

Review

Neonicotinoid Insecticide-Degrading Bacteria and Their Application Potential in Contaminated Agricultural Soil Remediation

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Abstract: Recent advances in the microbial degradation of persistent organic pollutants have the potential to mitigate the damage caused by anthropogenic activities that are harmfully impacting agriculture soil ecosystems and human health. In this paper, we summarize the pollution characteristics of neonicotinoid insecticides (NNIs) in agricultural fields in China and other countries and then discuss the existing research on screening for NNI-degrading functional bacterial strains, their degradation processes, the construction of microbial consortia, and strategies for their application. We explore the current needs and solutions for improving the microbial remediation rate of NNI-contaminated soil and how these solutions are being developed and applied. We highlight several scientific and technological advances in soil microbiome engineering, including the construction of microbial consortia with a broad spectrum of NNI degradation and microbial immobilization to improve competition with indigenous microorganisms through the provision of a microenvironment and niche suitable for NNI-degrading bacteria. This paper highlights the need for an interdisciplinary approach to improving the degradation capacity and in situ survival of NNI-degrading strains/microbial consortia to facilitate the remediation of NNI-contaminated soil using strains with a broad spectrum and high efficiency in NNI degradation.

Keywords: soil; neonicotinoid insecticides; degrading bacteria; microbial consortium; remediation

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1. Introduction

Pesticides play a crucial role in agricultural production by protecting crops from hazardous organisms and maintaining the safety of agricultural products and the environment [1]. The development of the first neonicotinoid compound, imidacloprid (IMI), by Bayer in the late 20th century marked the advent of a new generation of chemical insecticides. IMI significantly enhanced crop resistance to pests and propelled the rapid development and expansion of NNIs, making them one of the most widely used insecticide classes globally [2]. NNIs are commonly used to control pests affecting rice, maize, fruit trees, and vegetables [3]. After their introduction in the 1990s, NNIs rapidly occupied more than 25% of the global market share of pesticides [4] and are registered for use in the production practices of more than 140 crops in 120 countries, mainly in Asia, the Americas, and Europe [5]. China has registered about 3400 NNI products, including a diverse range of 38 dosage forms. Among these, wettable powders, emulsifiable concentrates, water-dispersible granules, and suspensions are the most prevalent varieties, accounting for 62.3% of total pesticide use [6]. Table 1 presents the information and physicochemical property parameters of seven currently commonly used NNIs.

Table 1. Chemical information and physicochemical property parameters of neonicotinoid insecticides.

Name	CAS	Abbreviation	CW	WS	VP	DT ₅₀ (d)	log K _{oc}	log K _{ow}
Imidacloprid	138261-41-3	IMI	255.70	610	4×10^{-7}	104–228	2.19–2.9	0.57
Thiamethoxam	153719-23-4	THIA	291.72	4100	6.6×10^{-6}	7–72	1.75	−0.13
Dinotefuran	165252-70-0	DIN	202.21	39,830	1.7×10^{-3}	50–100	1.41	−0.64
Acetamiprid	135410-20-7	ACE	222.67	2950	1.73×10^{-4}	2–20	2.3	0.80
Thiacloprid	111988-49-9	THI	252.72	184	3.0×10^{-7}	9–27	3.67	1.26
Clothianidin	210880-92-5	CLO	249.68	340	2.8×10^{-8}	13–1386	2.08	0.70
Nitenpyram	150824-47-8	NIT	270.71	590,000	1.1×10^{-3}	1–15	1.78	−0.66
Imidaclothiz	105843-36-5	IMID	261.69	500	NA	3.1	NA	NA

Notes: CW—chemical weight; WS—water solubility; VP—vapor pressure, DT₅₀(d)—half-life. log K_{oc} represents the octanol-water partitioning coefficient; log K_{ow} represents the soil organic carbon—water partitioning coefficient.

This study searched scientific articles published by the end of 2023 using the Web of Science (Thomson Reuters, New York, NY, USA) and Google Scholar (Google Inc., Mountain View, CA, USA) with the following search terms: (i) (neonicotinoid insecticides) and [soil or agriculture soil], and (ii) (bacteria degradation or biodegradation or microbial degradation) and [neonicotinoid insecticides].

