CHAPTER 2: LITERATURE REVIEW

2.1. Bioremediation

Microorganisms play an important role in the removal of harmful substances from the environment by using enzymes to metabolize environmental pollutants as a food source. Bioremediation is a viable alternative to traditional pesticide-treatment methods for pesticide-contaminated sites. Microbial activity has a significant impact on pesticide fate in the environment, with some microorganisms efficiently breaking down pesticides while others develop resistance. Mineralization and co-metabolism are the primary processes involved in pesticide degradation. Numerous elucidated mechanisms and pathways are involved in the biodegradation of different organic compounds (Wang et al., 2022). Figure 2.1 shows a flowchart of the different bioremediation techniques.

Bioremediation has the disadvantage of being slow, which leads to an extended period of treatment. This disadvantage might be brought about by physical, chemical, or biological constraints such as nutrients, pH, temperature, moisture, oxygen, soil properties, contaminant concentration, and the number and type/species of microorganisms. As a result, increasing the biodegradation rate is required to shorten the remediation time.

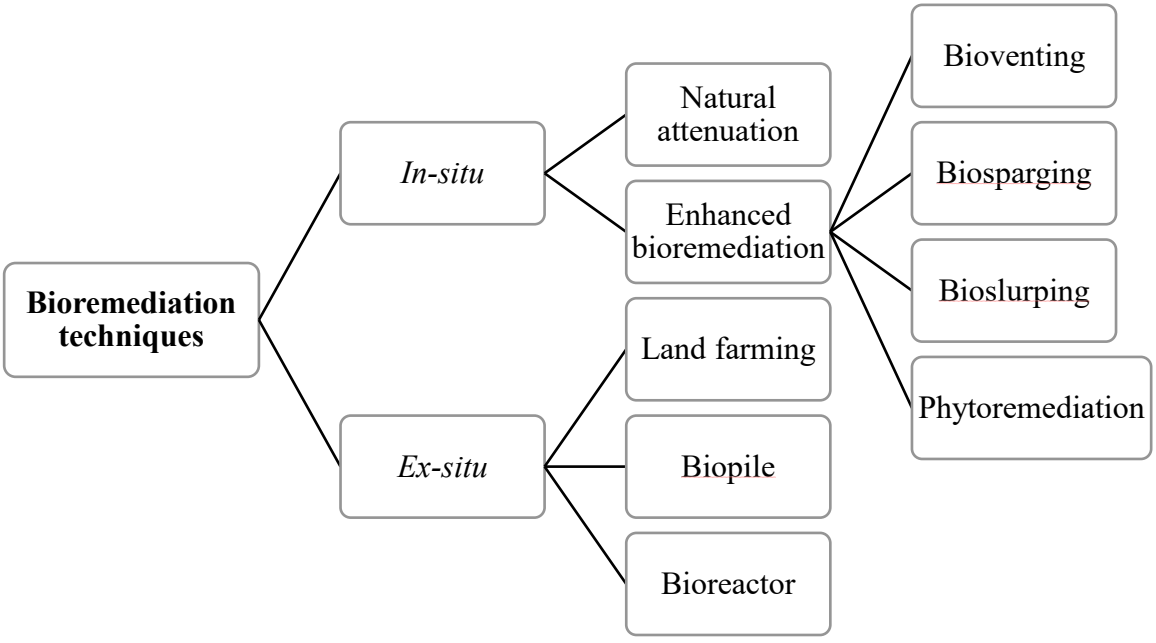

Figure 2.1: Bioremediation techniques

Enhanced bioremediation encompasses a wide range of technologies. These technologies may include the addition of electron acceptors or electron donors for stimulating naturally occurring microbial populations (biostimulation) or the addition of specific microbes (bioaugmentation) to enhance target pollutant biodegradation. Due to competition with autochthonous species, the addition of pure strains has been used primarily in lab-scale studies and has not been successful in full-scale situations (Tomei and Daugulis, 2013). In practice, mixed cultures containing a wide range of microorganisms are used. Fungi, anaerobic and aerobic sludge, and compost have been used as biomass sources and types.

The effectiveness of bioaugmentation strategies in the bioremediation of liquid and solid matrices has produced conflicting results. In a complex and dynamic matrix like soil, where many abiotic and biotic factors influence the survival and activity of the inoculated microorganisms, successful application is more difficult. Temperature, humidity, pH, nutrient content, and bioavailability are examples of abiotic factors. Biotic factors are more important, as evidenced by the ease with which added microorganisms grow in sterile soil. In addition to competition for substrates and nutrients with autochthonous microorganisms, the heterogeneity of the soil matrix can make homogeneous distribution of the added inoculum challenging. One of the most frequent concerns about bioaugmentation is that non-native species might not be able to survive in the contaminated conditions (Tondera et al., 2021). Another concern is that these species could continue to exist long after contaminants has been eliminated, thereby altering the environment.

The process of biostimulation involves supplying readily biodegradable nutrients (such as nitrogen, phosphorus, and trace elements) to the microorganisms to develop an environment that is conducive to the formation of metabolic pathways for the biodegradation of contaminants. By enhancing biomass activity, temperature can contribute to biostimulation; however, high temperature can also make pollutants more volatile, increasing the probability of their spread throughout the environment (Bonmatin et al., 2015). Even in some of the simplest *ex-situ* technologies, like landfarming and composting, controlling temperature is challenging. Only more complex systems, like bioreactors, can achieve more effective temperature control, but it adds to the operating costs.

