Biodegradation of Xenobiotics

By- Dr. Ekta Khare
Department of Microbiology,
Chhatrapati Shahu Ji Maharaj University, Kanpur

Xenobiotics

- A substance foreign to biological system is known as xenobiotic compound.
- Major sources of xenobiotic compounds enters into the environment are:
  - chemical and pharmaceutical industry of xenobiotics and synthetic polymers,
  - pulp and paper bleaching, which are the main sources of natural and manmade chlorinated organic compounds in the environment;
  - mining, which releases heavy metals into biogeochemical cycles;
  - fossil fuels (coal and petroleum), which may be accidentally released in large amounts into the ecosystem (oil spills)
  - intensive agriculture, which releases massive amounts of fertilizers, pesticides, and herbicides.
- These are some of the examples through which xenobiotic compounds enter into the environment.
- Xenobiotics have been released into the environment by human activities, and they often cause environmental pollution problems, since most such compounds cannot be readily degraded and have harmful effects on the natural ecosystem, including human beings.

Biodegradation

- Biodegradation is considered a type of degradation involving biological activity.
- Biodegradation is expected to be the major mechanism of loss for most chemicals released into the environment.
- This process refers to the degradation and assimilation of xenobiotics by living microorganisms to produce degradation products.
- The most important organisms in biodegradation are fungi, bacteria and algae. Biodegradable materials degrade into biomass, carbon dioxide and methane.
- Especially, most aerobic xenobiotic-degrading bacterial strains can use such chemicals as their sole sources of carbon and energy.
- Bacteria important in the biodegradation process include *Bacillus, Pseudomonas, Klebsiella, Actinomycetes, Nocardia, Streptomyces, Thermoactinomycetes, Micromonospora, Mycobacterium, Rhodococcus, Flavobacterium, Comamonas, Escherichia, Azotobacter* and *Alcaligenes*.
- Fungi active in the biodegradation process are *Sporotrichum, Talaromyces, Phanerochaete, Ganoderma, Thermoascus, Thielavia*, *Paecilomyces, Thermomyces, Geotrichum, Cladosporium, Phlebia, Trametes, Candida,Penicillium, Chaetomium,* and *Aerobasidium.*

...Biodegradation

- Microorganisms apply two modes of action for degradation of xenobiotics compound –
  - Aerobic biodegradation;
  - Anaerobic biodegradation.
- Example of aerobic degradative bacteria of xenobiotics are *Pseudomonas, Gordonia, Bacillus, Moraxella, Micrococcus, Escherichia, Sp hingobium, Pandoraea, Rhodococcus*, and anaerobic xenobiotics degradative bacteria are *Pelatomaculum, Desulphovibrio, Methanospirillum, Methanosaeta Desulfotomaculum, Syntrophobacter, Syntrophus*.
- Anaerobic habitats, including sludge digesters, groundwater, sediments, water-laden soils, gastrointestinal contents, feedlot wastes and landfill sites (Williams, 1977) and some xenobiotic compounds (e.g., **tetrachloroethylene, polychlorinated biphenyls (PCBs), and nitro-substituted aromatics**) can be effectively transformed or mineralized by anaerobic bacteria.

...Biodegradation

- 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.
- The **chemical structure** (responsible for functional group stability, reactivity, hydrophylicity and swelling behavior) is the most important factor affecting the biodegradability of materials.
- Other important factors are **physical and physicomechanical properties**, e.g., molecular weight, porosity, elasticity and morphology (crystalline, amorphous).
- 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.
- Type, number and position of bonds and substituent affect the xenobiotic character.

