Chapter 1 An Introduction to Bioremediation

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Chapter 1
An Introduction to Bioremediation

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1.1 Introduction

The explosive rise of global population has led to the increased exploitation of natural resources and sources to respond to the high demands of the population for food, energy, and all other requirements. Industrial revolution was a response to these requirements; however, it has resulted in the production of huge number of various organic and inorganic chemicals that have directly and indirectly led to the prolonged pollution of the habitats. The duration of the contamination is regarded to be because of their difficult biodegradability. The trend of environmental pollution is so fast and vast that the detectable rates of contamination are even encountered in the farthest ocean waters. Based on the estimations made by the environmental protection agency (EPA) only around 10 % of all wastes were safely disposed off (Chaudhry 1994; Reddy and Mathew 2001).

In addition to the pollutants and toxicants released from industries that continuously affect the environment, an abstracted review of news reveals the occasional but serious occurrence of environmental disasters such as the Exxon Valdez oil spill, the Union-Carbide (Dow) Bhopal disaster, large-scale contamination of the

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Rhine River, the progressive deterioration of the aquatic habitats and conifer forests in the Northeastern US, Canada, and some parts of Europe, or the release of radioactive material in the Chernobyl accident, and most recently the crises resulted from crude oil pollution of Mexico gulf waters and the leakage of the radioactive materials from Fukushima reactor in Japan. The contaminants known to be biologically degraded by microorganisms so far known and applied in bioremediation (bioremediants) have been categorized into five groups (Hickey and Smith 1996). These include:

(a) Halogenated aromatic hydrocarbons
(b) Munitions wastes
(c) Organic solvents
(d) Pesticides
(e) Polyaromatic hydrocarbon (PAH) (creosote oily wastes)

PAH, pentachlorophenols (PCP), polychlorinated biphenyls (PCB), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), 2-benzene, toluene, ethylbenzene, and xylene (BTEX), and trinitrotoluene (TNT) are persistent pollutants in the environment known to exert carcinogenic and/or mutagenic impacts and have been classified as priority pollutants by EPA. It has cost around one trillion USD to decontaminate toxic waste sites in the USA using traditional waste disposal methods such as incineration and landfilling (Reddy and Mathew 2001).

Bioremediation is regarded to be an effective and in the mean time an economic method for the decontamination of environment.

1.2 Role of Environmental Biotechnology in Pollution Management

Biotechnology can be used to assess the well being of ecosystems, transform pollutants into benign substances, generate biodegradable materials from renewable sources, and develop environmentally safe manufacturing and disposal processes.

Environmental biotechnology takes advantage of appropriately qualified living organisms and employs genetic engineering to improve the efficiency and cost, which are key factors in the future widespread exploitation of organisms to reduce the environmental burden of toxic substances.

In view of the urgent need of an efficient environmental biotechnological process, researchers have devised a technique called bioremediation, which is an emerging approach to rehabilitating areas fouled by pollutants or otherwise damaged through ecosystem mismanagement.

1.3 Bioremediation

The term of bioremediation has been made of two parts: "bios" means life and refers to living organisms and "to remediate" that means to solve a problem. "Bioremediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater. Bioremediation is the use of living microorganisms to degrade environmental pollutants or to prevent pollution. In other words, it is a technology for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution (Sasikumar and Papinazath 2003).

Bioremediation could simply be defined as a biological process of the decontamination of contaminated environment. The environment may be either terrestrial, aqueous, or both. However, a more comprehensive definition is presented below:

Bioremediation is a means of cleaning up contaminated environments by exploiting the diverse metabolic abilities of microorganisms to convert contaminants to harmless products by mineralization, generation of carbon (IV) oxide and water, or by conversion into microbial biomass (Baggott 1993; Mentzer and Ebere 1996).

A point to emphasize here is that bioremediation and biodegradation should not be confused with each other. Bioremediation as a technique may include biodegradation as only one of the mechanisms involved or applied in the process of bioremediation. Only some of contaminants are biodegradable, and only some of microorganisms can degrade a fraction of contaminants (Walsh 1999). Therefore, it would be of worth to study the biodegrading potential of microorganisms.

Despite the fact that microorganisms have been used for the treatment and transformation of waste products for at least a century, bioremediation is considered as a new technology for eco-friendly decontamination of polluted environment. As a popular case of the application of this technology, municipal waste water is microbiologically decontaminated under controlled conditions so that dependent upon the metabolic activities of microorganisms, different systems of activated sludge and fixed films are applied in waste water treatment facilities (King et al. 1992). Wastes and pollutions can be permanently eliminated. Also, lasting liabilities are eliminated taking advantage of less expensive but in the mean time more long-standing biological systems. Furthermore, bioremediation methods could be applied in an integrated manner together and coupled with other treatment approaches. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass.

Theoretically, there are enough bioremediants in nature that can be applied against a broad range of pollutants and bioremediation can be considered as a useful technique for the complete destruction of a wide variety of contaminants. Many compounds that are legally regarded as detrimental and dangerous can be

biotransformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible. Bioremediation can save life web and prohibit the passage of dangerous and risky contaminants from an ecosystem to another. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation. Bioremediation can prove less expensive than other technologies used for clean-up of hazardous waste (Vidali 2001).

However, bioremediation technology suffers from two drawbacks. One is that only a few bacteria and fungi act on a broad range of organic compounds. So far no organism has been known enough omnivorous to destroy a large percentage of the natural chemicals exist. This drawback may be bypassed through screenings for the discovery of new microbial species and detection of the potential of microorganisms in one hand, and the synchronized or successive application of the microorganisms that complete the bioactivity of each other.