While bioremediation is an effective method for insecticide remediation, it is important to note that it may not be suitable for all contaminated sites (Azubuike et al., 2016). Additionally, it is

crucial to consider the potential impact of bioremediation on the environment and human health, as well as the cost and feasibility of implementation. A bacterial strain with high efficiency in degrading pollutants is necessary for bioremediation to be successful.

As molecular biology advances, it may be possible to create genetically modified bacteria to improve the bioremediation of pollutants and pesticides. Recombinant microbial populations could be extremely valuable for bioremediation of different pesticides from the environment (Bhatt et al., 2021; Liu et al., 2019). Microorganisms can acquire the ability to degrade pesticides in soil by horizontal gene transfer from degradative plasmids, adjusting substrate specificity, or modifying the regulation of existing enzymes (Hussain et al., 2009). Furthermore, the use of genetic engineering has the potential to improve the efficiency of microorganisms with biodegradation or bioremediation capabilities (Liu et al., 2019).

Recent advances in omics-based technologies have made them useful instruments with novel insights into the biodegradation of pesticides. These insights could lead to the development of practical bioremediation strategies for environments contaminated with pesticides such as imidacloprid. Deeper insights into the microbial communities involved in various bioremediation initiatives have been obtained through the application of next-generation sequencing techniques. These methods greatly expand our understanding of the microbial processes that are intrinsic to bioremediation and provide insight into the results of different approaches used to clean up contaminants. Moreover, the integration of molecular biology and metagenomics has greatly expanded our comprehension of the biological systems found in these contaminated environments, making a substantial contribution to the general understanding of the microbial domain (Malla et al., 2018).

2.2. Application of indigenous bacteria for biodegradation

To achieve adequate remediation levels, some classes of contaminants (such as pesticides and chlorinated organic compounds) must have a prolonged reaction time and acclimatized microbial populations because they are toxic to the microbial agents used in their degradation and are not easily broken down biologically. The capacity of bacteria to degrade can be increased by acclimating to a contaminated environment (Wang et al., 2018). Furthermore, metabolites that are even more toxic than the parent compound may be formed during the biodegradation of complex molecules, and these metabolites may also suppress the growth of bacteria (Singleton, 1994).

Many native microbes have developed complex and effective metabolic pathways that enable them to biodegrade toxic compounds that are released into the environment (Abatenh et al., 2017; Vidali, 2001). The ability of these organisms to break down different xenobiotics is directly related to the time taken to adapt to their natural surroundings. Numerous studies have demonstrated how native microbial consortia, separated from polluted soils, can break down a variety of pesticides and pesticide mixtures. Some research suggests that populations acclimated to one type of pollutant can also break down other compounds with comparable structural properties (Wang et al., 2016; Wang et al., 2018). Microorganisms are the most commonly used biocatalysts in soil remediation, mainly because of their extraordinary capacity to adjust to the harsh environmental conditions present in contaminated sites. The complex interactions between the soil matrix, low levels of non-carbon nutrients, and wide fluctuations in temperature, pH, and moisture content are all included in these conditions. Notably, native microorganisms are often obtained from the soil that is intended for remediation, providing a clear benefit in hastening the adaptation process. Enzyme regulation and the selection and growth of specialized microorganisms are two of the most important phenomena that occur during the acclimation phase, which is critical in this context. Acclimatized cultures are used exclusively to break down specific compounds in various researches (Arora & Bae, 2014; Ye & Shen, 2004).

If a readily available carbon and energy source is provided, particularly through a co-metabolic pathway, the resulting microbial communities complete the mineralization of any compound (Fenner et al., 2021). When emphasis is placed on the full mineralization of toxic organics to CO_2, mixed cultures are especially important. Numerous studies conducted in pure cultures have demonstrated that toxic intermediates are formed during biodegradation because it may not be attainable for a single organism to mineralize the contaminants completely (Kumar et al., 2017; Zhang & Bennett, 2005). The interaction amongst all the species present is the main advantage of the microbial consortium formed by mixed culture. The chlorophenol mixture was broken down by acclimated activated sludge one to two orders of magnitude faster than pure strains taken from the acclimated consortium, according to Buitron et al. (1998). A consortium of Streptomyces sp. has been reported by Fuentes et al. (2017) to be able to eliminate an organochlorine pesticide mixture consisting of lindane, methoxychlor, and chlordane.

Recently, various mixed cultures were inoculated with mixed cultures of the bacteria *Streptomyces* spp. and the fungus *Trametes versicolor*, chosen for their known ligninolytic and pesticide-degrading abilities. For the first time, it was reported that an organophosphorus pesticide mixture consisting of chlorpyrifos and diazinon could be removed using a *Streptomyces* mixed culture from various environmental matrices, such as liquid medium, soil, and a biobed biomixture (Briceño, et al., 2018). Microbial consortia are typically more effective at degrading pesticides than single strains (Bhatt et al., 2021; Góngora-Echeverría et al., 2020). Furthermore, novel microbial communities can break down harmful chemicals as a source of energy through co-metabolism.

2.3. Factors affecting bioremediation

Microbial degradation relies not only on the availability of microorganisms with the right degradative enzymes but also on various environmental factors. The slow pace of degradation is a significant challenge in bioremediation. Therefore, extensive research is being carried out to increase the rate of degradation by choosing appropriate microorganisms and optimizing the degradation conditions.

