

MICROBIAL BIODEGRADATION OF XENOBIOTICS AND TOXIC SUBSTANCES

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Abstract

One of the biggest environmental problems is the accumulation of recalcitrant compounds that cause negative impacts on ecosystems. The concentration of xenobiotic compounds and toxic substances has increased considerably in recent decades due to products from different industrial processes. Xenobiotic compounds are a cause of concern mainly due to their carcinogenic, mutagenic and teratogenic effects and for their bioaccumulative and biomagnifying characteristics, therefore different strategies have been sought to reduce these effects. The degradation of xenobiotic compounds is a widely used strategy, it consists of a natural process carried out by diverse microorganisms that use said compounds as a form of survival, the microorganisms play the most important role in the degradation of recalcitrant compounds because they have the capacity to grow using them as the only carbon source, these types of organisms are a "greener" biocatalytic alternative. The metabolic routes of these microorganisms transform pollutants through specific systems, improving the environmental conditions that humans have been for two centuries altering violently.

Keywords: biodegradation, xenobiotics, microorganisms, environmental problems, biocatalysis.

Introduction

Xenobiotic compounds are chemicals present in the environment at unnatural concentrations. These compounds do not occur naturally, and if so, their concentrations are much lower than those produced by man. These products, mostly anthropogenic, are persistent in the environment due to their high thermodynamic stability, in addition, they are resistant to biodegradation, which is called recalcitrant. Xenobiotic compounds can have various toxic effects on human health, including their carcinogenic, mutagenic and teratogenic effects (Agrawal and Shahi, 2015).

The high lipophilicity of many of these xenobiotic compounds leads to bioconcentration/biomagnification, causing and increasing health problems in predators at high levels of the food chain. At present these persistent compounds are found in every part of the oceans, many in very low concentrations, but their effects on marine life and public health are not widely understood (Wu, 1999). Xenobiotic compounds include hydrophobic organic pollutants, which are persistent in the environment and tend to biomagnify, this group refers to compounds such as polychlorinated biphenyls, polycyclic aromatic hydrocarbons, chlorobenzenes, furans and some pesticides, mainly those used for some time such as DDT and dieldrin (Jantunen, 2010).

The potential for microbial communities to degrade many toxic compounds is significant. It has been shown that when many chemical compounds are repeatedly introduced into the environment, the period of adaptation of microorganisms to this substrate is much shorter. During this period, microorganisms are selected for their ability to degrade a given substrate. As a result, microbial populations arise naturally and can persist in the soil for several months after complete degradation of the toxic (Volova, 1999).

The advances in both chemical catalysis and biocatalysis are decisive to reduce the environmental footprint of chemical processes and technologies of this industrialized world, this was already seen with the development of chemical catalysis (that is, the use of catalysts of non-biological origin), but what biotechnology seeks is to develop catalytic media that are friendlier to the environment and especially to health. Biocatalysts (either enzymes or whole cells) constitute a more ecological alternative to traditional organic synthesis offering appropriate tools for the industrial transformation of natural products or synthetic materials under moderate reaction conditions, low energy requirements and minimization of isomerization and rearrangement problems.

Properties of xenobiotics that determine their toxicity

The toxicity of different substances is not the same. Since it manifests itself in the interaction of a xenobiotic with a biological system, its value depends on the properties of both the toxic and the biosystem, and is finally determined by:

- The ability of a substance to reach a target structure, the interaction of which initiates a toxic process;
- The nature and strength of the bond formed between the toxic and the target structure;
- The value of the target structure for maintaining homeostasis in the body (Salovarova, 2007).

The structure of a substance determines the size of the molecule, its mass, solubility, volatility, state of aggregation under normal conditions and chemical activity. All these properties affect the toxicity of the substance, however, none of them is the only significant one (Salovarova, 2007).

Capacity of xenobiotics for biodegradation.

Depending on the stability of pollutants in the environment, they can be divided into three groups:

Biodegradable toxicants, which are relatively easily destroyed in the environment under the influence of both abiotic and biotic factors. These include substances of biological origin and some organic compounds of non-biological origin (petroleum n-alkanes, alcohols, aldehydes, etc.). (Salovarova, 2007).

The environmental danger of this type of pollutant is determined by the speed of its entry into ecosystems, the ability to accumulate in the components of biogeocenoses, as well as the chronic effect of sublethal concentrations (Salovarova, 2007).

