ENVIRONMENTAL MICROBIOLOGY

Xenobiotics: Pollutants and their degradation-methane, benzene, pesticides, bioabsorption of metals

Indu Shekhar Thakur

School of Environmental Sciences,
Jawaharlal Nehru University
New Delhi-110 067

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Introduction

All substances originated into the environment either by biogenic or anthropogenic sources. Anthropogenic compounds describe synthetic compounds, and compound classes as well as elements and naturally occurring chemical entities which are mobilized by man's activities. A substance foreign to biological system is known as xenobiotic compound. Most of the xenobiotic compounds are degraded by microorganism may be defined as weak xenobiotic, however, few of them may persist longer in the environment and not easily degraded is known as recalcitrant compound (Figure 1). A xenobiotic is a chemical which is found in an organism but which is not normally produced or expected to be present in it. It can also cover substances which are present in much higher concentrations than are usual. The term xenobiotic is also used to refer to organs transplanted from one species to another. For example, some researchers hope that hearts and other organs could be transplanted from pigs to humans.
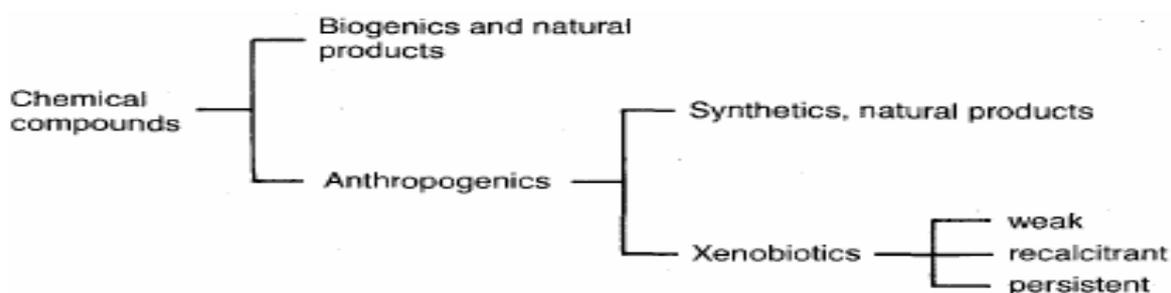

Figure 1: Origin of different types of chemical compounds in the environment

Xenobiotic substances are becoming an increasingly large problem in sewage treatment systems, since they are relatively new substances and are very difficult to remove from the environment. These substances are released into the environment in amounts that are unnatural due to human activity. The substances are composed of only about a hundred fundamental kinds of matter called element. Elements may be of environmental concern. The heavy metals e.g. lead, cadmium, mercury, are well recognized toxic substances in the environment. Elemental forms of essential elements may be very toxic or cause environmental damages. Elements are divided into metal and non-metals, a few elements with intermediate character are called metalloids. Two or more elements joined by chemical bonds are called compounds. Compounds may be inorganic or organic compounds. Inorganic and organic compounds may be divided depending upon presence of elements or group. Anthropogenic inorganic and organic pollutants are dispersed throughout the atmosphere, hydrosphere and lithosphere, and they have tendency to transform into another compounds which may be toxic, less toxic and not toxic to flora and fauna (Figure 2).

Elements and compounds undergo a number of processes in the environment viz. condensation, sedimentation, coagulation, reaction, diffusion, and scavenging. Condensation is the change in matter of a substance to a denser phase, such as a gas (or vapor) to a liquid. Sedimentation is the deposition by settling of a suspended material. Coagulation is the destabilization of colloidal particles brought about by the addition of a chemical reagent. Coagulation involves the interaction of a number of factors (coagulation factors) that lead to precipitation of suspended particles from a dispersed state. Diffusion is the movement of particles from an area where their concentration is high to an area that always has low concentration. Diffusion includes not only diffusion of particles, but all transport phenomena occurring within thermodynamic systems under the influence of thermal fluctuations. In

scavenging unwanted substances are removed from one place to another. Scavenger may be physical, chemical and biological in origin. During this processes chemicals react and transform into other compounds, persist longer in the environment, and not degraded easily, is known as recalcitrant compounds. Polyaromatic, nitrogen and halogen containing organic compounds are recalcitrant compounds which is difficult to degrade by microorganism. Such type of compounds have higher bioaccumulation and biomagnification potency when enters into the biotic entities. In bioaccumulation organism absorbs a substance at a rate greater than that at which the substance is lost. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. Biomagnification describes a process that results in the accumulation of a chemical in an organism at higher levels than are found in its food. It occurs when a chemical becomes more and more concentrated as it moves up through a food chain -- the dietary linkages between single-celled plants and increasingly larger animal species. Understanding the dynamic process of bioaccumulation and biomagnification is very important in protecting human beings and other organisms from the adverse effects of chemical exposure, and it has become a critical consideration in the regulation of chemicals.
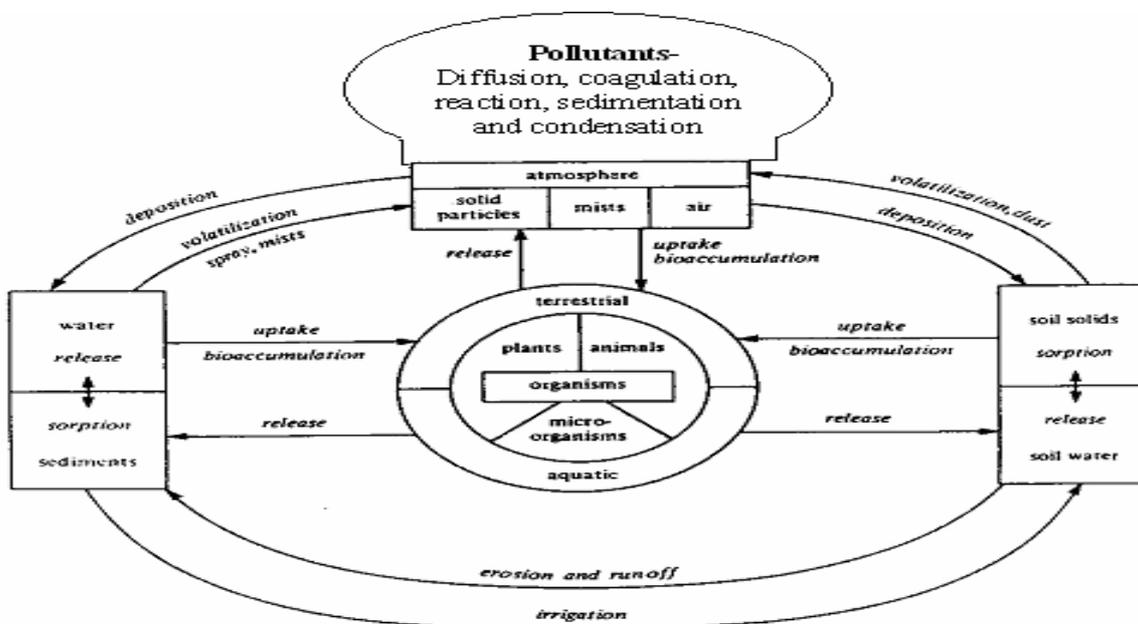

Figure 2: Interchange and transformation of pollutants in atmosphere, hydrosphere and lithosphere

Major sources of xenobiotic compounds enters into the environment are (i) chemical and pharmaceutical industries that produce a wide array of xenobiotics and synthetic polymers, (ii) pulp and paper bleaching, which are the main sources of natural and man made chlorinated organic compounds in the environment; (iii) mining, which releases heavy metals into biogeochemical cycles; (iv) fossil fuels (coal and petroleum), which may be accidentally released in large amounts into the ecosystem (oil spills) (v) 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. Due to their potential toxicity to both wildlife and humans, several persistent organic pollutants (POPs) have now been totally banned from production and use in many countries around the world.

In 2001, the Stockholm Convention under the auspices of United Nation Environmental Program (UNEP) specified a suite of POPs considered as potential endocrine disrupting chemicals (EDCs) (ecoestrogens) in the environment. Such concerns have heightened the need for novel and advanced bioremediation techniques to effectively remove POPs from a variety of contaminated environmental media including air, water, sediments and soils. Removal of such type of compounds is important.

Persistence of xenobiotic compounds and microorganism

The advent of modern chemical industry has resulted in the release of huge amounts of novel organic compounds, as industrial by-products, pesticides, other agrochemicals etc into the environment. These new compounds tend to resist biodegradation, with potential consequences such as persistence in the environment or bioaccumulation in food chains. The presence of 'artificial' groups such as chloro-, nitro- or sulfonate- in many synthetic chemicals makes them resistant to decomposition, as they are no longer recognized by the degrading microbes. The compounds are highly resistant to biodegradation is known as recalcitrant compounds.

Xenobiotic compounds are often toxic to life and are also often hard for microorganisms to metabolize (because they contain molecular arrangements that not normally encountered in nature). Thus, many of these compounds can accumulate in the environment and continue to be a hazard for many years. Microbes (bacteria and fungi) found in natural waters and soils have a very broad ability to utilize (catabolise) virtually all naturally occurring compounds as their sources of carbon and energy, thus recycling the fixed organic carbon back into harmless biomass and carbon dioxide. This capability of microbes has evolved over 3 billion years of the planets history and is responsible for the balance between photosynthesis (by plants and algae), fixing carbon dioxide into biomass, and respiration (by animals and bacteria), and converting organic compounds back to carbon dioxide by oxidation through natural detoxification processes. Recent advances in genetics and molecular biology in the last 20-30 years has shown that bacteria are genetically extremely adaptable, and in addition to the advantage due to their rapid growth rates, have a range of mechanisms which enable them to adapt to new environments. When compounds are persistent in the environment, their biodegradation often proceeds through multiple steps utilizing different enzyme systems or different microbial populations.

Degradation of chemical compounds

There are three requirements for biodegradation such as capable organisms, synthesis of requisite enzymes, suitable environmental conditions. In presence of recalcitrant compounds, the degradation initiated by induction of enzyme synthesis can be a problem, therefore, coordinate induction (through consortia of organisms) and co-metabolism i.e. transformation/degradation of a non-growth substrate in the obligate presence of another growth substrate are used. Co-metabolism is transformation of an organic compound by a microorganism incapable of using the substrate as a source of energy or of one of its constituent elements. The term "secondary substrate metabolism" is also in the literature - this means that the organism is actually growing on a second substrate and is transforming another substrate at the same without gaining benefit. This occurs, but is very difficult to detect and prove in natural environments, even though it can be demonstrated in pure cultures. It implies that many of the compounds co-metabolized are similar to normal substrates of the cells, differing sufficiently so that their immediate products cannot be further

metabolized. The bacterial population living together continues to resist their adverse condition to accomplish precise coordination of its functions to metabolize available substrate to use as sole carbon source. These microorganisms live in concerted way and coordinate physiological machinery through syntrophic relationship where one species of micro-organism (the recipient) uses, as a growth substrate, a waste product of the metabolism of another species. Syntrophy is the inability of either microorganism by itself to grow on a compound, yet when the two organisms work together they are able to both gain energy for growth from the compound. The syntrophic partnership is based on one partner maintaining the concentration of a product at an extremely low level so that product formation is not inhibited due to thermodynamic considerations.

