BIOTRANSFORMATION OF XENOBIOTICS FOR ENVIRONMENTAL BIOREMEDIATION

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Abstract:

Pesticides are agrochemicals used for crops prevention from pests. Their use has been largely increased in last few decades. The application of pesticides starts from the pre sowing stage. Different treatments include soil application, seed treatment, foliar spray, etc. Soil is the most important site of biological interactions. Microorganisms play an important role in the breakdown of pesticides, some of which are more resistant to decomposition than others. Catabolism and detoxification metabolism occur when a soil microorganism uses the pesticide as a carbon and energy source. The latter process is facilitated by resistant microorganisms. Amongst most of the important problems associated with pesticides application are their possible persistence in the environment and therefore, their possible incorporation into the food chain affects ecosystems and human beings. Another problem is the conversion of pesticides into the obsolete form, which may even show more harmful effects than the former. When the pesticides are not used within the given time of their efficacy, they become obsolete. They are decomposed into other chemical components, which sometimes become even more toxic than the original pesticides. Bioremediation therefore is the use of living organisms to minimize or eliminate the environmental hazards resulting from accumulation of toxic chemicals and other hazardous wastes. Bioremediation is a promising alternative to physico-chemical methods of remediation, because it is less expensive and can selectively achieve complete destruction of organic pollutants. The use of microorganisms for the degradation and detoxification of numerous toxic xenobiotics, especially pesticides, proved to be an efficient tool to decontaminate the polluted sites in the prevailing environment. Bioremediation methodology to treat xenobiotics such as pesticides in soil has gained considerable attention owing to its ecofriendliness and has been employed successfully in many countries. The degradation of xenobiotic compounds by members of the soil microflora is an important means by which these compounds are removed from the environment, thus preventing them from becoming pollution problems. Much work has been directed towards understanding the complexity of pesticide-microbial interactions in soil. Many studies have employed pure cultures of soil isolates or agar plate counts of soil populations. Microbial communities composed of several species are more likely to cause pesticide biodegradation in soil and rhizosphere environments than are single species. Biological decontamination methods are preferable to conventional approaches because, in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates. Biological monitoring has the advantage, over environmental monitoring, of determining the dose actually absorbed via any possible route: differences in absorption can be taken into account whether they are due to biological variability or to use of protective equipment. Different approaches for biodegradation are bacterial, fungal, and plant degradation. Further studies should be conducted to investigate the mechanisms by which the plants, microorganisms and their enzymes can assimilate these compounds. Knowledge of these enzymatic processes, especially concepts related to pesticides mechanism of action, resistance, selectivity, tolerance and environmental fate, has advanced our understanding of pesticide science and of plant and microbial biochemistry and physiology.

Keywords:

Bioremediation - biotechnology – biotransformation - environment - xenobiotics

INTRODUCTION

Soil microbial content plays a key role in soil. Microorganisms are essential for maintenance of soil structure, transformation and mineralization of organic matter, for availability of nutrients to plants. Soil microbial contents are able to metabolize and degrade a lot of organic pollutants such as xenobiotics and thus are of great concern for using in agricultural and soil biotechnology. Meanwhile, microbial degradation can lead to the formation of more toxic and persistent metabolites. Although soil microbial contents are characterized by fast flexibility and adaptability to change environmental condition, the application of pesticides especially for long-term application that can cause significant irreversible changes in the soil microbial population densities. Inhibition of species, which provide key process, can have a significant impact on function of whole terrestrial ecosystem especially the agroecosystem.

Soil biotechnology plays an increasing role in cleaning-up the environment from pollutants. To understand the underlying environmental mechanisms during soil bioremediation, a pragmatic approach using molecular tools is proposed. Subsequently, a series of new biotechniques for soils is examined. The uses of microorganisms to destroy, or reduce the concentration of hazardous wastes on a contaminated site are called bioremediation. Such a biological treatment system has various applications, including clean up of contaminated sites such as water, soils, sludges, waste streams, etc.

Sorption is the most important interaction between soil and for example xenobiotics and limits degradation and transport in soil. Pesticides bound to soil organic matter or clay particles are less mobile, bioavailable but also less accessible to microbial degradation and thus more persistent.

Degradation is a fundamental attenuation process for pesticides in soil. This process, catalyzed by soil microorganisms, is governed by both abiotic and biotic factors. Degradation is affected by a variety of interactions among microorganisms, various soil constituents, and the specific pesticide involved. Sorption is a similar key to control pesticide dispersive transport, transformation and bioaccumulation processes.

Bioremediation is the biological process of transformation or mineralization of toxicants introduced into the environment to less toxic or innocuous forms. It is an alternative, environmental friendly and relatively cost-effective alternative to conventional physico-chemical soil treatment technique. The success of bioremediation is largely determined by the metabolic potential of microorganisms to detoxify or utilize pollutants or mineralize them to useful forms. The process is dependent on biodegradability and bioavailability of pollutants to microorganisms, and environmental parameters including nutrient availability.