When NNIs are applied, there are few air deposits because of their low volatility. Additionally, only a small percentage, ranging from 1.6% to 28.0%, is absorbed by crops, while the majority remains as residues in the soil. This accumulation of NNIs in the soil leads to soil contamination [7,8]. Because of their high water solubility, the absorption of pesticide molecules in the soil is susceptible to passive plant intake, resulting in a decrease in the reproductive capacity and the massive death of non-target insects (bees, butterflies, etc.), a sharp decline in the number of insect-eating birds, the death of aquatic organisms, and even irreversible damage to the food chain and ecological environment [9–12]. At the same time, the degradation behavior of new alkaline pesticide fumes in soil is also an important process that leads to environmental regression, while the effectiveness of their degradation varies significantly under the influence of different environmental factors [13,14]. Therefore, understanding the status of NNI pollution in agricultural soils throughout China is important for the formulation of NNI pollution standards for agricultural soils and the development of pollution control technologies.

A large variety of functional bacteria with the ability to degrade pesticides are present in long-term pesticide-contaminated agricultural soils [15,16]. Currently, researchers have isolated a large variety of functional bacteria with an efficient ability to degrade NNI in contaminated agricultural soils, and their degrading characteristics, metabolic pathways, and critical functional genes have been well studied. Studies have shown that the direct application of functional bacteria into soil can effectively reduce the concentrations of NNIs in soil. In this study, we explore the current situation of NNI pollution in soil ecosystems in China. We also highlight current scientific and technological advances in the remediation of NNI-contaminated soil via the application of NNI-degrading bacteria, which we classify into three pillars: (1) the discovery and isolation of NNI-degrading bacteria; (2) advances in the understanding of the mechanisms and pathways through which microbes degrade NNIs; and (3) the development of efficient and persistent bioremediation technologies for NNI-contaminated soil.

2. Current Status and Risks of Neonicotinoid Insecticide Pollution in Agricultural Soils

NNIs are water soluble, and their half-life varies significantly across different compounds and soil types. More than 90% of the active ingredients of NNIs enter agricultural soil, making such soil the primary sink for NNIs in the environment [17]. At present, there are some differences in the residual concentrations of these compounds in agricultural soils in different countries, but most concentrations fall within the pollution range of ng g^{-1} [18]. Previous surveys have shown that the residual concentration of clothianidin in corn-growing soils in Canada is 0.91 ng g^{-1} [19], compared with the concentration

of 7.00 ng g^{-1} in the soils of the same crop in the United States [20]. NNI pollution of agricultural soils poses a threat to the environment and the quality of agricultural products in China [21–23]. The results reported in relevant published papers show that agricultural soils have been contaminated with NNIs to varying degrees in various parts of the country, and there are major differences in NNI concentrations in different regions and provinces; for example, the total NNI content in Hunan soils is $964.88 \text{ } \mu\text{g kg}^{-1}$, while the total content of NNI in Ningxia soils is only $33 \text{ } \mu\text{g kg}^{-1}$. Moreover, the types and concentrations of neonicotinoid pesticides found in farmland soils in China are higher than those in America ($2.3\text{--}4 \text{ } \mu\text{g kg}^{-1}$), Germany ($0.8\text{--}3.4 \text{ } \mu\text{g kg}^{-1}$), and England ($0.01\text{--}28.6 \text{ } \mu\text{g kg}^{-1}$) [24–26]. The main reasons for this phenomenon can be summarized as follows: First, these countries have implemented a strict ban on the use of some neonicotinoid pesticides that are still used in China. Second, China is a large agricultural country, with a wide crop-planting area, resulting in a corresponding increase in the use of neonicotinoid pesticides. The detection rates of various kinds of NNIs vary. Imidacloprid (IMI) and acetamiprid (ACE) were found in the soils from 31 areas in China. IMI residues were discovered at levels greater than those of ACE, reaching a maximum of $407 \text{ } \mu\text{g kg}^{-1}$ in Hunan. The above findings indicate that this pesticide is widely used for pest control and can remain in agricultural soils.