Bacteria thriving in the environment are exposed to a range of physical and chemical signals that need to be processed to achieve a positive or negative physiological response. Microorganisms have evolved towards ecological fitness rather than biotechnological efficiency; thus, it would take a long time for bacteria capable of cleaning up anthropogenic pollution to evolve by natural selection. Hence, studying the physiology, biochemistry and genetics of the catabolic pathways becomes crucial to recreate and accelerate natural processes in the test tube as well as to accomplish their rational manipulation to design more efficient biocatalysts for different biotechnological applications. Mixed microbial communities have the most powerful biodegradative potential because the genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas. The genetic potential and certain environmental factors such as temperature, pH, and available nitrogen and phosphorus sources, therefore, seem to determine the rate and the extent of degradation.

A huge number of bacterial and fungal genera possess the capability to degrade organic pollutants. Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds. It is based on two processes: growth and cometabolism. In the case of growth, organic pollutants are used as sole source of carbon and energy. This process results in a complete degradation (mineralization) of organic pollutants. Cometabolism is defined as the metabolism of an organic compound in the presence of a growth substrate, which is used as the primary carbon and energy source.

The basic sequences in biodegradation of xenobiotics compounds by bacteria are cellular uptake of compounds, manipulation of substrate by ring fission, ring cleavage, conversion of cleaved product into standard metabolites and utilization of metabolites. Enzymes initiate the significant mechanism for degradation, which depends upon the nature and type of substrates. Oxygenases contain a variety of factors such as hemes, flavins, pterins, copper, and manganese that activate O_2 to the singlet state. Oxygenases (catalyze the incorporation of oxygen from O_2 into organic compounds), monooxygenases (incorporate only one <O> as OH and the other goes off as H_2O, also called hydroxylases), dioxygenases (incorporate both <O's>), reductive ring cleavage (anaerobic-ring reduction followed by ring cleavage, presence of O_2 speeds up process), modified ring cleavage, and dehalogenation like reductive dechlorination (anaerobic), hydrolytic dehalogenation and oxygenolytic dehalogenation (O_2 followed by spontaneous halide loss) are involved in degradation. Bacteria consume metabolites preferentially usually confronted with alternative carbon sources and metabolizing less preferred substrates, such as pollutants. Bacteria have developed a physiological response (superimposed regulation) that controls and adjusts the specific regulation of catabolic Operons to the physiological and metabolic state of the cells The genes responsible for biodegradation pathways are usually arranged in clusters that comprise: (i) catabolic genes encoding the enzymatic steps of the catabolic pathway; (ii) transport genes

responsible for active uptake of the compound; and (iii) regulatory genes that adjust expression of the catabolic and transport genes to the presence of the compound to be degraded. The catabolic clusters are usually present in mobile genetic elements, such as transposons and plasmids, which facilitate their horizontal transfer of the respective genes and, therefore, rapid adaptation of microorganisms to the presence of new pollutants in a particular ecosystem.

Specialized enzyme systems and metabolic pathways for the degradation of man-made compounds have been found in microorganisms isolated from geographically separated areas of the world. The genetic characterization of an increasing number of aerobic pathways for degradation of xenobiotic compounds in different bacteria has made it possible to compare the similarities in genetic organization and in sequence, which exist between genes and proteins of these specialized catabolic routes and more common pathways. Evidence is presented that a range of genetic mechanisms, such as gene transfer, mutational drift, and genetic recombination and transposition, can accelerate the evolution of catabolic pathways in bacteria. However, there is virtually no information concerning the rates at which these mechanisms are operating in bacteria living in nature and the response of such rates to the presence of potential (xenobiotic) substrates. Quantitative data on the genetic processes in the natural environment and on the effect of environmental parameters on the rate of evolution are needed.

Biodegradation of xenobiotic compounds primarily dependent upon many of the enzymes used in the pathways for degrading unusual substrates catalyse novel reactions. The initial intracellular attack of organic pollutants is an oxidative process, the activation and incorporation of oxygen is the enzymatic key reaction catalyzed by oxygenases and peroxidases. After those peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism, e.g., the tricarboxylic acid cycle followed by biosynthesis of cell biomass from the central precursor metabolites, e.g., acetyl- CoA, succinate, pyruvate (Figure 3).
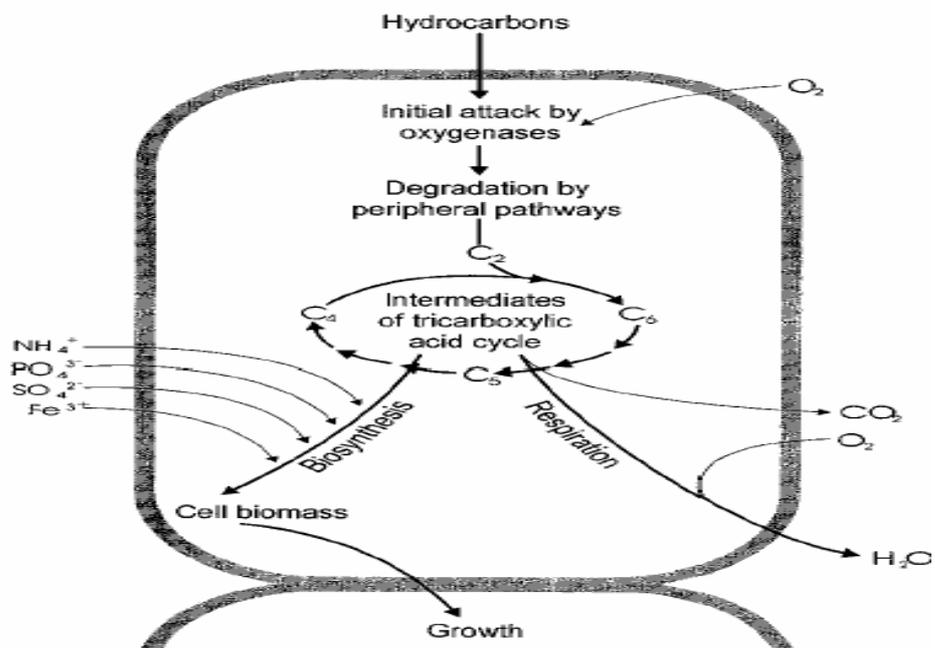

Figure 3: Fate of organic compounds in the uptake into the cells and degradation, assimilation and mineralization

Degradation of petroleum products

The most common sources of petroleum contamination are leaks in piping, leaks from corroded tanks, various equipment failures, overfill and spill while filling tanks and sudden accidental leakage. The petroleum products contaminate soil, ground water, surface water and air. The main components present in petroleum products are hydrocarbons, a significant portion of compounds contains nitrogen, sulphur and oxygen (NSO) and aromatic compounds. Some metals also present. In crude oil, chemical compounds may be divided into aliphatic hydrocarbon (e.g. alkanes, branched alkanes and alkenes), cycloaliphatic hydrocarbons (e.g. cyclohaxane), aromatic hydrocarbons and NSO compounds. These compounds entered in the environment not only from petroleum products but also agricultural, industrial, commercial, municipal, transportation and related activities. Some of them also formed unintentionally in the environment such as dioxin–like compounds formed in industrial and municipal sludge and in atmosphere. The compounds are degraded by aerobic and anaerobic microorganism contains catabolic enzymes and genes present either in plasmid or genomic DNA or both.

Degradation of n-alkanes

Alkanes originate from both biogenic and anthropogenic sources. Anthropogenic sources of *n*-alkanes include incomplete fossil fuel combustion, lubricant oils and biomass burning. Wind erosion of leaf epicuticular waxes, direct suspension of pollen, vegetation debris and microbial degradation are considered as the most important natural sources of particulate *n*-alkanes. Long-chain *n*-alkanes (C10 to C24) are degraded most rapidly. Short-chain alkanes (less than C9) are toxic to many microorganisms, but they evaporate rapidly from petroleum-contaminated sites. *Pseudomonas maltophilia, P. putida, Pseudomonas* sp. strain C12B, *Burkholderia cepacia, Acinetobacter* sp. strain ADP1 and *Acinetobacter* sp. strain M1 and *Nocardiodes* sp. strain CF8 are capable of efficient degradation of alkanes. Oxidation of alkanes is classified as being terminal or diterminal. The monoterminal oxidation is the main pathway. It proceeds via the formation of the corresponding alcohol, aldehyde, and fatty acid. ß-Oxidation of the fatty acids results in the formation of acetyl-CoA. *n*-Alkanes with an uneven number of carbon atoms are degraded to propionyl-CoA, which is in turn carboxylated to methylmalonyl-CoA and further converted to succinyl-CoA. Fatty acids of a physiological chain length may be directly incorporated into membrane lipids, but the majority of degradation products are introduced into the tricarboxylic acid cycle.
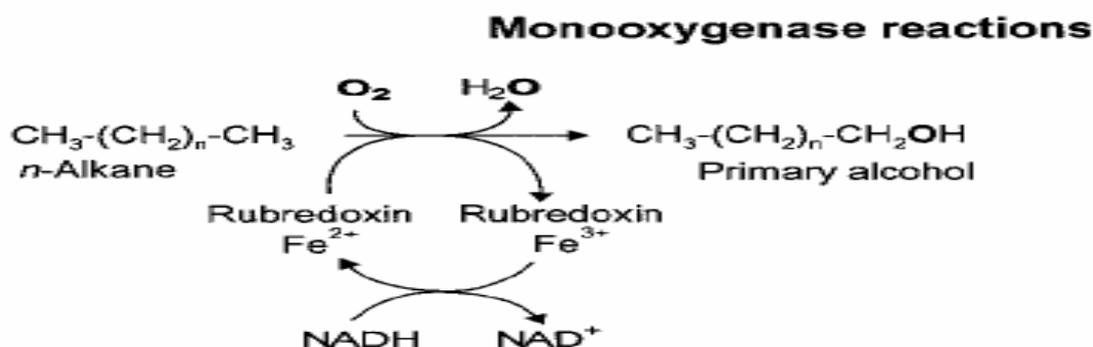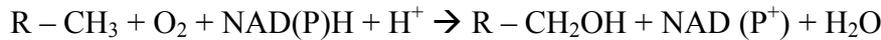

Figure 4: Degradation of n-alkane by monooxygenase enzyme initially to alcohol, which is converted to related aldehyde and acid, and finally to carbon dioxide
(Source: Ratledge, 1994)

