

**CHAPTER 6: IMIDACLOPRID
BIODEGRADATION IN SLURRY AND SOIL
MICROCOSM**

6.1. General

Pesticides may become airborne, get into soil, enter bodies of water, or be taken up by plants and animals. The environmental fate of pesticides depends on the physical and chemical properties of the pesticide as well as the environmental conditions. Once a pesticide has been released into the environment, it can be broken down by exposure to sunlight (photolysis), exposure to water (hydrolysis), exposure to other chemicals (oxidation and reduction), microbial activity (bacteria, fungi, and other microorganisms) and plants or animals (metabolism).

The presence of indigenous bacterial communities with natural remediation potential can be used to generate site-specific bioremediation of polluted soil. Laboratory microcosms are advantageous alternatives to field experiments, which are frequently expensive and subject to changes in environmental conditions. Standard laboratory tests are also less reliable, with results that may not accurately reflect real-world scenarios due to non-site-specific experimental conditions and microbial strains.

Soil microcosm studies in the laboratory permit the selection of suitable microorganisms that can survive for a longer period when introduced into polluted sites and that possess a high catabolic activity with which to degrade pollutants. Because of the smaller scale of microcosms, multiple replicates can be created, allowing for the study and establishment of relationships between different microorganisms and the environmental conditions conducive to efficient xenobiotic degradation. Laboratory studies also allow for the manipulation of various environmental factors and growth conditions that could favor the optimal activity of degradative microorganisms and facilitate effective biodegradation and bioremediation of polluted soils.

The present study gives an insight to the degradation of insecticide imidacloprid through different routes. During the biodegradation process, a number of experimental studies were carried out to understand the roles of indigenous bacteria existing within soil microcosms and those of isolated bacteria. In addition, experiments were also performed to study the amount of imidacloprid absorbed by *Cicer arietinum* plants.

6.2. Imidacloprid biodegradation in slurry

The most efficient bacteria, *Tepidibacillus decaturensis* strain ST1, was used for imidacloprid degradation in the present study. The bacterial treatment was found to be considerably effective in imidacloprid remediation over control (without bacterial inoculation) in soil slurry. The removal

of imidacloprid was least in the case of sterilized slurry (without bacteria and other microorganisms). The sterilized slurry, after inoculation with the isolated bacteria, was capable of degrading more than 80% of the imidacloprid within 10 days. Figure 6.1 shows the biodegradation of imidacloprid in slurry.

