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# Microbial Syntrophy- mediated Eco-enterprising

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# Microbial mediated remediation of pesticides: A sustainable tool

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## Abstract

In modern farming system, pesticide application partakes as most common practice in agricultural fields in which 2%–3% of pesticide is utilized and the rest persists in soil and water causing environmental pollution leading to toxicity (WHO, 1990. Diet, Nutrition, and the Prevention of Chronic Diseases, 797 pp). Pesticide residues remain in surface soil, leading to toxicity in the soil-water environment. A vast majority of the Indian population (56.7%) is engaged in agriculture and is, therefore, exposed to the pesticides used in agriculture. Moreover, microbial biodegradation of pesticides has foremost critical importance for modern agriculture and its environmental impact. Microorganisms occupy virtually every habitat on our planet, and their activities largely determine the environmental conditions of today's world. Indeed, they are heavily involved in biogeochemistry, metal precipitation, water purification and sustenance of plant growth that ensuring the recycling of elements such as carbon and nitrogen. In soil, microbes interact with the plant roots, a “hot spot” of microbial activity, with increased microbial numbers, microbial interactions and genetic exchange. In plant root, a narrow zone of soil that surrounds and is influenced by plant roots, known as rhizosphere, is home to an overwhelming number of microorganisms and invertebrates and is considered to be one of the most dynamic interfaces on Earth. The rhizosphere microbiome is dependent on the plant genotype, root exudates, and the environment. Therefore, the study of expressed microbial communities in pesticide-contaminated and uncontaminated rhizosphere is key to the investigation of the diverse roles played by microorganisms in respective niches and to the identification of the microbial genetic potential for biotechnological application in bioremediation of pesticide, including but not limited to: pharmaceutical, diagnostics, waste treatment, and renewable energy generation.

**Keywords:** Pesticide, Bioremediation, Rhizosphere, Enzymes, Bioengineering

## 1 Introduction

Pesticides, as a product of the progress of human civilization, have contributed to solving human food and clothing, enhancing social stability, and promoting social development, and have played a positive role in human health. Especially in the 1930s and 1940s, the successful discovery and production of organic pesticides provided effective means for controlling pest damage (Abubakar et al., 2020; Carvalho, 2017). The broad definition of pesticides refers to chemical synthesis used to prevent, eliminate or control diseases, insects, grasses, and other harmful organisms that harm agriculture and forestry, and to purposefully regulate the growth of plants and insects or derived from biological or

other natural substances. A substance or a mixture of several substances and their preparations. It refers to a class of drugs used to kill insects, sterilize, and kill harmful animals (or weeds) to protect and promote the growth of plants and crops in agricultural production (Yang et al., 2020). In particular, it is used in agriculture to control pests and regulate plant growth and weeding (Mahmood et al., 2016; Diwakar et al., 2008; Edwards, 1993; Madhun and Freed, 1990) (Table 1).

Depending on the chemical structure the most popular pesticides may be divided into the following groups:

1. Organochlorines (endosulfan, hexachlorobenzene)
2. Organophosphates (diazinon, omethoate, glyphosate)
3. Carbamic and thiocarbamic derivatives
4. Carboxylic acids and their derivatives
5. Urea derivatives
6. Heterocyclic compounds (benzimidazole, triazole derivatives, etc.)
7. Phenol and nitrophenol derivatives
8. Hydrocarbons, ketones, aldehydes, and their derivatives
9. Fluorine-containing compounds
10. Copper-containing compounds