The subterminal oxidation occurs with lower (C3 to C6) and longer alkanes with the formation of a secondary alcohol and subsequent ketone. n-Alkanes are readily degraded in both laboratory culture and the natural environment. The metabolic pathway initiated by a hydroxyloase (monooxygenose) enzyme to produce the corresponding alkane –1–ol:

$$R – CH_3 + O_2 + NAD(P)H + H^+ \rightarrow R – CH_2OH + NAD (P^+) + H_2O$$

n-Alkanes also metabolizedβ by dioxygenase enzyme but is less common. Subsequent metabolism may follow a number of pathways.
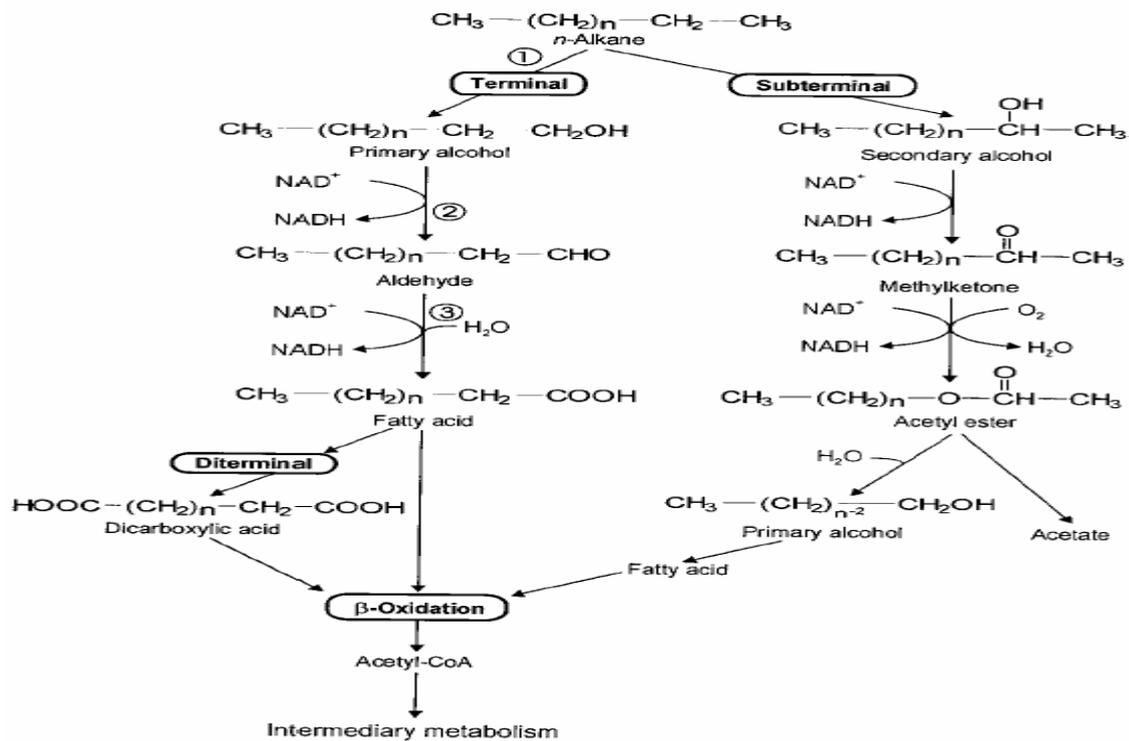

Figure 5: Degradation of n-alkane by terminal and sub terminal pathway
(Source: Ratledge, 1994)

Methanotrophic bacteria and methanogenic bacteria

Methanotrophs use oxygen to oxidize methane into carbon dioxide (CO_2). Methanotrophic bacterial systems have received a great deal of attention over the last ten years since it has been found that methane monooxygenase (the enzyme generated by Methanotrophs to react with methane) can degrade a wide variety of chlorinated hydrocarbons. The process is known as co-metabolism and is definitely an aerobic process. Methanogenesis is the process of degrading hydrocarbons with the end product being methane (CH_4) gas and carbon dioxide. The general reaction is as follows:

1. **$2C_{organic} + 2H_2O = CO_2 + CH_4$**

This is a strictly anaerobic process, methanogenic bacteria are poisoned by the presence of oxygen at levels as low as 0.18 mg/L of soluble oxygen (as O_2). The redox conditions under

which these two different microbial systems operate are literally at opposite ends of the spectrum i.e. Methanotrophic reactions occur at the Eh range of + 250 mV and Methanogenic reactions occur at the Eh range of - 200 mV. As an aside, methanogenic bacteria are one of the three classes of bacteria termed Archaea, which are representative of organisms that first appeared on Earth some 3.5 billion years ago. While their activity is inhibited by oxygen, these bacteria are robust enough to appear in a wide variety of natural locations such as: the intestinal tracts of ruminant mammals (cows etc.), sewage digesters, groundwater and soil. The precise mechanisms of hydrocarbon degradation under methanogenic conditions are not entirely understood by current researchers.

The genetics of aliphatic hydrocarbon degrading organisms are well characterized in the OCT plasmid that codes for a number of proteins involved in growth on n–alkanes ranging from C6 to C10. Chakrabarty and others reported initial studies in *Pseudomonas putida* Pp G6, and elucidated the fundamental pathways and genetic properties of the system. The metabolic pathway was found to involve an alkane hydroxylase (monooxygenase) and the alcohol so produced was further oxidized by an alcohol dehydrogenase and an aldehyde dehydrogenase to yield a fatty acid. The fatty acids are further oxidized by β-oxidation. *Psudomonas aeruginosa* containing OCT plasmid identified a number of loci on the plasmid and the chromosome that is involved in coding the pathway as indicated below:
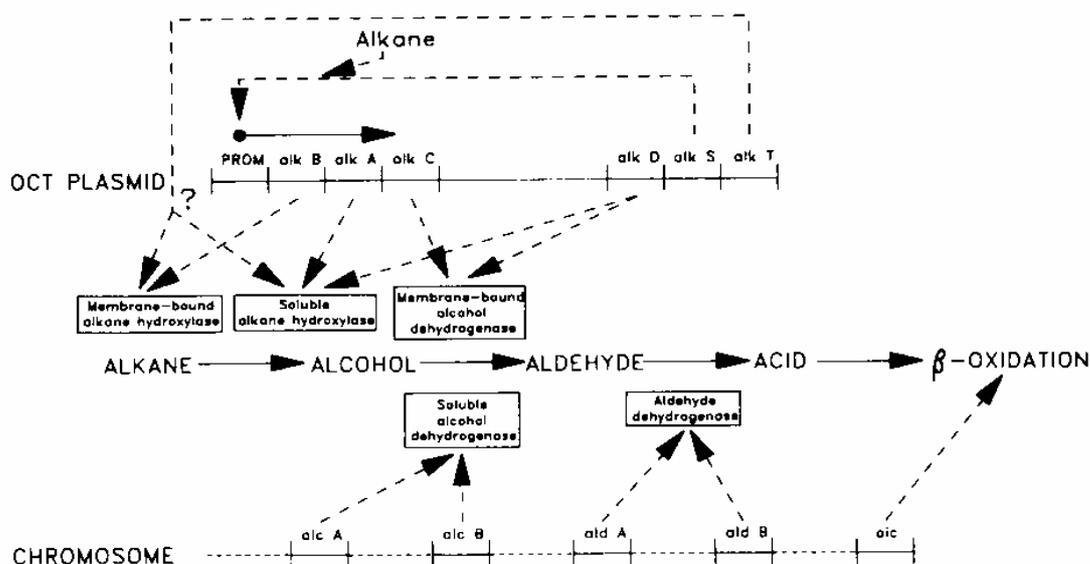

Figure 6: Role of genomic and plasmid DNA in degradation of n-alkane
(Ratledge, 1994)

Degradation of alkenes

Unsaturated 1- alkenes are oxidized at the saturated end of the chains. A minor pathway has been shown to proceed via an epoxide, which is converted to a fatty acid. Branching, in general, reduces the rate of biodegradation. Metabolic attack on saturated aliphatic hydrocarbons may be initiated either via attack on the double bond or by the same mechanism employed in n-alkane metabolism. Four main patterns of initial attack may be recognized:
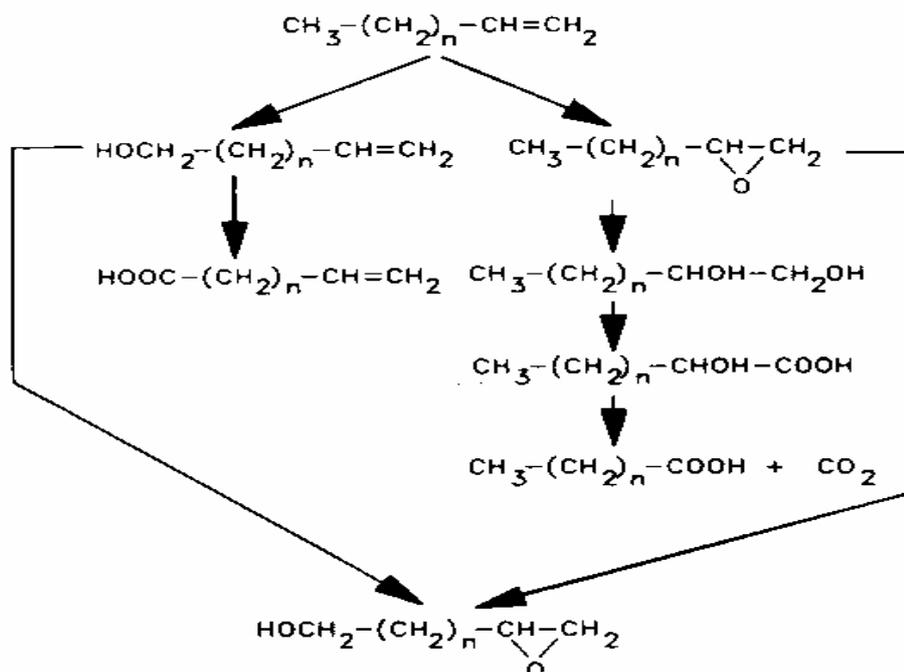
$$CH_3-(CH_2)_n-CH=CH_2$$

$$HOCH_2-(CH_2)_n-CH=CH_2 \qquad CH_3-(CH_2)_n-CH-CH_2$$
$$O$$

$$HOOC-(CH_2)_n-CH=CH_2 \qquad CH_3-(CH_2)_n-CHOH-CH_2OH$$

$$CH_3-(CH_2)_n-CHOH-COOH$$

$$CH_3-(CH_2)_n-COOH \ + \ CO_2$$

$$HOCH_2-(CH_2)_n-CH-CH_2$$
$$O$$

Figure 7: Basic metabolic pathways involved in the metabolism of alkenes
(Ratledge, 1994)

1. Oxygenase attack upon a terminal methyl group to produce corresponding alkene-1-ol,
2. Subterminal oxygenase attack to produce an alkenol with the hydroxyl group at a non-terminal carbon,
3. Oxidation across the double bond to give an epoxide,
4. Oxidation across the double bond to produce a diol.

