



## BIODEGRADATION OF XENOBIOTICS- A WAY FOR ENVIRONMENTAL DETOXIFICATION

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### ABSTRACT

Man-made compounds, also known as xenobiotics, are often refractory to degradation. The main reason is that they cannot be recognized by naturally present organisms and therefore do not enter common metabolic pathways. The physical and chemical characteristics of the compounds, as well as environmental factors, may influence their biodegradability. The main degraders in nature are microorganisms, mostly bacteria and some fungi. These organisms, due to their rapid rates of multiplication and great metabolic potential, are able to adapt to new substrates. Selection of degradative potent microorganisms and their successive adaptation to a naturally persistent compound might be a powerful means for environmental detoxification. Better understanding of metabolic pathways for the biodegradation of specific organic compounds as well as more thorough knowledge of degrading microorganisms will make purposeful application of biodegradation possible. This article is focused on the different properties of microbial degradation of xenobiotics.

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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. Major sources of xenobiotic compounds enters into the environment are (I) chemical and pharmaceutical industry of xenobiotics and synthetic polymers, (ii) pulp and paper bleaching, which are the main sources of natural and manmade 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. 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. Bacteria important in the biodegradation process include *Bacillus* (capable of producing thick-walled endospores that are resistant to heat, radiation and chemical disinfection), *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.

### **The biodegradation process can be divided into (1) aerobic and (2) anaerobic degradation**

If oxygen is present, aerobic biodegradation occurs and carbon dioxide is produced. If there is no oxygen, an anaerobic degradation occurs and methane is produced instead of carbon dioxide. When conversion of biodegradable materials or biomass to gases (like carbon dioxide, methane, and nitrogen compounds), water, salts, minerals and residual biomass occurs, this process is called mineralization. Mineralization is complete when all the biodegradable materials or biomass are consumed and all the carbon is converted to carbon dioxide. Biodegradable materials have the proven capability to decompose in the most common environment where the material is disposed, within one year, through natural biological processes into non-toxic carbonaceous soil, water or carbon dioxide. The chemical structure (responsible for functional group stability, reactivity, hydrophilicity and swelling behavior) is the most important factor affecting the biodegradability of materials. Other important factors are physical and physicochemical properties, e.g., molecular weight, porosity, elasticity and morphology (crystalline, amorphous). 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, most biogenic compounds are biodegraded rapidly. 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. However, it is not always easy to determine which structural moieties indeed are xenobiotic in the sense of 'foreign to life'. Some natural compounds show principally the same unusual structural features as xenobiotics, such as halogen substituent or nitro groups found in some antibiotics, or they contain stable chemical bonds like the ether and carbon-carbon bonds stabilizing lignin. Moreover, microorganisms throughout geological time have also been exposed to a variety of chemicals produced by abiotic natural processes: "Many of these compounds bear little relationship to the biological products from which they were originally derived. For example, soils and young sediments contain thousands of substituted polycyclic aromatic hydrocarbons.

### **Origin of xenobiotics in the environment**

Xenobiotic substances are becoming an increasingly large problem 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. 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. Elements and compounds undergo a number of processes in the environment viz. condensation, sedimentation, coagulation, reaction, diffusion, and scavenging. 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. 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. 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. 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. 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. 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. 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 biomagnifications 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. Biomagnifications 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 biomagnifications 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.