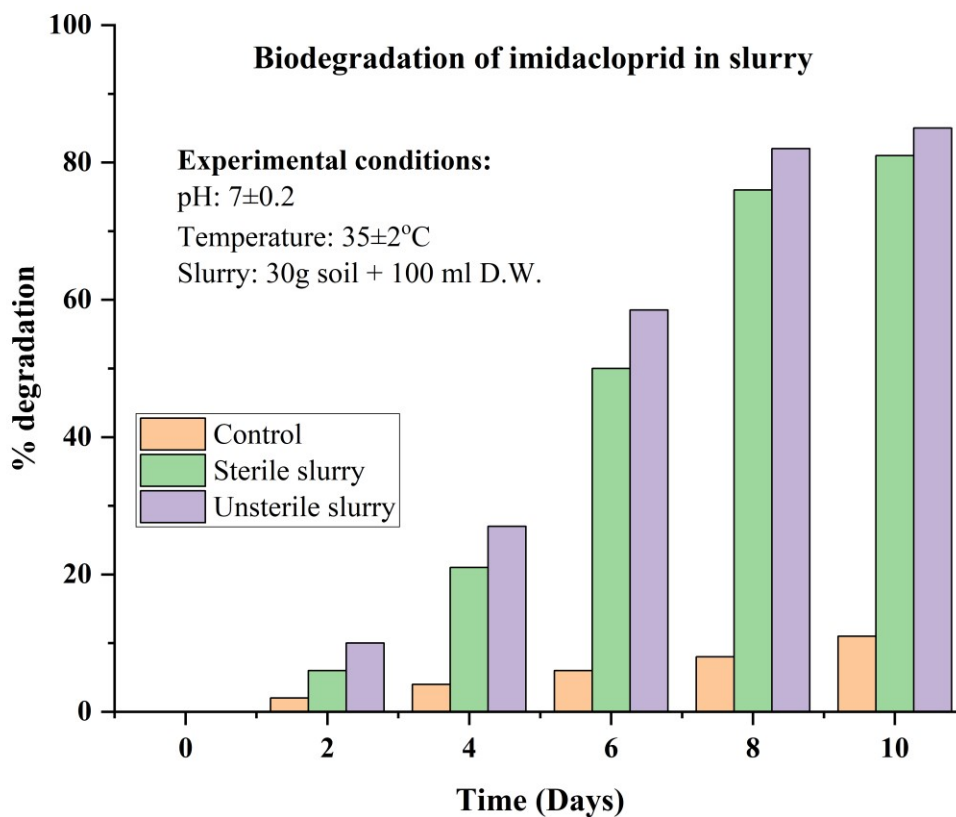


Figure 6.1: Biodegradation of imidacloprid in slurry

In unsterilized slurry, the degradation was maximum. The indigenous bacteria present in the slurry may be responsible for higher imidacloprid degradation in the unsterilized slurry. Several studies have been conducted on soil slurries for the degradation of various organic compounds, including insecticides. The study conducted by Kumar et al. (2007) for endosulfan biodegradation resulted in about 75% removal in 10 days using mixed cultures. In another study, cypermethrin degradation was studied by Bhatt et al. (2020) and reported more than 83% degradation in 15 days.

6.3. Imidacloprid biodegradation in soil microcosm

Pattern of imidacloprid degradation was as follows: Set 4 (82%) > Set 3 (77.5%) > Set 2 (11%) > Set 1 (5.5%) in case of sterile soil (A). The degradation of imidacloprid in case of unsterile soil sample (B) was: Set 4 (91%) > Set 3 (85%) > Set 2 (13.5%) > control (7.5%). The best percentage degradation of imidacloprid was observed when unsterile soil was used along with bacteria and plant (Set 4B). Figure 6.2 and Figure 6.3 represent the degradation patterns of imidacloprid in the case of sterilized and unsterilized soils respectively. Table 6.1 shows the % degradation of imidacloprid in sterile and unsterile soils in Set 1, Set 2, Set 3 and Set 4.

Table 6.1: % degradation of imidacloprid in sterilized and unsterilized soils in Set 1, Set 2, Set 3 and Set 4

Soil type	% degradation			
	Set 1	Set 2	Set 3	Set 4
Sterilized soil	5.5	11	77.5	82
Unsterilized soil	7.5	13.5	85	91

Tepidibacillus decaturensis strain ST1, an indigenous bacterium from the collected soil sample was identified as the predominant bacterium in the enrichment and was found to most effective isolate for imidacloprid biodegradation. In a similar study, conducted by Hu et al. (2013), indigenous bacteria were found to be effective for imidacloprid degradation. The selected bacterial strain was capable of degrading imidacloprid in liquid medium, slurry, and soil, in the present study. According to previous studies on pesticide degradation, microorganisms capable of degrading pesticides in culture media can also degrade pesticides in soil (Lu et al. 2013).

Results indicate that imidacloprid can be degraded to a larger extent in sterilized soil when inoculated with the isolated bacteria, indicating the efficacy of *Tepidibacillus decaturensis* strain ST1 in imidacloprid degradation. Imidacloprid degradation was observed to be higher in inoculated non-sterilized soils, with a plantation (91%) as compared to sterilized soils (82%). This study indicates that soil microflora facilitates imidacloprid degradation. Imidacloprid degradation was more pronounced in inoculated non-sterile soils than in inoculated sterile soils, demonstrating the potential of indigenous microorganisms for bioremediation. Higher degradation in unsterile

soil samples could be due to inoculation of degrading bacteria in the soil, which increases the catabolic potential of the soil. Furthermore, the presence of indigenous bacteria most likely performed a synergistic effect in biodegradation. This was also favored by previous studies that showed that soil bacteria play a vital role in pesticide degradation.