The ecotoxicological importance of many xenobiotics is determined not only by their toxicity and biodegradability, but also by the speed at which these pollutants enter ecosystems. If the rate of entry of decomposed pollutants exceeds the rate of their natural biodegradation, this can cause alterations in the composition and structure of ecosystems (Karasevich, 2002).

The bioaccumulation of contaminants, such as lipophilic compounds, in living organisms is a major hazard. As a result, its toxic effect may increase and the quality of the food supply for organisms of higher trophic levels may deteriorate. Many pollutants can accumulate in the ecosystem, be absorbed by soil particles and interact with humus. For example, some pesticides (thiophos), which are unstable in aqueous solution, bind to soil particles and persist for a long time in the natural environment. Then, as the humus mineralizes, the toxic molecules associated with it are released and can have a detrimental effect on organisms in this ecosystem.

Persistent xenobiotics are very stable compounds that break down extremely slowly. Among this group of compounds, the best known are organochlorine pesticides, in particular, DDT.

Recalcitrant xenobiotics are compounds that practically do not decompose, or in principle cannot be broken down at all. These include, first of all, heavy metals and radionuclides with a long half-life.

Under natural conditions, metals are found in the form of minerals. The smelting of metals from minerals and their use in a wide variety of branches of human activity have led to a significant increase in their content in the environment. Mercury, cadmium, chromium, arsenic, lead, beryllium, zinc, copper, thallium, etc. are of great toxicological importance. In addition to industrial activity, there is a natural influx of heavy metals into the biosphere due to weathering of rocks and their removal by rivers (Salovarova, 2007).

The following common characteristics of toxicants can be distinguished:

- Chemical pollution is extremely diverse and includes many classes of chemical compounds;
- The level of pollutants increases in regions of high population concentration;
- The biological effect of various pollutants can be non-additive and have a synergistic effect;
- From a biochemical point of view, several structural and functional systems of a cell: the genetic apparatus, biomembranes, proteins, can be exposed to toxic substances;
- The biotransformation of contaminants can lead to the emergence of compounds that are more persistent and more toxic than the parent substances;

- Many pollutants (hydrophobic compounds, heavy metals, radionuclides) can accumulate in organisms in higher concentrations than in the environment;
- Even low sublethal concentrations of pollutants, which can reduce reproduction and lead to population extinction, pose an environmental hazard.

Biodegradation of xenobiotics in the environment

The degradation of xenobiotics can occur as a result of physical and chemical processes and depends significantly on the type of soil, its structure, humidity, temperature, etc. The biological transformation of compounds that have entered the environment can proceed in different directions, leading to mineralization, accumulation or polymerization (Karigar and Rao, 2011).

Xenobiotics that undergo complete degradation, i.e. mineralize into carbon dioxide, water, ammonia, sulfates and phosphates, are used by microorganisms as the main growth substrates and undergo a complete metabolic cycle (Singh, 2008). The partial transformation of compounds occurs, as a rule, in the processes of cometabolism or cooxidation and is not associated with the inclusion of the products formed in the metabolic cycle by microorganisms. Finally, some aromatic hydrocarbons and synthetic polymers do not lend themselves to biological transformation at all.

The behavior of a xenobiotic in nature depends on many interrelated factors: the structure and properties of the compound itself, the physicochemical conditions of the environment, and its biocatalytic potential determined by the microbial landscape. All these factors together determine the speed and depth of xenobiotic transformation (Furukawa, 2010; Pieper, 2005). We must not forget that the biological degradation of xenobiotics is justified only when their complete mineralization, destruction and detoxification occurs. In the course of degradation, a series of successive modifications of the initial compound often occurs with the participation of several microbial species. Several types of microbial metabolism play an important role in removing xenobiotics from the environment.

When new substances enter the environment, natural genetic engineering can occur, as a result of which microbial forms with new catabolic functions emerge. Plasmids, extrachromosomal genetic elements, play a very important role in the processes of transfer of genetic information between organisms that lead to the biochemical variability of populations. Catabolic or degrading plasmids that encode the mineralization or transformation reactions of xenobiotics, give microorganisms the ability to redistribute a set of degrading genes among themselves.

Currently, a variety of natural catabolic plasmids is described, which are found in various representatives of the soil microflora. They are especially often identified among the genus *Pseudomonas* which are indicated in Table 1.

Tabla 1 - Pseudomonas plasmids, size and degradable *compounds*.