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

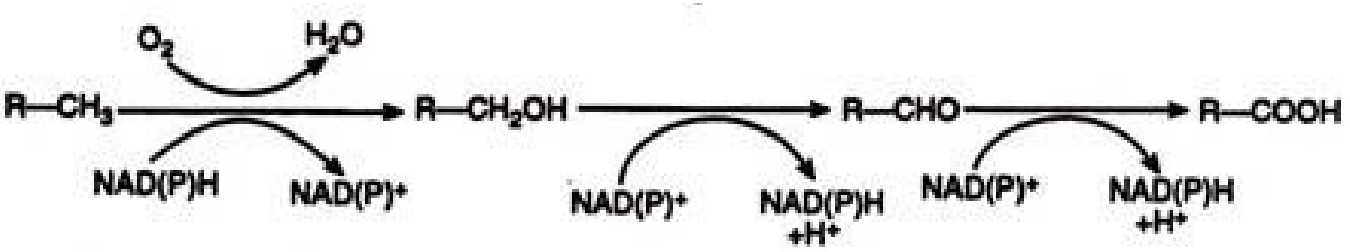
- They are not recognised as substrate by the existing degradative enzymes,
- They are highly stable, i.e., chemically and biologically inert due to the presence of substitution groups like halogens, nitro-, sulphonate, amino-, methoxy- and carbamyl groups,
- They are insoluble in water, or are adsorbed to external matrices like soil.
- They are highly toxic or give rise to toxic products due to microbial activity,
- Their large molecular size prevents entry into microbial cells,
- Inability of the compounds to induce the synthesis of degrading enzymes, and
- lack of the permease needed for their transport into the microbial cells.

Types of Recalcitrant Xenobiotic Compounds:

- (i) Halocarbons,
- (ii) Polychlorinated biphenyls,
- (iii) Synthetic polymers,
- (iv) Alkylbenzyl sulphonates,
- (v) Oil mixture
- (vi) Others: A number of pesticides are based on aliphatic, cyclic ring structures containing substitution of nitro-, sulphonate, methoxy-, amino- and carbomyl groups; in addition, they also contain halogens.
- Presence of halogens in the place of hydrogen in the molecule; the carbon-halogen bond is highly stable and its cleavage requires considerable energy.
- Substitution of H by other groups like nitro-, sulphonate, methoxy-, amino- and carbomyl groups.
- Cyclic structures, aromatic compounds, cycloalkanes and heterocyclic compounds are more recalcitrant than linear chain or aliphatic compounds.
- Branched linear chains resist biodegradation.
- In general, the more complex is the structure of a xenobiotic compound, the more resistant it is to biodegradation.
- Many other xenobiotics resist biodegradation due to their large molecular size and insolubility in water.

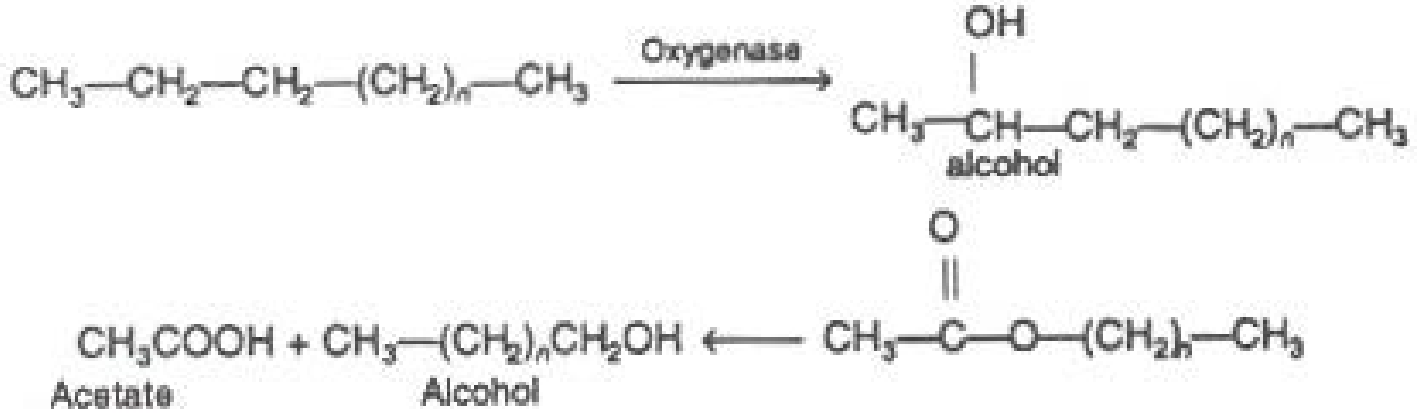
General Features of Biodegradation of Xenobiotics