The effectiveness of bioremediation depends on various factors such as the type of pollutant, the existence of other pollutants, the composition and activity level of microorganisms in the environment, and the physical and chemical conditions of the environment (e.g., pH, temperature, moisture). To make bioremediation successful, it is important to ensure that the environmental conditions are suitable for the growth and activity of microorganisms (Abatenh et al, 2017). This often involves adjusting the environmental parameters to promote microbial growth and degradation of pollutants at a faster rate. Therefore, the optimization of bioremediation requires considering different variables together to predict and understand the fate of environmental pollutants.

Bioavailability refers to the amount of pesticides that can be easily taken up by microbes in the context of bioremediation (Singh et al., 2008). If pesticides are not readily available to microbes, it may limit their ability to biodegrade the pesticides and their metabolites in soil, leading to longer half-lives. At low concentrations of pesticides, microbes are unable to produce enough energy to induce the catabolic gene systems responsible for biodegradation, which slows down the bioremediation process. Although the microbial cells may still degrade the pollutants present, the low levels of nutrients in the environment can reduce their growth rate, which eventually leads to

a decrease in the uptake of pesticides by the microbes. The bioavailability of pesticides is also dependent on various environmental factors such as pH, temperature, moisture content of the soil, solubility of the pesticide, presence of other toxic pollutants, and soil nutrients.

Numerous studies have suggested that pesticides present in soil are not easily degraded by microorganisms due to their low bioavailability (Singh et al., 2008). This is one of the major reasons why many pesticides persist in the environment. The uneven distribution of microorganisms and pesticides, the soil matrix that slows down substrate diffusion, and the solubility of the pesticide are some of the factors that contribute to the low bioavailability of pesticides (Odukkathil & Vasudevan, 2013). Therefore, it is essential to increase the bioavailability of pesticides in the topsoil to achieve complete bioremediation and prevent their leaching into groundwater and surface water.

Temperature has a significant effect on the degradation process because it affects the enzymatic function and activity (Pi et al., 2016). The rate of biodegradation can vary seasonally. Increased metabolic activity can raise the soil temperature due to its exothermic nature (Gangola et al., 2022). Soil temperature can increase due to increased metabolic activity, affecting the rate of biodegradation. The pH level of the soil can significantly affect pesticide absorption, adsorption, and abiotic/biotic degradation (Jaiswal & Verma, 2016). Each microorganism (e.g. bacteria, fungi, algae) has its own optimum pH range where it grows and performs best. Their metabolic activity can also decrease or increase when the pH is below or above the optimum pH range. Microorganisms prefer to consume the dissolved fraction of the substrate in their habitat; hence, the solubility of pesticides has a direct effect on their rate of biodegradation. Pesticides with low water solubility are more resistant to microbial transformation than those with high water solubility (Odukkathil & Vasudevan, 2013). The degradation efficiency of pesticides is greatly influenced by the type of soil in which they are present. This is because soil provides favorable conditions for the growth of microorganisms that play a crucial role in pesticide degradation. The rate of pesticide degradation is also affected by various soil properties such as organic matter content, clay content, moisture content, conductivity, pH, and other environmental conditions. The degradation efficiency of pesticides is greatly influenced by the type of soil in which they are present. This is because soil provides favorable conditions for the growth of microorganisms that play a crucial role in pesticide degradation. The rate of pesticide degradation is also affected by the various soil

properties such as organic matter content, clay content, moisture content, conductivity, pH, and other environmental conditions (Arias-Estévez et al., 2008; Müller et al., 2007).

The presence or lack of organic matter in the soil might influence the biodegradation pattern of pesticides. By promoting pesticide adsorption, soil organic matter (SOM) can either slow the rate of microbial-mediated pesticide degradation or enhance microbial activity and biodegradation via co-metabolism (Gangola et al., 2022). The rate of degradation may also be affected when bacteria and pollutants do not come in contact with each other. In addition to this, microbes and pollutants are not uniformly spread in the environment (Abatenh et al., 2017).

Studies on soils from agricultural fields and woodlands have shown that the rate at which pesticides like dicamba, metribuzin, linuron, glyphosate, alachlor, and 2,4-dinitroaniline break down is positively correlated with the biomass of soil microbes. Microbial biomass has been suggested as a potential measure of the ability of soil to degrade pesticides (Rodríguez-Cruz et al., 2006). Soil moisture content has a considerable effect on bioavailability and degradation of pesticides. Moisture is necessary for the cell growth and function of all soil microorganisms. Water availability has an impact on how soluble nutrients and water diffuse into and out of microbial cells. Supersaturated soil, with an excess of water, can have unfavorable effects, such as a decrease in oxygen content, which can impact aerobes. As a result, the environment will become anoxic, which will cause anaerobic respiration to occur. Anaerobic respiration uses less energy and slows down the rate of biodegradation (Odukkathil & Vasudevan, 2013).

Naseer et al. (2020) used *Bacillus* strain A2 bacteria to investigate the imidacloprid degradation efficiency at various pH levels (5, 6, 7, 8, and 9) and temperatures (25, 30, 35, and 40°C). In another study, Hu et al. (2013) used *Ochrobactrum* sp. strain BCL1 to study the effects of pH (5 to 9) and temperatures (20°C to 40°C) on imidacloprid degradation. Within two days, these bacterial strains were able to remediate 67% of 50 mg/L of imidacloprid; the ideal pH and temperature for degradation were found to be 8 and 30°C, respectively. Similarly, four bacterial isolates were used in a study by Shaikh et al. (2014) on imidacloprid remediation at various pH levels (4, 7, and 10) and temperatures (65, 25, and 5°C). For every bacterial isolate, the maximum imidacloprid degradation efficiency happened at a pH of 7 and a temperature of 25°C. The study revealed that imidacloprid degradation was more favorable in neutral pH conditions as opposed to alkaline and acidic ones. Additionally, the ideal temperatures for bioremediation were found to be

30°C and 35°C. Together, these results highlight the importance of biotic and abiotic environmental factors in promoting efficient bioremediation processes.