**Table 1 List of microbial enzymes involved in pesticide degradation.**

Pesticides	Microorganisms	Enzymes	References
1. Organochlorates or chlorinated hydrocarbons Aldrin DDT Alachor Dieldrin 1,4-Dichlorubenzene Endosulfan Heptachlor Methoxychlor Pentachlorontrobenzene BIS Pentachlorophenol (PCP)	<i>Pseudomonas</i> sp. strain ADP <i>Ancylobacter</i> sp. 515 <i>Agrobacterium</i> sp. CZBSAI <i>Pseudomonas</i> sp. <i>Bacillus</i> sp. <i>Micrococcus</i> sp. <i>Enterobacter aerogenes</i> <i>Enterobacter cloacae</i> <i>Klebsiella meumonia</i> <i>Bacillus</i> sp. <i>Pseudomonas putida</i> <i>E. coli</i> <i>Hydrogenomonas</i> sp. <i>Pseudomonas</i> sp. <i>Pseudomonas</i> sp. <i>Bacillus</i> sp. <i>Flawbacterizane</i> sp. <i>Clostridium</i> sp. <i>Bosea thucdants</i> <i>Sphingomonas paucimobilis</i> <i>E. aerogenes</i> <i>Cuprirvides</i> sp. strain	Dehalogenases	Katz et al. (2000) Ewida (2014) Patil et al. (1970) Sharma et al. (2016) Dimitrios et al. (2005) Sethunathan and Yoshida (1973) Fogel et al. (1982) Teng et al. (2017)

**Table 1 List of microbial enzymes involved in pesticide degradation—cont'd**

Pesticides	Microorganisms	Enzymes	References
2. Organo-phosphate	<i>P. putida</i>	Organophosphorus hydrolase (OPH), organophosphorus acid anhydrolase (OPAA), Laccase Aspergillus enzyme (A-OPH) Penicillium enzyme (P-OPH)	Dimitrios et al. (2005) Akbar and Sultan (2016) Tiware et al. (2019) Ishag et al. (2016) Dimitrios et al. (2005) Tiware et al. (2019) Abo-Amer (2007) Tiware et al. (2019)
Cadufos	<i>Flavobacterium</i> sp.		
Chlorpyrifos	<i>Achromobacter</i>		
Diazinon	<i>xylooxidans (JCp4)</i>		
Dimethoate	<i>Ochrobactrum</i> sp.		
Ethoprophos	( <i>FCpl</i> )		
Glyphosphate, acephate	<i>Pseudomonas capaciae</i>		
Malathion	<i>Bacillus cereus subtilis</i> , <i>Bacillus safensis</i>		
Monocrotophos	<i>Sphingomonas paucimoblis</i>		
Tetrachlorvinphos	<i>Clostridium</i> sp.		
	<i>Arthrobacter</i> sp.		
	<i>Pseudomonas aeruginosa</i> AA112		
	<i>Rhodococcus</i> sp.		
	<i>Stenotrophomonas</i> Ortiz-Hernández		
	<i>malihophilis</i> , <i>Proteus</i> and <i>Sánchezvulgaris</i> ,		
	<i>Vibrio metschinkouii</i> , <i>Serratia</i>		
	<i>ficaria</i> , <i>Serratia</i> sp.		
	<i>Yersinia enterocolitica</i>		
3. Carbamates	<i>Arthrobacter</i> sp.	Carbofuran hydrolase	Behki and Khan (1994) Chapalamadugu and Chaudhry (1994) Gunasekara et al. (2008) Chaudhry et al. (1988) Sharma et al. (2014a)
Aldicarb	<i>Rhodococcus</i> sp.		
Carbayl (1-naphthalenyl methyl carbamate)	<i>Pseudomonas</i> sp.		
Carbofuran	<i>Achromobacter</i> sp. and <i>Arthrobacter</i> sp.		
	<i>Xanthomonas</i> sp. and <i>Pseudomonas cepacia</i>		
	<i>Achromobacter</i> sp.		
	<i>Pseudomonas</i> sp.		
	<i>Flavobacterium</i> sp.		
	<i>Pseudomonas</i> sp.		
	<i>Flavobacterium</i> sp.		
	<i>Achromobacterium</i> , <i>Sphingomonas</i> sp.		
	<i>Arthrobacter</i> sp.		
Pyrethroid	<i>Serratia</i> , <i>Pseudomonas</i> , <i>Aspergillus niger</i>	Carboxyl esterase, pyrethroid hydrolase phosphotriesterase	Guo-liang et al. (2005)

11. Metal organic and inorganic compounds

12. Natural and synthetic pyrethroids and others.

WHO recommended classification of “Pesticides by Hazard” is shown in Table 2 and revised globally harmonized system (GHS) classification of pesticide is shown in Table 3.