More seriously, NNIs that remain in soil can enter crop organisms through the soil–root system, thus threatening the health of populations. An analysis by researcher on the residues of NNIs in vegetables and fruits consumed by students at a school in Zhejiang, China, indicated that all 123 samples contained at least one NNI. Commonly consumed foods, such as carrots, green vegetables, baby cabbages, and apples, were found to contain more than six NNIs. Although the estimated average daily intakes were below the current chronic reference dose (cRfD) of imidacloprid, there is a possibility of future downward revisions of the cRfD for NNIs [27]. The results of a study found that imidacloprid and thiamethoxam were the most commonly detected neonicotinoids in fruits and vegetables, with 66 and 51% detection rates in a study in Hangzhou, China, and 52 and 53% detection rates in a U.S. Congressional Cafeteria study, respectively [28]. The researchers' results showed that NNIs were detected in a variety of fruits and vegetables, with a residual concentration of 74 ng g^{-1} of thiamethoxam in cornflakes, 80 ng/g of imidacloprid in mangoes, and 10 ng g^{-1} of imidacloprid in strawberries [29]. In a recent research study carried out in 11 cities in Hainan, China, it was found that 31 novel transformation products of neonicotinoids were generated via innovative methods or a mix of several transformation processes. The average concentrations of neonicotinoids and nine of their transformation products (using legitimate standards) ranged from 0.0824 to 5.34 ng g^{-1} and from 0.0636 to 1.50 ng g^{-1} , respectively. The combined environmental degradation indices (EDIs) of the measured byproducts of such transformation processes were generally lower than those of the original neonicotinoid compounds, except for clothianidin desmethyl, which exhibited a ratio of 1157% [30]. In addition, new alkaline pesticide fumes also pose a potential threat to persons exposed to these pesticides for long periods of time [21]. The above results indicate that NNIs enter and accumulate in crops, and their risks should be included in regulatory management in the future.

In addition, NNIs that remain in crops can enter the human body through the food chain and accumulate. Several studies have detected residues of NNIs in human urine [31]. For example, a study examining the urine samples from 35 symptomatic patients with unknown histories in Japan and 50 non-symptomatic volunteers found that the concentration of N-methyl-Citrate in the urine samples had a certain correlation with typical symptoms, such as recent memory loss, finger tremors, fatigue, abdominal pain, headache, and chest pain [32]. Recent research findings also show that pesticides and their metabolites can pass freely through the human placenta. Studies have shown that NNIs pose risks of hepatotoxicity, developmental toxicity, genetic toxicity, neurotoxicity, and carcinogenicity [33].

In May 2022, the General Office of the State Council in China issued the Program of Action on Management of New Pollutants, which proposed that, with the production and use of toxic and harmful chemicals as the main source of new pollutants, there is a need

to improve the system of regulations on the management of environmental risks posed by harmful and toxic chemical substances and to significantly enhance the capacity for the management of new contaminants. As a new class of pollutants, NNIs are currently insufficiently managed to effectively control their risks, causing certain risks in terms of the ecological environment and the human body, and residues of NNIs in the environment pose new challenges for environmental protection. China is currently the leading producer, exporter, and consumer of NNIs [34]. However, research on the environmental contamination caused by NNIs is still in its early phases. There is an urgent need to strengthen the identification of the pollution characteristics of NNIs in the environment, to study in depth the laws that govern their migration and conversion, and develop removal techniques to safeguard human health and the ecological environment.

3. Neonicotinoid Insecticide–Degrading Bacteria

The reduction of NNIs in nature is divided into biological and non-biological reduction processes, whereby the biological reduction process occurs predominantly via microbial metabolism [35]. Microbial degradation is one of the main ways in which pesticides are degraded in soil. Studies have shown that a large number of naturally domesticated microorganisms with the ability to degrade NNIs are present in soils contaminated with NNIs [36]. Bacteria are considered to be the main microorganisms that naturally degrade NNIs in the environment. Currently, researchers in China studying NNI-degrading bacteria have mainly conducted research on the enrichment, screening, and identification of high-efficiency degrading strains; the construction of functional bacterial clusters for NNI degradation; and the pathways and mechanisms underlying bacterial degradation. In addition to the isolation of NNI-degrading bacteria from soil, some plant growth-promoting rhizobacteria (PGPR) have also been found to have the ability to degrade NNIs. For example, there are studies reported that the PGPR *Ensifer adhaerens* TMX-23 isolated from soybean plant degraded 37.5% of THIA after 25 days of incubation in a mineral salt medium (MSM) containing glucose [37].