According to Kumar et al. (2006), HCH isomer degradation peaked at 15% moisture content and declined as moisture content increased to 30%. The impact of a soil-to-water ratio ranging from 10 to 40 w/v% on *P. aeruginosa's* degradation of endosulfan isomers in soil slurry was investigated by Arshad et al. (2008). In the slurries with 15% soil, the biodegradation of endosulfan isomers decreased as soil moisture content increased. In a soil reactor kept at a moisture content of 38%, approximately 96.03% of endosulfan degradation was observed (Kumar & Philip, 2006). It is important to note that other factors, such as the presence of co-contaminants, nutrient availability, and the concentration of imidacloprid, can also affect the rate and efficiency of biodegradation. These factors should be considered when assessing the potential for biodegradation of imidacloprid in the environment, as they can impact the rate and efficiency of degradation. Numerous studies have been conducted for the optimization of environmental conditions for imidacloprid biodegradation. Some of the studies have been summarized in Table 2.1.

Table 2.1: Studies conducted on optimization of environmental conditions for imidacloprid biodegradation

S. No.	Organism	Parameters			References
		pH	Temperature	Concentration	
1.	*Ochrobactrum* sp.	5, 6, 7, 8, 9	20°C, 25°C, 30°C, 35°C and 40°C	-	(Hu et al., 2013)
2.	*Klebsiella pneumoniae* strain BCH1	3, 5, 7, 9, 11	10°C, 20°C, 30°C, 40°C, 50°C	50, 250 ppm	(Phugare et al., 2013)
3.	*Bacillus alkalinitrilicus*	-	37°C	50, 100, 150 mg/kg	(Sharma et al., 2014)
4.	*Bacterial strain* NUS1, NUS4	4, 7, 10	5°C, 65°C, 25°C	-	(Shaikh et al., 2014)

5.	*Enterobacter sp.* ATA1	1, 3, 5, 7, 9, 11	20℃, 37℃, 45℃, 50℃	25, 50, 75, 100 mg/kg	(Sharma et al., 2015)
6.	*Aspergillus terreus* YESM3	4, 6, 8	28℃	5, 10, 25, 50, 100, 200,400 mg/L	(Mohammed & Badawy, 2017)
7.	*Kocuria turfanesis* strain BS-J	6.4, 7.1, 7.2, 7.3	30℃	50, 100, 200, 300, 400, 500 ppm	(Dubey, 2017)
8.	*Bacillus* strain A2	5,6,7,8,9	25℃, 30℃, 35℃, 40℃	-	(Naseer et al., 2020)

2.4. Microorganisms used in bioremediation

According to Prabha et al. (2017), microbial communities are crucial to the complete mineralization, transformation, or degradation of pesticides. Pesticides are usually broken down by broad-spectrum enzymes for detoxification or involuntary metabolism in Eukaryotes (plants, animals, and fungi), while Prokaryotes (bacteria) metabolize pesticides for consumption as vital nutrients and energy. The breakdown of pesticides and their byproducts is primarily facilitated by bacteria and fungi within the microbial population (Prabha et al., 2017). The most crucial element in the bioremediation process for the efficient removal of pollutants is the selection of appropriate microorganisms. Pesticide biodegradation follows distinct pathways depending on its nature, and environmental condition and primarily depends on microbe type. Most pesticides in the soil can be degraded by many different types of soil microorganisms that possess bioremediation capabilities (Cycon & Piotrowska-Seget, 2016). Bacteria and fungi play an important role in the degradation process, but fungi basically biotransform pesticides into nontoxic substances by introducing structural changes and then releasing them into the soil, where it is receptive to further degradation by bacteria (Adithya et al., 2021).

2.4.1. Bacteria assisted biodegradation

Bacteria are the most popular microbes for biodegradation because they can readily transform into mutant strains with different biochemical pathways in the adaptive environment (Bhatt et al., 2021). Due to their capacity for rapidly developing new enzymes and metabolic pathways, bacteria

are more likely to contain enzymes that break down pesticides (Gangola et al., 2022). The majority of research on the breakdown of pesticides by microbes has concentrated on bacteria; cyanobacteria, actinomycetes, fungi, and other types of microbes have received relatively little attention (Prabha et al., 2017). Numerous bacteria are capable of breaking down a variety of pesticides, such as pyrethroids, carbamates, and organophosphates.