The harm of pesticides to the human body is mainly manifested as acute toxicity and chronic toxicity. Pesticides enter the human body in large quantities through the mouth, inhalation and exhalation tract

**Table 2 WHO recommended classification of pesticides.**

WHO class		LD <sub>50</sub> for rats (mg/kg body wt.)		Examples
		Oral	Dermal	
I <sub>a</sub>	Extremely hazardous	< 5	< 50	Parathion, Dieldrin, Phorate
I <sub>b</sub>	Highly hazardous	5–50	50–200	Aldrin, Dichlorvos
II	Moderately hazardous	50–2000	200–2000	DDT, Chlordane
III	Slightly hazardous	Over 2000	Over 2000	Malathion
U	Unlikely to present acute hazard	5000 or higher		Carbetamide, Cycloprothrin

**Table 3 GHS Classification of pesticides.**

GHS category	Classification criteria			
	Oral		Dermal	
	LD <sub>50</sub> (mg/kg bw)	Hazard statement	LD <sub>50</sub> (mg/kg bw)	Hazard statement
Category 1	< 5	Fatal if swallowed	< 50	Fatal in contact with skin
Category 2	5–50	Fatal if swallowed	50–200	Fatal in contact with skin
Category 3	50–300	Toxic if swallowed	200–1000	Toxic in contact with skin
Category 4	300–2000	Harmful if swallowed	1000–2000	Harmful in contact with skin
Category 5	2000–5000	May be harmful	2000–5000	May be harmful

or contact, and the acute pathological reaction shown in a short time is acute poisoning (Singh et al., 2017). Acute poisoning often leads to nerve paralysis and even death, and even causes large-scale deaths, becoming the most obvious pesticide hazard. According to reports from the World Health Organization and the United Nations Environment Program, more than 3 million people worldwide are poisoned by pesticides each year, and 20 million of them die. Today, due to the wide application of pesticides in various aspects, it is impossible for any person living in modern life to avoid daily exposure to various pesticides at very low concentrations, either through food or through drinking water. The resulting possible harm to human health is continuous low-level exposure, which is a potential chronic toxic effect. (Kovach et al., 1992; van der Werf and Hayo, 1996).

However, from the current stage, the use of pesticides is inevitable. For human beings to survive more healthily and safely, to understand, avoid, slow down and solve this increasingly serious problem, it is necessary and necessary to explore and study the environmental pollution mechanism of pesticides.

## 2 Bioremediation technology of pesticide-contaminated soil

At present, pesticides are seriously polluting the soil all over the world, which not only affects the growth and development of plants but also affects human health through the food chain. It has become a serious problem that restricts agricultural products and food safety and sustainable agricultural development (Singh et al., 2017). Countries around the world have invested a lot of manpower and material resources. Research and develop technologies for remediation of pesticide soil pollution.

At present, there are many methods for remediation of pesticide-contaminated soil, and the establishment of ecological rapid environmental remediation technology is the fundamental solution to this problem. Bioremediation technology has become the most active field of soil environmental protection technology due to its obvious advantages of low consumption, high efficiency, and environmentally safe pure ecological process (Minsheng and Xin, 2004; Gavrilesu, 2005).

The research of bioremediation technology began in the mid-1980s, and there have been successful applications in the 1990s. In a broad sense, contaminated soil bioremediation technology refers to the use of various organisms in the soil (plants, animals, and microorganisms alone or in combination) to absorb, degrade and transform pollutants in the soil to reduce the content of pollutants in the soil to an acceptable level or the process of converting toxic and harmful pollutants into harmless substances. Under this concept, soil bioremediation technology can be divided into three types: phytoremediation, animal remediation, and microbial remediation. Narrowly contaminated soil bioremediation refers to microbial remediation technology, which uses soil microorganisms to use organic pollutants as carbon sources and energy sources to degrade harmful organic pollutants in the soil into harmless inorganic substances (CO and HO) or another harmless material process (Gavrilescu, 2005; Uqab et al., 2016; Niti et al., 2013; Alkorta and Garbisu, 2001).