Contamination of biospheric elements (e.g., agricultural and forestay soils, groundwater, sediments, surface water, air, etc.) with hazardous and toxic chemicals is one of the major problems facing the industrialized and urbanized world today. Bioremediation is the use of microorganisms or microbial processes to degrade environmental contaminants throughout new biotechnologies. Bioremediation frequently must address multiphasic, heterogenous environments, such as agricultural lands in which the contaminant is present in association with the soil particles, dissolved in soil liquids, and in the soil atmosphere. Because of these complexities, successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology, chemistry, etc.

Soil is an extremely complex, dynamic and living medium, formed by mineral particles, organic matter, water, air and living organisms. It establishes the interface between earth, air and water and performs many vital functions both from an ecological as non-ecological point of view. While the physical basis of anthropogenic activities, source of raw materials and the geogenic and cultural heritage are among the non-ecological functions, the soil ecological services include biomass production, filtering, buffering and transformation capacity between the atmosphere, the groundwater and the plant cover. Soils and sediments serve as a gene reservoir, because the soil provides a biological habitat for a large variety of organisms [1].

The main sources of soil contamination in the World are spills during industrial and commercial operations, municipal and industrial waste treatment, oil extraction and production, inadequate storage of goods, etc. Heavy metals and mineral oil are regarded as the main soil and groundwater pollutants in the World. Other pollutants, such as polycyclic aromatic hydrocarbons (PAH), aromatic hydrocarbons (BTEX), phenols, chlorinated hydrocarbons (CHC) and others.

One of the most important key word of contaminated soil is the physico-chemistry, which describes the concentrations of the pollutant by itself represent a challenge, as too high concentrations may be toxic for the microorganisms and too low concentrations may be below the threshold limit. Moreover, the appropriate electron acceptors and donors have to be present. Conditions such as pH, redox potential and temperature have to be suitable and the presence of easier to degrade substrates can also represent an obstacle, as these compounds will be preferred by the microorganisms [2].

The bioremediation methods depend on having the:

- **right** microorganisms
- in the **right** place
- with the **right** environmental factors for carrying out the biodegradation.

There are some questions that have to answer before using bioremediation techniques are:

- Is the contaminant biodegradable?
- Is biodegradation occurring in the site naturally?
- Are environmental conditions appropriate for biodegradation?
- If the waste does not completely biodegrade, where will it go?

Physiology of biodegradative microbes

- A bioremediation process is **based** on the **activities of aerobic or anaerobic heterotrophic microorganisms**.
- Microbial activity is affected by a number of physico-chemical environmental parameters.

The factors that directly affect on bioremediation are:

- Electron donors (energy sources),
- Electron acceptors,
- Nutrients,
- pH,
- Temperature,
- Inhibitory substrates or metabolites, etc.

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Xenobiotics: those compounds that are alien to a living organism and have a ability to accumulate in the environment. Xenobiotics include:

- pesticides,

- fuels,
- solvents,
- alkanes,
- polycyclic hydrocarbons (PAHs),
- antibiotics,
- synthetic azo dyes,
- pollutants (dioxins and polychlorinated biphenyls),
- polyaromatic,
- chlorinated and
- nitro-aromatic compounds.

- The main concern with xenobiotic compounds is the toxicity.
- It is quite shocking that some xenobiotic compounds e.g.,
    1. phenols,
    2. biphenyl compounds,
    3. phthalates, etc. act as endocrine disruptors.

Biodegradation is one of the natural processes that help to remove xenobiotics from the environment by microorganisms. Most organisms have **detoxifying** abilities (that is, mineralization, transformation and or immobilization of pollutants), and microorganisms, particularly bacteria, play a crucial role in **biogeochemical cycles** for sustainable development of the **biosphere.**

- **Microbial degradation** of petroleum and other hydrocarbons is incredibly course of action that mainly **depends** on the:
    - composition of community and
    - its adaptive response to the presence of these compounds.
- Such degradation of petroleum in marine sites is restricted principally by the availability of **P and N**.
- **pH, moisture, oxygen and temperature** are prime **factors influencing the degradation rate**

ROLE OF MICROORGANISMS

- Microorganisms represent half of the biomass of our planet, yet we know as little as 5% of the microbial diversity of the biosphere.
- Microbes offer a simpler, economical and more environmental friendly strategy to reduce environmental pollution and to help in biodegradation of xenobiotics.
- Bacterial genera aid efficiently in biotransformation processes, evaluation of the xenobiotic contaminated areas is quite essential and involves the enumeration and detection techniques of the xenobiotic degraders that generate quick and safe consequences.
- The enumeration and monitoring of xenobiotic degrading bacterial populations in contaminated environment with traditional microbiological methods can acquire an inordinate length of time

The *in-situ* bioremediation process consists of three fundamental steps

- **Bioattenuation**: monitoring natural progress of degradation to ensure that contaminant decreases with time of sampling.
- **Biostimulation**: intentional stimulation of resident xenobiotic-degrading bacteria by electron acceptors, water, nutrient addition, or electron donors.
- **Bioaugmentation**: addition of laboratory grown bacteria that have appropriate degradative abilities.