Another drawback to bioremediation is that it takes a long period of time to act and impose its effect. There are some solutions to get rid of such a limitation. Genetic manipulation techniques have led to an invaluable opportunity to obtain new strains of enhanced or new bioremediation activity. Another solution may be the addition of some enhancers in an environment or in a formulation to biochemically fortify certain bioremediation pathway(s). The third solution is the synchronous use of one or more microorganisms that directly and/or indirectly increase the bioactivity of a bioremediant. Increase of the bioremediant population is the fourth and the simplest solution.

Bioremediation is limited to those compounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation, and there are some concerns that the products of biodegradation may be more persistent or toxic than the parent compounds. Biological processes are often highly specific. Important site factors required for successful bioremediation include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants. It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases. Bioremediation often takes longer than other treatment options, such as excavation and removal of soil or incineration. Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation.

There is no accepted definition of “clean,” evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments (Vidali 2001).

Bioremediation although considered as a reliable technique in the middle of present environmental problems, however, it can also be considered problematic because, while additives applied to promote the activity of the particular microorganism(s) may disrupt other organisms inhabiting same environment when done in situ. Even if genetically modified microorganisms are released into the environment after a certain point of time it becomes difficult to remove them. Bioremediation is generally very costly, is labor intensive, and can take several months for the remediation to achieve acceptable levels. Another problem regarding the use of in situ and ex situ processes is that it is capable of causing far more damage than the actual pollution itself.

Nutrient imbalance can hinder biodegradation. Inadequate provision of nitrogen, phosphorus, potassium, and sulfur (which is probably the most important and the most easily modified of all the factors) could limit the rate of hydrocarbon degradation in the terrestrial environment (McGill and Nyborg 1975).

In the agreement to the United States Office of Technology Assessment (1990), the amendment of limiting nutrients to the spill site is necessary. There are sufficient hydrocarbon-utilizing microorganisms in soil to start bioremediation as soon as nutrient limitation is alleviated (Stone et al. 1942). The natural phospholipids, soybean lecithin and ethyl allophanate, are respectively the best available phosphorus and nitrogen sources, for the microbial bioremediants of oil contaminations (Olivieri et al. 1978).

From the view point of future prospects of bioremediation, it seems that the development of our knowledge of microbial populations, their interactions to the natural environment and contaminants, the increase of their genetic capabilities to degrade contaminants, the long-term field studies of new economical bioremediation techniques can increase the potential for significant advances. There is no doubt that bioremediation is the need of present world and can lead to protection and preservation of natural resources we have browsed from the next generations.

1.4 Types of Bioremediation

On the basis of place where wastes are removed, there are principally two ways of bioremediation:

1.4.1 In Situ Bioremediation

Most often, in situ bioremediation is applied to eliminate the pollutants in contaminated soils and groundwater. It is a superior method for the cleaning of contaminated environments because it saves transportation costs and uses harmless microorganisms to eliminate the chemical contaminations. These microorganisms are better to be of positive chemotactic affinity toward contaminants. This feature

increases the probability of the bioremediation in close points where bioremediants have not distributed. Also, the method is preferred as it causes the least disruption of the contaminated area. This would be of much relevance either where the least investment and pollution are favored (for example in factories) or in areas contaminated with dangerous contaminants (for example in areas contaminated with chemical or radioactive materials).

Another advantage of in situ bioremediation is the feasibility of synchronous treatment of soil and groundwater. However, in situ bioremediation posses some disadvantages: the method is more time-consuming compared to other remedial methods, and it leads to a changed seasonal variation in the microbial activity because of the direct exposure to the variations in uncontrollable environmental factors, and the use of additives may lead to additional problems. The yield of bioremediation is determined by the kind of waste materials, namely if wastes could provide the required nutrients and energy, then microorganisms would be able to bioremediate. However, in the absence of favorable wastes, the loss of bioactivity may be compensated through stimulation of native microorganisms. Another choice of less preference is to apply genetically engineered microorganisms.

Two types of in situ bioremediation are distinguished based on the origin of the microorganisms applied as bioremediants:

(i) *Intrinsic bioremediation*—This type of in situ bioremediation is carried out without direct microbial amendment and through intermediation in ecological conditions of the contaminated region and the fortification of the natural populations and the metabolic activities of indigenous or naturally existing microfauna by improving nutritional and ventilation conditions.
(ii) *Engineered in situ bioremediation*—This type of bioremediation is performed through the introduction of certain microorgansims to a contamination site. As the conditions of contamination sites are most often unfavorable for the establishment and bioactivity of the exogenously amended microorganisms, therefore here like intrinsic bioremediation, the environment is modified in a way so that improved physico-chemical conditions are provided. Oxygen, electron acceptors, and nutrients (for example nitrogen and phosphorus) are required to enhance microbial growth.

1.4.2 Ex Situ Bioremediation

The process of bioremediation here takes place somewhere out from contamination site, and therefore requires transportation of contaminated soil or pumping of groundwater to the site of bioremediation. This technique has more disadvantages than advantages.

Depending on the state of the contaminant in the step of bioremediation, ex situ bioremediation is classified as:

(i) Solid phase system (including land treatment and soil piles)—The system is used in order to bioremediate organic wastes and problematic domestic and industrial wastes, sewage sludge, and municipal solid wastes. Solid-phase soil bioremediation includes three processes including land-farming, soil biopiling, and composting.
(ii) Slurry phase systems (including solid–liquid suspensions in bioreactors)—Slurry phase bioremediation is a relatively more rapid process compared to the other treatment processes.

Contaminated soil is mixed with water and other additives in a large tank called a bioreactor and intermingled to bring the indigenous microorganisms in close contact with soil contaminants. Nutrients and oxygen are amended, and the conditions in the bioreactor are so adjusted that an optimal environment for microbial bioremediation is provided. After completion of the process, the water is removed, and the solid wastes are disposed off or processed more to decontaminate remaining pollutants.