Many microorganisms display more than one of these pathways. Alkynes can also be degraded aerobically but have been little studies. It is thought that hydratases are responsible for the initial metabolic attack.

Degradation of cycloaliphatic compounds

Cycloaliphatic compounds are present in large proportion in petroleum product and some oils. Cometabolism of cycloaliphatics has been reported using whole cells and cell extracts of alkanes and other hydrocarbon degraders. The initiation of co-metabolic involves the conversion of cycloaliphatics to alcohol or ketones by low specificity monooxygenase enzymes. The mechanism of cyclohexane degradation is shown in Figure 8.

Degradation of aromatic hydrocarbon

Aromatic hydrocarbons are ubiquitous in nature. The commercial, industrial, natural activities generate huge amount of aromatic hydrocarbons causes a great concern due to potential hazard to flora and fauna. Benzene, toluene, ethyl benzene, styrene and the xylenes are among the 50 largest-volume industrial chemicals produced with production figures of millions of tones per year. Diversity of aerobic and anaerobic biodegradation of aromatic

compounds has several common features. Aromatic compounds degradation is initiated by conversion of complex aromatic compounds to a "starting substrate". The initial reaction may involve the introduction of oxygen atoms by a monooxygenase and then formation of catechol. This phenomenon occurs in the aerobic catabolic funneling, and then most peripheral pathways involve oxygenation reactions carried out by monooxygenases and/or hydroxylating dioxygenases that generate dihydroxy aromatic compounds (catechol, protocatechuate, gentisate, homoprotocatechuate, homogentisate, hydroquinone, hydroxyquinol). These intermediate compounds are the substrates of ring-cleavage enzymes that use molecular oxygen to open the aromatic ring between the two hydroxyl groups (ortho cleavage, catalyzed by intradiol dioxygenases) or proximal to one of the two hydroxyl groups (meta cleavage, catalyzed by extradiol dioxygenases). A dioxygenase breaks open the aromatic ring of catechol, producing cis,cis-muconate, an unsaturated dicarboxylic acid. This product is then oxidized to acetyl-CoAs by the aforementioned beta-oxidation path. Catabolic plasmids occur naturally in aromatics hydrocarbons such as camphor, naphthalene, salicylate and other compounds. Most of the catabolic plasmids are self-transmissible and have a broad host range.
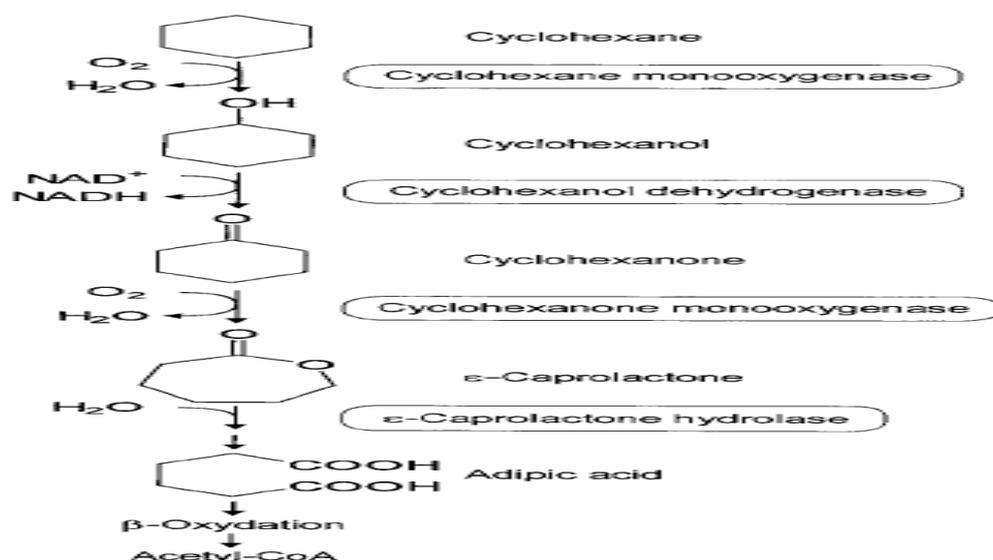

Figure 8: Metabolic pathway involved in the degradation of cyclohexane. The hydroxylase enzyme responsible for the initial metabolic step was detected in *Pseudomonas* sp.
(Source: Ratledge, 1994)

Benzene

There are few reports on the bacterial degradation of benzene. The excellent studies carried out in the previous three decades elucidated the pathway identified the intermediates and characterized the enzyme systems. The first step of benzene oxidation is a hydroxylation catalyzed by a dioxygenase. The product, a diol, is then converted to catechol by a dehydrogenase. These initial reactions, hydroxylation and dehydrogenation, are also common to pathways of degradation of other aromatic hydrocarbons. The introduction of a substituent group onto the benzene ring renders alternative mechanisms possible to attack side chains or to oxidize the aromatic ring. Two divergent pathways employed both share the same initial mode of attack resulting in formation of catechol which is further degraded by either catechol 1,2-dioxygenase (ortho or intradiol-cleavage) and subsequently via β-Ketoadipate pathway,

or catechol 2,3-dioxygenese (meta or extradiol cleavage). Both routes have been described in different benzene initializing strains. Benzene intilizing bacteria, *Acinetobacter calcoacetions* RJE74, carries a large plasmid (pww174) encoding the enzyme for the metabolism of benzene via the β -Ketoadipate pathway.
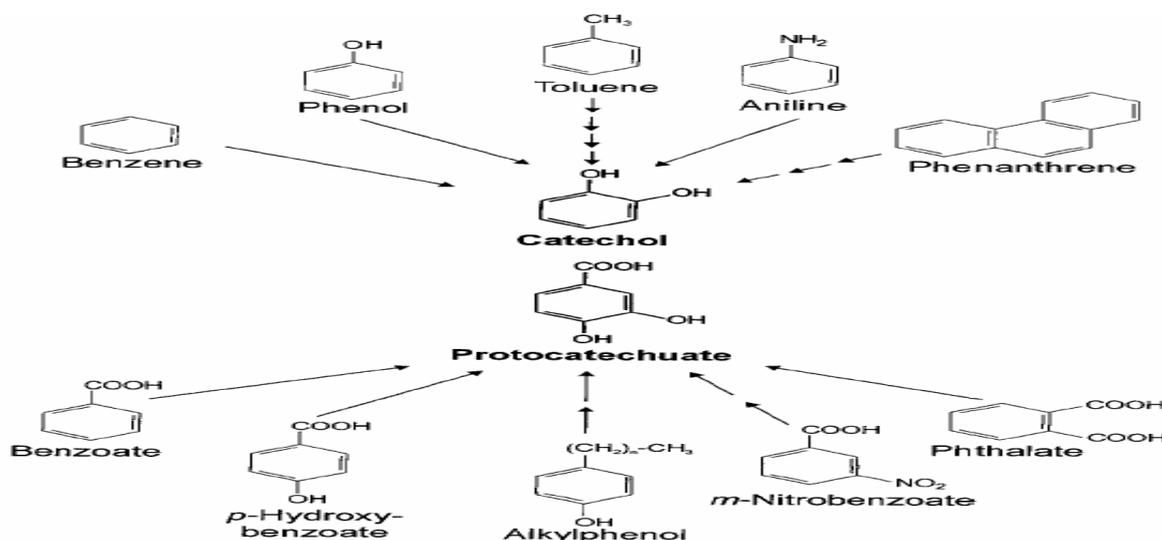

Figure 9: Degradation of aromatic compounds by funneling pathway and central pathway
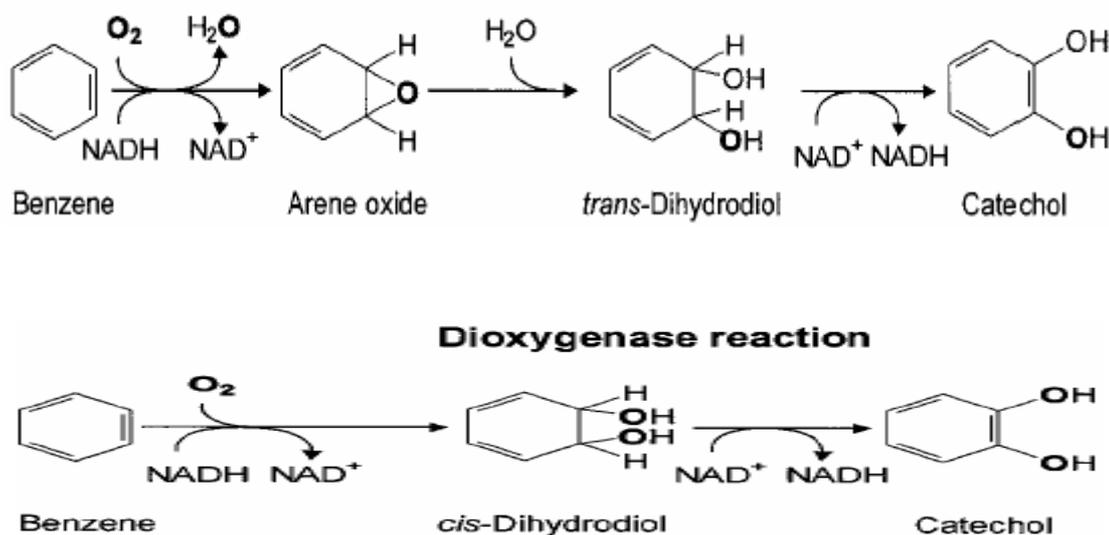