- **Degradation of alkanes and aromatic hydrocarbons generally occurs as follows:**
- (i) An oxygenase first introduces a hydroxyl group to make the compound reactive,
- (ii) The hydroxyl group is then oxidised to a carboxyl group,
- (iii) The ring structure is opened up (in case of cyclic compounds),
- (iv) The linear molecule is degraded by β-oxidation to yield acetyl CoA which is metabolised in the usual manner. For example, an n-alkane is oxidised as follows.



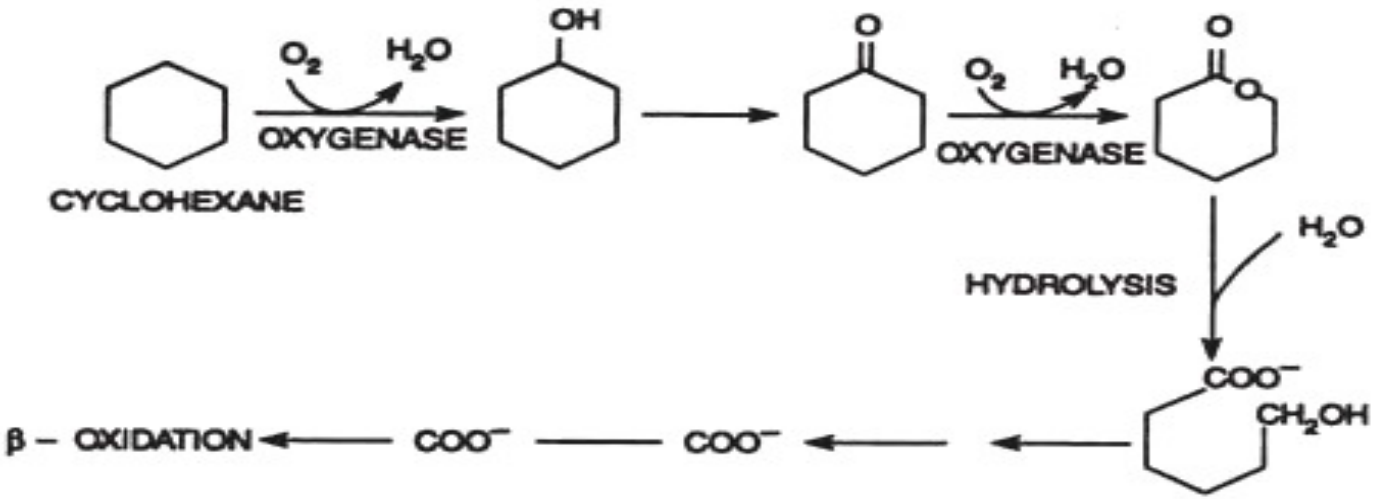
Aliphatic Hydrocarbons

- Aliphatic Hydrocarbons may be saturated or unsaturated, n-Alkanes of 10-24 carbons are the most readily biodegraded.
- Similarly saturated aliphatics are easier to degrade than unsaturated ones and branched chains show decreased biodegradation.
- Biodegradation of n-alkanes is catalysed by oxygenases to produce carboxylic acid, which is then degraded by β-oxidation.
- Oxidation may involve the methane group at one end of n-alkane molecule, or it may occur at a β-methylene group.
- Sometimes, both terminal methyl groups are oxidised to yield a dicarboxylic acid; this reaction is used by many microorganisms for the biodegradation of branched chain n-alkanes.