1.5 Bioremediation Techniques

There are several bioremediation techniques, some of them have been listed as follows (Baker and Herson 1994):

1.5.1 Bioaugmentation

The addition of bacterial cultures to a contaminated medium used frequently in in situ processes. Two factors limit the use of added microbial cultures in a land treatment unit: (a) nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and (b) most soils with long-term exposure to biodegradable waste have indigenous microorganisms that are effective degraders if the land treatment unit is well managed (Vidali 2001).

1.5.2 Biofilters

The use of microbial stripping columns used to treat air emissions.

1.5.3 Bioreactors

The use of biological processes in a contained area or reactor for biological treatment of relatively small amounts of waste. This method is used to treat slurries or liquids. Slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume.

Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil-bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pretreatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor (Vidali 2001). Bioreactors have been used to treat soil and other materials contaminated with petroleum residues (McFarland et al. 1992; Déziel et al. 1999).

1.5.4 Biostimulation

The stimulation of the indigenous microbial populations in soils and/or ground water. This process may be done either in situ or ex situ.

1.5.5 Bioventing

The process of drawing oxygen through the contaminated medium to stimulate microbial growth and activity.

Bioventing is the most common in situ treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Vidali 2001). In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few centimeters to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases (Vidali 2001).

1.5.6 Composting

An aerobic and thermophillic process that mixes contaminated soil with a bulking agent. Composting may be performed using static piles, aerated piles, or continuously fed reactors. Composting is a technique that involves combining

contaminated soil with nonhazardous organic amendments such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting (Vidali 2001). Composting is a process by which organic wastes are degraded by microorganisms, typically at elevated temperatures. Typical compost temperatures are in the range of 55–65 °C. The increased temperatures result from heat produced by microorganisms during the degradation of the organic material in the waste. Windrow composting has been demonstrated using the following basic steps. First, contaminated soils are excavated and screened to remove large rocks and debris (Antizar-Ladislao et al. 2007, 2008).

The soil is transported to a composting pad with a temporary structure to provide containment and protection from weather extremes. Amendments (straw, alfalfa, manure, agricultural wastes, and wood chips) are used for bulking agents and as a supplemental carbon source. Soil and amendments are layered into long piles, known as windrows. The windrow is thoroughly mixed by turning with a commercially available windrow turning machine. Moisture, pH, temperature, and explosives concentration are monitored. At the completion of the composting period, the windrows would be disassembled and the compost is taken to the final disposal area.

1.5.7 Landfarming/Land Treatment/Prepared Bed Bioreactors

Solid phase treatment system for contaminated soil that may be applied as an in situ process or ex situ in a soil treatment cell. Landfarming is a simple bioremediation technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil (Vidali 2001).

Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative (Vidali 2001). Spilled oil and wood-preserving wastes have been bioremediated by landfarming treatments (Haught et al. 1995; Margesin and Schinner 1999).

1.5.8 Biopiling

Biopiles are a hybrid of landfarming and composting. Essentially, engineered cells are constructed as aerated composted piles. Adding compost to contaminated soil enhances bioremediation because of the structure of the organic compost matrix (Kästner and Mahro 1996). Compost enhances the oxidation of aromatic

contaminants in soil to ketones and quinones, which eventually disappear (Wischmann and Steinhart 1997).

Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms (Vidali 2001).

Biopile treatment is a full-scale technology in which excavated soils are mixed with soil amendments, placed on a treatment area, and bioremediated using forced aeration. The contaminants are reduced to carbon dioxide and water. The basic biopile system includes a treatment bed, an aeration system, an irrigation/nutrient system, and a leach ate collection system. Moisture, heat, nutrients, oxygen, and pH are controlled to enhance biodegradation. The irrigation/nutrient system is buried under the soil to pass air and nutrients either by vacuum or positive pressure. Soil piles can be up to 20 ft high and may be covered with plastic to control runoff, evaporation, and volatilization, and to promote solar heating. If volatile organic compounds (VOCs) in the soil volatilize into the air stream, the air leaving the soil may be treated to remove or destroy the VOCs before they are discharged into the atmosphere. Treatment time is typically 3–6 months (Prasad Shukla et al. 2010; Wu and Crapper 2009).

1.6 Organisms Involved in Bioremediation Process

Organisms that are due to be applied in bioremediation shall fulfill the following requirements (Alexander 1994) (a) The organisms will have the effective enzymes important in bio-remediation; (b) The organism shall be able to live and demonstrate its bioactivity under conditions of pollution; (c) The organism must be able to get access to the contaminant that may be not soluble in aqueous environments or severely adsorbed to solid surfaces; (d) the substrate site of the contaminant must be accessible for the active site of the enzyme of role in bioremediation; (e) contaminant and the enzymatic system must come in close contact somewhere in or out of the cell; and finally (f) appropriately favorable environmental conditions must exist or be provided to arise the population of the potential bioremediant.

The successful occurrence of bioremediation would be dependent on the provision of the conditions mentioned above; however, various types of uni-/multicellular organisms have the required potentials to be applied in bioremediation processes. Indeed species of plants, bacteria, and fungi may be used to eliminate pollutants. However, microorganisms are of the highest bioremediation potentials as they are natural decomposers in different ecosystems and can easily proliferate. Microorganisms as fungi and bacteria degrade and break down the molecules of natural and or synthetic origins. Their high rate of generation, chemotactism, complex enzymatic, and secretion systems make them valuable replacements for other chemical and or physical remediation agents. A continuous study to identify

and select new species and strains for bioremediation processes is highly required. The recent discovery of a bacterial species *Geobacter metallireducens* is a good proof for the necessity of such studies. The bacterium reduces and removes radioactive uranium from drainage waters in mining operations and from contaminated ground waters. Even dead microbial cells can be useful in bioremediation technologies. The bacterium reduces and removes radioactive uranium from drainage waters in mining operations and from contaminated ground waters. Even dead microbial cells can be useful in bioremediation technologies. Highly toxic heavy metals are reduced and fixed by secretions of some bacteria and algae and are omitted from the flow of food materials in the ecosystem. Similarly, plants like locoweed can absorb and bioaccumulate high amounts of selenium in their tissues in a form that is not hazardous till consumption of plant tissues.