Figure 10: Monooxygenase and dioxygenase reaction in degradation of benzene
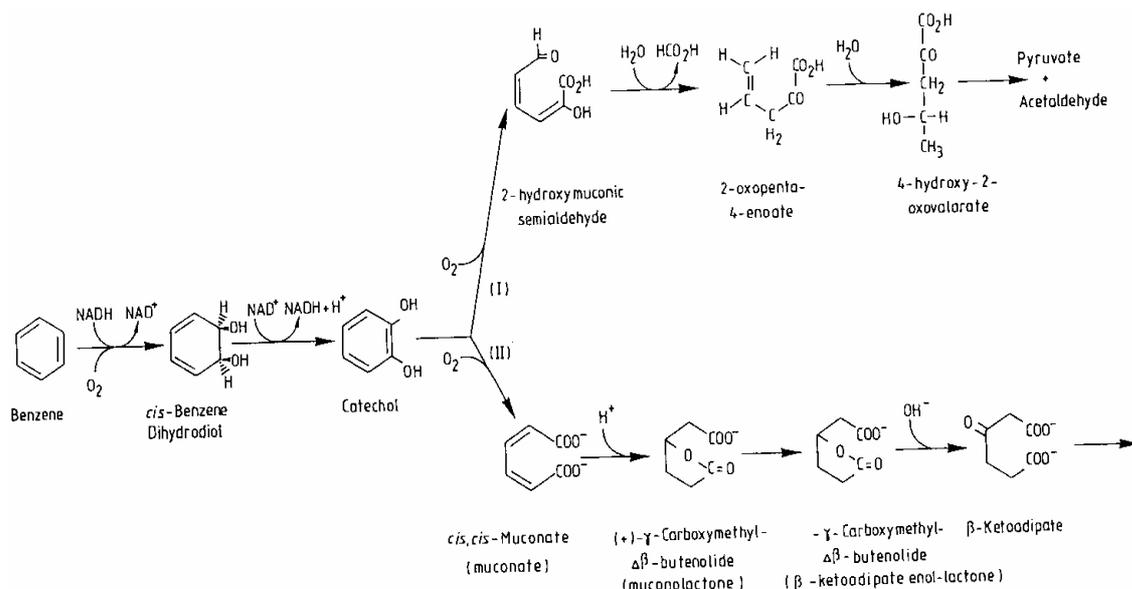*(Ratledge, 1994)*

Figure 11: The biodegradation route of benzene. Benzene is converted into Catechol via cis-Benzene Dihydrodiol to catechol which is finally degraded by ortho (II) or meta ring cleavage (I) *(Source: Ratledge, 1994)*

Degradation of chlorinated organic compound

The recalcitrance of organic pollutants increases with increasing halogenation. Substitution of halogen as well as nitro and sulfo groups at the aromatic ring is accomplished by an increasing electrophilicity of the molecule. These compounds resist the electrophilic attack by oxygenases of aerobic bacteria. Halogenated organic compounds constitute one of the largest groups of environmental chemicals including pesticides. The industrial production of new halogenated organic compounds has increased throughout the last century and these compounds are integral to a variety of industrial applications.

A critical step in the degradation of organohalides is cleavage of the carbon-halogen bond, and microorganisms have evolved a variety of metabolic strategies for dehalogenation. The natural production and anthropogenic release of halogenated hydrocarbons into the environment has been the likely driving force for the evolution of an unexpectedly high microbial capacity to dehalogenate in presence of different classes of xenobiotic haloorganics.

Dehalogenation of aromatic compounds

There are three classes of dehalogenation:
1. Oxidative dehalogenation

In this process the halogen substituents are lost fortuitously during oxygenation of the ring. A number of proteobacterial pure culture isolates of the genera Thauera, Pseudomonas, and Ochrobacterium completely mineralized chlorinated aromatic compound as a sole source of carbon and energy conditions has been repeatedly observed for mixed cultures
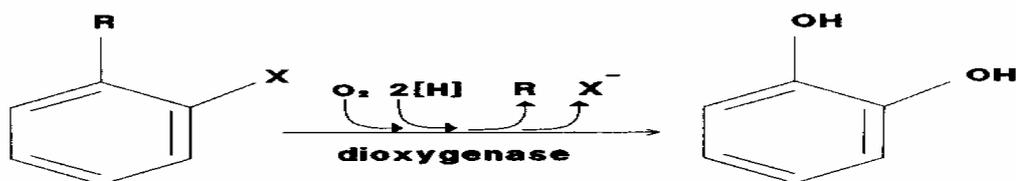
Figure 12: Oxidative dehalogenation processes *(Source: Ratledge, 1994)*

2. *Hydrolytic dehalogenation*

In this case a hydroxyl group specifically replaces halogen substituent. The source of the oxygen atom in the hydroxyl group is water instead of oxygen. This reaction can occur under both aerobic and denitrifying conditions. e.g. dehalogenation of 4-chlorobenzoate to form 4-hydroxybenzoate by *Arthrobacter* and *Pseudomonas*.
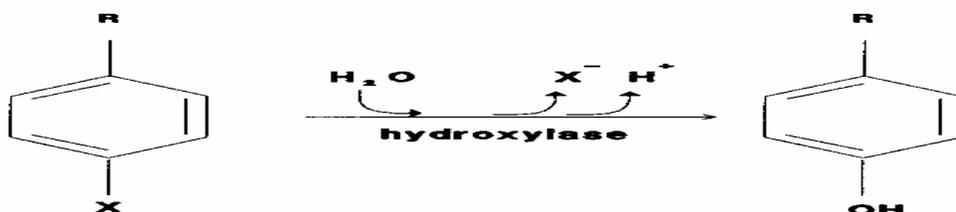

Figure 13: Hydrolytic dehalogenation processes *(Source: Ratledge, 1994)*

3. *Reductive Dehalogenation*

Microbial means to dehalogenate organohalides under anaerobic conditions by a reductive mechanism can be largely divided into abiotic, or cometabolic, and metabolic conversion. While the latter is found in halorespiring bacteria (HRB), which couple the reductive dehalogenation reaction by specific, high-affinity biocatalysts to microbial growth, the former is proposed to be catalyzed mostly by metal ion-containing heat-stable tetrapyrroles or enzymes, in which these compounds are incorporated as cofactors. More than a decade ago, the isolation and characterization of the δ-proteobacterium *Desulfomonile tiedjei*, able to couple the reductive dehalogenation of 3-chlorobenzoate to energy conservation, set the stage for the unraveling of this novel type of energy metabolism in anaerobic microorganisms.
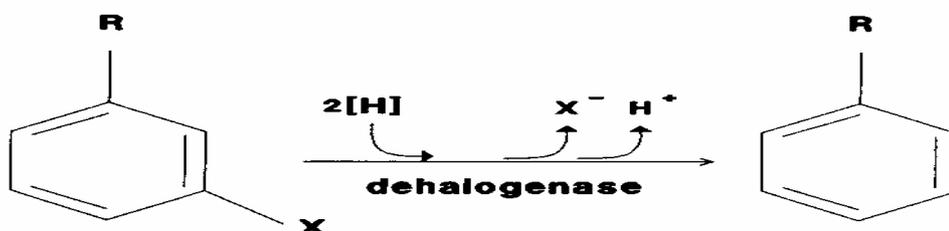

Figure 14: Reductive dehalogenation reaction
(Source: Ratledge, 1994)

Dehalogenation in phototrophic bacteria

A few examples that phototrophic bacteria, including *Rhodospirillum* and *Rhodopseudomonas* spp., can grow phototrophically under anaerobic conditions using halocarboxylic acids or 3-chlorobenzoate have been reported .

Degradation of halogenated aromatic compounds

Chlorinated aromatic compounds are widely used as pesticides, industrial applications, and produced unintentionally as trace contaminants during the industrial production of chlorinated compounds and incineration of chlorine-containing waste. Brominated aromatic compounds have found as flame-retardants, and florinated and iodinated aromatic compounds have pharmaceutical applications. The chemical inertness and hydrophobicity of these compounds has resulted in widely distribution in the environment. The important compounds are halogenated benzoic acid halogenated benzene, halogenated phenols, halogenated anilines, halogenated phenoxyacetic acids, halogenated biphenyls and halogenated dibenzo-p-dioxins and dibenzofurans.

Degradation of halogenated benzoic acids

Aerobically, halogenated benzoates are degraded by initial dioxygenation of the aromatic ring to yield halocatechol. Ring cleavage of these compounds takes place most efficiently by othro, or β-ketoadipate, route yielding halo-cis, cis-muconates. Meta, or extradiol, cleavage of halocatechol is formed by catechol- 2,3-dioxygenase to yield degradable metabolites. Meta cleavage of 3-halocatechols gives productive halogenated semialdehydes and 5-chloroformyl-2-hydrooxypenta-2,4-dieonates. The latter compounds probably find irreversibly to basic groups of the dioxygenese, which results in inactivation of the enzyme. Under anaerobic conditions halobenzoates are reductively halogenated. Genes for the complete mineralization of 3-chlorobenzoate via the modified ortho cleavage pathway are known to be located on the plasmid pWR1 (pB13) from *Pseudomonas* sp. strain B13 of molecular size 11 kb, plasmid pAC25 or pAC27 from *Pseudomonas putida* AC and on the plasmid pJP4 from *Alcaligenes eutrophus* JMP134.
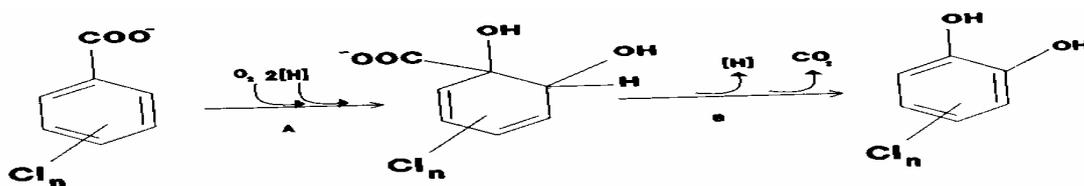

Figure 15: Aerobic degradayion of chlorobenzoate to chlorocatechol. The enzymes benzoate dioxygenase (A) and benzoate dihydrodioldehydrogenase (B) involved in the reactions *(Source: Ratledge, 1994)*

Degradation of halogenated phenols

Chlorinated phenols are used on large scale as wood preservatives, fungicides, herbicides, and general biocides. They may be mono, di, tri, tetra, penta and hexa chlorophenols. The biodegradation of chlorophenols takes place by three main pathways. In general mono and di-chlorophenols are converted into chlorocatechol by monooxygenase, whereas higher chlorinated phenols are hydroxylated to form chlorinated hydroquinones. Under anaerobic

conditions, chlorophenols undergo initial reductive dechlorination. The first three enzymes of the pentachlorophenol (PCP) degradation pathway in *Sphingobium chlorophenolicum* (formerly *Sphingomonas chlorophenolica*) have been characterized, and the corresponding genes, *pcpA*, *pcpB*, and *pcpC*, have been individually cloned and sequenced. To search for new genes involved in PCP degradation and map the physical locations of the *pcp* genes, a 24-kb fragment containing *pcpA* and *pcpC* was completely sequenced. The four gene products PcpB, PcpC, PcpA, and PcpE were responsible for the metabolism of PCP to 3-oxoadipate.
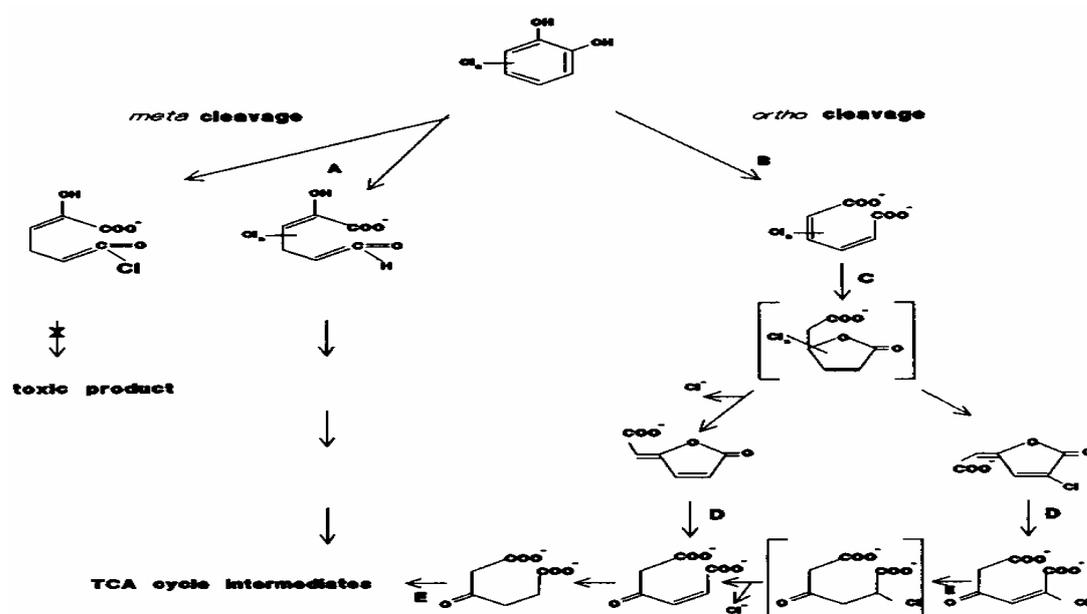