Several bacterial isolates that can use specific pesticides as the sole source of carbon, nitrogen, or phosphorus were identified. Bacteria capable of degrading pesticides include *Bacillus, Pseudomonas, Klebsiella, Burkholderia, Flavobacterium, Streptomyces, Arthrobacter, Mycobacterium, Azotobacter* etc. Hydrolytic enzymes found in *Pseudomonas* sp. and *Klebsiella* sp. break down neonicotinoids and organophosphorus compounds (Sun et al., 2020). It has been demonstrated that bacterial strains from the genera *Pseudomonas, Alcaligenes, and Sphingomonas* can break down carbamates and organophosphates. Herbicides containing s-triazine, like atrazine, can be broken down by *Pseudomonas* sp. and *Klebsiella pneumoniae*. Additionally, herbicide 2,4-D and organochlorine pesticides such as endosulfan, lindane, and organophosphorus insecticide chlorpyrifos can be broken down by *Pseudomonas* and *Alcaligenes* sp. *Pseudomonas putida* have demonstrated a high capacity for the biodegradation of permethrin and cypermethrin (Kumar & Sachan, 2021; Kumar et al., 2019; Mir et al., 2020). It has been reported that a strain of *Enterobacter aerogenes* breaks down various pesticides, such as bifenthrin, cypermethrin, and others. The ability of various strains of *Bacillus* and *L-proteobacteria* to degrade organophosphate pesticides was also assessed. It has been discovered that *Bacillus* species work well to degrade malathion (Geed et al., 2022; Khan et al., 2016). The bacteria *Stenotrophomonas maltophilia* have endosulfan and DDT degrading capability (Ozdal et al., 2017; Pan et al., 2016). *Sphingomonas* is a type of gram-negative bacteria that is highly effective in degrading DDT (Kumar & Sachan, 2021).

Pseudomonas putida and *Acinetobacter rhizosphaerae* were able to degrade the organophosphate fenamiphos. Different microbes were isolated with the ability of biodegradation of carbamate pesticides. Several strains of *Pseudomonas*, *Flavobacterium*, *Achromobacterium*, *Sphingomonas*, and *Arthrobacter* are capable of degrading carbofuran. A bacterial consortium of six bacterial strains, i.e., *Stenotrophomonas maltophilia*, *Proteus vulgaris*, *Vibrio metschinkouii*, *Serratia ficaria*, *Serratia* spp., and *Yersinia enterocolitica*, possess tetrachlorvinphos degradation capacity

(Ortiz-Hernández & Sánchez-salinas, 2010). *Fusarium solani*, *Fusarium oxysporum*, *Pseudomonas striata*, *Aspergillus ustus*, *Achromobacter* sp., *Aspergillus versicolor*, *Penicillium chrysogenum*, *Penicillium rugulosum*, *Penicillium janthinellu* and *Trichoderma viride* have also been found to be effective in degradation of different types of pesticides (Bhalerao & Puranik, 2007; Guillén-Jiménez et al., 2012; Kataoka et al., 2010).

2.4.2. Fungi-assisted biodegradation

The capacity to utilize pesticides as a source of energy has been found in numerous fungi. A wide variety of pesticides are degraded by *Phanerochaete chrysosporium*, one of them (Fulekar et al., 2013). Various pesticide classes are broken down to varying degrees by white-rot fungi, including lindens, atrazine, diuron, terbuthylazine, metalaxyl, DDT, gamma-hexachlorocyclohexane, dieldrin, aldrin, heptachlor, chlordane, and mirex. Parallel to this, a number of additional fungi, including *Hypholoma fasciculare, Coriolus versicolor, Agrocybe semiorbicularis, Auricularia auricula*, and *Dichomitus squalens* (Mukherjee et al., 2018). Several fungi, including white-rot fungi, Trichoderma and Aspergillus, have the capability to degrade pyrethroids, lindane, and other pesticides (Oliveira et al., 2015; Tortella et al., 2005).

2.5. Bacteria used for biodegradation of imidacloprid

Imidacloprid is metabolized by a wide range of bacteria, including those belonging to the genera *Bacillus, Pseudoxanthomonas, Mycobacterium, Rhizobium, Alcaligenes, Flavobacterium, Pseudomonas*, and *Stenotrophomonas*. In 2007 it was first reported that *Leifsonia* strain PC-21 co-metabolized imidacloprid and degraded 37–58% of 25 g/L of imidacloprid in a trypsin solution containing 1 g/L succinic acid and d-glucose in 3 weeks at 27^{o}C. This was the first evidence of the biodegradation of imidacloprid by bacteria (Anhalt et al., 2007). *Pseudomonas* sp. 1G can convert imidacloprid into urea metabolites as well as denitrification products (Pandey et al., 2009).

It has been observed that Pseudomonas has a major effect on imidacloprid degradation. Through soil enrichment, *Pseudomonas* sp. PRT 52 can be isolated from fields and is capable of metabolizing imidacloprid, endosulfan, and coragen, three distinct chlorinated pesticides. It was demonstrated that this biological strain could break down 46.5% of imidacloprid in 40 hours when imidacloprid was the only carbon source (Gupta et al., 2016). The *Pseudomonas mosselii* Strain NG1 can efficiently break down imidacloprid residue found in mango orchards (Bhattacherjee et al., 2020)

Imidacloprid and acetamprid can be reduced by *Sphingomonas* sp. TY and *Acinetobacter* sp. TW, which are isolated from tobacco waste, in a broad range of pH and temperature values (Wang et al., 2011). About 71% of imidacloprid could be degraded in 11 days *Bacillus thuringiensis* strain that was isolated from contaminated marine sediments (Kaur et al., 2011). *Klebsiella pneumonia* strain BHC1 breaks down 78% of imidacloprid at 30°C in 7 days, producing metabolites like 6-chloronicotinic acid, imidacloprid guanidine, and nitroguanidine. (Sabourmoghaddam et al., 2015). It has been documented that imidacloprid is degraded by several bacterial species, producing a variety of secondary metabolites that are more toxic and persistent as compared to the parent compound. Olefin, 4-hydroxy and 5-hydroxy imidacloprid are the principal metabolites of imidacloprid. *Stenotrophomonas maltophilia*, a bacterial isolate, was shown to convert imidacloprid via the dehydrogenation process into olefin by Dai et al. (2010). *Leifsonia* species converted imidacloprid into guanidine and urea metabolites when glucose and succinate were present (Anhalt et al., 2007). Similarly, *Bradyrhizobiaceae* SG-6C could convert imidacloprid to 6-chloronicotinic acid (Shettigar et al., 2012). A summary of the various studies conducted for imidacloprid biodegradation using different microorganisms has been enlisted in Table 2.2.