Various microorganisms like various corynebacteria, mycobacteria, pseudomonads, and some of yeasts have been known to act as nontoxic bioemulsifiers and to eliminate oil slicks and petroleum pollutions through biodegradation of oil hydrocarbons metabolized as sources of energy and carbon. Similarly, some microorganisms have the potential to degrade and or metabolize synthetic compounds (as remnants of pesticides in agroecosystems) collectively called as xenobiotics. Fungi as well as some anaerobic bacteria can degrade dyes. Bioremediation is a process performed through different mechanisms such as biosorption, biodegradation, bioaccumulation, and metabolism (biotransformation, detoxification, and . . .) of the contaminant molecules. Dependent on the type of the organism, some terms are used to specify the bioremediation. Phytoremediation is referred to the type of bioremediation that is relied on plants and algae as bioremediants. Mycoremediation is a type of bioremediation where fungi serve as bioremediants. In this book and in the following of this chapter, mycoremediation will be the only subject of consideration.

1.7 Mycoremediation

The history of fungal bioremediation or mycoremediation goes back to only a couple of decades ago. Despite of youth of the science of mycoremediation, this new branch of environmental biotechnology attracts a daily increasing attention of scientists that is because of the exceptional characteristics of mycoremediants, themselves. Fungi are equipped with a well-developed enzymatic system that awards them the ability to grow well on a broad range of natural as well as synthetic substrates. Fungi produce and secrete higher rates of different extracellular enzymes into their peripheral environment and degrade various substrates to small molecules that can be absorbed by and metabolized in their cells.

The fact that fungi are exogastric creates an opportunity for metabolism of new substrates such as many nonpolar, nonsoluble toxic compounds that are not amenable to intracellular processes such as cytochrome P450 (Reddy and Mathew 2001; Levin et al. 2003).

The evolutionary notable characteristic of fungal morphology, production, and ramification of cylindrical strands (hyphae, in singular form: hypha) of tip growth enables them to search for new yet noncolonized sources of material and energy and penetrate the useable substrates. Beside its physically positive impact in the enforced occupation of the inner parts of the penetrated substrates, the hyphal morphology of fungi with strong flow of cytoplasmic stores of material and energy toward hyphal tips enables fungi to get access to the inner layers of the substrates that are not in direct contact with an aqueous environment. The diversity of the produced and secreted enzymes besides hyphal growth of fungi gives them the ability to overcome the problem that is often encountered with the substrate sites covered with other molecules that inhibit fungal enzyme active site from reaching the substrate site. Another advantage of mycoremediants is that the enzymes of importance in bioremediation are stimulated under nutrient deficiency conditions (Mansur et al. 2003; Aust et al. 2003). Fungal growth rate is also reasonably enough fast for applications in bioremediation processes. In addition to the ability to penetrate contaminating substrates, fungi are regarded superior to bacteria in that they can grow under environmentally stressed conditions (low pH and poor nutrient status), where bacteria are expected to be of limited bioactivity (Davis and Westlake 1979). Fungi are able to survive, grow, and develop under toxic conditions intolerable for most bacteria (Aust et al. 2003).

Most fungi are of short life cycles and higher rates of sporulation and therefore can raise their populations in a rather short time span. For instance, yeast populations in a fresh water stream increased by several orders of magnitude in the 5 days after an oil spill (Jones 1976). Fungal degradation may proceed more rapidly than bacterial degradation, with complexation suggested as the main mechanism of calcium mobilization (Gadd 2010). Moreover, the fungi can be easily transported, genetically engineered, and scaled up.

All these features encourage us to consider fungi as the organisms preferred for applications in bioremediation. Fungal bioremediation is subject to the prevailing temperature, moisture, and soil conditions (Kearney and Wauchope 1998). The soil pH, water availability, nutritional status, and oxygen levels vary and may not always are optimal for the growth of white rot fungi (Philippoussis et al. 2002; Zervakis et al. 2001) or extracellular enzyme production for pollutant transformation (Gadd 2001). Hence the kinetics of pesticides degradation in the soil is commonly biphasic with a very rapid degradation rate in the beginning followed by a very slow prolonged dissipation. The remaining residues are often quite resistant to degradation (Alexander 1994). Among environmental parameters, the availability of water in soil may be a very important factor affecting the success of bioremediation, as water availability affects oxygen supply and thus fungal growth and enzyme production (Philippoussis et al. 2001; Marin et al. 1998). In addition to affecting microbial behavior, water availability affects contaminant binding and distribution in the soil. The behavior of organic compounds in water plays a very important role in their accessibility for microbial utilization in the environment (Atagana et al. 2003). Other factors effective on biodegradation in soil include chemical nature, concentration of the contaminant, soil type, amount of soil organic

matter, and microbial community structure and activity (Schoen and Winterlin 1987). Reportedly, degradation of a diverse group of organic contaminants is dependent on the nonspecific and non-stereoselective ligninase not specifically induced by the pollutants and produced under substrate limiting growth conditions (Singh and Kuhad 2000).