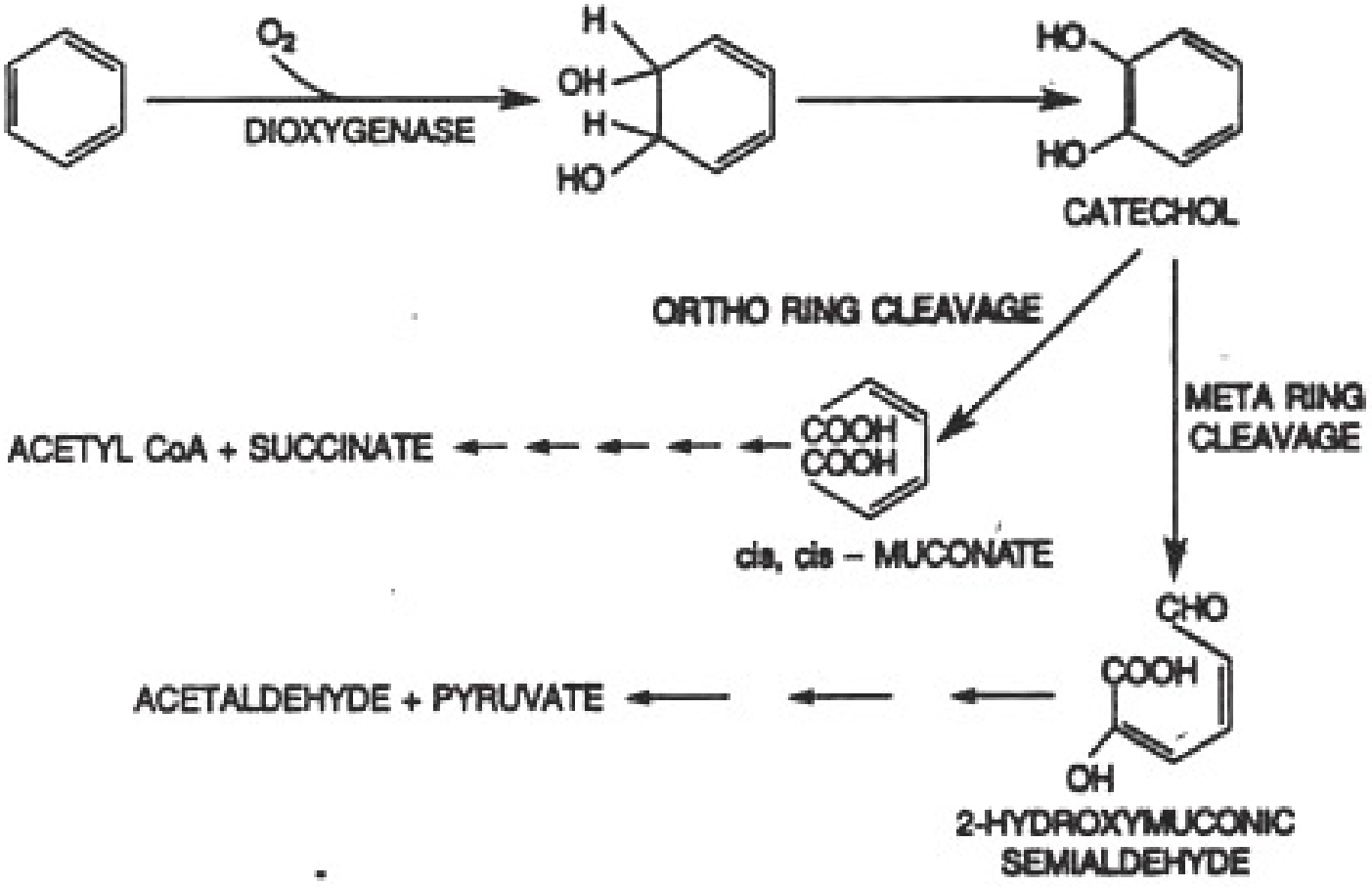
Alicyclic hydrocarbon, e.g., cyclohexane, is oxidised as follows:

- Alicyclic hydrocarbons are present naturally in waxes from plants, crude oil, microbial lipids etc. and are represented by xenobiotics used as pesticides and also in petroleum products.
- (i) First an oxygenase adds an —OH group in the ring,
- (ii) Then another oxygenase forms an ester in the form of a lactone,
- (iii) Which is then hydrolysed to open the ring structure to yield a linear molecule.
- In both these oxidations mono-oxygenases are involved which add oxygen to a single position in the molecule.