In recent years, researchers have isolated a large number of bacterial strains with an NNI degradation function via the separation and purification of these species from different environmental media (Table 2). Currently, the strains obtained via isolation are mainly derived from bacterial genera such as *Bacillus*, *Mycobacterium*, *Pseudoxanthomonas*, *Rhizobium*, *Rhodococcus*, and *Stenotrophomonas*. As shown in Table 2, different bacterial genera exhibit different degrading effects and degradation spectra for NNIs.

Table 2. Neonicotinoid insecticide-degrading bacteria.

Microorganism	Source	Reaction Condition	Degradation Rate	References
Imidacloprid				
<i>Klebsiella pneumoniae</i> BCH1	Agricultural soil, India	30 °C, pH 7, 7 d	50 mg L ⁻¹ , 78%	[38]
<i>Pseudomonas</i> sp. RPT52	Agricultural soil, India	37 °C, 200 r min ⁻¹ , 24 h	128 mg L ⁻¹ , 46.5%	[39]
<i>Pseudoxanthomonas indica</i> CGMCC 6648	Rhizosphere soil, Chian	28 °C, pH 7, 6 d	311 mg L ⁻¹ , 70.1%	[40]
<i>Bacillus aerophilus</i>	Sugarcane field soils, India	Sandy loam soil, 60 d	150 mg kg ⁻¹ , 96.1%	[41]
<i>Pseudomonas</i> sp. 1G	Soil, Australia	28 °C, microaerophilic	50 mg L ⁻¹ , about 70%	[42]
<i>Rhizobium</i> sp.	Oil field soil, Malaysia	28 °C, 120 r min ⁻¹ , 25 d	25 mg L ⁻¹ , 45.48%	[43]
<i>Bacillus alkalinitrilicu</i>	Sugarcane field soils, India	28 °C, 56 d	50 mg/kg, 98.02%	[44]

Table 2. Cont.