Table 1 List of some pesticides and degrading bacteria genera

Target compounds	Bacteria degrading the compounds	References
	Pesticides	
Endosulfan	*Mycobacterium* sp.	Sutherland et al. 2002 [3]
Endosulphate	*Arthobacter* sp.	Weir et al., 2006 [4]
HCH	*Pseudomonas putida*	Benezet and Matusumura, 1973 [5]
2,4-D	*Alcaligenes eutrophus*	Don and Pemberton, 1981 [6]
DDT	*Dehalospirilum multivorans*	Chaudhry and Chapalamadugu, 1991 [7]
	Halogenated organic	
Atrazine	*Pseudomonas* sp.	Bruhn et al., 1988 [8]
Vinylchloride	*Dehalococcoides* sp.	Heiss et al., 2003 [9]
PCE	*Dehalococcoides ethenogenes*195	Magnuson et al., 2000 [10]
	PAH compounds	
Napthalene	*Pseudomonas putida*	Habe and Omori, 2003 [11]
PCP	*Pseudomonas* sp.	Yen and Serdar, 1988 [12]
2,3,4-chloroaniline	*Pseudomonas* sp.	Spain and Nishino, 1987 [13]
	Phthalate compounds	
Phthalate	*Burkholderia cepacia*	Chang and Zylstra, 1999 [14]
	Other compounds	
PCB	*Rhodococcus*RHA1	Kimbara, 2005 [15]
Benzene	*Dechloromonas* sp.	Coates et al., 2001 [16]
	Petroleum products	
	Achromobacter sp. *Acinetobacter* sp. *Micrococcus* sp. *Nocardia* sp. *Bacillus* sp. *Flavobacterium* sp.	Austin et al., 1977 [17]
	Azo dyes	
	Bacillus sp. *Pseudomonas* sp. *Sphingomonas* sp. *Xanthomonas* sp.	Dykes et al., 1994 [18] Stolz, 2001 [19] Stolz, 2001 [19] Reife and Freeman, 2000 [20]

(Source of this table: Sinha et al. [21]).

BIOCHEMICAL PATHWAYS

- The pathways of degradation are mainly of two broad options:
    1. either aerobic
    2. or anaerobic.
- Wide phylogenetic diversities of bacteria are capable of degrading contaminants aerobically; they degrade PCB (Polychlorinated Biphenyls) very efficiently e.g: *Pseudomonas*, *Bacillus* JF8.
- As anoxic conditions, several bacterial species that are anaerobic or facultative aerobic use natural organics as carbon and energy sources e.g.:
    1. Methanogens,
    2. Denitrifying microbes,
    3. Reducers
- Co-metabolism: occur widely in microbial metabolism.
- Microorganisms transform the desired xenobiotic compound even though the compound itself cannot serve as the primary energy source for those organisms.
- To degrade the contaminant, the microbes require the presence of other compounds (primary substrates) that can support their growth.
- The enzymes or coenzymes produced to degrade the primary substrate may display some activity for other substrate which is significantly known as co-substrate.
- The co-substrate is not the physiologically intended substrate but is just 'accidentally' transformed or in other words, the process occurs in a much randomized manner
- the aerobic organisms overcome the problem of degradation with oxygenases that initially reduce elemental oxygen to activate it, permitting it to insert into inert xenobiotic molecule.
- Under anaerobic conditions oxygen is inserted by some other source that is, organic acids are added onto xenobiotic molecule with synthases; O_2 in water is inserted into double bonds with hydratases and carbonic acid is added onto molecules with carboxylases.
- Xenobiotic compounds are oxidized and mineralized under anaerobic conditions if they are able to serve as electron donating substrates of primary metabolism.
- Phenols, phthalates and hydrocarbons including benzene-toluene-ethyl benzene-xylene (BTEX) fall into this category.
- Several xenobiotic compounds act as terminal electron acceptors (TEA), supporting growth of microorganisms by gaining energy from the oxidation of simple substrates (e.g. H_2).
- Pesticides, during anaerobic degradation, undergo dechlorination, hydrolysis, nitro-reduction and dealkylation, which have a metabolic shift from one pathway to another.
- Reductive dechlorination is common to all halogenated pesticides including aliphatic, cyclic aliphatic, aromatic, aniline-based henoxyalkanotes and cyclodiene types as the terminal electron accepting the process.
- This metabolism is referred to as halorespiration or dehalorespiration.