Figure 16: Metabolic route for aerobic degradation of chlorocatechols. A, catechol-2,3-dioxygenase; B, catechol-1,2-dioxygenase; C, muconate cycloisomerase; D, dienelactone hyrolase, and E, maleylacetate reductase involved in degradation of chlorocatechol
(Source: Ratledge, 1994)

Degradation of halogenated phenoxyacetic acid

The herbicides 2,4 dicholorphenoxy acetic acid (2,4 D) and 2, 4, 5-trichlorophenoxyacetic acid (2, 4, 5-T) have been used for more than 40 years. In general, biodegradation of 2,4-D takes place via initial cleavage of other bond, followed by hydroxylation of the resulting dichlorophenol to chlorocatechols. The chlorocatechol intermediates formed from the chlorinated compounds are degraded by the ortho cleavage. The genes encoded this pathway are located on a 80kb plasmid. The genes for 2,4-D degradation is present in three operon: tdf A gene coding for 2,4-dicholophenoxyacetic acid monoxygenese, the tdf B gene coding for 2,4-dichlorophenol hydroxylase and the tdf CDEF genes coding for the modified ortho cleavage pathway.

Degradation of halogenated anilines

Chlorinated anilines are used as intermediates in the synthesis of pesticides. Degradation of these compounds proceeds by initial dioxygenation to form holocatechols catalyzed by an aniline oxygenase. Further degradation is via the modified ortho cleavage pathway. Reductive dehalogenation of chloroanilines takes place under anaerobic conditions.

Degradation of halogenated benzenes

Chlorobenzenes are used as solvents, fumigants and as intermediates in dye production and pesticides. Chlorobenzene is degraded via dioxygenation to form chlorocatechol that is further degraded by the ortho cleavage pathway. The genes involved in degradation of chlorobenzene in strain P51 are located in two clusters on a 110kb plasmid. The tcbA and tcbB genes are encoding the degradation of chlorocatechol present on a transposable element. The second cluster consists of tcbC, tcbD and tcbE genes, coding for type II catechol 1,2-dioxygenase, cycloisomenase and hydroxylase enzymes. The transposable elements may have played a role in the transfer of the first two genes and the evolution of this catabolic pathway.

Degradation of halogenated biphenyls

Polychlorinated biphenyls are used as non-inflammable heat transfer finds as dielectric fluids in capacitors and transformers, hydraulic fluids and plasticizers in paints. The ability to degrade PCB is found in several genera of Gram positive and Gram negative aerobic bacteria. In most cases, chlorobenzoates accumulate as end products. Biphenyl degraded by meta cleavage of the resulting chlorocatechol can lead to toxic end products, only when enzymes for the ortho cleavage route of chlorocatechols are induced then it involved in the degradation. The enzymes involved in the degradation of chlorinated biphenyls to benzoate are encoded in genes located on both on chromosomes and on plasmids. At least four genes are involved in the degradation of PCBs. Gene bphA codes for the biphenyl dioxygenase, gene bphb encodes the dihydrodial dehydroxygenase, gene bphc encodes the 2,3-dihydroxybipheyl dioxygenase and gene bphd codes for the 2-hydroxy-6-oxo-6 phenyl hexa – 2,4-dieonate hydrolase. The evidences for anaerobic degradation of PCBs by dechlorination have been reported.

Degradation of halogenated dibenzo-p-dioxins and dibenzofurans

Dioxin like compound is formed unintentionally in the environment that is in highly toxic and recalcitrant. Very slow oxidative degradation of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (2,3,7, 8- TCDD) has been reported. Dibenzo-p-dioxin-utilizing bacteria, *Sphingomonas* sp. strain RW1 was isolated from enrichment cultures inoculated with water samples from the river Elbe. The isolate grew with both the biaryl ethers dibenzo-p-dioxin and dibenzofuran (DF) as the sole sources of carbon and energy. Biodegradation of the two aromatic compounds initially proceeded after an oxygenolytic attack at the angular position adjacent to the ether bridge, producing 2,2',3- trihydroxydiphenyl ether or 2,2',3-trihydroxybiphenyl from the initially formed dihydrodiols, which represent extremely unstable hemiacetals. A key enzyme in the degradation pathways of dibenzo-p-dioxin and dibenzofuran, namely, 2,2',3-trihydroxybiphenyl dioxygenase, which is responsible for meta cleavage of the first aromatic ring, has been genetically and biochemically analyzed. The dbfB gene of this enzyme has been cloned from a cosmid library of the dibenzo-p-dioxin- and dibenzofuran-degrading bacterium *Sphingomonas* sp. strain RW1.

Bioabsorption of metals

Bioabsorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals. Bioabsorbents are prepared from naturally abundant or waste biomass of algae, fungi, moss or bacteria that have been killed. Biosorption is possible by both living and nonliving

biomass. Biosorption is a rapid phenomenon of passive metal sequestration by the non-growing biomass. Bioaccumulation is a growth dependent process and biosorption involves mechanisms like ion exchange, chelation, and complexation inorganic precipitation may occur by hydrolysis and inorganic deposition via adsorption by physical forces and ion entrapment in inter and intra-fibrillar capillaries and spaces of the structural polysaccharide network as a result of diffusion through cell walls and membranes. Several active groups of cell constituents like acetamide group of chitin, structural polysaccharides of fungi amine, sulphahydral and carboxyl group in protein, phosphodiester (teichoic acid), phosphate, hydroxyl in polysaccharides participate in biosorption. Although biosorption is promising, its mechanism is not well elucidated. Microbial cells can accumulate heavy metals, radionuclides and organmetalloid compounds by a variety of process both physicochemical and biological. There are various mechasim through which microorganism uptake heavy metals (Figure 17).
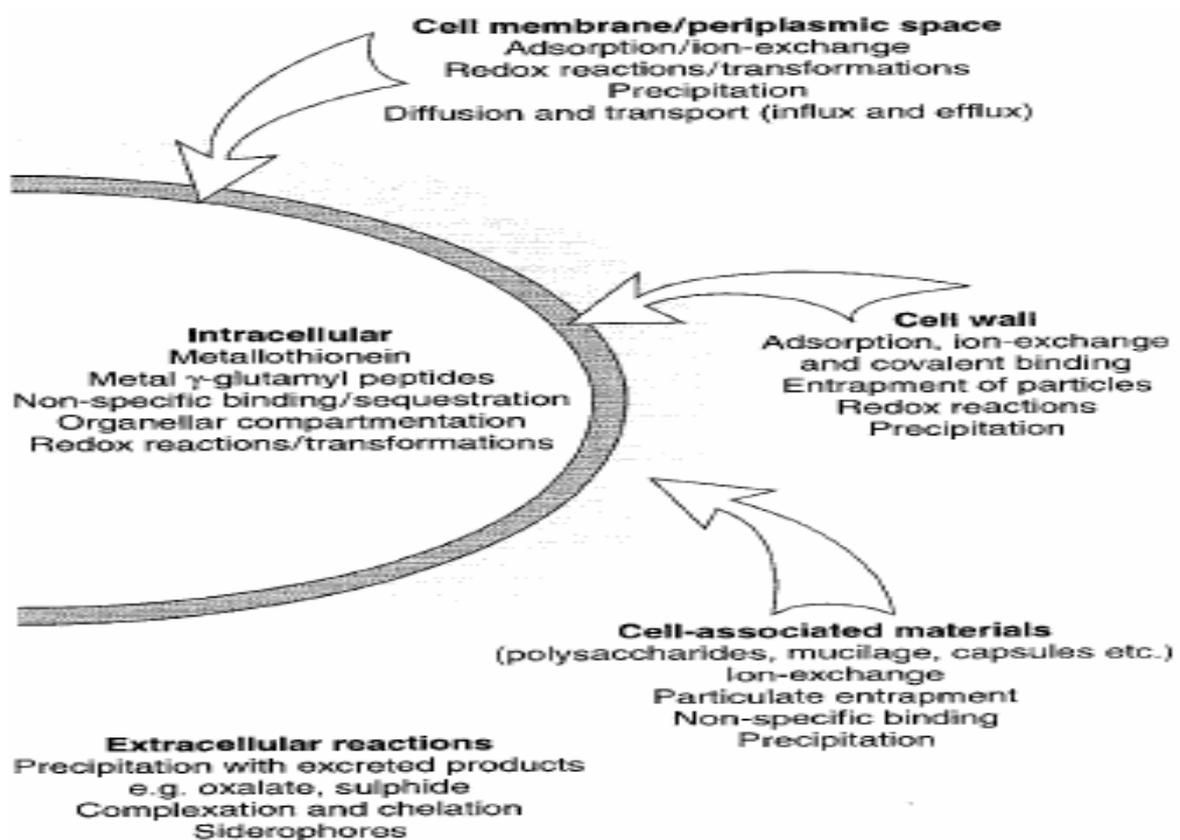

Figure17: Role of microorganism in uptake of metal and detoxification
(Source: Thakur, 2006)

Heavy metals

There is no general definition of heavy metals. "Heavy metals" are chemical elements with a specific gravity that is at least 5 times the specific gravity of water. There are 53 metals with a density above 4-5 g/cm^3 reported as heavy metals, but it's better to consider them from their physiological effects and toxicity. At high concentrations, metals form unspecific complex compounds in the cell, which lead to various toxic effects depending on the metal and the micro-organism considered.