Aromatic hydrocarbon is oxidised as follows:

- Aromatic hydrocarbons are rather stable.
- **These are oxidised by di-oxygenascs (Fig.) to catechol which is further metabolised by two separate pathways:**
- (i) In case of ortho-ring cleavage pathway, a 1, 2- dioxygenase cleaves the ring between the two adjacent hydroxyl groups and sequential catabolism of the product cis, cis-muconate yields succinate + acetyl CoA.
- (ii) Alternatively, the enzyme 2, 3-di- oxygenase cleaves the ring between the carbon atom having an OH group and an adjacent carbon lacking an OH group (meta-cleavage); the products at the end of reaction sequence are acetaldehyde and pyruvate.
- Both ortho and meta pathways are involved in degradation of aromatic hydrocarbons.
- Benzene is degraded by the meta pathway.


O2
DIOXYGENASE
H
OH
H
HO
HO
HO
CATECHOL
ORTHO RING CLEAVAGE
META RING CLEAVAGE
ACETYL CoA + SUCCINATE
COOH
COOH
cis, cis – MUCONATE
CHO
COOH
OH
ACETALDEHYDE + PYRUVATE
2-HYDROXYMUCONIC SEMIALDEHYDE

Polycyclic Hydrocarbons

- Polycyclic hydrocarbons contain two or more rings. Generally, one of the terminal rings is attacked by a di-oxygenase, leading to ring cleavage and degradation so that in the end a single ring remains which is catabolised in a manner similar to that described for aromatic hydrocarbons.

Degradation of complex molecules containing aliphatic, aromatic, alicyclic or heterocyclic components is difficult to generalise but the following features are observed:

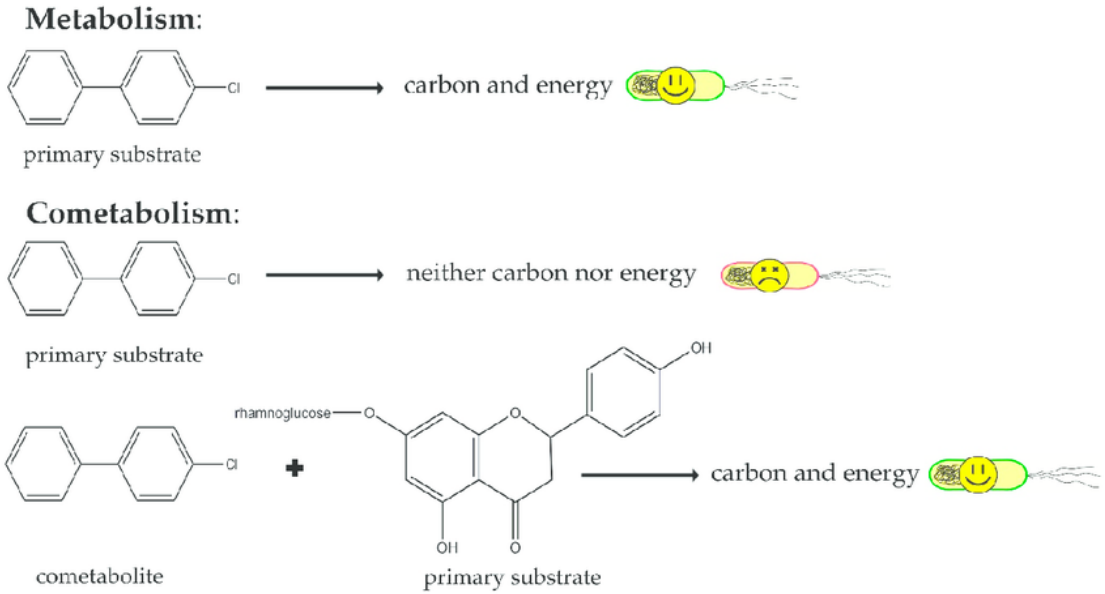
- Amide-, ester- or ether bonds are first attacked and further degradation of the products so generated takes place;
- If these bonds are absent or inaccessible; aliphatic chains are degraded;
- If aliphatic chains are branched, the aromatic component of complex molecules may be attacked,
- The site and mode of attack depends on the molecular structure, the micro-organism involved and the environmental conditions,
- In general, recalcitrance of various benzene derivatives increases with the substituent groups (at meta position) as follows: COOH = OH < $-NH_2$ < $-O—CH_3$ < $-SO_3^-$ < $-NO_2^-$.
- Further, the greater the number of substituent groups on the benzene ring, the higher the degree of recalcitrance.
- The position of substitution also affects recalcitrance as meta > ortho > para in recalcitrance.