Microbial remediation technology is the use of microbial life metabolism activities to degrade organic pesticides to restore contaminated soil to a healthy state (Singh et al., 2020). The microorganisms used mainly include indigenous microorganisms, foreign microorganisms, and genetically engineered bacteria. Microbial remediation technology can be divided into in situ remediation, on-site remediation, and ex-situ remediation. In-situ remediation is not only simple in operation and low in cost but also does not damage the soil environment required for plant growth. The pollutant oxidation is safe, and there is no secondary pollution. Good effect, it is an environmentally friendly technology that is efficient, economical and ecologically sustainable (Odukkathil and Vasudevan, 2013).

There are two ways to degrade pesticides by microorganisms: one is that microorganisms directly act on pesticides, using pesticide components as the only carbon source or nitrogen source, and phosphorus source to degrade pesticides through enzymatic reactions. The microorganisms isolated from Pakistani soil such as Fulthorpe can mineralize 2,4-D, and found that adding nitrate, potassium ion, and phosphate can increase the degradation rate (Fulthorpe et al., 1996).

Canada's Stauffer Management company has developed some pesticide-contaminated soil bioremediation technologies for several years. They achieve the purpose of remediation by stimulating the function of degradable indigenous microbial communities in specific environments (Gray et al., 1999). The application of inorganic nitrogen fertilizer and phosphate fertilizer significantly promoted the digestion of atrazine. The digestion rate of atrazine in different treatments was as follows: combined application of nitrogen and phosphorus fertilizers > single application of nitrogen fertilizers > single application of phosphate fertilizers > no fertilizer treatment (Tao et al., 2019). The other is to co-metabolize pesticides with other organic matter. Microbial remediation is different from phytoremediation. Usually, a single microorganism can degrade a variety of pesticides, such as *Pseudomonas* can degrade DDT, aldrin, toxaphene, and dichlorvos. From 1993 to 1995, Spadaro conducted field trials on the bioremediation of 2,4-D in the soil in Poland. After 7 months of adding anaerobically digested sludge in an anaerobic environment, the 2,4-D in the soil increased from 1 to 100 mg/kg was reduced to 18 mg/kg, and the feasibility of bioremediation was confirmed in large-scale trials (Struthers et al., 1998; Spadaro et al., 1998). In addition, microorganisms can also reduce the effectiveness of pesticides by changing the

physical and chemical characteristics of the soil, thereby indirectly playing a role in the remediation of contaminated soil.

Nowadays, bioremediation of pesticide pollution through microbes has entered the genetic level, and the ability of microorganisms to degrade pesticides is improved through genetic recombination and the construction of genetically engineered bacteria. The current research on microbial remediation technology is quite mature. Researchers from all over the world have isolated and screened a large number of degradable microorganisms. (Spadaro et al., 1998; Ortiz-Hernández and Laura, 2013).

### 3 Metabolic pathways of microbial degradation of pesticides

Microbial pesticide degradation can be divided into enzymatic degradation and non-enzymatic degradation (Struthers et al., 1998; Spadaro et al., 1998). The enzymatic degradation effects are as follows:

(1) Microorganisms use pesticides or certain parts of their molecules as energy and carbon sources, and some microorganisms can use certain pesticides as their sole carbon or nitrogen source. Some can be used immediately by microorganisms, while others cannot be used immediately. Special enzymatic hydrolysis is required before the pesticides can be degraded (Singh, 2008; Fragoeiro and Magan, 2005). (2) Microorganisms degrade pesticides through co-metabolism. Many studies have shown that due to the complex structure of certain chemical pesticides, a single microorganism cannot degrade it, and it needs to be metabolized and degraded by two or more microorganisms (Bollag, 1991; Sharma et al., 2014b; Wang et al., 2010). This field is a hot spot of current research. (3) Detoxification and metabolism. Microorganisms do not obtain nutrients or energy from pesticides but develop detoxification to protect their own survival. Non-enzymatic degradation: Microbial activity changes the pH and causes degradation of pesticides, or produces some auxiliary cofactors or chemical substances participate in the transformation of pesticides, such as dehalogenation, de-hydrocarbonization, hydrolysis of amines and esters, reduction, ring cleavage, etc. (Horvath, 1972; Munnecke et al., 2018; Koushik et al., 2016; Alexander, 1999). The aerobic/anaerobic biodegradation pathways of many refractory pesticides have been clarified. The Biodegradation and Biocatalyst Database of the University of Minnesota in the United States collected 139 metabolic pathways, 910 reactions, and 577 kinds of pesticides and other compounds. Enzymes, 328 microbial entries, 247 biotransformation rules, 50 organic functional groups, which include many pesticides degradation and metabolism pathways and enzymes, such as parathion, atrazine, 2,4-D, 4-the metabolic pathways and degradation mechanisms of nitrophenol, tetrahydrofuran, S-triazine, DDT and other pesticides have been listed in detail (Mohn and Tiedje, 1992; Gao et al., 2010).

### 4 Influencing factors of microbial remediation of pesticide

The nature of the pesticide itself, especially the internal chemical bond, concentration, water solubility, molecular polarity, bioavailability, compound adsorption (Ellis et al., 2001) and environmental factors (temperature, salinity, pH, soil type, redox potential, nutrition substances) (Rieger et al., 2002; Arbeli and Fuentes, 2007) are the main factors affecting the biodegradation and restoration of pesticides. Whether microorganisms can restore environmental pollutants ultimately depends not only on their degradability itself, but also on other factors such as the bioavailability of the pollutants and the ability of bacteria to compete with indigenous microorganisms. Increasing the solubility and bioavailability



of pollutants is a necessary condition for successful restoration by biological methods (Aislabie and Lloyd-Jones, 1995; Karthikeyan et al., 2004). The degradation efficiency of pesticides in the soil is also closely related to the activity of microorganisms in the soil, and the activity of microorganisms in the soil is affected by many factors, such as pesticide concentration, soil physical and chemical properties, organic matter types and content, microbial flora composition, etc. (Nam and Kim, 2002; Luthy et al., 1997; Zacharia, 2011).

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## 5 Plant rhizosphere microdomains are an important place to degrade organic pollutants