Microorganism	Source	Reaction Condition	Degradation Rate	References
<i>Mycobacterium</i> sp. MK6	Soil, Egypt	28 °C, 14 d	150 mg L ⁻¹ , 99.7%	[45]
<i>epidibacillus decaturensis</i> . ST1	Agricultural field, India	30 °C, 120 rpm, 20 d	200 mg L ⁻¹ , 90%	[46]
<i>Ochrobactrum</i> sp. BCL-1 Acetamiprid	Rhizosphere soil, China	30 °C, pH 8, 48 h	50 mg L ⁻¹ , 67.67%	[47]
<i>Fusarium</i> sp. CS-3	Soil, China	25–30 °C, pH 5–7, 96 h	50 mg L ⁻¹ , 98%	[48]
<i>Ensifer meliloti</i> CGMCC 7333	Rhizosphere soil, China	30 °C, pH 7.5, 220 r min ⁻¹	500 mg L ⁻¹ , 65.1%	[49]
<i>Pigmentiphaga</i> sp. AAP-1	Industrial soil, China	30 °C, pH 7, 2.5 h	100 mg L ⁻¹ , 100%	[50]
<i>Pseudoxanthomonas</i> sp. AAP-7	Industrial soil, China	30 °C, pH 7, 60 h	200 mg L ⁻¹ , 95% 300 mg L ⁻¹ , 93% 400 mg L ⁻¹ , 87% 600 mg L ⁻¹ , 73%	
<i>Ensifer adhaerens</i> CGMCC 6315	Soil, China	30 °C, 12 h	200 mg L ⁻¹ , 94.4%	[51]
<i>Ochrobactrum</i> sp. D-12	Agricultural soil, China	30 °C, pH 7, 14 h °C	3000 mg L ⁻¹ , 39.27%	[52]
<i>Rhodococcus</i> sp. BCH-2	Contaminated soil, India	35 °C, pH 7, 8 d	50 mg L ⁻¹ , 84.65%	[53]
<i>Penicillium oxalicum</i> IM-3	Soil, China	30 °C, 14 d	500 mg L ⁻¹ , 41.6%	[54]
<i>Streptomyces canus</i> CGMCC 13662	Soil, China	30 °C, pH 7, 4 d	200 mg L ⁻¹ , 87.6%	[55]
<i>Pseudomonas</i> sp. FH2	Agriculture field soil, China	30 °C, pH 7.0, 14 d	800 mg L ⁻¹ , 96.7%	[56]
Imidaclothiz <i>Stenotrophomonas maltophilia</i> CGMCC 1.1788	Soil, China	30 °C, 84 d	500 mg L ⁻¹ , 36.2%	[57]
Clothianidin <i>Pseudomonas stutzeri</i> smk Thiacloprid	Agricultural Soil, China	30 °C, pH 7, 14 d	10 mg L ⁻¹ , 62.0%	[58]
<i>Variovorax boronicumulans</i> J1	Agricultural soil, China	30 °C, pH 7.2, 60 h	200 mg L ⁻¹ , 62.5%	[59]
<i>Ensifer meliloti</i> CGMCC 7333	Rhizosphere soil, China	30 °C, 60 h	200 mg L ⁻¹ , 86.8%	[60]
<i>Microvirga flocculans</i> CGMCC 1.16731	Soil, China	30 h	159 mg L ⁻¹ , 90.5%	[61]
<i>Rhodotorula mucilaginosa</i> IM-2 Thiamethoxam	Soil, China	30 °C, 20 d	200 mg L ⁻¹ , 59.9%	[62]
<i>Ensifer adhaerens</i> TMX-23	Rhizosphere soil, China	30 °C, 10 d	200 mg L ⁻¹ , 21.6%	[63]
<i>Bacillus aeromonas</i> IMBL 4.1	Soil, India	37 °C, pH 6.0 °C–6.5, 15 d	50 mg L ⁻¹ , 45.28%	[64]
<i>Pseudomonas putida</i> IMBL 5.2	Soil, India	37 °C, pH 6.0 °C–6.5, 15 d	50 mg L ⁻¹ , 38.23%	[64]
<i>Acinetobacter</i> sp. <i>Enterobacter</i> sp. <i>Bacillus</i> sp.	Agricultural soil, India	15 d	50 mg L ⁻¹ , 94.72% 50 mg L ⁻¹ , 90.78% 50 mg L ⁻¹ , 82.06%	[65]

A strain of *Ensifer adhaerens* CGMCC 6315, which was isolated from NNI-contaminated soil, was found to have the ability to degrade acetamiprid effectively; it degraded 94.4% of 200 mg L⁻¹ of ACE in 12 h and quickly eliminated 87.8% of 5 mg kg⁻¹ of residual soil ACE within 2 d [51]. After 72 h of incubation, a nitenpyram-degrading bacterium known as *Rhodococcus ruber* CGMCC 17550, which was isolated from a sewage treatment tank, was used for the degradation of nitenpyram. The nitenpyram degradation rate increased as the biomass of resting *Rhodococcus ruber* CGMCC 17550 cells increased, reaching 98.37% at an optical density of 600 (OD600) of 9 in a transformation broth that contained 100 mg L⁻¹ of nitenpyram [65]. The above results show that long-term use of NNIs in soils can naturally domesticate bacterial strains that can degrade NNIs and show soil bioremediation potential. Soil contaminated with NNIs is an important source of microorganisms that are capable of degrading these pollutants, and there are differences in degradation behavior in contaminated soil, as well as differences in metabolic pathways and the resulting metabolic intermediates.

