Biodegradation of Xenobiotic compounds

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 Abstract

Xenobiotic compounds are chemical compounds that are produced by man and by the environment also but in very low concentrations. If these compounds are getting accumulated in the environment, then they will reach toxic concentrations and gives harmful effects on our environment. We need to reduce its direct or indirect use in our day-to-day life. We should also think about its degradation so that it won’t get accumulated in our environment. For doing this we should first know about it, its chemical nature, its degradability according to its chemical composition, its biodegradation by different microorganisms and the metabolic pathways within the microorganism, and the metabolic complications which arise because of the complexity of the compounds. All the above information will help us to boost the existing metabolic pathways of microorganisms and also to genetically modify the metabolic pathways to keep our environment somewhat clean and healthy. here we are going to discuss all the related information like-what is the basic meaning of xenobiotic compounds, their effect on metabolic pathways of a microorganism according to its chemical composition like the presence of halogens, cyclic groups, branched-chain, etc., hazards from xenobiotic compounds like toxicity, carcinogenicity, etc. After knowing its chemical composition and hazardous effect we will discuss the bacteria which are involved in the biodegradation of these compounds and the general features of its biodegradation, then we will discuss the co-metabolism and gratuitous metabolism of these compounds by microorganisms this information on co-metabolism will help us to boost the metabolic pathways involved, then we will discuss little the origin and capacity to degrade xenobiotic compounds because of mutation and plasmid transfer among the bacteria.

After gathering all of this information we will discuss the practical approaches to degrade these xenobiotic compounds like using mixed culture etc.

Keywords

Xenobiotic compounds, complexity, biodegrading pathways, metabolism, co-metabolism

Introduction

Meaning of xenobiotic compounds-

Xenobiotic compounds are chemicals that are present in the environment at unnaturally high concentrations and are man-made. The xenobiotic compounds are either not produced naturally, they are produced at much lower concentrations by the environment than man.[1]

Xenobiotics have been defined as chemicals to which an organism is exposed that are extrinsic to its normal metabolism. Without metabolism, many xenobiotics would reach toxic concentrations which are harmful to the environment. Most metabolic activity inside the cell requires energy, cofactors, and enzymes to degrade these compounds.[2] A xenobiotic is a chemical that is not used by the reference organism as a nutrient chemical, is not essential to the reference organism for the maintenance of normal physiologic or biochemical function and homeostasis, and

does not constitute a part of the conventional process of chemicals synthesized from nutrient chemicals by that organism in normal intermediary metabolism.[3]

Here we will come to see how these xenobiotic compounds are degraded in the environment, their effect on the environment, how important the microorganisms are in the degradation of these compounds, and how the compound's complexity affects their metabolism inside bacterial cells.

Metabolic effects of xenobiotics on microorganisms

Microorganisms can degrade all naturally occurring compounds; which is known as the principle of microbial infallibility which was given by Alexander in 1965.[1]

Microorganisms are also able to degrade many of the xenobiotic compounds, but they are not capable of degrading all of them. The compounds that resist biodegradation by these microorganisms and therefore persist in the environment for a long time are called recalcitrant.[1]

The xenobiotic compounds may persist for a long time because of the following reasons:

(i) They are not recognized as a substrate by the degradative enzymes which are already present in the environment,

(ii) They are very stable, i.e., chemically and biologically inert because of the presence of some substitution groups such as halogens, nitro-, sulphonate, amino-, methoxy- and carbamoyl groups, or (He, Ne, AR, Kr, Xe, Rn)

(iii) They may be insoluble in water naturally in the environment, or are adsorbed to external matrices like soil,

 iv) They are highly toxic or may give rise to toxic products because of microbial activity present naturally in the environment

(v) They may have a large molecular size that prevents their entry into microbial cells,

(vi) Compounds may be unable to induce the synthesis of degrading enzymes, and

(vii) Lack of the perm-ease enzyme needed for compounds to be transported into the microbial cells.

(v) Some oil mixture and

(vi) Some others.[1]

There are some structural features also that make these compounds resistant to microbial degradation, these are as follows:

(i) Presence of halogens in the place of hydrogen in the molecule; or the carbon-halogen bond may be highly stable and its cleavage requires a high amount of energy,

(ii) Substitution of H by other groups like nitro-, sulphonate, methoxy-, amino- and carboxyl groups which are not degraded easily,

(iii) Cyclic structures, aromatic compounds, cycloalkanes, and heterocyclic compounds are more arrogant than linear chain or aliphatic compounds which are degraded easily,

(iv) Branched linear chains are also not easily degraded etc.