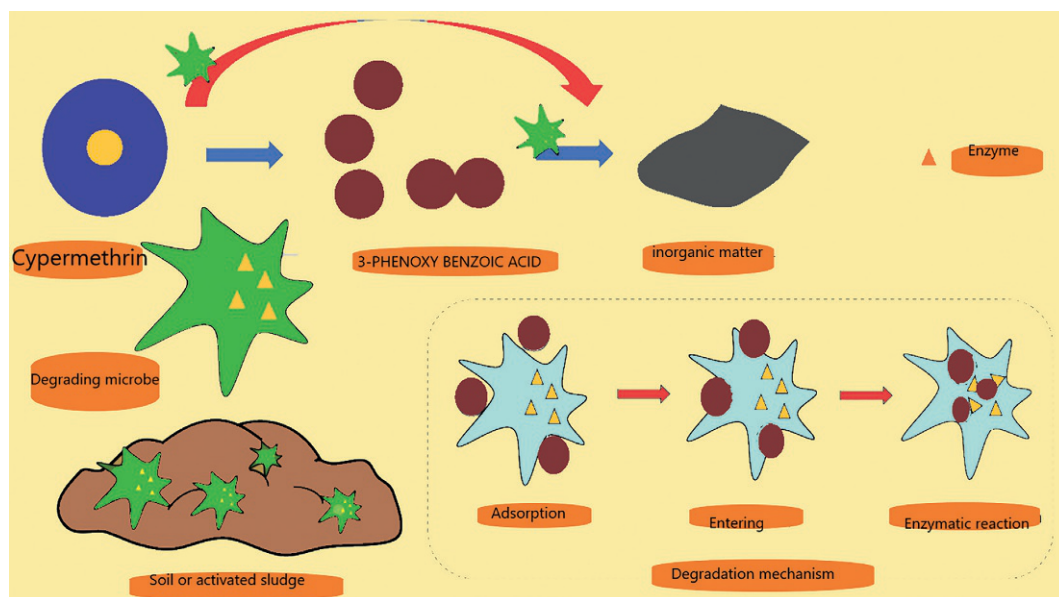
In 1904, Lorenz Hiltner proposed the concept of the rhizosphere. The rhizosphere is a micro-area of the root-soil interface affected by plant roots, and it is also a place where plants-soil-microbes interact with their environmental conditions (Arias-Estévez et al., 2008). In this micro-region, a large number of plant root secretions are concentrated, including high-molecular-weight secretions and low-molecular-weight secretions. The former mainly includes viscose and extracellular enzymes, and the latter mainly consists of low-molecular organic acids, sugars, phenols, and various amino acids (Singh et al., 2019). The possible mechanism for the rapid degradation of organic pollutants in the rhizosphere microdomains is the catalytic degradation of enzymes released from the roots and the degradation of rhizosphere microorganisms (Kah et al., 2007).

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## 6 Enzymes released from roots can catalyze the degradation of organic pollutants

The enzymes released by plant roots into the soil can directly degrade related compounds, sometimes very fast, making the desorption and mass transfer of organic pollutants from the soil a rate-limiting step. After plant death, enzymes released into the environment can continue to play a decomposing role (Yang et al., 2019). The degradation of organic pollutants by plant-specific enzymes provides strong evidence for the potential of phytoremediation. The EPA laboratory in Athens, Georgia, USA, identified five enzymes from freshwater sediments: dehalogenase, nitric acid Reductase, peroxidase, laccase, and nitrilase, these enzymes all come from plants. Nitrate reductase and laccase can decompose explosive waste (TNT) and combine the broken ring structure into plant material or organic residues to become part of the deposited organic matter. The plant-derived dehalogenase can reduce the chlorine-containing organic solvent trichloroethylene to chlorine ions, carbon dioxide, and water. Although these isolated enzymes can degrade TNT and other organic pollutants, experience has shown that in vitro enzymes have high environmental requirements. Unsuitable acidity, high metal concentrations, or bacterial toxins can all inactivate or destroy enzymes. However, enzymes can be protected in plant tissues or near the root zone, and after being released into the soil, they can maintain their degradation activity for several days (Anderson et al., 1993; Anderson and Coats, 1995). Therefore, phytoremediation depends on the entire plant body to achieve (Fig. 1).

Microbial degradation of pesticides is an important field of environmental restoration science and technology. It is the process by which pesticides is transformed into environmentally compatible substance. The physical and chemical forces are acting upon the pesticides but microorganisms play important role in the degradation of pesticides and convert it into simpler non-toxic compounds. During

**FIG. 1**

Pesticide degradation mechanism.

degradation the carbon dioxide and water are formed by the oxidation of the parent compound and by this process energy is produced which help in the metabolism of the microbes the intracellular and extracellular enzyme of the microbes play a major role in the degradation of the chemical compounds.