Table 2.2: Microorganisms used for biodegradation of imidacloprid

S. No.	Microorganisms used	Experimental conditions	Findings	References
1.	*Leifsonia strain PC21*	30–35 °C, 25 mg/L	37–58 % degradation	(Anhalt et al., 2007)
2.	*Pseudomonas sp. 1G*	28 °C, 50 ppm, 14 days	70%	(Pandey et al., 2009)
3.	*Acinetobacter* sp. TW and *Sphingomonas* sp. TY	25–37°C pH 7.0–8.0 for strain TW, 6.0–7.0 for strain TY, 1.0 g/L, 12 and 18 h for TW and TY, respectively	100% degradation	(Wang et al., 2011)
4.	*Brevundimonas* sp. MJ15	125, 250, 500, and 1000 ppm of imidacloprid, 28 days		(Shetti & Kaliwal, 2016)

5.	*Ochrobactrum sp.*	30°C, pH 8, 50 mg/L, 48 h	67.67% degradation	(Hu et al., 2013)
5.	*Klebsiella Pneumoniae BCH1*	30°C, pH 7, 7 days	78.0% degradation	(Phugare et al., 2013)
6.	*Stenotrophomonas maltophilia* CGMCC 1.1788	30°C, pH 7.2	Metabolites: 5-hydroxy imidacloprid (Sucrose co-substrate), olefin imidacloprid (Succinate co-substrate)	(Liu et al., 2013)
7.	*Bacillus sp.*	30–35°C, pH 7, 48–72 h	50.0% degradation	(Shaikh et al., 2014)
8.	*Bacillus alkalinitrilicus*	37±1°C, 120 rpm, 150 mg/kg 56 days	86.21% degradation	(Sharma et al., 2014)
9.	*Pseudoxanthomonas indica*	1.22 mm/L, 6 days	70.1% degradation	(Ma et al., 2014)
10.	*Rhizobium sp.*	25 mg/L	45.48% degradation	(Sabourmoghaddam et al., 2015)
11.	*Mycobacterium sp. Strain MK6*	150 µg/ml, 15 days	99% degradation	(Kandil et al., 2015)
12.	*Enterobacter sp.* ATA1	37°C, pH 7, 120 rpm 50 mg/L 20 days	74.0 % degradation	(Sharma et al., 2015)
13.	*Bacillus Aerophillus*	100 mg/kg 30 days	58.0% degradation	(Akoijam & Singh, 2015)
14.	*Pseudomonas PRT 52*	37°C, 0.5 mm, 40 h	46.5% degradation	(Gupta et al., 2016)

15.	*Kocuria turfensis BS-J*	30°C, 150 rpm 200 ppm 7 days	85–91% degradation	(Dubey et al., 2019)
16.	*Pseudomonas mosselii NG1*	30°C, 0.606 µg/g, 67 days	91.42% degradation	(Bhattacherjee et al., 2020)
17.	*Rhodopseudomonas capsulata*	1000 mg/L, 5 days	97.0% degradation	(Wu et al., 2020)
18.	*Hymenobacter latericoloratus CGMCC*	30°C, 100 mg/L	64.4% degradation	(Guo et al., 2020)
19.	*Paracoccus Achromobacter*	37°C, 200 rpm, 15 days	85.67% degradation	(Gao et al., 2021)
20.	*Ochrobactrum thiophenivora, Sphingomonas melonis*	COD, BOD_5, TOC, pH and DO were determined for two weeks	100.0% degradation	(Erguven & Demirci, 2021)
21.	*Pseudomonas plecoglossicida* MBSB-12	25,000 ppm, 90 days	87% Metabolites: imidacloprid guanidine, olefin, imidacloprid urea and a dechlorinated degraded product of imidacloprid	(Borah et al., 2023)
22.	*Sphingobacterium sp. and Agrobacterium sp.*	95 mg/L Imidacloprid-guanidine	81.02%-84.87% degradation	(Gautam & Dubey, 2022)

23.	*Phanerochaete chrysporium*	7 days	91% -93%% degradation	(Shang et al., 2023)

2.6. Toxicity assessment

Pesticide toxicity analysis is crucial for maintaining environmental sustainability, maintaining human health, and promoting environmentally friendly agricultural practices. It is essential for reducing the adverse effects that pesticides cause on ecosystems.

The LBTA (Luminescent Bacteria Toxicity Assay) is widely identified as an acute toxicology test, because of the short time of exposure. The measurement of acute (5-30 min) as well as chronic (12-24 h) toxicity can be done using luminescent bacteria. Acute toxicity assay is based on the inhibition of light caused by interruption in photosensitization, whereas chronic toxicity is based on variations in bacterial growth or viability. In the bioluminescence reaction, the bacterial luciferase is crucial. Since cellular metabolism and light emission are closely associated, the bacterial metabolic state is reflected in the light intensity. Exposure of luminescent bacteria to toxic compounds may result in the inhibition of bacterial luciferase, leading to a rapid decrease in light intensity. To determine the toxicity to luminescent bacteria, the inhibition can be estimated by measuring the light intensity of the bacteria exposed to the testing sample and comparing it to that of the control.