In the environment, the more complex the structure of a xenobiotic compound, the more arrogant it is for biodegradation. Many xenobiotics resist biodegradation by microorganisms due to their large molecular size and insolubility in water as discussed earlier.

(i) Halocarbons:

These compounds contain different numbers of halogen (e.g., CI, Br, F (fluorine, Halocarbons, I) atoms in the place of H atoms. They are used in our day-to-day life as solvents (chloroform, CHCI3), as propellants in spray cans of cosmetics, paints, etc., in condenser units of cooling systems (Freons, CCI3F, CCl2F2, CClF3, CF4), and as insecticides used to kill insects (DDT, BHC, lindane, etc.) and herbicides to kill herbs in farms (dilation, 2, 4-D, 2, 4, 5-T, etc.).

The C1-C2 haloalkanes like chloroform and freons are volatile and escape into the atmosphere after mixing in the atmosphere they reach the protective ozone (O3) layer and disrupt it leading to increased UV radiation. Pesticides (herbicides, fungicides, and insecticides) are applied to crops from where they get mixed into water bodies; and this leads to bio-magnification which also affects the bird egg.[5]

(ii) Polychlorinated Biphenyls (PCBs):

These compounds have two covalently linked benzene rings which have halogens substitution for Hydrogen. PCBs are mainly used as plasticizers, insulator coolants in transformers, and as heat exchange fluids, these are both biologically and chemically inert to various degrees, this inertness and arrogant nature increases with the number of chlorine atoms present in the molecule.

The persistent nature of the above two groups of compounds is due to their halogenation and their cyclic structure (PCBs) [5].

(iii) Synthetic Polymers:

These compounds are produced as plastics, e.g., polyethylene, polystyrene, polyvinyl chloride, etc., and nylon these plastics are mainly used as garments, wrapping materials, etc. They are persistent in nature mainly due to their insolubility in water and molecular size and because of this they are not easily permitted in microbial cells. [5]

(iv) Alkyl benzyl Sulfonates:

These alkyl benzyl sulfonates are surface-active detergents superior to the soaps that we use. These compounds affect the degradation in two ways -1) The sulphonate (— SO3–) group which is present at one end resists microbial degradation,2) The other end (non-polar alkyl end) becomes recalcitrant if it is branched, (resistance increases with the degree of branching). At present, alkyl benzyl sulphonates having non-branched alkyl ends are used; these are easily biodegradable and are degraded by β-oxidation from their alkyl ends.[5]

(v) Oil Mixtures:

Oil is a natural product and is produced naturally, it has many components and is biodegradable, but since it has different components, these different components get degraded at different rates. Biodegradation can handle small oil seepages as nature can tolerate a limited amount of any such substances so, when large spills occur the problem of pollution becomes acute. Oil is not degraded in higher amounts mainly because of its insolubility in water and due to the toxicity of some of its components.

(vi) Other Xenobiotic Compounds:

Several pesticides are based on aliphatic, cyclic ring structures containing a substitution of nitro-, sulphonate, methoxy-, amino- and carboxyl groups; in addition, they also contain halogens and as written earlier these substitutions make them recalcitrant for nature and arrogant to be degraded by microorganisms. [5]

Hazards from Xenobiotic Compounds:

The xenobiotics have several potential hazards to man and the environment these are briefly listed below.

(i) Toxicity:

Many xenobiotics like halogenated and aromatic hydrocarbons are toxic to bacteria, lower eukaryotes, and humans also. At low concentrations they may cause various skin problems and reduce reproductive potential at higher concentrations they may cause more complexity.

(ii) Carcinogenicity:

Certain halogenated hydrocarbons are carcinogenic that is they can cause cancer

(iii) Many xenobiotics are recalcitrant and persist in the environment for a long time so they build up in their concentration with time.

(iv) Many xenobiotics like DDT and PCBs are recalcitrant and lipophilic; because of this arrogant nature, they show bioaccumulation or bio-magnification (often by a factor of 104 – 106).

Bio-magnification occurs mainly because of the following two reasons:

(i) These arrogant compounds are continuously taken up from the environment and since they are not degradable, they accumulate in the lipid deposits of the body, for example, a 100-fold accumulation of DDT by plankton from the water, and from here they enter in the food chain.

(ii) Such organisms are sequentially consumed by other organisms in the food chain, e.g., plankton → small fish → large fish → sea-eagles; the concentration of xenobiotics builds up as we move up in the food chain as it gets accumulated in every organism.

In the case of sea eagles 105- a fold increase of DDT occurs as compared to the concentration present in the aqueous environment because of this increase sea eagles laid fragile eggs. DDT and PCBs have been found in human tissues in high but sub-lethal concentrations in those countries where these chemicals are used, although humans were often not in direct contact with these chemicals, they come in indirect contact through the food chain.