Many fungal genera have been identified that include species able to metabolize hydrocarbons and some of them may be applied in bioremediation of oil-polluted regions. These fungal genera include: *Acremonium* (Llanos and Kjøller 1976), *Aspergillus* (Bartha and Atlas 1977; Obire et al. 2008), *Aureobasidium* (Bartha and Atlas 1977), *Candida* (Bartha and Atlas 1977; Obire et al. 2008), *Cephalosporium* (Bartha and Atlas 1977; Obire et al. 2008), *Cladosporium* (Walker et al. 1973; Bartha and Atlas 1977; Obire et al. 2008), *Cunninghamella* (Bartha and Atlas 1977), *Fusarium* (Llanos and Kjøller 1976; Obire et al. 2008), *Geotrichum* (Obire et al. 2008), *Gliocladium* (Llanos and Kjøller 1976), *Graphium* (Llanos and Kjøller 1976), *Hansenula* (Bartha and Atlas 1977), *Mortierella* (Llanos and Kjøller 1976), *Mucor* (Obire et al. 2008), *Paecilomyces* (Llanos and Kjøller 1976), *Penicillium* (Llanos and Kjøller 1976; Bartha and Atlas 1977; Obire et al. 2008), *Rhodosporidium* (Ahearn and Meyers 1976; Bartha and Atlas 1977), *Rhodotorula* (Bartha and Atlas 1977; Obire et al. 2008), *Saccharomyces* (Bartha and Atlas 1977), Sphaeropsidales (Llanos and Kjøller 1976), *Sporobolomyces* (Bartha and Atlas 1977), *Torulopsis* (Bartha and Atlas 1977), *Trichoderma* (Hadibarata and Tachibana 2009; Llanos and Kjøller 1976; Obire et al. 2008), *Trichosporon* (Ahearn and Meyers 1976; Bartha and Atlas 1977).

Obire (1988) found several oil-degrading aquatic yeast species belonged to the genera *Candida*, *Rhodotorula*, *Saccharomyces*, and *Sporobolomyces* (yeasts), and among filamentous fungi, *Aspergillus niger*, *Aspergillus terreus*, *Blastomyces* sp., *Botryodiplodia theobromae*, *Fusarium* sp., *Nigrospora* sp., *Penicillium chrysogenum*, *Penicillium glabrum*, *Pleurofragmium* sp., and *Trichoderma harzianum*. Annual application of various fungicides, herbicides, and insecticides at practical doses has a negative impact on the dynamics of nitrate nitrogen, mobile phosphorus, and exchangeable potassium in different soil layers (Ivanov 1974). The ecological targets include microorganisms involved in nitrogen and carbon transformations: low application doses of some agrochemicals such as simazine, atrazine, and zeazine decrease activity of urease and other soil enzymes and promote the accumulation of phytotoxic bacteria and fungi (Grodnitskaya and Sorokin 2006). *Trichoderma viride* degrades insecticides fenitrothion and fenitrooxon (Baarschers and Heitland 1986), and some of its strains are able to degrade malathion through carboxylesterase(s) (Matsumura and Boush 1966). Mukherjee and Gopal (1996) compared the potential of two soil fungi, *Aspergillus niger* and *Trichoderma viride* for the degradation of chlorpyrifos and found that *T. viride* was more active, 95.7 of chlorpyrifos degraded in the presence of *T. viride* as compared to 72.3 % in the presence of *A. niger* by day 14. The toxic metabolite of chlorpyrifos, 3,5,6-trichloropyridinol was not detected during the 14-day incubation period. *Trichoderma harzianum* has been found to degrade DDT, dieldrin, endosulfan, pentachloronitrobenzene, and pentachlorophenol but not

hexachlorocyclohexane. The fungus degrades endosulfan under various nutritional conditions throughout its growth stages. Endosulfan sulfate (the first fungal metabolite) and endosulfan diol have been detected as the major fungal metabolites of endosulfan mainly resulted from oxidative system (Katayama and Matsumura 1993). Askar et al. (2007) have indicated the possibility of the herbicide bromoxynil biodegradation through treatments with *T. harzianum* and *T. viride* aimed to prohibit underground and surface water sources. The ability of *Trichoderma* species to survive and grow in the agar media amended with the recommended doses of prevalent herbicides (Pakdaman et al. 2002) seems to be very promising in the cleanup of agroecosystems and omission of agrochemical xenobiotics that otherwise could hurt future agricultural crops. The application of *Trichoderma* species would not only clean the agricultural ecosystems but also would lead to increased yields of future crops as the result of the biological control of plant pathogens (Pakdaman and Goltapeh 2006) and induction of plant defensive system. Furthermore, *Trichoderma* can be applied in the bioremediation of soils contaminated with toxic metabolites from toxigenic microorganisms (Grodnitskaya and Sorokin 2006). *Trichoderma* is regarded as a well-known biological control agent that can be applied instead of a range of agrochemicals from fungicides, nematicides, acaricides, and insecticides, while the fungus is also a mycoremediant. The fungus while it enhances plant growth and development, behaves friendly to the free-living plant growth-promoting fungi *Piriformospora indica* and *Sebacina vermifera* (Ghahfarokhy et al. 2011) indicating the possibility of the use of all three fungi in integrated programs for organic agricultural systems.

Lentinus edodes, the gourmet mushroom has been shown to possess the capacity for removing more than 60 % of pentachlorophenol from soil (Pletsch et al. 1999) and appears to remain active at lower temperatures typically encountered with temperate soils of central and Northern Europe (Okeke et al. 1996).

A number of fungal strains (in the parenthesis) have been isolated from oil refinery soils in Poland (IETU 1999; Ulfig et al. 1997, 1998): *Aphanoascus reticulisporus* (3), *Aphanoascus keratinophilum* (6), *Candida famata* (14), *Exophiala* sp. (6), *Fusarium* sp. (11), *Geomyces pannorum* (3), *Geotrichum candidum* (3), *Microsporum gypseum* (5), *Paecilomyces lilacinus* Fungi (3), *Penicillium* sp. (4), *Phialophora* sp. (2), *Phoma* sp. (3), *Pseudallescheria boydii* (4), *Scopulariopsis brevicaulis* (1), *Trichophyton ajelloi* (15), and *Trichophyton terrestre* (4).

