromobacter, Alcaligenes, Bacillus* spp., *Flavobacterium,Pseudomonas, Candida*

Biodegradation of Different Compounds

- ***Halomethanes:*** Methane mono-oxygenase enzyme, transform it into methanol. Alternatively, a glutathione dependent hydrolase catalyses oxidative dechlorination of halomethanes into methanol; oxygen is derived from water as reaction is anaerobic. Methanol is oxidised to $CO_2 + H_2O$ via formaldehyde and formic acid.
- ***Cyanide:*** High concentrations are toxic even to degrading microbes. HCN is degraded as follows:



Aliphatic hydrocarbons: n-Alkanes of 10-24 carbons are readily biodegraded. However, unsaturated and branched chains show decreased biodegradation. Biodegradation of n-alkanes is catalysed by oxidation of methasne group at one end or β-methylene group by oxygenases to produce to carboxylic acid, which then undergoes β-oxidation.



Alicyclic hydrocarbons: Are present naturally in waxes from plants, crude oil, microbial lipids etc. and are represented by xenobiotics used as pesticides and in petroleum products.

Aromatic hydrocarbons: Oxidised by di-oxygenase to catechol, which is further metabolised by two separate pathways:

- Ortho-ring cleavage pathway: A 1,2-dioxygenase cleaves the ring between two adjacent hydroxyl group and sequential catabolism of the product yields succinate + acetyl CoA.
- Meta cleavage: Enzyme 2,3-dioxygenase cleaves the ring between carbon atom having an OH group and an adjacent carbon lacking an OH group and sequential catabolism of the product yields acetaldehyde and pyruvate. Benzene is degraded by meta cleavage.

- ***Polycyclic hydrocarbons:*** One of the terminal ring is attacked by the di-oxygenase, leading to ring cleavage and degradation. At end single ring remains which is cleaved by ortho-ring cleavage pathway.
- ***Complex molecules:*** During degradation of complex molecules amide, ester or ether bonds are first attacked followed by degradation of aliphatic chains. If aliphatic chains are branched the aromatic component of the complex molecule may be attacked
- In general, the recalcitrance of various benzene derivatives increases with the subsituent group as follows COOH= OH<-NH_2<-O-CH_3<-SO_3^-<-NO_2^-. The position of substitution also affect recalcitrance as meta> ortho> para. Further the greater the number of subsituent groups on the benzene ring the higher the degree of recalcitrance.

Biodegradation of halogenated compounds: Involves elimination of the halogen groups: It may occur directly by removal of hydrogen halide e.g. HCl etc. or it may involve the substitution of halogen by –H or –thio group resulting in formation of double bond. Degradation of the non-halogenated product molecule.

Degradation of halogenated aromatic compounds can occur by two ways:

Halogen elimination after ring cleavage

- Addition of –OH group by a di-oxygenase to yield chlorinated catechol. In case of phenols the reaction is catalysed by a hydroxylase which adds another –OH group to yield the catechols.
- Ring cleavage by ortho/meta cleavage pathway
- Elimination of halogen from the straight chain
- Degradation of the aliphatic hydrocarbon so obtained.

Halogen elimination before ring cleavage: This occurs rarely, usually by the removal of the halogen group by its substitution with –H or –OH group followed by degradation of the compound so obtained.

***Recalcitrant*:** The compounds that resist biodegradation and thereby persist in the environment are called as recalcitrant. The recalcitrant xenobiotic compounds can be grouped into following types:

- ***Halocarbons:*** Compounds containing one or more halogen atoms (F, Br, Cl, I) in place of H atom. Mainly used as solvent ($CHCl_3$), propellants (CCl3F, CCl2F2, CClF3, CF4), insecticides (DDT etc.) and herbicides (dalapon, 2,4-Detc.).
- ***Polychlorinated Biphenyls:*** Compounds having two covalently linked benzene rings having one or more halogen atoms (F, Br, Cl, I) in place of H.
- ***Synthetic polymers:*** These compounds are produced as plastics, e.g. polyethylene, polystyrene, polyvinyl chloride etc.
- ***Alkylbenzyl Sulphonates:*** These are surface active detergents superior to soaps. The sulphonate group and non- polar alkyl group present are responsible for recalcitrant nature.
- ***Other Xenobiotic compounds:*** Like Oil mixtures, pesticides etc.

The xenobiotic compounds may be recalcitrant due to the one or more of the following reasons:

- Not recognised as substrate by the existing degradative enzymes.
- Highly stable due to presence of substitution groups like halogens, nitro, carbamyl, sulphonate etc.
- Decreased bioavailability due to insolubility or adsorption
- Excessive toxicity of the parent compound or its product
- Large molecular size or lack of suitable permeases prevent their entry into the cell
- Inability of the compound to induce the synthesis of degrading enzymes.

RULES OF BIODEGRADATION PREDICTION

- Large molecules are usually broken into smaller molecules at bonds that can be easily cleaved.
- Compounds that contain repeating structures tend to degrade first into these substructures.
- Small compounds are preferentially converted to intermediary metabolites in the smallest number of catalytic steps.
- Pathways are likely to be optimised for maximum yield of metabolic energy.
- Under aerobic conditions, compounds containing only carbon and hydrogen are initially metabolised by oxygenases.
- Chemically facile hydrolysis reactions generally have metabolic priority, e.g., ester, amide and nitrile hydrolysis.

- Polymers are generally poorly metabolised unless they contain readily accessible, chemically facile hydrolysable group.

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