Pathway of degradation of different xenobiotic compounds

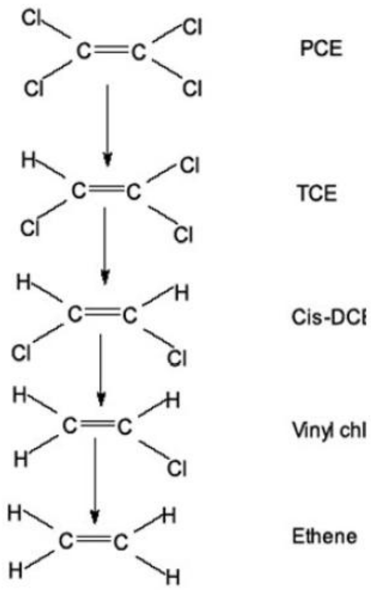
For examples:

a. Aliphatic compound;

b. Aromatic monocyclic compound;

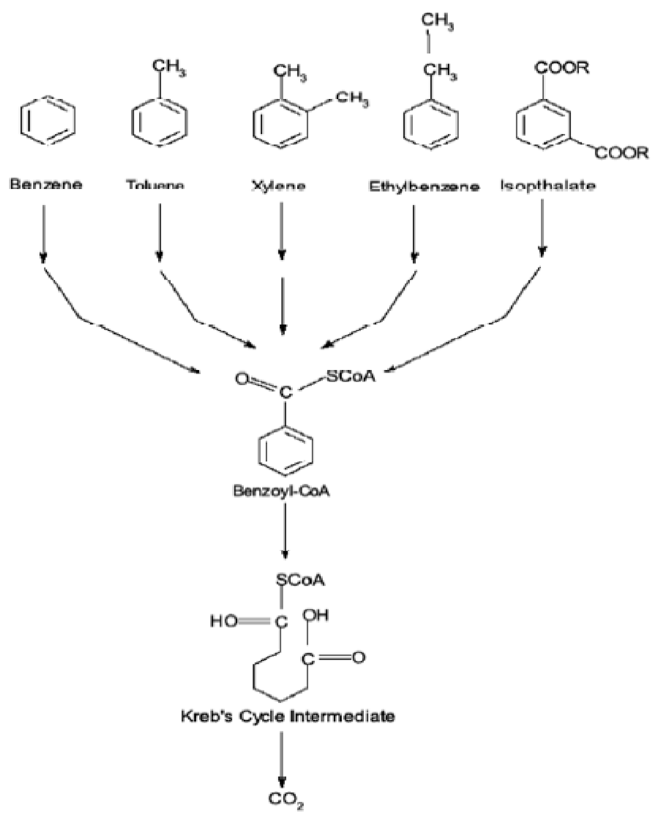
c. Aromatic polycyclic compound.

a. Aliphatic compound [21]:

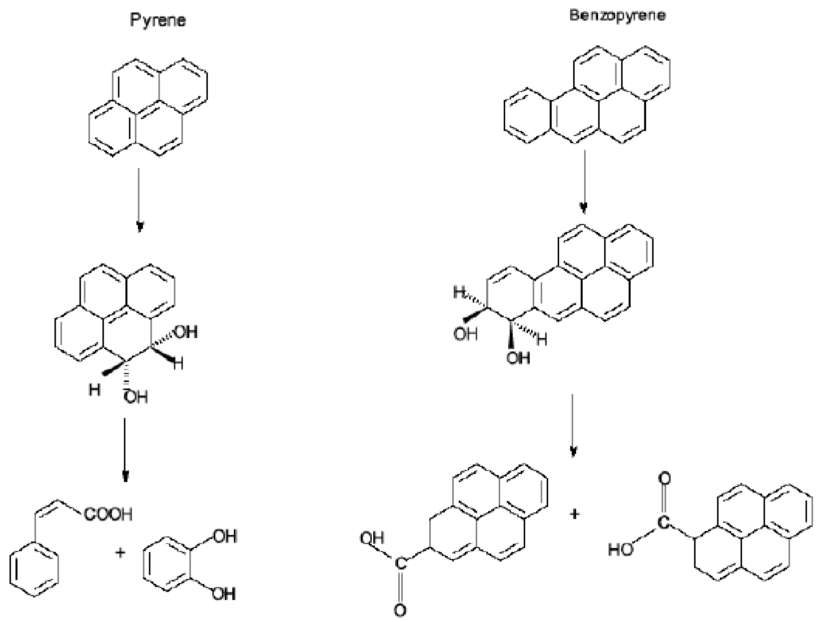


a

b. Aromatic monocyclic compound [21]:



***c. Aromatic polycyclic compound** [21]:*



MOLECULAR APPROACHES

- Most of the xenobiotic degrading bacteria have plasmids which code for catabolic genes.
- To characterize the appropriate genes and to enhance the process of degradation through improved constructed strains, a proper management is required.
- Degradation technology is spanning the spectrum from environmental monitoring ultimately to biodegradation as well as bioremediation.
- Molecular approaches are being used to characterize the nucleic acids of various bacteria from environmental samples.
- Comparing with standard microbiological methods, the molecular techniques provide more comprehensive interpretation of the in situ microbial community and its response to both engineered bioremediation and natural attenuation processes.