4. Neonicotinoid Insecticide-Degrading Bacterial Consortia

Currently, functional bacteria selected for separation are mainly selected for their capacity to degrade one or a few NNIs, but in an actual polluted environment, there is often a coexistence of several NNIs, the actual environmental conditions are more complex, and indigenous bacterial clusters are more competitive; so, the efficacy of a single degrading strain for NNI degradation is often not ideal [66]. Degrading bacteria often exist in the natural environment in the form of consortia, using the synergies between different bacteria to completely degrade organic pollutants in the environment, thereby providing energy for their own growth and reproduction. Thus, researchers have attempted to capitalize on the synergies of different strains of bacteria present in broad-spectrum functional bacterial consortia to increase the efficiency of NNI degradation. As shown in Table 3, some NNI-degrading consortia have been well studied. For example, an NNI-degrading consortium named N1, consisting of bacterial strains from the genera *Paenibacillus*, *Rhodococcus*, *Microbacterium*, *Kocuria*, *Paraburkholderia*, and *Pseudoacidovorax*, and a yeast strain closely related to the genus *Rhodotorula*, was isolated from pesticide-contaminated agricultural soil. Under optimal conditions, the degradation rate of N1/2 for imidacloprid and thiamethoxam was 60.1% and 33.4%, respectively [67]. Researchers have separated two NNI-degrading bacteria from contaminated soil, named *Bacillus Aerophilus* and *Bacillus Alkaninitricus*. The combination of these two strains into a consortium resulted in a higher capability for pollutant degradation. This consortium is particularly effective in degrading imidacloprid in soil and can adapt to various degrees of soil pollution [68]. It is evident that bacterial consortia can somewhat overcome problems such as incomplete and narrow spectrum degradation inherent in the use of single bacterium. Therefore, the construction of more effective bacterial consortia is expected to economically increase the efficient removal of NNIs from agricultural soil, with a wider application prospect.

Table 3. Neonicotinoid insecticide-degrading bacterial consortia.

Name	Source	Reaction Condition	Degradation Rate	References
N1/2	Contaminated soil, Costa Ric	Imidacloprid, thiamethoxam, 160 rpm, 25 °C, 5 d	50 mg L ⁻¹ , 60.1% (imidacloprid), 33.4% (thiamethoxam)	[67]
/	Sugarcane growing soils, India	Imidacloprid, 25 ± 2 °C, 56 d	50 mg kg ⁻¹ soil, 93.6% 100 mg kg ⁻¹ soil, 94.2% 150 mg kg ⁻¹ soil, 93%	[68]
SCAH	Contaminated soil, China	Clothianidin, 150 rpm, 30 °C, 15 d	500 mg L ⁻¹ , 79.3%	[69]
ACE-3	Acetamiprid-contaminated soil, China	Acetamiprid, pH 6.0–8.0, 20–42 °C, 144 h	50 mg L ⁻¹ , 100%	[70]
/	Wastewater disposal site, Greece	Thiabendazole, 28 d	5 mg kg ⁻¹ soil, 100% 50 mg kg ⁻¹ soil, 100% 100 mg kg ⁻¹ soil, 100%	[71]

5. Neonicotinoid Insecticide Degradation Pathway

To date, the majority of functional degrading bacteria obtained from the environment have been used to catalyze biological degradation processes, and the efficiency of these microbes depends on a variety of factors, such as the pesticide type used, the soil microbes present, and the soil water content [72,73]. The process of microbial degradation of NNIs can be classified according to the type of reaction and the group of effects occurring during the process of transformation of substances, such as (mixed-circle) opening, (de)methylation, side-chain rupture/hydrolysis, (re)nitrification, and dechlorination (carbon) [74]. In these processes, mercury hydroxylase and P450 ca-oxygenase are two known key enzymes, which have been speculated to play the functions of water-resistant antioxidant cluster molecules [$-C\equiv N$] and hybrid oxidation openings (mercury, microxylate, etc.), respectively [75].

The pathways of and the products generated from microbial degradation of NNIs vary depending on the structure of the pesticides used and the metabolic activity of degrading

microbes. Imidacloprid is the earliest and most comprehensively studied NNI to date. In 2007, researchers first reported the biological degradation of imidacloprid by different strains of bacteria [76]. There are two main pathways of IMI microbial degradation. In the first pathway, IMI is first converted to 5-Hydroxy IMI and then to Olefin-IMI via the action of dehydratase; olefin-IMI contains unsaturated double bonds, which makes it more easily degraded and eventually converted to carbon dioxide. The second pathway involves 2-Nitroso restorative (IMI) action generated through IMI (Nitroso), guanidine, and urea (Figure 1). Both pathways produce 6-chlorofluorocarbic acid and 6-phosphate-based phosphoric acid, which are easily degradable organic substances that are oxidized to produce H_2O and CO_2 [77]. Amidase and its encoding genes, such as *aceA* and *aceB*, play a leading role in imidacloprid degradation [78].