Phanerochaete chrysosporium and other white rot fungi are able to degrade a broad range of structurally diverse xenobiotics ranging from the insecticides DDT and lindane to wood-preserving chemicals (Kirk et al. 1992), including PCP and the creosote components anthracene and phenanthrene, to polychlorinated biphenyls and dioxins. Other compounds degraded by white rot fungi include 2,3,7,8-TCDD, 3,4,3′,4-TCB, benzo(α)pyrene, Aroclor 1254, 4-chloroaniline, 3,4-dichloroaniline, chloroaniline-lignin conjugates, benzo(α)pyrene, pentachlorophenol, triphenylmethane dyes, crystal violet, pararosaniline, cresol red, bromophenol blue, ethyl violet, malachite green, brilliant green, 2,4,5-trichlorophenoxyacetic acid, phenanthrene, polycyclic aromatics, anthracene, fluoranthene, benzo(*β*) fluoranthene, benzo(*k*)fluoranthene, benzo(α)pyrene, indeno(*ghi*)pyrene,

benzoperylene, azo and heterocyclic dyes, Orange II, Tropaeolin O, Congo red, Azure B, and trinitrotoluene (Bumpus et al. 1985; Eaton 1985; Arjmand and Sandermann 1985, 1986; Haemmerli et al. 1986; Bumpus and Aust 1987; Kohler et al. 1988; Mileski et al. 1988; Lamar et al. 1990; Lin et al. 1990; Bumpus and Brock 1988; Ryan and Bumpus 1989; Bumpus 1989; Huttermann et al. 1989; Cripps et al. 1990; Fernando et al. 1990). In a series of experiments, from laboratory bench-scale to full-scale field demonstrations, Haught et al. (1995) demonstrated the potential of *Phanerochaete chrysosporium* and *P. sordida* in PAH biodegradation. However, white rot fungi are of no considerable ability in the removal of high-molecular weight PAHs (five rings and above). A pilot-scale reactor system was developed that combined extraction of PAH-contaminated soil with a physically separate fungal bioreactor containing *P. chrysosporium* (May et al. 1997). The extraction of high-molecular weight PAHs from the soil led to their further bioavailability for the fungus and to high degradation rates. In another study, *P. sordida* transformed PAHs with three and four rings in creosote-contaminated soil, but five- and six-ring PAHs were not degraded (Davis et al. 1993).

The edible oyster mushroom *Pleurotus ostreatus* is able to degrade 80 % of the total PAHs in soil within 35 days (Bogan and Lamar 1999). The comparison of the abilities of *P. ostreatus*, *P. chrysosporium*, and *Trametes versicolor* in the biodegradation of PAHs, and in the in solidum production of ligninolytic enzymes revealed the superiority of *P. ostreatus* in the colonization of sterilized soil from straw-grown inocula and in the degradation of anthracene, phenanthrene, and pyrene. *P. ostreatus* and *T. versicolor* produced similar rates of manganese peroxidase and laccase in soil but *P. chrysosporium* produced only extremely very low rates of these enzymes (Novotny et al. 1999). In aged soil contaminated with creosote, *P. ostreatus* degraded approximately 40 % of the benzo[α]pyrene present after 12 weeks of incubation (Eggen and Majcherczyk 1998; Eggen and Sveum 1999). However, degradation severely came down to around 1 % when spent mushroom compost containing *P. ostreatus* was supplemented with fish oil and used for a soil contaminated with creosote. After 7 weeks, approximately 89 % of the three-ring PAHs, 87 % of the four-ring PAHs, and 48 % of the five-ring PAHs had been degraded (Eggen 1999). Removal of 86 % of the priority PAHs was reported. However, the use of ligninolytic fungi for remediation of PAH-contaminated soil has not always given promising results.

In a bench-scale test, *P. ostreatus* effectively decreased the decreasing concentrations of the pesticide lindane from 345 to 30 mg l^{-1}, within 45 days (http://www.earthfax.com). Subsequent pilot-scale tests utilizing macroscale plots with capacities of about 2 cubic yards, lindane concentrations decreased from 558 to 37 mg l^{-1} in 274 days. Following performance of the pilot-scale tests, approximately 750 tons of contaminated soil was excavated. The contaminated soil was mixed with 16 % (w/w) fungal inoculum (i.e., sawdust and cottonseed hulls thoroughly colonized with *P. ostreatus*). Initial lindane concentrations ranged from 7.1 to 37 mg l^{-1} averaging 21 mg l^{-1}. After 24 months of treatment, lindane concentrations decreased by 97 % to 0.57 mg l^{-1}, achieving the industrial treatment goal of 4.4 mg l^{-1} and almost also reaching the residential risk-based concentration

of 0.49 mg l^{-1} (http://www.earthfax.com). The fungal mycelium effectively colonizes natural soil (Lang et al. 2000) and its temperature requirements are considerably lower than that of *P. chrysosporium* (Hestbjerg et al. 2003), as it is active at 8 °C (Heggen and Sveum 1999). Novotny et al. (1999) has described *P. ostreatus* as a suitable candidate to apply for the clean-up of soils contaminated with recalcitrant pollutants because of its capability of robust growth and efficient extracellular enzyme production in soil even in the presence of pollutants such as PAHs. They suggested that mycelial growth through contaminated soil and efficient enzyme expression were the key to removal of the pollutant molecules from the bulk soil. The production and activity of these enzymes in contaminated soil under field conditions are two prerequisites for successful application of white rot fungi in soil bioremediation (Lang et al. 1998). Beyond introduction of white rot fungi in natural soil, enhanced degradation of pesticide molecules requires effective growth and competition with indigenous microorganisms (Canet et al. 2001). Therefore, *P. ostreatus* as a mycoremediant applicable in the chemical preparation of pesticide-contaminated agricultural lands, and in the meantime as a reliable fungal biological control agent for anti-nematode disinfestations (Palizi et al. 2006, 2007, 2009) can be regarded as an effective control tool that can be practically applied instead of abrogated nematicides.