### **Bioavailability of xenobiotics**

The term of bioavailability is defined as the total volume of the contaminant found in the soil or bottoms in free state which is or can be absorbed by the organism [10]. The scope and rate of all transformations of xenobiotics depends on the chemical structure and concentration of the xenobiotic, type and number of microorganisms capable of degrading or transforming the xenobiotic, as well as the physico-chemical properties of the environment to which the xenobiotic is released or in which it accumulates [3, 10, 11]. The bioavailability of a xenobiotic depends on its state (solid, liquid or gas), water solubility, capability of adsorbing and adhering to solid particles of soil or sediment [3]. It is generally accepted that only the water-dissolved fraction of the xenobiotic is available to the microorganisms and the direct contact of the xenobiotic with the microorganism's cell is the condition of the biological

transformation of the xenobiotic. At the same time it should be pointed out that the vast majority of xenobiotics exhibit significant hydrophobicity and thus, after release into the environment, they are immobilized on the solid particles of the matrix by sorption or in the structures of the organic matter by occlusion. Among various environments into which xenobiotics are released the soil appears to be the most diverse system, comprising solid, liquid and gas phases. The solid phases are mineral (fragments of rocks and minerals), organic (humus, animal and plant remnants) and mineral-organic particles. The liquid phase is water with dissolved mineral and organic substances, as well as gases, retained by capillary forces between soil aggregates and lumps. The mineral and organic compounds dissolved in the water constitute the soil water retention. The soil air is saturated by vapor and contains approximately 10-times more carbon dioxide than atmospheric air, and fills the soil spaces between solid particles that have not been taken by water. The process of desorption, that is, releasing the contamination, is the result of a collaboration between the physico-chemical factors, such as a change of humidity, reaction or surface properties of the sorbent, and biological factors, i.e. it is caused not only by the activity of microorganisms, but also plants and animals. The released contaminants are transported by way of diffusion and dispersion, which may lead to the xenobiotic coming into direct contact with the microorganism's surface. Passing the physiological barrier of cellular membranes of microorganisms is the key stage in the process of transformation of xenobiotics, taking place with the participation of more or less specialized enzymes of xenobiotic decomposition pathways.

### Parameters Influencing Bioavailability and the Rate of Biodegradation

In laboratory as well as environmental systems, only the fraction of the xenobiotic pollutant that is dissolved in the aqueous phase is generally assumed to be available to the microorganisms for degradation. The extent of biodegradation and the rate at which it occurs depend on the chemical structure and concentration of the compound being degraded, the type and number of microorganisms present, and the physicochemical properties of the environment. Bioavailability is controlled by parameters such as the physical state of the pollutant compound (solid, liquid, gaseous), its solubility in water, and its tendency to adsorb or bind to soil or sediment particles. In soil aggregates or other solids, microbes may be excluded from entering the smaller micro pores. Xenobiotics present in the micro pores are thus unavailable to the microorganisms and must diffuse through pore water to the grain surface in order to be degraded. However, diffusion in soil systems may well be sorption-limited. Actually, sorption, immobilization and micro pore entrapment are major causes for the persistence of many xenobiotics. 'Aging', i.e. the length of time a soil or sediment has been exposed to contamination, also affects bioavailability. Pollutants may undergo reactions that lead to strong binding to soil and sediment material, becoming increasingly unavailable to microorganisms with the progress of time. Many xenobiotics, for example the polycyclic aromatic hydrocarbons and the polychlorinated biphenyls, are poorly soluble in water, and tend to adsorb to and be immobilized by the soil matrix and sediment material. As mentioned above, the structure of xenobiotic molecules is characterized by 'unphysiological' substituents and stable chemical bonds, which impede or even prevent biodegradation. Unfavorable concentrations of the xenobiotic

compound also affect biodegradation. In high concentrations, many xenobiotics are toxic to organisms, including the degradative bacteria. When determining biodegradation rates, it is important to keep in mind that an observed 'disappearance' of a xenobiotic from an ecosystem does not necessarily mean that it was biodegraded, since loss can also occur by partial degradation, biotransformation, or by volatilization, leaching, or chemical conversion (polymerization, modification, breakdown). In monitoring the environmental fate of a chemical, one must also monitor the products formed, not simply the disappearance of the parent compound. The rates of xenobiotic biodegradation in the environment may range from days and weeks to years and decades. The organophosphate insecticide malathion disappears from soil within approximately one week, and the herbicide 2, 4-D (2, 4-dichlorophenoxyacetic acid) is degraded within four to six weeks in soil. Modern herbicides are designed to undergo biodegradation within one cropping season. On the other hand, there are recalcitrant xenobiotics that persist in the environment for many years.