Many luminescent bacteria including *Pseudomonas fluorescens, Vibrio fischeri*, and *Vibrio harvey* have been employed conventionally in bioluminescent assay for analysis of toxicity (Chaturvedi et al., 2021; Ma et al., 2014). The spectral range of the bioluminescence is between 420 nm and 660 nm. Bioluminescence is produced by the action of luciferase enzyme, which converts flavin mononucleotide (FMN) to reduced flavin mononucleotide ($FMNH_2$). The following reaction illustrates the further oxidation of reduced flavin mononucleotide and a long-chain aliphatic aldehyde by molecular oxygen.

$$FMNH_2 + R\text{-}CHO + O_2 \rightarrow FMN + R\text{-}COOH + H_2O + light \qquad (2.1)$$

Various studies have been conducted by researchers that provide data on the toxic effects of pesticides and their metabolites. Ecotoxicity assessment including phytotoxicity as well as bacterial toxicity analyses have been done extensively. In a study conducted by Rodríguez-Castillo

et al. (2019), 95% of neonicotinoid insecticides could be degraded successfully using microbial consortia in a stirred tank reactor. Seed germination and bioluminescence inhibition assays were also conducted. In another study, cytotoxicity assessment was done in case of the herbicide atrazine, where 1000 mg/L of atrazine could be degraded in 7 days. Toxicity decreased after bacterial degradation (Kolekar et al., 2019). Organophosphorus pesticides were degraded by Briceño et al. (2020) using mixed culture resulting in more than 90% degradation. In the study moderate inhibitory effect was found in radish. Exposure of *Phaseolus vulgaris* to high imidacloprid concentration results in reduction of plant photosynthetic pigments (Ajermoun et al., 2022). In another study, white-rot fungus was used for degradation of malathion, acetamiprid and imidacloprid. Microtox tests were conducted using *Vibrio fischeri.* Slight increase in toxicity of treated solutions was observed due to formation of toxic metabolites (Hu et al., 2022). Chlorpyriphos degradation was conducted by Lara-Moreno et al. (2022) and toxicity assessment was conducted using *Vibrio fischeri* as test organism. In a recent study, Elango et al. (2023) conducted a study on degradation of neonicotinoid insecticide, acetamiprid using bacteria. It resulted in 89.72% of acetamiprid under optimized conditions. Cytotoxicity assessment was done on earthworm and zebrafish embryo. Toxicity assessment for imidacloprid study have also been conducted by Ni et al., (2024). A significant reduction in residual toxicity was observed after carrying out degradation of imidacloprid.

2.7. Metagenomic analysis

The genome-independent study of entire microbial communities is known as metagenomics. In this emerging field, molecular biology and genetics are combined to analyze genetic materials extracted straight from soil samples, enabling the identification and characterization of soil microorganisms. Metagenomics makes the pool of genomes from a given environment easier to access. Soils are complex ecological niches that contain one of the largest reservoirs of a wide variety of microorganisms. However, because most microbes in nature cannot be grown, they are generally inaccessible. According to Kennedy et al. (2011), metagenomics techniques enable the evaluation of new, valuable genetic resources, such as unique enzymes, and the creation of a wide range of biotechnological applications. An increasingly comprehensive picture of the diversity and abundance of soil can be obtained thanks to the growing amount of data collected using these methods. Metagenomic analysis in biodegradation of pesticides is a new field with limited studies conducted. The metagenomic study report reveals that prolonged imidacloprid application shifts

the diversity of beneficial soil microbes and the identification of potent bioremediation. The metagenomic study of imidacloprid degradation in different niches has been summarized in Table 2.3

Table 2.3: Studies conducted on metagenomic analysis of insecticide biodegradation

S.no.	Sample type	Findings	References
1.	Agricultural soil	The study found an increase in *Actinobacteria, Firmicutes*, and *Bacteriodates*, and a reduction in *Proteobacteria* in soil contaminated with pesticides compared to uncontaminated soil.	(Gangola et al., 2021)
2.	Soil	The OTU number of gammaproteobacterial decreased by 33%. The genus Gemmata totally disappeared in imidacloprid-treated soil.	(Garg et al., 2021)
3.	Soil	An increase in *Chloroflexi* and *Nitrospirae* genera occurred, while *Gemmatimonadetes* and *Parcubacteria* decreased. The pesticide-exposed soil exhibited a wide increase in the genera *Methylotenera, Ramlibacter, Rubrivivax*, and *Nitrospira*.	(Yu et al., 2020)
4.	Soil sample	A total of 18 genes related to biodegradation and 5 genes related to pesticide degradation, including cytochrome p450 were identified.	(Wu et al., 2021)
5.	Pesticide-contaminated soil	Consortium ACE-3 had a bacterial population composed of *Sphingobium, Acinetobacter, Afipia, Stenotrophomonas*, and *Microbacterium*, accounting for 3.07%, 10.01%, 24.45%, and 49.12%, respectively.	(Sun et al., 2020)
6.	Soil sample	Increase in actinobacteria abundance after individual treatment of imidacloprid, metribuzin, and benomyl. The combination of these three pesticides reduces the	(Astaykina et al., 2020)

		relative abundance of proteobacteria and affects the fungal community.	
7.	Soil	The metagenomic study revealed the presence of a variety of biodegradation genes (BDGs) including polyphenol oxidase and laccase (PPO), Cytochrome p450 protein (CYP), and Lip gene lignin peroxidase. Additionally, several pesticide degradation genes (PDGs) were identified, such as *mhel, opd, mpd, atz, trzN, chd, hdx, hdl1*, and *fmo*.	(Malla et al., 2022)
8.	Agricultural soil	Metagenomic techniques were used to identify genes that are resistant to pesticides and to increase the rate at which pesticides are biodegraded.	(Sundaram et al., 2022)

