



Study on Degradation Mechanism of Immobilized Mixed Microbial Agents on Compound Contaminated Soil

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Abstract

Deltamethrin, as a widely used pyrethroid insecticide, has caused significant environmental pollution due to its persistence and non-degradability in soil. Therefore, the immobilized microbial technology has shown good application potential due to its low cost and quick effect. Among them, *Aspergillus niger* as the representative of 17 kinds of bacterial mixed agents has strong tolerance and decomposition effect, can secrete a variety of degradation enzymes, can play a good degradation effect on deltamethrin, and has a broad application prospect, but there are many limitations in the degradation of deltamethrin, and its degradation characteristics still need to be further explored. Therefore, this paper reviewed the current situation of pesticide compound contaminated soil in China, the harm and biological toxicity of pyrethroid pesticides, the application and mechanism of immobilized mixed microbial agents with *Aspergillus niger* as the main body in the degradation of pyrethroid pesticides, in order to lay a theoretical foundation for the treatment of soil pyrethroid pesticide residues, help realize the practical application of immobilized compound microbial agents in the remediation of soil pollution, and promote the green development of agricultural ecology in China.

Subject Areas

Biomedical & Life Sciences

Keywords

Pyrethroids, Immobilization, Mixed Microbial Agents, Contamination, Degradation Characteristics

1. Introduction

Pyrethroids (pyrs) are a class of highly effective biomimetic pesticides, which are widely used in agricultural production. Compared with the direct pollution and toxic effects of pyrethroid pesticides on the environment, animals and plants, the secondary pollution caused by intermediate metabolites deserves more attention. Among them, 3-phenoxybenzoic acid (3-PBA) is one of the necessary degradation intermediates of most pyrethroids (such as deltamethrin, Cypermethrin, permethrin and fenvalerate). The study found that 3-PBA is a potential environmental estrogen with reproductive toxicity; And its hydrophobicity is weaker than that of the parent pesticide [1], it is difficult to degrade under natural conditions, prone to migration and bioaccumulation [2], and its half-life can reach 180 d [3], which is more potentially harmful. Therefore, how to eliminate 3-PBA residue is the key to solving pyrethroid pollution.

Microbial remediation has a good development prospect in the research of bioremediation of soil. The use of microbial metabolism to comprehensively treat harmful substances in the surface is simple and effective. Among them, 17 kinds of degrading bacteria, mainly *Aspergillus niger*, show great potential in the degradation of pyrethroid organic pollutants, but the activity of the strains in the free state is unstable and difficult to reuse. In recent years, immobilized microbial technology has been widely used in the treatment of environmental pollutants, which effectively solves the problems of strains in free state. Different kinds of microorganisms have different characteristics, which have a great impact on microbial immobilization. Therefore, understanding the characteristics, application status and immobilization principle of different degradation bacteria has important theoretical and practical significance for soil remediation by immobilized microorganisms. In this paper, the degradation characteristics and application status of 3-PBA degrading bacteria with *Aspergillus niger* as the main body were reviewed, and the related research on the remediation of pyrethroid pollution in soil by using immobilized mixed microbial agents was introduced, with a view to large-scale application of immobilized mixed microbial agents to treat pesticide residues in the future, maintain the stability of the ecosystem, and promote the rapid development of green agricultural economy.

2. Dynamic Study on Compound Pollution of Pesticides

2.1. Current Situation of Soil Pesticide Pollution in China

While pesticides have brought great benefits to human society, the environmental and safety problems caused by them are becoming increasingly serious. According to the research, during the use of pesticides, only 10% - 20% are adsorbed on crops, and the rest float in the air and enter the soil with atmospheric precipitation, causing serious soil pollution. According to the statistics of the food and Agriculture Organization of the United Nations, since the 1940s, the worldwide production of chemical pesticides has increased at an annual rate of 11%, from about 200,000 tons in the early 1950s to nearly 6 million tons in the early 21st century.

As a large pesticide country, China has a high degree of dependence on pesticides, with a total use of millions of tons, ranking first in the world. The average amount of soil per unit area is 2.5 - 5 times higher than that of developed countries, far exceeding the internationally recognized safe upper limit of $7.5 \text{ kg}\cdot\text{hm}^{-2}$. Therefore, the area of crops suffering from pollution is more than 1 billion Mu every year. The pollution of agricultural residues in soil has caused the imbalance of soil microbial community, the reduction of various enzyme activities, and the enrichment of toxic substances through the food chain, which has seriously threatened China's ecology and human health.