HERBICIDE DEGRADATION BY MICROBES

Degradation may be by:

- Photochemical decomposition predominates in air and water, only a small percentage of pesticides is decomposed in that way in soil.
- Chemical decomposition of herbicides in soil evolves through hydrolytic and non-hydrolytic transformations and oxidation.
- Microorganisms are decomposers of aliphatic and hydroxyl compounds but decompose aromatic substances at a slower rate.
- Compounds contain O, S or N in ring are slowest to decompose

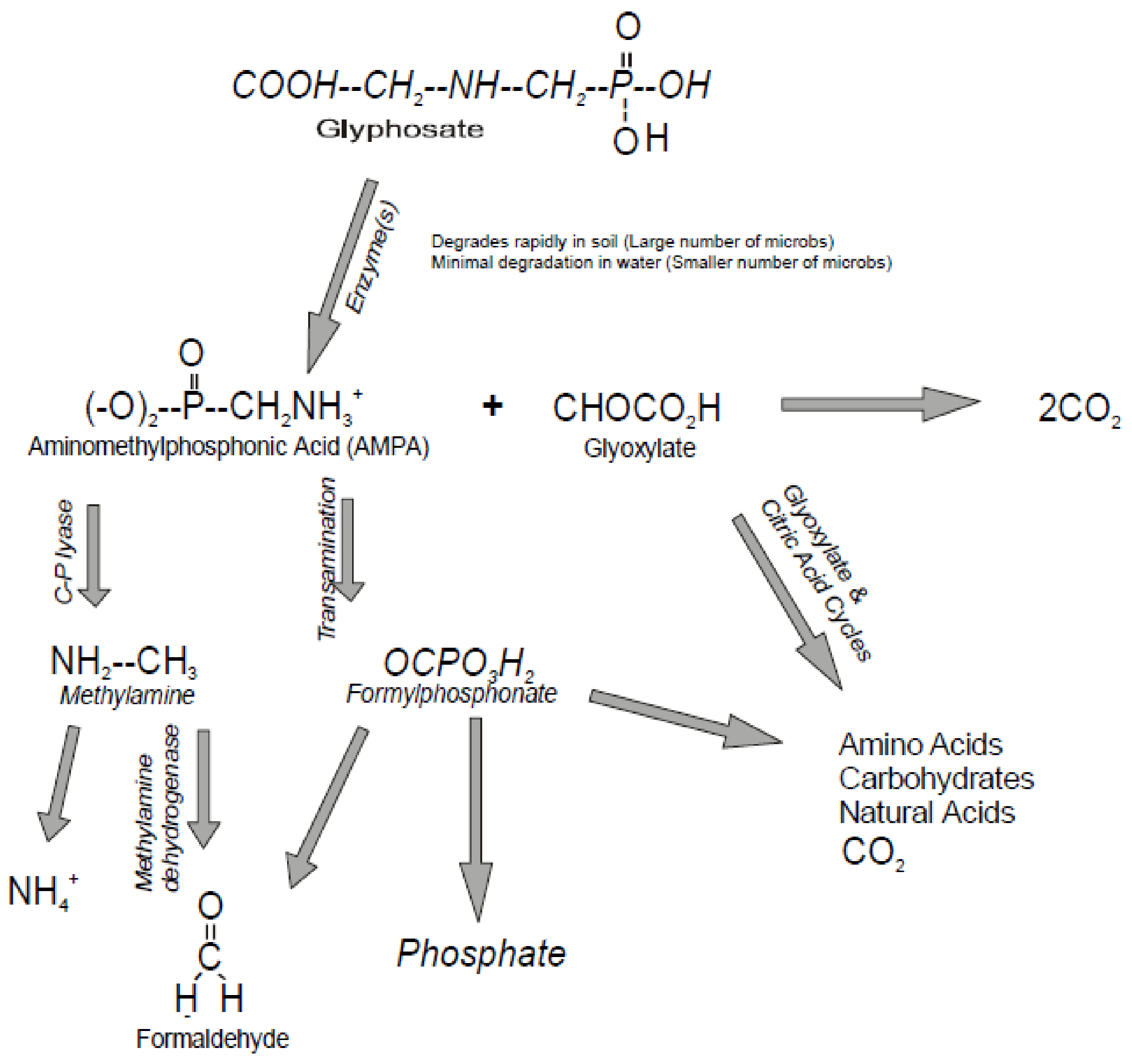
Herbicides affect microbes physiologically by:

- **Changing their biosynthetic mechanism**

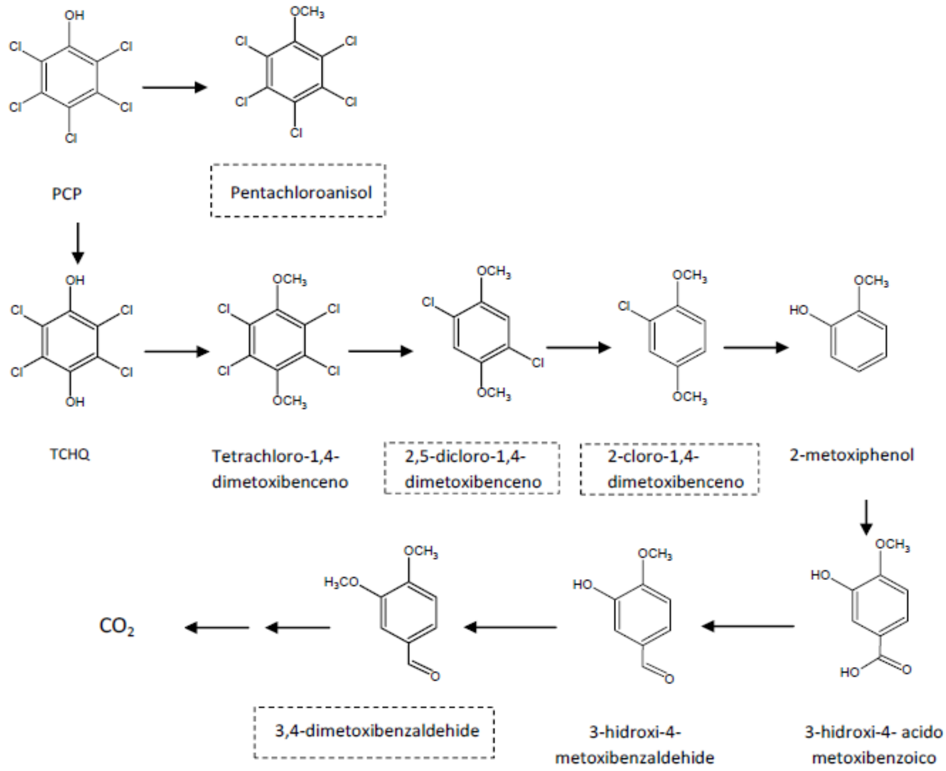
(Changing level of protein biosynthesis is reflected on the ratio of extracellular and intracellular enzymes);

- **Affecting protein biosynthesis**
  (Induction or repression of certain enzymes synthesis);
- **Affecting the cellular membranes**
  (Changes in transport and excretion processes);
- **Affecting plant growth regulators**
  (Transport of IAA, GA synthesis and ethylene level);
- **Applied in high doses**,
  (May kill microorganisms).

Glyphosate Degradation Pathway



Proposed pathways for the degradation of pentachlorophenol by *Anthracophyllum discolor* in soil (Rubilar et al [22]).



Problems cause limitation in soil bioremediation technology

In situ and ex situ methods

Bioremediation technologies can be broadly classified as *ex situ* and *in situ*. *Ex situ* technologies are those treatments which involve the physical removal of the pollutants for treatment process. In contrast, *in situ* techniques involve treatment of the pollutants in soil. Some of the examples of *in situ* and *ex situ* bioremediation are given below:

1. *Land farming*: Solid-phase treatment system for contaminated soils: may be done *in situ* or *ex situ*.
2. *Composting*: Aerobic, thermophilic treatment process in which pollutant is mixed with a bulking agent; can be done using static piles or aerated piles.
3. *Bioreactors*: Biodegradation in a container or reactor; may be used to treat liquids or slurries.
4. *Bioventing*: Method of treating contaminated soils by drawing oxygen through the soil to stimulate microbial activity.
5. *Biofilters*: Use of microbial stripping columns to treat air emissions.
6. *Bioaugmentation*: Addition of bacterial cultures to a contaminated medium; frequently used in both *in situ* and *ex situ* systems.
7. *Biostimulation*: Stimulation of indigenous microbial populations in soils by providing necessary nutrients.

8. *Intrinsic bioremediation*: Unassisted bioremediation of contaminant; by regular monitoring.

9. *Pump and treat*: Pumping ground water to the surface, treating, and reinjecting.

Advantages and disadvantages of bioremediation

The bioremediation methods depend on having the right microorganisms in the right place with the right environmental factors for carrying out the biodegradation. The right microorganisms are e.g., bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Bioremediation offers several advantages over conventional techniques such as land filling or incineration. Bioremediation can be done on site, is often less expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be coupled with other physical or chemical treatment methods. Bioremediation has its limitations. Some chemicals are not amenable to biodegradation, e.g., heavy metals, radionuclides and some chlorinated compounds. In some cases, microbial metabolism of contaminants may produce toxic metabolites. Bioremediation is a scientifically intensive procedure which must be tailored to the site-specific conditions, which means one has to do treatability studies on a small scale before the actual clean-up of the sites. One of the primary distinctions between surface soils, which typically receive regular inputs of organic material from plants, will have higher organic matter content. The high organic matter content is typically associated with high microbial populations with great diversities. The organic matter serves as main source of carbon and energy as well as a other macronutrients such as nitrogen, phosphorous, and sulfur. Subsurface of soils and ground water sediments have lower levels of organic matter and thus lower microbial communities than soil surface [23]. Bacteria become more dominant in the microbial community with increasing depth in the soil profile and the numbers of other organisms such as fungi or actinomycetes decrease. This is attributed to the ability of bacteria to use alternative electron acceptors to oxygen. Other factors that control microbial populations are moisture content, dissolved oxygen, and temperature.