Plasmid	Degradable compound	Plasmid size, kb
WILL	Salicylate	60 60
WILL	Salicylate	72
WILL	Salicylate	83
TOLL	Xylene and Toluene	113
pjP1	2,4-Dichlorophenoxyacetic acid	87
pjP2	2,4-Dichlorophenoxyacetic acid	54
pjP3	2,4-Dichlorophenoxyacetic acid	78
TAKES	Camphor	225
XYL	Xylene	15
pAC31	3,5-diclorobenzoato	108
pAC25	3-clorobenzoato	102
pWWO	Xylene and Toluene	176
NEAR	Naphthalene	69
XYL-K	Xylene and Toluene	135

Source: O.V. Turkovskaya
 Elaborated: by the authors.

Figure 1 shows the biodegradation of a very famous pesticide, atrazine. This substance is a herbicide and inhibits the growth of weeds. But, unfortunately, its biological activity extends to animals and humans.

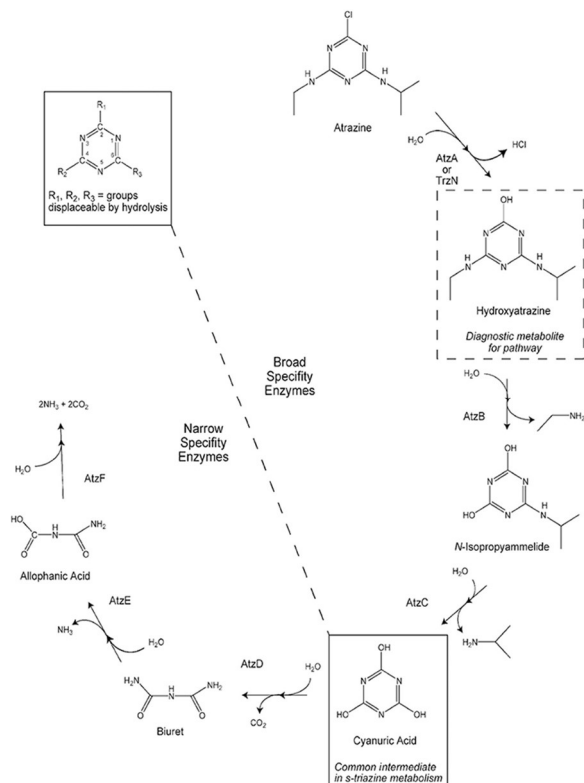


Figure 1 - Biodegradation of atrazine.

The breakdown of atrazine is carried out by the bacterial strain *Pseudomonas* sp. All stages, except the breakdown of the intermediate biodegradation product, cyanuric acid, are carried out by specific enzymes encoded by the plasmid genes *atzA*, *atzB* and *atzC*.

Bacteria that destroy non-halogenated aromatic compounds, as a rule, convert them into catechol or protocatechoate, and then, in the course of various oxidative degradation reactions, into acetyl-CoA and succinate or pyruvate and acetaldehyde. The latter compounds are metabolized by almost all microorganisms. Halogenated aromatic compounds, the main components of most pesticides and herbicides, are destroyed by the same enzymes in catechol, protocatechoate, hydroquinone or their halogenated derivatives, and the rate of their degradation is inversely proportional to the number of halogen atoms in the parent compound. Dehalogenation (cleavage of a substitute halogen atom of an organic molecule), which is necessary to detoxify a compound, is often carried out in the course of a non-specific dioxygenase reaction, replacing a halogen in the benzene ring with a hydroxyl group. This reaction can occur both during the biodegradation of the initial halogenated compound and later (Glick, Pasternak, 2002).

Often, the biodegradation of a substance that does not occur in nature is accompanied by the formation of other, also unnatural, substances. For example, the explosive RDX under anaerobic conditions (in activated sludge) is reduced to a mixture of nitrous derivatives. One of them, dimethylnitrosamine, is especially interesting because, as a result of a greater metabolic reduction, it dimerizes, transforming into two isomeric dimethylhydrazines: symmetric and asymmetric (Fig. 2).