2.2. Harm and Biological Toxic Effect of Pyrethroid Pesticides

Pyrethroid pesticides that emerged in the last century have gradually replaced organochlorine and organophosphorus by virtue of their advantages of high insecticidal efficiency, low toxicity to mammals, and low residue. There are many kinds of pyrethroid pesticides, which are widely used at present: deltamethrin, bifenthrin, fenvalerate, permethrin, fenprothrin, Cypermethrin, etc. Due to its strong fat solubility, it is easy to cause bioaccumulation, and is not easy to be decomposed naturally for a long time, and even produces more toxic intermediate products due to incomplete decomposition, such as 3-phenoxybenzoic acid (3-PBA), protocatechuic acid, etc., which directly pose a serious threat to biosafety and human health. In recent years, the excessive use and improper treatment of pyrethroids have led to excessive pesticide residues and damage to aquatic and terrestrial ecosystems. The residues of these pesticides and their intermediates have been widespread in rivers, lakes, reservoirs and other water environments, and increase with the increase of usage. Pyrethroids are the first neurotoxins found to act through voltage gating to control sodium channels. In addition, some of them also have cytotoxicity, genotoxicity and genotoxicity, reproductive toxicity, growth and development toxicity. Pyrethroid pesticides and their degradation intermediates are highly toxic to aquatic animals, silkworm, bee and other non-target organisms, which can cause teratogenesis, carcinogenesis and mutagenesis. Although these substances are not highly toxic to mammals, they will be rapidly absorbed in the human body and difficult to metabolize. As the main metabolite, 3-phenoxybenzoic acid has the characteristics of longer half-life, high biological toxicity, fast migration rate in soil, and is an estrogen substance, which will lead to endocrine disorders.

2.3. Control Technology of Pyrethroid Pesticide Pollution

According to the migration law and influencing factors of pyrethroids in the environment, the current treatment methods of pyrethroid pollution can be roughly divided into physical method, chemical method and bioremediation method. Physical method refers to using the physical properties of pyrethroids to separate them from water by precipitation, extraction, adsorption and other means. This kind of method generally has the defects of high cost, low removal efficiency and

small scope of application. Chemical methods can be divided into chemical oxidation method and chemical reduction method. Chemical oxidation refers to the treatment of solid pollutants or high concentration wastewater by incineration, landfill and other means, which easily cause secondary pollution; The chemical reduction law converts pollutants into substances with weak toxicity or easy degradation through some reducing substances. This kind of method has a good effect in some cases, but it is difficult to deal with large-scale pollution. Bioremediation is a process that uses biological functions such as absorption, metabolism and degradation of pollutants to promote the decomposition and transformation of pollutants, generate carbon dioxide, water and simple inorganic substances, and effectively repair the polluted environment [4]. Among them, microbial remediation demonstrates notable efficacy: microorganisms degrade pesticides directly through enzymatic reactions, such as oxidation, dehydrogenation, reduction, hydrolysis, synthesis, or indirectly by changing the chemical and physical properties of the environment [5]. This method has the advantages of high efficiency, economy, wide application range, and can avoid secondary pollution. It is considered to be an important technical means for pyrethroid pesticide degradation and pesticide residue pollution control [6].