Other factors that influence biodegradation involve environmental conditions such as temperature, pH, water content and salinity, presence of inhibitory chemicals, availability of electron donors and nutrients, and availability of oxygen or other electron acceptors. In soil, for example, oxygen availability is very often the limiting factor of aerobic biodegradation processes. Moreover, the presence of competing microorganisms, or of predators grazing on the microbial consortium, also affects biodegradation.

### Role of microbes in biodegradation

According to Curtis and Reinhard (1994) microorganisms represent half of the biomass of our planet. Human activity disturbs the environment; they introduce xenobiotic chemicals in the biosphere. Microorganism exhibit capability to degrade xenobiotics by their metabolic pathways in consideration of exploiting as new carbon sources to detoxify toxic compounds (Copley, 2000). Microbes show ecofriendly behavior to overcome environmental pollution and to help in biodegradation of xenobiotic compounds. Microorganisms apply two modes of action for degradation of xenobiotics compound –

- Aerobic biodegradation;
- Anaerobic biodegradation.

Aerobic biodegradation processes require excess O<sub>2</sub> delivery systems, because it is necessary to supply continuous O<sub>2</sub> due to biofouling in subsurface remedial applications (Baker and Herson, 1994), when bioreactors are applied its energy costs and sludge production are high (McCarty and Smith, 1986; Jewell, 1987). 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 (Zhang and Bennett, 2005). According to Chowdhury *et al.* (2008) and Varsha *et al.* (2011) example of aerobic degradative bacteria of xenobiotics are *Pseudomonas*, *Gordonia*, *Bacillus*, *Moraxella*, *Micrococcus*, *Escherichia*, *Sphingobium*, *Pandoraea*, *Rhodococcus*, and anaerobic xenobiotics degradative bacteria are *Pelatomaculum*,

Desulphovibrio, Methanospirillum, Methanosaeta Desulfotomaculum, Syntrophobacter, Syntrophus. Microbes apply xenobiotics as their substrates and grow on them, degrading or fragmenting them, which is highly beneficial in case of bioremediation (Iyovo *et al.*, 2010; Surani *et al.*, 2011; Varsha *et al.*, 2011). Effective Microorganism (EM) is the consortia of valuable microorganisms which secretes organic acids and enzymes for utilization and degradation of xenobiotic compounds (Monica *et al.*, 2011). Microbes are collected from the contaminated sites like waste water, residual sites and distillery sludges; they are excessively resistance to higher concentrations of xenobiotics (Narasimhulu *et al.*, 2010).

Some of toxic organic pollutants and Heavy metals which show resistance to some of the microbes can be degraded using tolerant microbes (Tripathi, 2011). For the removal of solid waste effluent activated sludge and aerated lagoons are used they are the richest source of microbial consortium (Priya *et al.*, 2011). *Pseudomonas* sp. is most efficiently useful in the degradation of xenobiotics such as aromatic and aliphatic hydrocarbon of oils. Wasi *et al.* (2010) reported *Pseudomonas fluorescens* SM1 strain is a good candidate for remediation of some heavy metals and phenolics in heavily polluted sites. According to Hadad *et al.* (2005) plastics are manufactured by polyethylenes are degraded by *Brevibaccillus borstelensis* and *Rhodococcus ruber*. The scientist has been made an attempt to characterize bacterial communities and their responses to xenobiotic pollutants, to isolate potential degraders and to identify the genes involved in biodegradation processes (Greene *et al.*, 2000; Watanabe *et al.*, 2002). The detailed analysis of microbial diversity, in an environment can be divided into two broad categories: culture-dependent studies and culture independent studies (Juck *et al.*, 2000). A wide range of unidentified pollutant-degrading microorganisms can be identified by culture independent techniques that can be harbored in contaminated environments (Margesin *et al.*, 2003). Over the last two decades the increasing attention of xenobiotic biodegradation under anaerobic conditions such as in groundwater, sediment, landfill, sludge digesters and bioreactors, has been observed. Generally, anaerobic bacteria able to degrade xenobiotics are present in various anaerobic habitats, inter alia sediments, water laden soils, reticuloruminal contents, gastrointestinal contents, sludge digesters, feedlot wastes, groundwater, and landfill sites [20].