Metabolic processes

Primary metabolism of an organic compound has been defined as the use of the substrate as a source of carbon and energy. This substrate serves as an electron donor resulting in microbial growth. Application of *co-metabolism* to polluted site with xenobiotics is required when the compound cannot serve as a source of carbon and energy by nature of the molecular structure, which does not induce the required catabolic enzymes. The term *co-metabolism* has been defined as the metabolism of a compound that does not serve as a source of carbon and energy or as an essential nutrient which can be achieved only in the presence of a primary (enzyme inducing) substrate. Aerobic processes are characterized by metabolic activities involving oxygen as a reactant. Dioxygenases and monooxygenases are two of the primary enzymes employed by aerobic organisms during transformation and mineralization of xenobiotics. Anaerobic microbes take advantage of a range of electron acceptors, which, depending on their availability and the prevailing redox conditions, include nitrate, iron, manganese, sulfate, and carbon dioxide.

Factors limiting the bioremediation

Energy sources

One of the primary variables affecting the activity of microorganisms is the ability and availability of reduced organic materials to serve as energy sources (Table 2). Whether a contaminant will serve as an effective energy source for an aerobic heterotrophic organism is a function of the average oxidation state of the carbon in the material. In general, higher oxidation states correspond to lower energy yields which thus provide less energetic incentive for microorganism degradation.

Table 2 The summary of the major factors limiting the bioremediation

Microbial	
	Growth until critical biomass is reached
	Mutation and horizontal gene transfer
	Enzyme induction
	Enrichment of the capable microbial populations
	Production of toxic metabolites
Environmental	
	Depletion of preferential substrates
	Lack of nutrients
	Inhibitory environmental conditions
Substrate	
	Too low concentration of contaminants
	Chemical structure of contaminants
	Toxicity of contaminants
	Solubility of contaminants
Biological aerobic vs. anaerobic process	
	Oxidation/reduction potential
	Availability of electron acceptors
	Microbial population present in the site
	Growth substrate vs. co-metabolism
Type of contaminants	
	Concentration
	Alternate carbon source present
	Microbial interaction (competition, succession, and predation)
Physico-chemical bioavailability of pollutants	
	Equilibrium sorption
	Irreversible sorption
	Incorporation into humic matters
Mass transfer limitations	
	Oxygen diffusion and solubility
	Diffusion of nutrients
	Solubility/miscibility in/with water

The outcome of each degradation process depends on microbial properties (biomass, population diversity, enzyme activities), substrate (physico-chemical characteristics, molecular structure, and concentration), and a range of environmental factors (pH, temperature, moisture content, Eh, availability of electron acceptors and carbon and energy sources). These parameters affect the acclimation period of the microbes to the substrate. The molecular structure and contaminant concentration have been shown to strongly affect the feasibility of bioremediation and the type of microbial transformation occurring, and whether the compound will serve as a primary, secondary or co-metabolic substrate.

Bioavailability

The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolism and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor [24]. This appears to be the case in most contaminated soils and sediments. For example, the contaminating explosives in soil did not undergo biodegradation process even after 50 years.

Treatments involving rigorous mixing of the soil and breaking up of the larger soil particles stimulated biodegradation drastically [25]. The bioavailability of a contaminant is controlled by a number of physico-chemical processes such as sorption and desorption, diffusion, and dissolution. A reduced bioavailability of contaminants in soil is caused by the slow mass transfer to the degrading microbes. Contaminants become unavailable when the rate of mass transfer is 0. The decrease of the bioavailability in the course of time is often referred to as aging or weathering.

It may result from:

1. Chemical oxidation reactions incorporating contaminants into natural organic matter,
2. Slow diffusion into very small pores and absorption into organic matter, and
3. The formation of semi-rigid films around non-aqueous-phase liquids (NAPL) with a high resistance toward NAPL-water mass transfer.

These bioavailability problems can be overcome by the use of food-grade surfactants [26], which increase the availability of contaminants for microbial degradation.

Bioactivity and biochemistry

The term bioactivity is used to indicate the operating state of microbiological processes. Improving bioactivity implies that system conditions are adjusted to optimize biodegradation ([27]. For example, if the use of bioremediation requires meeting a certain minimum rate, adjustment of conditions to improve biodegradation activity becomes important and a bioremediation configuration that makes this control possible has an advantage over one that does not.

In nature, the ability of organisms to transfer contaminants to both simpler and more complex molecules is very diverse. In light of our current limited ability to measure and control biochemical pathways in complex environments, favourable or unfavourable biochemical conversions are evaluated in terms of whether individual or groups of parent compounds are removed, whether increased toxicity is a result of the bioremediation process, and sometimes whether the elements in the parent compound are converted to measurable metabolites. These biochemical activities can be controlled in an in situ operation when one can control and optimize the conditions to achieve a desirable result.

Non-technical criteria

In addition to technical obstacles to bioremediation, some of the non-technical criteria that affect bioremediation are ability to achieve the required clean-up target, acceptable cost relative to other remediation options, acceptable risks in residual contaminants remaining after bioremediation, favourable public perception, favourable regulatory perception, ability to meet time limitations, and the ability to conform to space limitations.