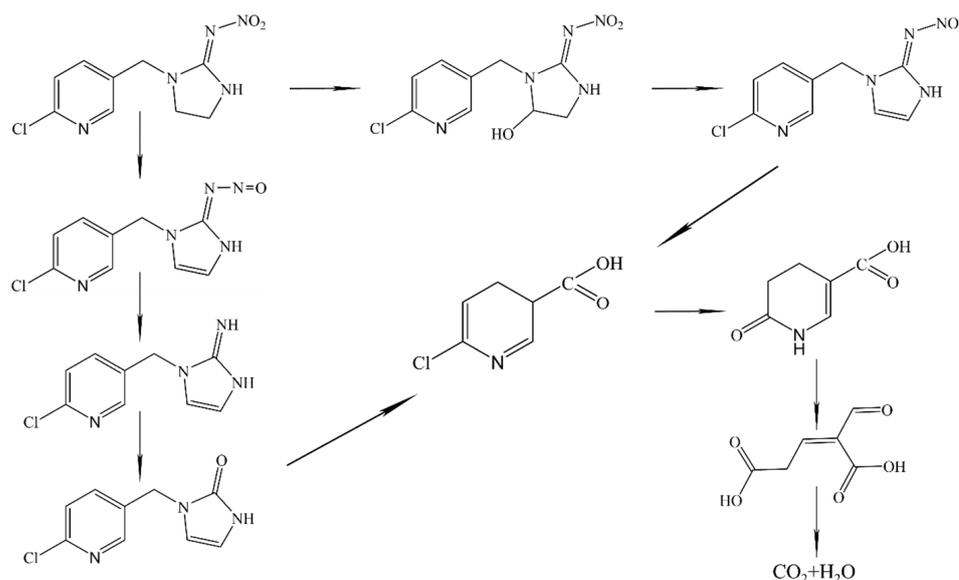


Figure 1. Microbial degradation pathways of imidacloprid.

As research progresses, the pathways of acetamiprid biodegradation are better understood (Figure 2). Generally, the $C\equiv N$ of acetamiprid is produced through the oxidation breakdown of N-amide derivatives, which are dissolved asymmetrically to produce two intermediates, with one intermediate rapidly producing 6-chlorophyllic acid and eventually being mineralized to H_2O and CO_2 [51]. *Rhodococcus* sp. BCH-2 can degrade acetamiprid to Ac-4 and rapidly oxidize it to produce 6-chloropyric acid, which is eventually mineralized to CO_2 and H_2O after multi-stage oxidation [53]. Furthermore, studies have demonstrated that acetamiprid does not produce an intermediate in some microbial degradation processes, i.e., it is directly dechlorinated and de-methylated to the final product [79], and that micro-organisms cannot convert this final product into other products, although studies have found that it can continue to be degraded in animal and plant systems [80]. During the degradation of acetamiprid, genes like *anhA*, *anhC*, *anhB*, *anhD*, and *anhE* play important roles [55].

Thiamethoxam can be degraded by microorganisms in a variety of ways, one of which is through the nitro-reduction metabolic pathway to produce nitroso, guanidine, and urea. This pathway is the main pathway for microbial degradation of thiamethoxam. It can also be converted to methyl-thiamethoxam via the demethylation pathway [74]. (Figure 3). In a recent study, a thiamethoxam degrading functional strain *Ensifer adhaerens* TMX-23 was isolated via screening from rhizosphere soil bacteria, could biotransform 96% of thiamethoxam in soil samples [37], thereby accelerating the degradation of thiamethoxam residues in the soil.

of 95.7% was exhibited, resulting in the near-complete removal of clothianidin from the soil over 45 days [69].