Fragoeiro (2005) studied the bioremediative potential of eight isolates (*Phanerochaete chrysosporium* R170, *Pleurotus ostreatus* R14, *Trametes versicolor* R26 and R101, *Polystictus sanguineus* R29, *Pleurotus cystidiosus* R46, *Pleurotus sajor-caju* R139, *Trametes socotrana* R100) of white rot fungi on soil extract agar amended individually and as a mixture with 0, 5, 10, and 20 mg l^{-1} simazine, trifluralin and dieldrin under two different water regimes (−0.7 and −2.8 MPa water potential). She found that the best isolates were *T. versicolor* (R26 and R101) and *P. ostreatus*, exhibiting good tolerance to the pesticides and water stress and the ability to degrade lignin and produce laccase in the presence of these pesticides. As a result, the activity of those three isolates plus *P. chrysosporium* (well described for its bioremediation potential) was examined in soil extract broth in relation to differential degradation of the pesticide mixture at different concentrations (0–30 mg l^{-1}) under different osmotic stress levels (−0.7 and −2.8 MPa). Enzyme production, relevant to P and N release (phosphomonoesterase, protease), carbon cycling (β-glucosidase, cellulase) and laccase, involved in lignin degradation was quantified. The results suggested that the test isolates have the ability to degrade different groups of pesticides, supported by the capacity for expression of a range of extracellular enzymes at both −0.7 and −2.8 MPa water potential. *P. chrysosporium* and *T. versicolor* R101, were able to degrade this mixture of pesticides independently of laccase activity, whereas *P. ostreatus* and *T. versicolor* R26 showed higher production of this enzyme. Complete degradation of dieldrin and trifluralin was observed, while about 80 % of the simazine was degraded regardless of osmotic stress treatment in a nutritionally poor soil extract broth. The results with toxicity test (Toxalert®10), suggested the pesticides were metabolized. Therefore the capacity for the degradation of high concentrations of mixtures of pesticides and the production of a range of enzymes, even under

osmotic stress conditions suggested potential applications in soil. Subsequently, microcosm studies of soil artificially contaminated with a mixture of pesticides (simazine, trifluralin, and dieldrin, 5 and 10 mg kg^{-1} soil) inoculated with *P. ostreatus*, *T. versicolor* R26 and *P. chrysosporium* and grown on wood chips and spent mushroom compost (SMC) were examined for biodegradation capacity at 15 °C. The three test isolates successfully grew and produced extracellular enzymes in soil. Respiratory activity was enhanced in soil inoculated with the test isolates and was generally higher in the presence of the pesticide mixture, which suggested increased mineralization. Cellulase and dehydrogenase were also higher in inoculated soil than in the control especially after 12 weeks incubation. Laccase was produced at very high levels, only when *T. versicolor* R26 and *P. ostreatus* were present. The greatest degradation for the three pesticides was achieved by *T. versicolor* R26, after 6 weeks with degradation rates for simazine, trifluralin, and dieldrin 46, 57, and 51 % higher than in natural soil and by *P. chrysosporium*, after 12 weeks, with degradation rates 58, 74, and 70 % higher than the control. The amendment of soil with SMC also improved pesticide degradation (17, 49, and 76 % increase in degradation of simazine, trifluralin, and dieldrin compared with the control).

The bracket-like polypore fungus, *Ganoderma australe* can also degrade lindane in liquid agitated cultures (Dritsa et al. 2005). The enzymes produced by these fungi are lignin peroxidase, manganese peroxidase, and laccases, which are frequently referred to as lignin-modifying enzymes (LMEs), and are highly induced in the presence of wheat bran. Rigas et al. (2007) studied the bioremediation of a soil contaminated by lindane utilizing the fungus *Ganoderma australe via* response surface methodology and identified and evaluated five parameters of determinative impacts on the bioremediation process effectiveness of the solid-state system and concluded that the most important response for bioremediation purposes was biodegradation/biomass maximized at the factors levels: temperature 17.3 °C, moisture 58 %, straw content 45 %, lindane content 13 ppm, and nitrogen content 8.2 ppm.

The degradation of simazine by *Penicillium steckii* in soil samples from areas of the herbicide application has been reported (Kodama et al. 2001).

Species from the genera *Aspergillus*, *Alternaria*, and *Cladosporium* are able to colonize samples of concrete applied as radioactive waste barrier in the Chernobyl reactor, can leach iron, aluminum, silicon, and calcium, and re-precipitate silicon and calcium oxalate in their microenvironments (Fomina et al. 2007). A study concerning the metabolism of polyphosphate in *Trichoderma harzianum*, a biocontrol agent with innate resistance against most chemicals used in agriculture, including metals, when grown in the presence of different concentrations of cadmium, has indicated the biomass production is affected by the concentration of metal used. Control cultures were able to accumulate polyphosphate under the conditions used. Moreover, the presence of cadmium induced a reduction in polyphosphate content related to the concentration used. The morphological/ultrastructural aspects have been characterized by using optical and scanning electron microscopy and were affected by the heavy metal presence and

concentration. The efficiency of cadmium removal has revealed the potential of *Trichoderma harzianum* for use in remediation. The data indicate the potential for polyphosphate accumulation by the fungus, as well as its degradation related to tolerance/survival in the presence of cadmium ions (De Freitas et al. 2011).