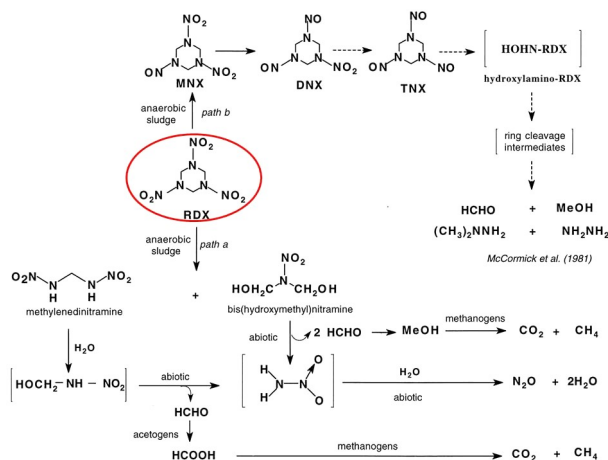


Figure 2 - anaerobic biodegradation of RDX

The biosphere can neutralize xenobiotics and include them in its composition in the form of biogenic elements, a number of xenobiotics contain many valuable atoms, for example, nitrogen, which is necessary for all living things. The ability of the biosphere to use and neutralize xenobiotics is based on the fact that most of them are not xenobiotics. That is, they are formed in small quantities in the biosphere and are constantly present in it.

For example, carbon tetrachloride (carbon tetrachloride) is degraded by *Pseudomonas* sp. KC with the formation of extremely toxic intermediate metabolites: phosgene (carbonic acid dichloride) and thiophosgene. Of course, these dangerous molecules exist for a very short time, a fraction of a second, and are instantaneously hydrolyzed (Fig. 3).

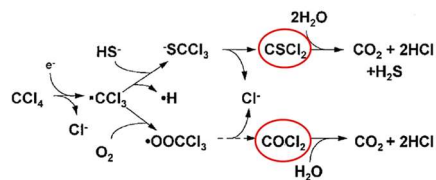


Figure 3 - Aerobic biodegradation of carbon tetrachloride

Enzymes as decontaminating agents

The use of enzymatic proteins may represent a good alternative to overcome most of the disadvantages related to the use of microorganisms (Nannipieri, 1991). Enzymes have several

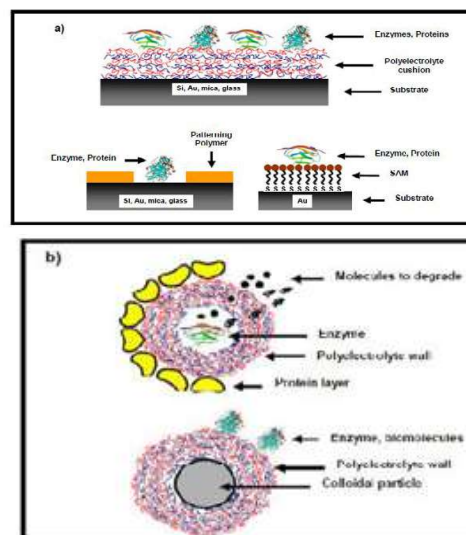
beneficial characteristics. They are the main effectors of all transformations that occur in biota. They are catalysts with narrow (chemo, region and stereo selectivity) or wide specificity and can therefore also be applied to a wide range of different compounds in mixture. They can produce major transformations of the structural and toxicological properties of contaminants, and even their complete conversion into harmless inorganic end products.

Enzymes can act intracellularly, i.e. in the presence of or *within* their cells of origin; extracellularly, i.e. both in the *presence* and *absence* of their cells of origin; free, i.e. *soluble* in solution and catalysis will be *homogeneous*; or immobilized, that is, *linked through* different links to a solid *matrix* and the catalysis will be *heterogeneous* (Gianfreda, 2006).

Rodríguez Couto and Toca Herrera (2006) illustrated very interesting examples of structures and methods for the immobilization of biomolecules, including enzymes, with specific reference to laccase, an enzyme widely used in the decontamination of pollutants (Gianfreda, 1999).

Figure 4 - Supramacromolecular structures usable to immobilize biomolecules. a) 2D and b) 3D structures.

2D and 3D supramacromolecular structures can be used to immobilize biomolecules or to build microreactors. In the first case, multiple layers of polyelectrolytes, micropatterns, and self-assembled monolayers (SAMs) appear to be suitable for scope (Figure 4a); whereas hollow layers of polyelectrolytes or colloidal particles covered by polyelectrolytes (and phospholipids) can host proteins within and/or other types of functional molecules, allowing diffusion of molecules across the shell wall (Figure 4b).