Manickam et al. (2010) studied the microbial diversity in three sites contaminated with chlorinated pesticides. They found biodegradative genes such as linA that can help in the bioremediation of these pesticides. Fan et al. (2012) studied hydrolases to degrade pyrethroid pesticides. No thermostable pyrethroid esterases were identified despite multiple purified and characterized pyrethroid esterases from different sources.

Researchers analyzed soil samples from an HCH-dumpsite to evaluate the microbial community responsible for in-situ bioremediation using shotgun metagenomics sequencing. They found that *Chromohalobacter, Marinimicrobium, Idiomarina, Salinosphaera, Halomonas, Sphingopyxis, Novosphingobium, Sphingomonas,* and *Pseudomonas* (bacteria), *Halobacterium, Haloarcula,* and *Halorhabdus* (archaea), and *Fusarium* (fungi) were dominant genera. Abundant HCH degradation genes (*lin* genes) were also detected. (Sangwan et al., 2012). Fang et al. (2014) assessed six datasets from freshwater and marine sediments to determine the abundance and diversity of biodegradation genes and pathways for DDT, HCH, and atrazine. Gene abundance and diversity varied by sample source and location. Lip and mnp genes were dominant for organic pollutant degradation. The *hdt*, *hdg*, and *atzB* genes were most abundant for DDT, HCH, and ATZ degradation respectively. Complete pathways were mapped for DDT and ATZ, but only limited HCH degradation pathways were found. A study conducted by Itoh et al. (2014) aimed to

understand the impact of fenitrothion, an organophosphorus insecticide, on the microbial diversity of soil. The study observed a significant difference in the microbial communities after the application of fenitrothion. The application of fenitrothion led to a higher rate of proliferation of MEP-degrading microbes such as *Burkholderia* bacteria. This reflects the succession and adaptation strategies of microbial communities under insecticide application. Another study by Chaussonnerie et al. (2016) reported the discovery of two new Citrobacter isolates capable of reproducing chlordecone transformation, an organochlorine insecticide, through metagenomics studies of soils contaminated by chlordecone or other organochlorines and from the sludge of a wastewater treatment plant.

In soil treated with imidacloprid, the presence of new bacterial phyla such as *Planctomycetes*, *Verrucomicrobia* and *Chloroflexi* was observed, while *Crenarchaeota* and *Acidobacteria* were destroyed. This indicates that the use of pesticides has a direct impact on the diversity of soil microbial communities (Gangola et al., 2021). Astaykina et al. (2020) used metagenomics to study the effects of benomyl, metribuzin, and imidacloprid on soil microbial populations. The combined application of these pesticides resulted in the dominance of Proteobacteria in the soil, which has high hydrolytic activity and can be used as a bioremediating agent. However, a study conducted by Astaykina et al. (2022) showed that even at the lowest concentration, these three pesticides had negative effects on the diversity of earthworm gut microbiota. The combined application of these pesticides resulted in the dominance of *Proteobacteria* in the soil, which has high hydrolytic activity and can be used as a bioremediating agent.

2.8. Objectives of the study

Based on the review of the literature, it can be deduced that bioremediation is a highly effective method for the degradation of recalcitrant chemicals. However, this method tends to be slower compared to other techniques. To address this limitation, studies could focus on accelerating the degradation process by selecting suitable microorganisms or through the application of bioreactors. Pesticide residues, in particular, tend to remain bound to the soil for extended periods, making their removal essential. For the removal of pesticides from agricultural lands, ex-situ techniques are not practically feasible; therefore, in-situ methods should be adopted in such cases. Additionally, the metabolites formed after bioremediation can sometimes be toxic, making it important to evaluate the toxicity of insecticides and their degradation products. Insecticides also

have a significant impact on soil microflora, so it is necessary to monitor and account for these effects.

While numerous lab-scale studies have been conducted by researchers, there has been no commercialization of biological methods for insecticide removal, primarily because lab studies are conducted under controlled conditions. This highlights the need to find feasible, practical methods for large-scale applications. Soil microcosm studies could serve as a suitable intermediate step for experimentation before proceeding to large-scale applications. Furthermore, maintaining specific bacteria capable of degradation can be challenging in the long term, so preserving these potential bacteria could be crucial for sustained bioremediation efforts. Based on these insights from the literature review, the following objectives have been set for the present study.

- Isolation and identification of imidacloprid-degrading bacteria
- Degradation of imidacloprid in water, slurry and soil
- Toxicity assessment of samples before and after bioremediation using isolated bacteria
- Microcosm study in the soil to determine the effect of volatilization, the role of indigenous bacteria, uptake by plants and degradation by isolated bacteria
- Metagenomic analysis of collected soil, untreated and treated soil samples and comparative analysis
- Application of lyophilized and encapsulated bacteria for degradation of imidacloprid