Biodegradation of Halogenated Compounds

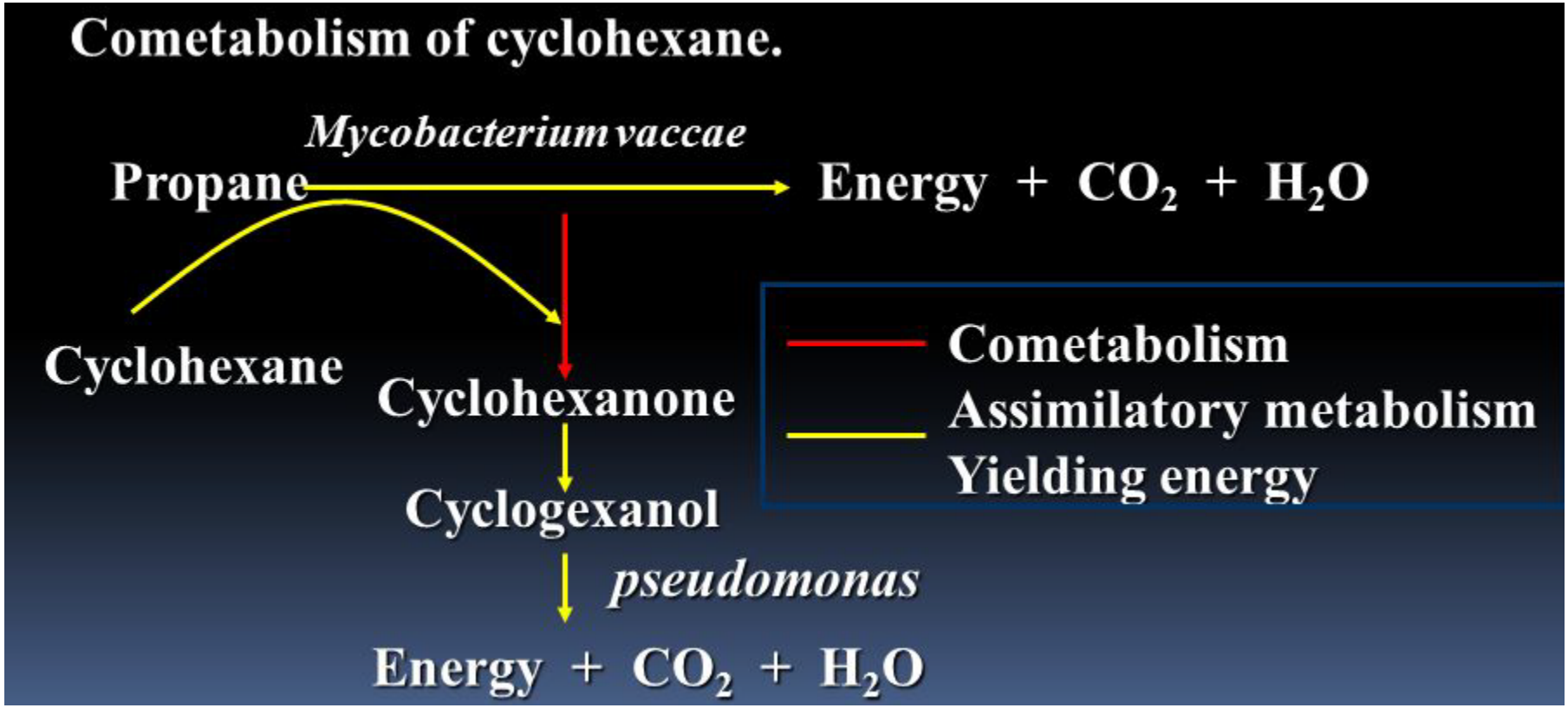
- **Biodegradation of such compounds involves two distinct steps:**
- (i) Elimination of the halogen groups, and
- (ii) Degradation of the non-halogenated product molecule.
- Removal of halogen molecule may occur either directly involving the removal of hydrogen halide (e.g., HCl), or it may involve the substitution of halogen by —H, -OH or a -thio group.
- The direct halogen removal produces a double bond and is relatively rate in nature.
- The mechanism involving halogen substitution, especially by —OH, is far more common particularly for fully reduced aliphatics or aromatics.