Anaerobes use natural organics such as proteins, carbohydrates, and many others as carbon and energy sources. *G. metallireducens*, *D. acetonicum*, *Acidovorax*, *Bordetella*, *Pseudomonas*, *Sphingomonas*, *Variovorax*, *Veillonella alkalescens*, *Desulfovibrio* spp., *Desulfuromonas michiganensis*, and *Desulfitobacterium halogenans* are the major groups of anaerobic microorganisms involved in biodegradation of xenobiotic compounds.

### Biodegradation of Xenobiotic Compounds

The predominant degraders of organo pollutants in the oxic zone of contaminated areas are chemo-organotrophic species which use a huge number of natural and xenobiotic compounds as carbon sources and electron donors for the generations of energy. Many bacteria are able to metabolize organic pollutants. A single bacterium does not possess the enzymatic capability to degrade all or even most of the organic compounds in a polluted soil. The latest work in

biodegradation has shown that many compounds are found in nature and are potentially biodegradable; the microbes that degrade them usually have small populations and are limited by environmental conditions. Considerable interest has been focused on microbes able to degrade polycyclic aromatic hydrocarbons (PAHs), which are a persistent component of petrochemical wastes, organonitrogen compounds such as nitrotoluenes and chlorinated organic compounds like pentachlorophenol, polychlorinated biphenyl and chlorinated dioxin like compounds. The enzymes used by microbes to degrade allowing the degradation of similar chemicals. Thus, bacteria adapted to grow on phenol (a disinfectant) may also degrade synthetic chlorophenols such as the broad-spectrum pesticide pentachlorophenol (PCP). Bacteria that grow on toluene (a common solvent and fuel) may also attack nitrotoluenes, such as the explosive trinitrotoluene (TNT). Specialized enzyme systems and metabolic pathways for the degradation of man-made compounds such as chlorobiphenyls and chlorobenzenes 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 (substituted) aromatic 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 mechanism, such as gene transfer, mutational drift, and genetic recombination and transposition, can accelerate the evolution of catabolic pathways in bacteria.

### Summary

Xenobiotics (greek xenos= strange, foreign, foreigner) are chemically synthesized compounds that do not occur in nature and thus are 'foreign to the biosphere'. They have 'unnatural' structural features to which microorganisms have not been exposed to during evolution. Xenobiotics may resist biodegradation, or they undergo incomplete biodegradation or just biotransformation. Depending on their fate in air, water, soil, or sediment, xenobiotic pollutants may become available to microorganisms in different environmental compartments. Actually, the dominant means of transformation and degradation of xenobiotic compounds on Earth resides in microorganisms. In natural habitats, the physicochemical properties of the environment may affect and even control biodegradation performance. Sorption to soil and sediment as well as micropore entrapment are major causes for the persistence of many xenobiotics. Fungi and aerobic as well as anaerobic bacteria are involved in the degradation of xenobiotics. On the other hand, microorganisms may use xenobiotic compounds as a source of energy, carbon, nitrogen, or sulfur. Degradation of many xenobiotic chemicals requires microbial communities. Some xenobiotics, however, appear to resist microbial attack. In the near future, the collective knowledge in the field of microbial degradation may enable scientists to establish rules to predict the biodegradability and the biodegradation pathways of xenobiotic compounds. 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.

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