Generally speaking, bacterial strains or consortia that are currently domesticated from soil have a greater potential for application in the remediation of NNI-polluted soil [81]. However, it is regrettable that most of the existing studies have been conducted only in the laboratory. Thus, the application of functional bacteria in actual agricultural soil pollution management is unclear, and there are still many constraints. For example, nutrients are an essential factor in microbial growth, and in the process of using microbes to repair polluted agricultural soil, although some of the pollutants can be exploited by microbes, they still cannot satisfy the energy required for the growth of microbes, which affects the reproduction and survival of bacteria. In addition, under purely cultivated conditions, the strains of compound bacteria can work together, but the competitiveness of indigenous microorganisms in *in situ* soil is stronger. This has a greater impact on the stability of the added exogenous bacteria, which may even be eliminated due to their disadvantage in the competition with indigenous microbes. Furthermore, under *in situ* conditions, the interaction of exogenously applied microbes in polluted soil varies greatly under climatic conditions, which also poses limitations and challenges to using bacteria for insecticide-contaminated soil remediation.

With the rapid development of genetic engineering and molecular biology technologies, the biodegradation efficiency of NNIs can be improved through the application of gene editing or synthetic biology technology. For example, based on genome sequencing and using comparative genomics and comparative transcriptomics, the specific genetic architecture of nicotine metabolism in the nicotine-degrading bacterial strain JY-Q was resolved [80]. However, in a complex of tobacco waste water extract, the effect of JY-Q on nicotine degradation was poor due to the lack of bacterial tolerance and nicotine conversion ability. To break through this bottleneck, researchers first identified the regulatory factors and promoters of the three modules (upper, middle, and downstream) of the nicotine metabolism in JY-Q and then applied metabolic engineering strategies for combined replacement of the endogenous promoters, and the nicotinic degradation rate of the modified bacterial strains increased by 67% and 69%, respectively, compared with the wild type [82,83].

7. Summary and Outlook

This paper provides a summary of NNIs' pollution status, the degradation ability of functional bacteria, and their application in agricultural soil. NNI-degrading bacteria that are richly filtered from NNI-polluted agricultural soil have different degradation characteristics. Compared to single functional strains, naturally domesticated or artificially constructed functional consortia have higher NNI degradation efficiency, a broader spectrum, and wider prospects of application in actual polluted agricultural soil remediation. In the future, research will be needed in the following areas:

- (1) At present, most research studies on bacterial degradation of NNIs have been conducted in the laboratory setting, rather than on the remediation of real polluted agricultural soil. Since the environment in agricultural soil is very complex, the use of NNI-degrading bacteria for the remediation of real farm soil needs more testing.
- (2) Composite consortia are a research hotspot for the degradation of NNIs using bacteria. In the future, modern molecular biology methods, such as high-flow sequencing, stable isotope tracing, macro-genomics, and macro-transcriptomics, can be used to clarify the mechanisms underlying the synergistic interaction between different strains of bacteria in bacterial groups and to filter such strains for the construction of bacterial consortia with targeted and efficient NNI degradation for application in actual agricultural soil remediation.
- (3) In the future, NNI-degrading functional bacteria or consortia can be prepared as immobilized bacterial agents to improve the survival rate of degrading bacteria under

in situ conditions and harsh climatic conditions, promote the biodegradation of NNIs, and achieve efficient and safe remediation of NNI-contaminated soil.

Therefore, in future research, it is necessary for microbiologists, bio-geochemists, agronomists, soil scientists, and modelers to engage closely in interdisciplinary cooperation and continue in-depth research for a better understanding of the environmental adaptability of NNI-degrading bacteria, their degradation enzymes, and the synergies of degrading bacterial strains and genes, as well as to combine engineering, high-flow sequencing, and genomics methods to establish the appropriate quantitative models to build functional bacteria with strong environmental adaptation, broad degrading spectra, and better degradation ability. In summary, the use of functional bacteria to control NNI pollution in agricultural soils is a low-cost, green, and feasible method that can provide an important technical reference for the resourcing and reuse of polluted farm soils, while ensuring the safety of agricultural products and human health.

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