Fe, Mo, Mn	Low toxicity
Zn, Ni, Cu, V, Co, W, Cr	Average toxicity
As, Ag, Sb, Cd, Hg, Pb, U	High toxicity

Microorganism in metal absorption

Biosorption by Fungi

Among microorganisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal binding properties. Polysaccharides, in association with lipids and proteins, represent the main constituent of fungal cell wall. In filamentous fungi outer cell wall layers mainly contain neutral polysaccharides (glucans and mannans). While the inner layers contain more of glucosamines (chitin and chitosan) in a microfibrillar structure, ligands within these matrices include carboxylate, amine phosphate, hydroxyl, sulphydral and other functional groups. Proteins are also found to be associated with metal binding. *Rhizopus, Aspergillus, Streptoverticillum* and *Saccharomyces* are important fungi used for metal biosorption.

Biosorption by algae and moss

Photoautotrophs marine algae have bulk availability of their biomass from water bodies. Special polysaccharides are present in the algae cell wall contained potential metal ion binding sites. The number and kind of binding sites depend on the chemical composition of the cell wall. In Pheophycean members, algin is present and contributes significantly to metal binding. It was been suggested that the polysaccharides of cell wall could provide amino and carboxyl group as well as the sulphate. The amino, carboxyl group and the nitrogen and oxygen based moieties could also form coordinated band with metal ion. Metal ion could also be electrostatically bonded to unprotonated carboxyl oxygen and sulphate covalent bonding between divalent cation and algae cell wall proteins has also been reported.

Mechanisms such as entrapment of metal both in the form of insoluble micro deposits in the inter and intra-fibrillar capillaries and paracrystalline regions of polysaccharides and the binding to other biopolymers (RNA, Polyphosphates) can contribute to the metal binding**.** The photoautotrophs, eukaryotic algae cell wall are mainly cellulosic and potential metal binding groups are carboxylate, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. Of these amine and imidazoles are positively charged when protonated and build negatively charged metal complexes. The amino and carboxyl groups and nitrogen and oxygen of the peptide bonds are also available for coordination bonding with metal ions such as lead (II), copper (II) and chromium (IV).

Biosorption by bacteria

Bacteria may carry determinants of resistance to a number of heavy metals. Bacterial resistance to heavy metals is conferred by specific resistance determinants, which are often, but not always, carried on plasmids or transposons. Resistance is specific to one or a few metals and the mechanisms of resistance include efflux of the metal, modification of the speciation of the metal, sequestration of the metal, or a combination of these mechanisms. Cell walls of bacteria and cynobacteria are principally composed of peptidoglycans, N

acetylglucasamine, β 1-4 acetyl muramic acid with peptide chains. Cell wall of gram-negative bacteria is not heavily cross-linked. They have an outer membrane which is composed of an outer layer of lipopolysaccharides (LPS), phospholipids and proteins. Gram-negative bacteria are more widespread in metal contaminated soils then gram-positive bacteria. The anionic nature of bacterial surface enables them to bind metal cations through electrostatic interactions. Because of their thickness and anionic character which is mainly due to peptidoglycan, teiochoic acid and teichuronic acids the cell wall of gram positive bacteria has high capacity for metal binding. *Bacillus subtilis, B. licheniformis, Pseudomonas* sp., *Serratia mercascens, Pseudomonas aeruginosa, Zooglea ramigera* and *Streptomyces* sp. are widely used for metal removal from effluent.

Mechanisms of bacterial metal resistance

There are 4 mechanisms of bacterial metal resistance
1. Keeping the toxic ion out of the cell (reduced uptake)
2. Highly-specific efflux pumping (i.e. removing toxic ions that entered the cell by means of transport system evolved for nutrient cations or anions). Efflux pumps can be either ATPases or cheiosmotic driven. ATPases are enzymes that use the chemical energy from cleavage of the high-energy phosphodiester bond of ATP to drive the formation of concentration gradients;
3. Intra or extracellular sequestration by specific mineral-ion binding components (e.g. metallothioneins) and/or segregation into complex compounds.
4. Enzymatic detoxification (oxydoreductions) which convert a more toxic ion to a less toxic one.

The first two mechanisms can be grouped under the term avoidance, whereas the last two are known as sequestration mechanisms. It is important to notice that mechanisms of metal tolerance might be adaptations of the processes of normal homeostasis. Homeostasis describes the fact that cells have processes to monitor and maintain intracellular concentrations of metals. So, metal homeostasis must involve uptake of sufficient essential metals while providing protection against their toxicity.

Mechanism of uptake
The first barrier to penetration is the wall, which provides some protection for the cytoplasmic membrane. Cell walls, especially those of fungi, can be used as biosorbents. However, walls cannot act as a perfect barrier to entry of some ions that are essential trace elements for micro-organisms. So, the metal ion is first transported into the cytoplasm in spite of its high concentration, which is the first reason why metal ions are toxic.

Mechanism of efflux
Efflux pumps reduce the intracellular concentration of metals by means of transport systems, without any enzymatic transformation. This mechanism is more widespread than enzymatic detoxification. Uptake and efflux mechanisms can be classified in 8 protein families approximately: the most important are the ABC family (ATP Binding Cassette), the P- and A-type ATPases family, the RND family (Resistance, Nodulation and cell Division) and the MIT family (Metal Inorganic Transport).

Plasmid resistance
Bacterial plasmids contain genes that provide extra functions to the cells, among which resistances to toxic metals. Plasmids are small circular DNA molecular that can move from

one cell to another. Thus, the transfer of toxic metal resistance from one cell to another is facilitated. This is why, most of the time, resistance systems are found on these plasmids, but some systems are determined by chromosomal genes in other organisms. Plasmid-determined resistance systems are very specific.

Metallothioneins and binding
Bacterial metallothioneins (MT) are commonly grouped in 3 classes. Class I and II are gene-encoded, whereas class III is not. Metallothioneins are low molecular mass, cysteine-rich metal-binding proteins, homologous to mammalian metal binding proteins. They have bind divalent cations, such as Ca^{2+}, Cu^{2+} and Zn^{2+} so tightly that harm is avoided (sequestration).

Metal binding peptides
Enhance bioabsorbents has been developed by creating a repetitive metal-binding motif consisting of (Glu-Cys) Gly. These peptides emulate the structure of phytochelatin, metal chelating molecules that play a major role in metal detoxifications. The phytochelatin analogues were presented on bacterial surfaces, enhancing Cd2+ and Hg2+ bioaccumulation by 12 fold to 20 fold respectively.

Genetic engineering for specific protein
The highly specific metal absorbing proteins is the result of a clearly designed genetic circuit that is tightly under the control of gene which are regulatory proteins used for controlling the expression of enzyme. The high affinity and selectivity of MerR toward mercury has been exploited for the construction of microbial biosorbent specific for mercury removal. Presence of surface-exposed MerR on an engineered strain enabled 6 fold higher Mg2+ binding. Similarly, cell overexpressing ArsR accumulated 5- and 60-fold higher level of arsenate and arsenite.

Factors effecting bioabsorption

Effect of pH
It is now well established that heavy metals are taken up from water predominantly by ion exchange. Carboxyl and sulphate groups have been identified as the main metal-sequestering sites in seaweed and, as these groups are acids, its availability is pH dependent. At pH in the range 3.5-5.5 these groups generate a negatively charged surface, and electrostatic interactions between cationic species and this surface can be responsible for metal biosorption.

Effect of temperature
Temperature has not been studied as a relevant variable in biosorption experiments. The tests are usually performed at approximately 25-30ºC. A slight increase in cation uptake by seaweed in the range of 4 to 55ºC has been reported.

Size of bioabsorbent
The influence of biosorbent size on metal biosorption has been evaluated. The experimental results indicate that the biosorbent size influence the capacity and rate of biosorption. This is so because size grading of ground biomass particle by standard sieves works on the length and width dimensions. Larger biomass particles of *Sargassum fluitans* and *Ascophylum nodosum* had higher metal uptake than smaller particles in the case of cadmium, copper, nickel, lead and zinc. Then, the influence of biosorbent size on metal uptake seems to be a function of both the type of biomass and the metal ion.

Inorganic salts

A mineral salt solution added to the medium enabled growth of bacteria at higher concentrations of added metal. This could be interesting to compare their results for another metal, or to find other suitable salts. 4 different cadmium salts, $Cd(NO_3)_2$, $CdCl_2$, $CdSO_4$ and CdO on the resistance of different Gram-positive and Gram-negative species has been studies in which $CdCl_2$ was the most toxic cadmium salt whereas $Cd(NO_3)_2$ was efficiently absorbed.

Biorectors in bioabsorption

The use of freely suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficulty in separating biomass and effluent. Therefore, process parameters are optimized for bioabsorption of heavy metals with various types of bioreactor. Various modifications are made by using immobilized biomass particles in the bioreactors, and based on that bioreactors are divided into following types:

Packed bed reactor (PBR)

The most important characteristic of a PBR is that material flows through the reactor as a plug; they are also called plug flow reactors (PFR). Ideally, all of the substrate stream flows at the same velocity, parallel to the reactor axis with no back -mixing. All material present at any given reactor cross -section has had an identical residence time. The longitudinal position within the PBR is, therefore, proportional to the time spent within the reactor; all product emerging with the same residence time and all substrate molecule having an equal opportunity for reaction. There are three substrate flow possibilities in the reactors downward flow, upward flow and recycling flow method. The conversion efficiency of a PBR, with respect to its length, behaves in a manner similar to that of a well -stirred batch reactor with respect to its reaction time. Immobilized, living biomass has primarily taken the form of biofilms on supports prepared from a range of inert materials. Supports include agar, cellulose, alginates, cross-linked ethyl acrylate, ethylene glycol dimethylacrylate, polyacrylamide and silica gel. The biomass may be used in its 'natural state', or modified, for example, by alkali treatment, to improve biosorption efficiency. Small-scale systems may be adequate for low-volume waste containing valuable elements such as gold.
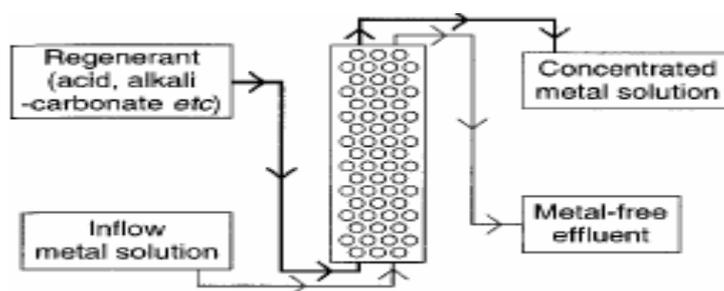

Figure 18: Packed bed reactor in bioabsorption of heavy metals. The recycling method is upward flow where concentrated metals can be recovered
(Source: Thakur, 2006)