Co-Metabolism and Gratuitous Metabolism of Xenobiotic Compounds

- Some xenobiotic compounds, e.g., cyclohexane, halogenated compounds etc., are degraded by microbes, but these compounds are rarely, if ever, used as sources of energy and carbon by them.
- Degradation of such compounds, therefore, depends on the presence of another compound which induces the necessary enzymes, and metabolism of which provides both energy and reducing equivalents for the degradation of xenobiotic compounds (and the C, energy etc. needed for microbial growth).
- Clearly in such cases, degradation of xenobiotic compounds depends on the presence and metabolism of a suitable substrate called co-metabolite, such a degradation is called co-metabolism.



Co-Metabolism



… Co-Metabolism and Gratuitous Metabolism of Xenobiotic Compounds

- In contrast, several xenobiotic compounds are degraded by an existing pathway and are used by microbes as sources of energy and reducing equivalents; this is known as gratuitous metabolism.
- In such a metabolism, the necessary enzymes are already induced by another compound which is not needed as a co-metabolite.
- The xenobiotics degraded by both gratuitous and co-metabolism are very similar to the natural substrates of the enzymes involved in their degradation.
- Often a xenobiotic compound may not be completely degraded by gratuitous metabolism, but the product may be less polluting or may be used as substrate by some organisms.

The Origin of Capacity to Degrade Xenobiotics

- Continued exposure of microorganisms to xenobiotic compounds can often lead to the evolution of metabolic processes needed to wholly or partly degrade the xenobiotic.
- **These capabilities may arise due to:**
- (i) Mutation, and
- (ii) Transfer of plasmid borne genes.

Practical Approaches to Xenobiotic Degradation

- Biodegradation of xenobiotic compounds depends on their concentration (too high concentration may be toxic), pH of the medium, temperature, availability of water and other nutrients and presence of organic compounds (these may be co-metabolites, inhibitors or preferred substrate, in place of the xenobiotic, by microorganisms).
- In general, the xenobiotic compound should be available in an acceptable concentration and toxic levels should not occur.
- In a treatment system, a constant supply of the compound should be available for selective maintenance of microbes capable of its degradation.
- In addition, interfering organic compounds should not be present in the environment.
- **Practical application of microbes for xenobiotic degradation is facilitated by:**
- (i) Supply of sufficient nutrients or co-metabolites,
- (ii) Maintenance of the xenobiotic compounds to non-toxic levels and
- (iii) Provision of microbial population or inoculum.

Use of Mixed Populations

- **The use of mixed populations of microbes for degradation of xenobiotic compounds is desirable due to the following reasons:**
- (i) Two different microbes can together degrade a xenobiotic completely, while either of them alone is incapable of this feat. In such a case, the product of degradation by one microorganism serves as the substrate for the other.
- (ii) One microorganism may produce growth factor/nutrient required by the other. For example, *Nocardia* sp. degrades cyclohexane but is unable to produce biotin. A *Pseudomonas* sp. strain produces biotin but can not degrade cyclohexane.
- (iii) Co-culture may lead to plasmid transfer into a faster growing species thereby creating a faster growing species capable of degrading the xenobiotic.