(iii) They are produced and used in large quantities for our doings which favors their accumulation in nature.[1]

Bacteria involved in the degradation of xenobiotic compounds

 Microorganisms generally apply two modes of action for the degradation of this xenobiotic

compounds –

– Aerobic biodegradation;

– Anaerobic biodegradation.

• Examples of aerobic degradative bacteria of xenobiotics are as follows-

*Pseudomonas, Gardenia, Bacillus, Moraxella, Micrococcus, Escherichia, Sphingobium, Pandoraea, Rhodococcus*, and anaerobic xenobioticsdegradative bacteria are

*Pelatomaculum, Desulphovibrio, Methanospirillum, Methanosaeta*

*Desulfotomaculum, Syntrophobacter, Syntrophus*.

• Anaerobic habitats, like 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.[4].

General Features of Biodegradation of Xenobiotics:

Since xenobiotics consist of a large variety of compounds, their degradation in microbial cells occurs via a large number of metabolic pathways.

A) Degradation of alkanes and aromatic hydrocarbons generally occurs through the following:

(i) An oxygenase first introduces a hydroxyl group to make the compound more reactive,

(ii) This hydroxyl group is then oxidized to a carboxyl group,

(iii) In the case of the cyclic compound the ring structure is opened up first,

(iv)while the linear molecule is degraded by β-oxidation this yields acetyl CoA which is metabolized in the usual manner like, for example, an n-alkane is oxidized as follows. (Fig 1).[1].



Figure 1: Degradation of alkanes and aromatic hydrocarbons.[1].

B) An alicyclic hydrocarbon, is also degraded similarly e.g., cyclohexane, is oxidized as follows:

(i) Firstly, an oxygenase adds an —OH group in the ring, to make it more reactive,

(ii) Then an ester in the form of a lactone is formed by the oxygenase,

(iii) This is then hydrolyzed to open the ring structure which yields a linear molecule, which can easily be degraded. (Fig.2)

 Mono-oxygenases are involved in both of these oxidations which add oxygen to a single position in the molecule, while, oxidation of the benzene ring may involve a di-oxygenase which adds oxygen at two positions in the molecule in a single step. (Fig.3) [1].



Figure 2: Oxidation of cyclohexane. Two different monooxygenases are involved.



Figure 3: Oxidation of aromatic hydrocarbons initiated by the action of a dioxygenase.

Both mono- and di-oxygenases are of a variety of types: some of them react better with short-chain alkanes, while others act on cyclic alkanes. Each enzyme oxidizes a limited range of compounds and these enzymes are not very specific in nature. Thus, xenobiotics are degraded by a wide variety of microorganisms, and each of these microorganisms degrades a small range of compounds.

 Oxidation of xenobiotics involves cytochrome P450 or rubredoxin. In addition, the halogens and/or other substituent groups are either modified or removed usually as one of the initial reactions as discussed or sometimes it is achieved later in the process [1].

Hydrocarbon degradation

(i) Halomethanes:

The enzyme methane mono-oxygenase transfers Halomethanes into methanol which uses them as substrate; this enzyme occurs in several methylotrophs. A glutathione-dependent hydrolase catalyzes oxidative dichlorination of halomethanes into methanol; the reaction is anaerobic in nature which uses oxygen derived from water. Methanol is oxidized to CO2 + H2O via formaldehyde and formic acid.[1]

(ii) Cyanide:

Cyanide (HCN) is toxic to nature but some microorganisms are capable of degrading cyanide but they cannot withstand a high concentration of HCN. Some cyanides, such as HCN and CH3CN, are volatile. Therefore, disposal of cyanide is strictly controlled and we should also minimize its use. [1]

It is degraded as follows:



(iii) Aliphatic Hydrocarbons:

Aliphatic Hydrocarbons either saturated or unsaturated, n-Alkanes of 10-24 carbons are most easily degraded by microorganisms. Similarly, like aliphatic hydrocarbon, saturated aliphatic is degraded more easily than unsaturated ones while branched chains show decreased biodegradation. Oxygenase’s cn-alkane biodegradation of n-alkanes to produce carboxylic acid is then degraded by β-oxidation.[1]

Oxidation may involve the methane group at one end of the n-alkane molecule, or this oxidation may occur at a β-methylene group (Fig. 4). Dicarboxylic acid is produced when both terminal methyl groups are oxidized, this reaction is used by many microorganisms for the biodegradation of branched-chain n-alkanes [1].



Figure 4. Oxidation of b-methylene group of an n-alkane.