3. Microbial Degradation Characteristics of Pyrethroids

3.1. Degradation Pathways of 3-Phenoxybenzoic Acid

Compared with pyrethroid itself, there are less studies on how its metabolites are degraded, but such studies are also of great significance. Among them, the most discussed metabolite is 3-PBA. *Aspergillus niger* initiates 3-PBA degradation via cytochrome P450 monooxygenases, producing protocatechuic acid. *Pseudomonas* then cleaves protocatechuic acid via the ortho-pathway into tricarboxylic acid (TCA) cycle intermediates. Simultaneously, *Bacillus* secretes esterases that hydrolyze residual pyrethroids, reducing competitive inhibition on 3-PBA degradation. This cross-feeding minimizes metabolic bottlenecks and enhances degradation efficiency by 37% compared to monocultures. Dehmel *et al.* [7] preliminarily speculated that 3-PBA was degraded to phenol or protocatechuic acid by studying the degradation of 3-PBA by strains *Pseudomonas* ET1 and *Pseudomonas pseudoalcaligenes* pob310. Tallur *et al.* [8] isolated *Micrococcus* sp. PN1 from soil, and speculated the possible degradation pathway through different kinds of degradation enzyme activities and degradation intermediates (3-PBA, protocatechuic acid, phenol, etc.). Chen *et al.* [9] detected the degradation products of 3-PBA by *Bacillus* sp. dg-02 and found that 3-PBA generated 3-(2-methoxybenzene) benzoic acid under the action of oxygenase, and then underwent the cleavage of diaryl ether bond to obtain protocatechuic acid, phenol and 3,4-dimethoxyphenol. In addition, there are differences in the degradation pathways of 3-PBA by different microorganisms. Through the analysis of the degraded metabolites, we can only preliminarily infer the pathway of 3-PBA degradation to produce monophenyl compounds (such as phenol). There are few reports on the specific research of

pyrethroid pesticide degrading bacteria that can degrade its hydrolysis intermediate monophenyl compounds. Tallur *et al.* [8] isolated from soil a *Micrococcus* cpn1 that degrades cypermethrin through mineralization. This bacterium can not only use cypermethrin as the only carbon source and energy, but also uses fenvalerate, deltamethrin, phenol, protocatechuic acid, catechol and other substances as its only carbon source and energy material.

3.2. Soil Remediation Approaches and Application Prospects of Mixed Microbial Agents

Mixed microbial agents refer to biological agents composed of a variety of microorganisms, which can improve the degradation ability of specific pollutants and soil remediation effect through mutual cooperation. The mixed agent is mainly composed of *Aspergillus niger* as the core component, combined with 17 kinds of bacteria such as *Pseudomonas*, *Micrococcus*, *Bacillus* and *Aeromonas*, and formed by immobilization technology. *Aspergillus Niger* has a strong degradation ability and can secrete a variety of degradation enzymes, which can effectively decompose pyrethroid pesticides. *Pseudomonas* has a wide range of metabolic capacity and tolerance to a variety of environmental conditions, which can enhance the overall degradation effect of mixed microbial agents. *Micrococcus* and *Bacillus* can further promote soil health and microbial community stability. *Pseudomonas* contributes to broad-spectrum metabolic flexibility and enhances tolerance to fluctuating environmental conditions. *Micrococcus* specializes in breaking down aromatic intermediates like phenol, while *Bacillus* secretes surfactants that improve bioavailability of hydrophobic pesticides. *Aeromonas* thrives in aerobic environments, accelerating oxidative degradation. This consortium ensures sequential degradation of pyrethroids and their metabolites, minimizing residual toxicity. *Aeromonas* showed excellent degradation performance in aerobic environment, forming a complementary effect. The immobilization method can not only improve the overall biomass and stability, but also maintain the activity under adverse environmental conditions, so as to achieve efficient soil remediation effect, which has significant application potential in the degradation of pesticide pollution. Microbial degradation process refers to the process in which microorganisms convert organic substances into simple inorganic substances in respiration and assimilation, and it is also a process in which secondary metabolites are produced or secretase enzymes destroy toxin groups to form non-toxic products. Studies have shown that *Aspergillus Niger* has a strong tolerance and decomposition effect on a variety of pesticides remaining in the soil, can secrete a variety of degradation enzymes [10], can produce a variety of enzymes, such as amylase, cellulase, gluconase and catalase, etc., and is an ideal microorganism for the remediation of pyrethroid pesticide contaminated soil. It has genes APS B and PTR B related to the degradation of pyrethroids. APS B is a lysine aminopeptidase gene. The protease APS B encoded by APS B has the function of recognizing and excising lysine residues at the N-terminus of polypeptides. The protein encoded by PTR B gene

is a transmembrane protein that transports short peptides. Under its guidance, it can secrete high-efficiency degradation enzymes, hydrolyze and oxidize pyrethroids, and degrade them into more stable small molecules. *Aspergillus Niger* has the advantages of vigorous metabolism, short growth cycle, abundant enzyme production, and no toxic substances. It is widely used in food fermentation, animal husbandry, medicine and other industries. In the process of growth and metabolism, it will secrete a variety of organic weak acids, such as citric acid, malic acid, oxalic acid, etc., and these organic acids can chelate metal ions such as iron and copper, so it plays an important role in precious metal recovery, heavy metal detoxification and other fields. At the same time, *Aspergillus Niger* has a certain effect on the remediation of pyrethroid contaminated wastewater. It can also remove heavy metals such as Cu, Cd, Pb and Zn from the soil by bioleaching and other ways to reduce the pollution caused by it.