Fluidized bed reactors

It is very important to process engineering because of its excellent heat and mass transfer characteristics. In a fluidized-bed reactor, the substrate is passed upward through the bed at a high enough velocity to lift the particles. This causes some mixing, more than the piston-flow

model in the packed-bed reactor, but complete mixing as in the CSTR model. This type of reactor is ideal for highly exothermic reactions because it eliminates local hot-spots, due to its mass and heat transfer characteristics. It is most often applied in immobilized-enzyme catalysis where viscous, particulate substrates are to be handled. Fludized beds of alginate- and polyacrylamide-immobilized algae; for example, *Chlorella vulgaris* and *Spirulina platensis,* have been used to remove a variety of metals, including Cu^{2+}, Pb^{2+}, Zn^{2+} and Au^{3+}, from mixtures, and several schemes for selective recovery. The localization of some of the processes, especially enzymes and redox reactions is uncertain (postulated locations are indicated) and may vary between groups, strains and species of both prokaryotic and eukaryotic organisms.
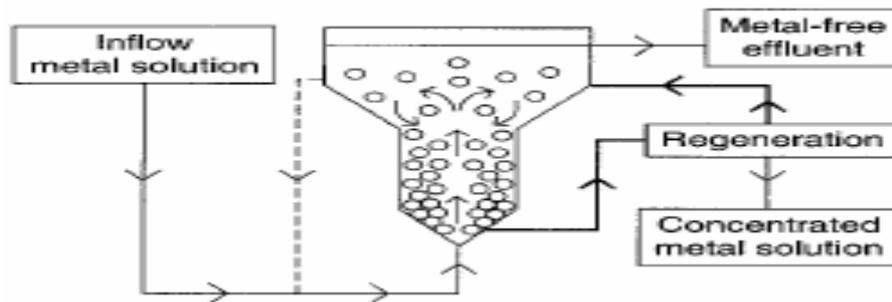

Figure 19: Fludized bed reactor in bioabsorption of metals. A fluidized-bed reactor is a combination of the packed-bed and stirred tank, continuous flow reactors
(Source: Thakur, 2006)

Rotating disc reactor (RDR)
A large-scale commercial process treats effluents from metal mining and milling using rotating-disc biofilm-contacting units for simultaneous degradation of cyanide, thiocyanate and ammonia, as well as metal removal by biosorption. The RDR as biocontactor combines advantages of the aerobic rotating biological contactor: high biomass concentration, short hydraulic retention time, low-energy consumption, operational simplicity, and advantages of the anaerobic process: no oxygen transfer limitation, low quantities of waste biological solids.
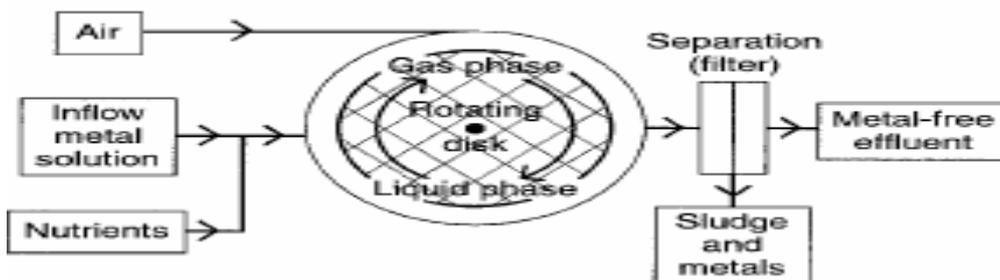

Figure 20: Rotating disc reactor (RDR) in bioabsorption of heavy metals. The constant rotation of the disc causes mixing of the liquid, and at the same time the disc surface alternately comes into contact between air and waste water acting as an aeration device for waste water treatment and removal of metals
(Source: Thakur, 2006)

Single blanket reactor

Hydrogen sulphide is produced by sulphate-reducing bacteria (SKBs), such as *Desulphovibrio* and/or *Desulphotomaculum* sp. Sulphate-reducing activity can occur as a result of anaerobic biomass decay in biosorption systems, and thus act as a useful auxiliary metal-removing mechanism. This plant successfully removed toxic metals and sulphate from contaminated ground water at a long-standing smelter site by precipitation of the metal as metal sulphides.
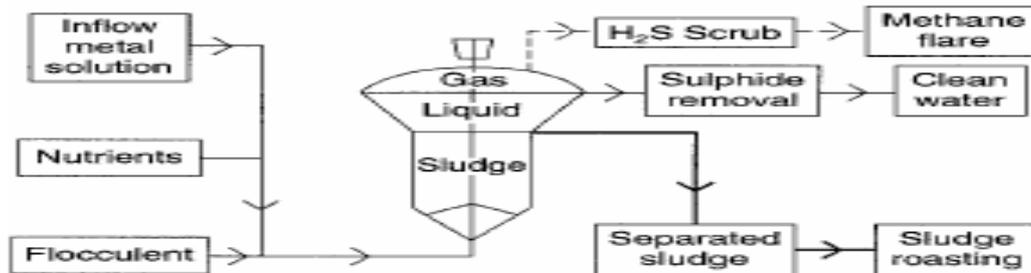

Figure 21: Sludge blanket reactor for bioabsorption of heavy metals in which flow of metal solution in upward flow combined with the settling action of gravity suspends the blanket with the aid of flocculants. Small sludge granules begin to form whose surface area is covered in aggregations of bacteria and sulphide is removed
(Source: Thakur, 2006)

Sequential reactor

In sequential bioreactor two or more bioreactor may be connected to increase the efficiency of the metal absorption. Sometime reactor-types can be mixed i.e. the use of a biofilm reactor in conjunction with a tandem stirred-tank reactor. Living-cell in its 'natural state', or modified are used to improve biosorption efficiency. Small-scale systems may be adequate for low-volume waste containing valuable elements such as gold.
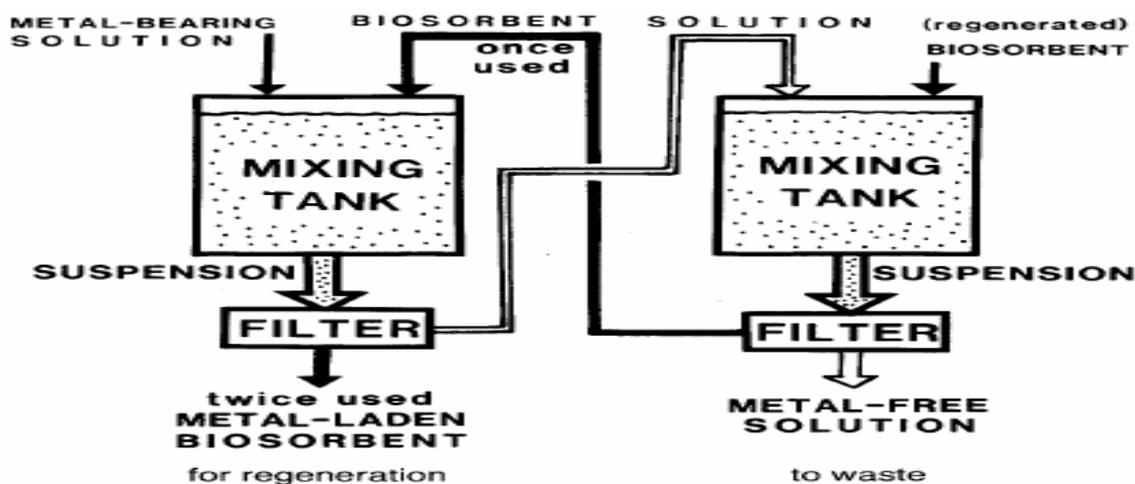

Figure 22: Sequential bioreactor for absorption of heavy metals in which metal is mixed in presence of biosorbent (1st reactor) and regenerated biosorbent is used in 2nd reactor for efficient recovery of metals
(Source: Thakur, 2006)

Phytoremediation

Phytoremediation of metal contaminated soils offers a lower cost method for soil remediation and some extracted metals may be recycled for value. Both the phytoextraction of metals and phytovolatilization of Se or Hg by plants offer great promise of commercial development. Natural metal hyperaccumulator phenotype is much more important than high yield ability when using plants to remove metals from contaminated soils. The hypertolerance of metals is the key plant characteristic required for hyperaccumulation; vacuolar compartmentalization appears to be the source of hypertolerance of natural hyperaccumulator plants. Alternatively, soil Pb and Cr^{6+} may be inactivated in the soil by plants and soil amendments (phytostabilization). Little molecular understanding of plant activities critical to phytoremediation has been achieved, but recent progress in characterizing Fe, Cd, and Zn uptake by *Arabidopsis* and yeast mutants indicates strategies for developing transgenic improved phytoremediation cultivars for commercial use. Because the costs of growing a crop are minimal compared to those of soil removal and replacement, the use of plants to remediate hazardous soils is seen as having great promise; other recent reviews on many aspects of soil metal phytoremediation are available. Phytoremediation is the use of plants to make soil contaminants non-toxic and is often also referred to as bioremediation, botanical-bioremediation, and Green Remediation. The idea of using rare plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced in 1983, gained public exposure in 1990, and has increasingly been examined as a potential practical and more cost effective technology than soil replacement, solidification, or washing strategies presently used. Categories of phytoremediation include phytoextraction (the use of plants to remove contaminants from soils), phytovolatilization (the use of plants to make volatile chemical species of soil elements), rhizofiltration (the use of plant roots to remove contaminants from flowing water) and phytostabilization (the use of plants to transform soil metals to less toxic forms, but not remove the metal from the soil). The use of plants and associated rhizosphere organisms or bioengineered plants to metabolize toxic organic compounds also appears promising. Phytostabilization appears to have strong promise for two toxic elements, chromium and lead. Reduction of Cr^{6+}, which poses an environmental risk, to Cr^{3+} which is highly insoluble and not demonstrated to pose an environmental risk, by deep rooted plants can be very effective

Conclusion

Human activities have brought about widespread pollution of the natural environment in the form of heavy metals, and inorganic and organic compounds. A number of organic pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated aromatic compounds and nitrogen containing aromatic compounds are resistant to degradation and represent an ongoing toxicological threat to both wildlife and human beings. Over recent years, a growing number of potential hazards linked to the ubiquitous presence of POPs in the environment have been reported. Bioremediation is an attractive alternative to traditional physicochemical techniques for the remediation of these POPs at a contaminated site, as it can be more cost-effective and it can selectively degrade the pollutants without damaging the site or its indigenous flora and fauna. However, despite being hailed as a panacea to the safe and effective solution to contaminated environmental media, bioremediation technologies, to date, have had limited applications due to the challenges of substrate and environmental variability, as well as the limited biodegradative potential and viability of naturally occurring microorganisms. Microorganism with suitable and stable genetic traits, and efficient and effective biodegradation processes would be helpful for clean and green environment.

Suggested Reading

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