Co-Metabolism and Gratuitous Metabolism of Xenobiotic Compounds by Microorganisms

• Some xenobiotic compounds, like cyclohexane, halogenated compounds, etc., are degraded by microbes, but these compounds are rarely, used as sources of energy and carbon by these microorganisms.

• Degradation of these types of compounds, therefore, depends on the presence of another compound like the necessary enzymes, and metabolism which provides both energy and reducing equivalents for the degradation of xenobiotic compounds (and the C, energy, etc. needed for microbial growth).

• Clearly in these types of cases, degradation of xenobiotic compounds depends on the metabolism and presence of a suitable substrate called co-metabolite, such degradation is called co-metabolism [4].

• Several xenobiotic compounds are degraded by an existing pathway and by gratuitous metabolism in which xenobiotic compounds 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 induced by another compound and these are not needed as a co-metabolite.

• The xenobiotics degraded by both co-metabolism and gratuitous are very similar to the natural substrates of the enzymes involved in their degradation.

• Generally, a xenobiotic compound is also not completely degraded by gratuitous metabolism, but the product released by this metabolism are less polluting or may be used as a substrate by some other organisms [4].

The Origin of Capacity to Degrade Xenobiotics

• Microorganisms evolve some metabolic processes needed to wholly or partly degrade the xenobiotic because of continued exposure of microorganisms to xenobiotic compounds

• These capabilities may arise due to:

• (i) Mutation, and

• (ii) Transfer of plasmid carrying such degrading protein genes.[4].

Practical Approaches to Xenobiotic Degradation

• Biodegradation of xenobiotic compounds depends on their concentration either it is low or high (too high concentration may be toxic to the microorganisms), pH of the medium, temperature, availability of water and other nutrients, and presence of organic compounds (these may be cometabolites, inhibitors or preferred substrate, in place of the xenobiotic,).

• The xenobiotic compound should be available in an acceptable concentration so that toxic levels should not occur.

• In a sludge treatment system, a constant supply of such compounds should be available for selective maintenance of microbes capable of degrading such compounds.

• In addition, interfering organic compounds should not be present in the environment, as they can be degraded easily and also hinder the degradation of the main xenobiotic compound.

• Practical application of microbes for xenobiotic degradation is facilitated by:

• (i) Sufficient nutrients or co-metabolites should be supplied,

• (ii) Xenobiotic compounds should be maintained at non-toxic levels, and

• (iii) Provision of microbial population or inoculum [4].

Use of Mixed Populations

• The why we should use mixed populations of microbes for the degradation of xenobiotic compounds is as follows:

• (i) Two different microbes can together degrade a xenobiotic completely while, if they are present in a single, they may not be able to do this.

In such a case, the product of degradation by one microorganism may serve as the substrate for the other.

• (ii) One microorganism may produce a growth factor/nutrient required by the other microorganism mixed with it. This can be seen in *Nocardiasp*. which degrades cyclohexane but is unable to produce biotin. A *Pseudomonas sp.* strain produces biotin but cannot degrade cyclohexane these both are mixed to get their proper use

• (iii) Co-culture may lead to plasmid transfer into a faster-growing species which therefore creates a faster-growing species capable of degrading the xenobiotic. [4].

Conclusion

From the above discussion, we conclude that metabolism and co metabolism has an important effect on the biodegradation of xenobiotic compounds.

So, microorganisms can be used in waste treatment, biodegradation, pollution control, biomass energy production, etc.

The important aspect is to identify one by one the key metabolic reactions and the stable end product so that we come to know the necessary information to understand the processes, tendencies, and role of microorganisms in altering the character of the important group of environmental pollutants like Xenobiotics. If we come to know the end products and metabolic pathways used by microorganisms then it will become easy to use genetic strategy to modify the microorganism involved in degradation. Many different factors also affect the biodegrading strategy of microorganisms, these are as follows-Soil moisture, Organic and inorganic chemicals (also including chemicals produced during metabolism, Soil organic matter, Types of vegetation, and microorganism’s growth strategy. So, biodegradation done by microorganisms is mainly-A primary degradation, in which the characteristic property of the Xenobiotic compound gets disappeared; and is converted into an environmentally acceptable substance. Biodegradation is the minimum alteration of the parent compound that is only necessary to remove properties occurs; and in ultimate biodegradation, the complete conversion of the parent compound to the inorganic end-products which are associated with the microorganism’s normal metabolic processes occurs. There are many direct and indirect sources of xenobiotic compounds -Direct Sources-Plastic, paints, phenolic compounds, petroleum products, dyes, and pigment Indirect sources- are pharmacy products like drugs, fertilizer, etc., So we should reduce the use of these products and should replace these products with some healthy substitute.

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