## 7 Pesticide degradation via microbial associated plant rhizosphere

In the rhizosphere microdomains of plants, root exudates and decomposition products provide nutrients for the reproduction of microorganisms, so that there are a large number of microorganisms near the roots, which promotes the degradation of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons, and pesticides in the rhizosphere microdomains. For example, the research results of Arthur et al. showed that the half-life of atrazine in plant root zone soil was about 75% shorter than that in non-plant control soil, and the number of atrazine degrading bacteria in root zone soil was higher than that in the control soil. The number is nine times more (Esteve-Núñez et al., 2001). Nichols et al. confirmed that the number of microbial communities in the root zone of the same plant is more in contaminated soil than in uncontaminated soil (Schnoor et al., 1995). The mineralization rate of several surfactants in rhizosphere soil is 1.4–1.9 times faster than that of non-rhizosphere soil (Arthur et al., 2000). These studies fully show that the continuous reduction of organic pollutants in the rhizosphere is caused by microbial activities (Nichols et al., 1997; Knaebel et al., 1992; Diez et al., 2017; Nawaz et al., 2011). However, when Fang et al. studied phytoremediation of herbicide pollution, they found that the planting

of some plants under herbicide stress did not affect the number of degrading bacteria in the root zone soil, and the mineralization rate of atrazine in the root zone soil was even higher than that of unplanted plants. It may be that the exfoliated roots of the experimental plants do not contain substances that can promote the growth of degrading bacteria, nor can they induce the possible degradation pathways of microorganisms (Sun et al., 2004).

## 8 Several issues that need further study in bioremediation

The use of organic pollutants caused by microorganisms and phytoremediation pesticides to pollute the soil environment is currently the most promising organism for development and application. Remediation technology, but with the continuous expansion of the scope and connotation of bioremediation, especially for complex contaminated soil ecosystems, each remediation technology must not only overcome its own original shortcomings but also need to further understand and solve the problems in the process of remediation. New phenomena and new problems, such as the discovery of new types of pollutants, the soil remediation process of pollutants and ecological/health risks, the composite mechanism of remediation technologies, and efficient applications.

Therefore, the bioremediation of contaminated soil still faces great challenges and the task is very arduous. Given this, future biological restoration should pay special attention to the following aspects:

1. Remediation process of contaminated soil and ecological risk assessment, strengthen the understanding of the chemical/biological process of microbial metabolism in the remediation of contaminated soil, such as the key links of bioremediation, intermediate processes, enzymology, and co-metabolism mechanisms, rhizosphere effects, etc. Intermediate products, structure, properties and characterization of pollutants in soil, pay close attention to secondary pollution in the process of bioremediation; construct ecotoxicological and biological evaluation methods for the process of bioremediation of contaminated soil, such as ecotoxicological diagnostic indicators and diagnosis mechanism and ecological toxicology standard system; Establish corresponding laws and regulations for bioremediation technology to ensure the public recognition and promotion of remediation technology.
2. Due to the micro-regional, dynamic and complex characteristics of the rhizosphere environment, there are still some difficulties in the research on the rhizosphere stress and rhizosphere repair of organic pollutants. For the dynamics of the rhizosphere under the stress of organic pollutants. The regulation process, especially the root exudates, the leading factor that affects the rhizosphere environment, and the mechanism of the most active biological phase in the soil environment, microbes, in the organic matter rhizosphere pollution ecosystem is currently lacking a systematic understanding. The research work in this area needs to be further strengthened.
3. Carry out research on the screening technology of plant-microorganism complexes to efficiently repair the soil environment contaminated by organic pollutants. This research area includes the screening indicators of plants enriched in specific organic pollutants, the establishment of methods, and the use of molecular biology techniques to study the microbial diversity of plant rhizosphere micro-domains from the genetic level, such as the application of RAPD DNA molecular biology detection technology, as well as ARDRA (amplified rDNA restriction analysis)

and PCR-DGGE technology based on 16s rDNA to accurately discover microorganisms that degrade specific organic pollutants.

4. Use molecular biology technology and genetic engineering technology to locate and clone genes that control the secretion of specific secretions from plants to create efficient bioremediation plants. In the long run, it is entirely possible to apply molecular biology and genetic engineering technology to construct efficient and safe genetically engineered bacteria or genetically modified plants and to use plant-microbe joint remediation technology to achieve the goal of remediation of contaminated soil.

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