Fungi not only lonely but also in association with other organisms (algae, bacteria, plants) can exert their positive bioremediative impacts on the environment. As an example, mycorrhizal associations may be applied in metal cleanup in the general area of phytoremediation (Van der Lelie et al. 2001; Rosen et al. 2005; Gohre and Paszkowski 2006). Mycorrhizas can increase phytoextraction directly or indirectly by increasing plant root growth and development. Plants inoculated with mycorrhizas isolated from metal-contaminated environments indicate increased phytoaccumulation of metals. However, many complicating determinant factors such as metal tolerance of fungal strains, their mycorrhizal status, and the nutritional status of polluted soils affect the final output of mycorrhization (Meharg 2003). Mycorrhizas decrease the absorption of metals by plants (Tullio et al. 2003). Arbuscular mycorrhizas (AM) reduce the transfer of heavy metals like zinc to shoots of their host plants in moderately zinc-contaminated soils, where metals are bound and trapped in mycorrhizal structures and immobilized in the mycorrhizosphere (Christie et al. 2004). However, such mutual relationships between mycobiont and phytobiont in metal-contaminated regions are not always of beneficial consequences and dependant on the local conditions may lead to neutral and or even detrimental results (Meharg and Cairney 2000). A protective metal-trapping effect of ectomycorrhizal fungi has been postulated (Leyval et al. 1997). A copper-adapted *Suillus luteus* isolate has been shown to protect pine seedlings against elevated toxic concentrations of copper ions. Such a metal-adapted *Suillus–Pinus* combination would expectedly be useful in large-scale reclamation under phytotoxic conditions enfaced in metal-contaminated and industrial sites (Adriaensen et al. 2005). Persistent fixation of Cd (II) and Pb (II) through the formation of an efficient ectomycorrhizal barrier has been indicated to reduce the translocation of the heavy metals into tissues of birch trees (Krupa and Kozdroj 2004). Such findings may be of practical applications in soil mycoremediation and re-vegetation (Gadd 2010). Natural soil organic compounds such as the insoluble glycoprotein glomalin abundantly produced on the hyphae of arbuscular mycorrhizal fungi can sequester and stabilize potentially toxic metals such as copper, cadmium, lead, and manganese and therefore, may be regarded as a useful biostabilizer in mycoremediation of heavy metal contaminated regions (Gonzalez-Chavez et al. 2004). Increased uranium concentration and content in roots and decreased concentrations of uranium in shoots have been reported as the effect of *Glomus intraradices*. AM fungi and root hairs improve not only phosphorus acquisition but also root absorption of uranium, and the mycorrhiza generally decreases root to shoot transfer of uranium (Rufyikiri et al. 2004; Chen et al. 2005a, b). With ericaceous mycorrhizas in *Calluna*, *Erica*, and *Vaccinium* spp. grown on Cu- and Zn-contaminated and/or naturally metalliferous soils, the fungi clearly prevent upward transfer of metals to plant shoots (Bradley et al. 1981, 1982). As ericaceous plants commonly grow on nutrient-deficient soils, the

mycorrhiza may additionally benefit these plants through enhanced absorption of soil nutrients (Smith and Read 1997). Thus, development of stress-tolerant plant–mycorrhizal associations may be a promising strategy for phytoremediation and soil amelioration (Schutzendubel and Polle 2002). The efficiency of such symbiotic associations in ericaceous plants is so high that these plants can naturally colonize harshly polluted areas (Cairney and Meharg 2003). The ectomycorrhizal fungi *Suillus granulatus* and *Pisolithus tinctorius* can promote the release of cadmium and phosphorus from rock phosphate (Leyval and Joner 2001) while the ericoid mycorrhizal fungus *Oidiodendron maius* can solubilize zinc oxide and phosphate (Martino et al. 2003). Experimental studies on ericoid mycorrhizal and ectomycorrhizal fungi have showed that many species are able to solubilize zinc, cadmium, copper phosphates, and lead chlorophosphate (pyromorphite) and release phosphate and metals (Fomina et al. 2004). However, it has been demonstrated that mycorrhization is sometimes of neutral effects, for example non-mycorrhizal *Pinus sylvestris* and pines infected with the ectomycorrhizal *Paxillus involutus* are able to enhance zinc phosphate dissolution, resist to metal toxicity, and acquire the mobilized phosphorus, increase the amount of phosphorus in shoots while zinc phosphate is present in the growth matrix (Fomina et al. 2006).

Synergistic effects have been recorded between fungi and bacteria during bioremediation processes, for example, besides direct degradation of hydrocarbons, fungal mycelia can penetrate oil and increase the surface area available for biodegradation and bacterial attack. Also, it has been reported that despite of initial degradation of a synthetic petroleum mixture by bacteria, the rate of biodegradation increased up to twice as the result of the synchronous activity of both bacteria and fungi. Martens and Zadrazil (1998) screened a variety of wood-rotting fungi for their ability to degrade PAHs in a bioreactor containing straw and soil. A higher degradation rate (40–58 % of the applied [^{14}C]-PAH as $^{14}CO_2$) was observed in microcosms containing fungal strains that did not colonize the soil than in those inoculated with the soil-colonizing fungi. An explanation for the difference was that the indigenous soil bacteria were stimulated by compounds produced during the lysis of straw by non-colonizing fungi, which provided carbon sources to enhance bacterial growth and PAH degradation.

1.8 Conclusion and Perspective

With the increasing population of the world, the science and technology of bioremediation is going to become a necessity of today modern life. Fortunately considering the youth of this interdisciplinary science, there have been significant progresses in the field. The diversity of bioremediants, the multiplicity and diversity of available techniques, the variation of the substrates used by bioremediants in different types of aqueous and terrestrial habitats all seem as good signs of this well-promising science and technology. Therefore, bioremediation will expectedly be of rising number of applications in different environments from battle fields to

rural, urban, and industrial areas. Fungi as a huge group of unicellular–filamentous microorganisms of high rate of biodiversity being isolated from different environments are rightfully considered as a potent group of bioremediants and with an eye to the recent advances in genetic and metabolic engineering, it seems that fungi will have much more share in the bioremediation of pollutants and wastes.

Based on what mentioned above, it is well expectable that bioremediation and, in a narrower sense, mycoremediation would expand to more specific scientific branches in near future in order to be able to quickly respond to the challenges of current and future world.

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