Biodegradation methods of xenobiotic compounds

Aerobic degradation

One of the most common types of bioremediation is the mineralization of toxic organic pollutants to non-toxic products such as carbon dioxide. Oxygen is the most common electron acceptor during microbial respiration and degradation of xenobiotic compounds, in addition, a wide diversity of microorganisms capable of degrading these compounds do so under aerobic conditions, such as the species *Pseudomonas*, which has been widely studied for its ability to degrade very different pollutants (Lovley, 2003). Most of the research that has been done to evaluate the removal of contaminant is carried out under aerobic conditions because these microorganisms are easier to grow than anaerobes, in addition to having a more versatile metabolism (Singleton, 1994). The degradation of xenobiotic compounds depends largely on the

enzymes used in the metabolic pathway to degrade unusual substrates. The initial reaction for degradation occurs intracellularly and is an oxidative process that occurs by the activation and incorporation of oxygen, this reaction is catalyzed by oxygenases and peroxidases mainly. After this, peripheral metabolic pathways convert pollutants into intermediates of subsequent central intermediate metabolisms, such as the tricarboxylic acid cycle, followed by a biosynthesis of cell biomass from central precursor metabolites, e.g., acetyl-CoA, succinate, pyruvate (Thakur, 2008).

Anaerobic degradation

The anaerobic degradation of xenobiotic compounds becomes very important, especially to carry out the mineralization of xenobiotics with little volatility, that is, more complex structures and weights. In some cases, the degradation of some compounds does not occur aerobically if there was no previous anaerobic treatment (Master et al., 2002).

Many polluted ecosystems such as aquatic sediments, stratified lakes, wetlands and some soils are characterized by a deficiency or absence of oxygen. In these environments microorganisms can use nitrate, iron, manganese sulfate and carbonate as electron acceptors, the reduction of these acceptors results in denitrification, iron reduction, sulfurogenesis, manganese reduction and methanogenesis respectively. An example of this is anaerobic bacteria that reduce hydrocyanic acid to methane and ammonia, aerobic microorganisms hydrolyze it to formamide and then to formic acid and ammonia. In mammals, the enzyme rhodase converts cyanide into less toxic thiocyanate, or is included in the non-proteinogenic amino acid β -cyanalanine.

The anaerobic degradation of organic compounds is carried out by microorganisms that use metabolic pathways essentially different from those that perform an aerobic degradation, in addition, their performance in contaminated environments depends on the electron acceptors that are available (Vázquez-Rodríguez et al., 2008).

Among the transformations that can occur by these microorganisms are hydrolysis, decarboxylation, dechlorination, demethoxylation and deamination. A large number of studies have employed specific reactors to obtain greater removal of the contaminant, the most common being upflow anaerobic reactors (UASBs) and continuous stirring tank reactors (CSTR) (Mogensen et al., 2003).

Biofiltración

The biofiltration process is based on the entry of contaminated air into bioreactors in which the microbial flora converts volatile organic pollutants into carbon dioxide, water and biomass. Since microorganisms develop their activity in liquid medium, biodegradation must take place in the aqueous phase.

Bioventing or air injection

Arroyo et al. (2001) define bioventing as an in situ biorecovery treatment consisting of forced ventilation of the soil by injection of oxygen at pressure into the unsaturated soil zone through injection wells. Due to the aeration of the soil, the degradation of hydrocarbons will be favored for two reasons: by volatilization, facilitating the migration of the volatile phase of pollutants, and by biodegradation, since by increasing the oxygenation of the soil the microbial activity will be stimulated.

Biosparging

Biosparging is defined by Wilson (1999) as an in situ method that combines the effect of ventilation with the use of native microorganisms to degrade organic compounds absorbed by the soil in the saturated zone. In biosparging, air and nutrients are injected into the saturated zone to enhance the activity of the microorganisms present. This technique is used for cleaning organic compounds in soil and groundwater. The efficiency of biosparging for the degradation of herbicides such as atrazine has been demonstrated (Crawford et al. 2000).

Conclusions

Biodegradation is the process of destruction by microorganisms of substances that pollute the environment. Microbial degradation plays an essential role in detoxifying persistent pollutants that pollute the environment as a result of human activity.

Many bacteria of the genus *Pseudomonas* carry plasmids encoding enzymes that catalyze the degradation of aromatic and halogenated organic compounds. In most cases, a plasmid contains genes for enzymes of a specific catabolic pathway. By combining plasmids from different strains in one host, it is possible to create an organism capable of degrading various compounds. In addition, with the help of genetic manipulations, it is possible to expand the range of substrates degraded by a certain enzymatic pathway.

Listing examples of biodegradation, many of which have already been firmly established in practice, one cannot help but admire the flexibility of metabolic pathways. The biosphere is capable of processing almost any chemical. Microbial populations create new, previously non-existent enzymes (and at the same time the plasmid genes that encode them), in an order unique to any product of the chemical industry. Understanding this gives hope that the current environmental crisis will one day be overcome.

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