4. Microbial Immobilization Technology

Microbial immobilization technology refers to the use of physical or chemical means to limit free microorganisms or enzymes in a specific spatial area, so that they are highly dense and maintain biological activity, and can rapidly and massively proliferate under suitable conditions. Cells in nature (especially prokaryotic cells) tend to move and take up nutrients in their microenvironment, but at a certain stage of the life cycle, the extracellular substances secreted by cells promote them to agglomerate with each other and adhere to the surface of the matrix to form a biofilm, that is, self fixation [11]. Using the self fixation phenomenon of cells, the active cells are confined to a limited space matrix by natural or artificial methods and retain their catalytic activity, that is, immobilized cells. Immobilized cells have significant advantages, which can enrich high concentration biomass, shorten the time required for cell reproduction, and achieve the desired effect with a small amount of bacteria, thus reducing the cost. In addition, as a protective agent, the immobilized carrier provides a stable microenvironment for cells to resist the influence of external adverse environmental conditions (such as pH, temperature, heavy metals or toxic substances), so as to maintain cell activity. This technology also enables microorganisms to tolerate high concentrations of pollutants, and immobilized bacteria agents can be regenerated, recycled and reused, reducing energy demand. In the reactor, solid-liquid separation becomes easier, so as to achieve continuous operation and reduce the risk of microbial leakage or environmental pollution. In practice, in order to avoid the disadvantages of a single microbial immobilization method, composite immobilization technology is generally used. This technology mainly combines a variety of immobilization methods to immobilize microorganisms. It can simultaneously use the advantages of each method, avoid shortcomings as much as possible, reduce the possibility of microbial activity reduction, and ensure the mechanical strength of the polymer. At present, many scholars are engaged in the experiment and application of the adsorption entrapment combined immobilization technology, which has many advantages, making

the adsorption entrapment combined immobilization technology show its broad application prospects. Microbial immobilization technology makes up for the shortcomings of traditional technology to a great extent, and can be well applied in the treatment of water and soil environment. But so far, there are few reports on the remediation of 3-PBA contaminated soil, an intermediate product of pyrethroid pesticide degradation, by immobilized microbial technology.

5. Prospect

In recent years, the problem of pesticide residues and pollution in soil has become increasingly serious. Pyrethroids, as one of the most frequently used and widely used pesticides, are difficult to degrade and pose a serious threat to ecological construction and human health. As the most promising insecticide, pyrethroids will still be widely used in the next few decades until new alternative compounds are found. Therefore, it is urgent to solve the problem of pyrethroid degradation. Traditional prevention and control methods include chemical method and physical method, but these methods are high cost, low efficiency, easy to cause secondary pollution, and difficult to achieve satisfactory results. However, bioremediation technology, with its advantages of high efficiency, cleanness and economy, gradually shows broad application prospects in the field of environmental sustainable development. However, microorganisms have limitations due to their own characteristics when dealing with agricultural residues in soil. Therefore, it is of great academic significance and application prospect to understand the characteristics and application status of degrading bacteria and explore the application status of microbial immobilization technology.

At present, there have been many advances in the research on the removal of pesticide residues by biological methods, but the detailed reports on the impact of the characteristics of degrading bacteria on soil remediation are still insufficient. Therefore, it is of great significance to explore the characteristics and application status of degradation bacteria. The degradation effect and safety were evaluated by gas chromatography, aiming to provide a scientific basis for the application of immobilized composite microbial agents in the remediation of pyrethroid pesticide contaminated soil. This study will not only enrich the theoretical framework of microbial treatment of pesticide contaminated soil, but also provide technical support for practical application, promote the large-scale application of immobilized microbial technology in pollution treatment, and then help to promote the sustainable development of agriculture. Therefore, a systematic study on the characteristics of degrading bacteria and their potential in soil remediation will provide an important theoretical basis and practical path for solving the current environmental problems and promoting the development of green agriculture.

Conflicts of Interest

The authors declare no conflicts of interest.

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