Non-scientific factors affecting bioremediation

Several non-scientific factors hinder the development of bioremediation technologies and some of them are discussed below:

Regulatory factors

Regulations both drive and constrain the use of bioremediation. Regulation creates the bioremediation market by dictating what must be cleaned up, how clean it must be and which clean-up methods may be used [28]. The use of genetically engineered microorganisms (GEMs) presents additional regulatory hurdles. There is much debate over whether to use natural or GEMs in bioremediation. The advantages of naturally-occurring microbes currently outweigh those of GEMs.

Regulation can have an impact on bioremediation in three different ways:

1. Creating markets: Federal environmental programs require treatment of recurring wastes and remediation of existing wastes contaminating soils and groundwater [29].
2. Controlling the product: Environmental laws and regulation may specify health and safety criteria for products before they can be marketed in USA.
3. Toxic substances control act (TSCA) inventory: All new chemicals marketed in US must be listed in this inventory. Naturally-occurring microbes are already considered on the TSCA inventory. There are no organism-specific TSCA regulations on naturally-occurring microbes; but, regulations specific for GEMs are under development.
4. Other regulatory programs: The Food and Drug Administration and the US Department of Agriculture control the introduction of human-food and soil pathogens. The EPA regulates the use of microbes as pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act [29].
5. Controlling the process: Environmental Laws and regulations may specify how a product or equipment can be used to accomplish specific waste-management objectives. Some of the major US environmental laws which control the bioremediation process are listed below:
   - Toxic Substances Control Act of 1976.
   - Federal Water Pollution Control Act of 1972 amended by the Clean Water Act of 1977 and the Water Quality Act of 1987.
   - Solid Waste Disposal Act of 1965 amended by the resource conservation and Recovery Act of 1976 and the Hazardous and Solid Waste Amendments of 1984.
   - Comprehensive Environmental Response, Compensation and Liability Act of 1980 amended by the Superfund Amendments and Reauthorization Act of 1986.
   - Clean Air Act of 1970 amended by CAA Amendments of 1977 and of 1990.
   - National Environmental Policy Act of 1969.

Research and technical factors

Although there are a number of contaminants that are biodegradable, including petroleum hydrocarbons, alcohols and solvents, many widely used industrial chemicals such as PCBs, pesticides, coal tars, chlorinated solvents, and polynuclear aromatic hydrocarbons are not degraded so readily. So more intensive research is needed, but funding for this kind of basic research is diminishing.

Unlike the conventional treatment technologies, bioremediation technique must be tailored specifically to each polluted site. Each waste site has unique characteristics, and thus requires individual attention. As yet, official criteria for evaluating the success or failure of a particular strategy have not been established.

Human resource factor

Because bioremediation is a new technology, there is a lack of trained human resources in this field. A successful bioremediation program requires a multidisciplinary approach, integrating fields such as microbiology, engineering, geology, hydrogeology, and soil science and project management. Universities do not offer qualifications in bioremediation engineering and such combined expertise can be acquired only through experience and training on the job.

Economic and liability factor

Unlike other industries, bioremediation does not result in the production of high value-added products. Thus, venture capital has been slow to invest in the technology and, as a consequence, commercial activity in R and D has lagged far behind other industrial sectors.

As bioremediation is considered innovative technology, clients and regulatory agencies often scrutinize bioremediation more closely than conventional technologies. Consequently, tighter restrictions and performance standards are frequently imposed on bioremediation than on other remediation technologies. This can ultimately lead to a greater risk from a liability standpoint if the bioremediation program does not accomplish the predetermined goals.

FUTURE PERSPECTIVE

Throughout the past decade, there has been a great deal of progress in the study of the biodegradation of xenobiotic compounds. Several new genetically modified microorganisms with bioremediation potential have been isolated and many new biodegradation pathways have been elucidated. Nevertheless, this knowledge is far from complete. The biotransformation of organo-compounds of generally xenobiotics e.g., sulphur, phosphorus and more halogenic organo-compounds are still to be explored. The efficiency of xenobiotic biotransformation can be significantly improved by addressing key issues as tolerance to various xenobiotics, constitutive expression of the catabolic genes and the substrate-specificity, kinetics and the stability of the encoded enzyme. However, the utility of constructed organisms in dealing with problems related to environmental pollution in nature is yet to be investigated.

CONCLUSIONS

All the factors are positive in some cases where bioremediation technology has been successfully completed. Knowledge of the sensitivity to biodegradation of some pollutants is still lacking and toxicity testing is becoming more important. Many reports indicate that bioremediation of petroleum hydrocarbons can lead to reduced toxicity and have been taken as evidence of favourable biochemistry in these cases. There are many factors that limit bioavailability and have the effect of slowing the transport of specific compounds into aqueous phase where biological uptake occurs readily. The importance of bioavailability is strongly dependent on the nature of the pollutant and the soil biochemistry. Bioactivity includes consideration of those parameters that have long been recognized as influencing the rate of bioremediation. With current bioremediation configurations, only certain parameters can be manipulated. This suggests that certain sites may be particularly favourable for *in* *situ* strategies, because the bioactivity may be easily maintained. The trend is slowly changing and for bioremediation using both indigenous and non-indigenous, naturally occurring microorganisms, the regulatory hurdles are decreasing.

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