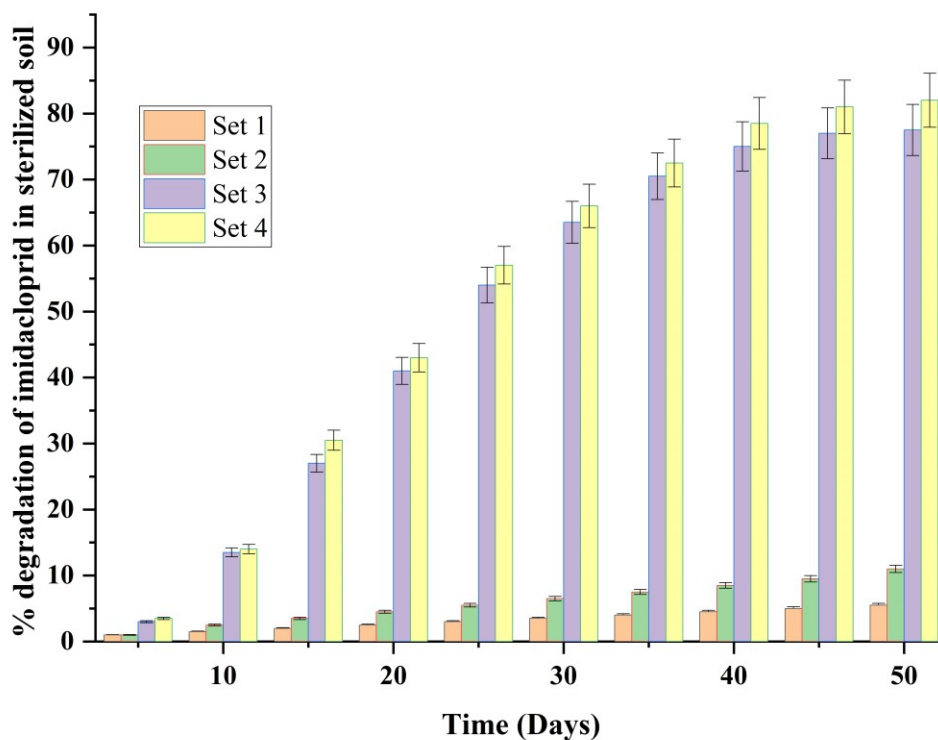


Figure 6.2: Degradation pattern of imidacloprid with time in sterilized soil in Set 1, Set 2, Set 3, and Set 4

According to previous researches, the involvement of native soil microorganisms in pesticide bioremediation processes is variable. For example, Bidlan et al. (2004) reported that the degradation of HCH isomers by the indigenous microflora was limited during the bioremediation of soils using known bacterial consortium polluted with hexachlorocyclohexane. In contrast, Cycoń et al. (2013) investigated the biodegradation of a mixture of organophosphorus pesticide by *Serratia marcescens* in soils of different textures, and they reported that the indigenous microflora

present in each type of soil was capable of degrading the pesticides under investigation. According to the study conducted by Fuentes et al. (2017), degradation of pesticides could be achieved by actinobacteria even in the presence of native microflora.

A lower degradation rate was observed in the case of control i.e., Set 1 and Set 2, in both sterile as well as unsterile soils. The residual level of imidacloprid was 18 mg/kg in the case of unsterile soil after 45 days when treated with the isolated bacteria in combination with plantation. In control, imidacloprid residue in plant parts was 9.5 and 12.5 $\mu\text{g/g}$ in sterile soil and unsterile soil respectively. Self-degradation of imidacloprid was 5.5 and 7.5% in sterile and unsterile soil, respectively, when spiked with 200 ppm imidacloprid. The half-life of imidacloprid in different soil samples has been given in Figure 6.4. The half-life of imidacloprid in soil can be approximated to 300. In the present study, the half-life of imidacloprid reduced to 12 days after bioremediation using the bacterial isolate *Tepidibacillus decaturensis* strain ST1.

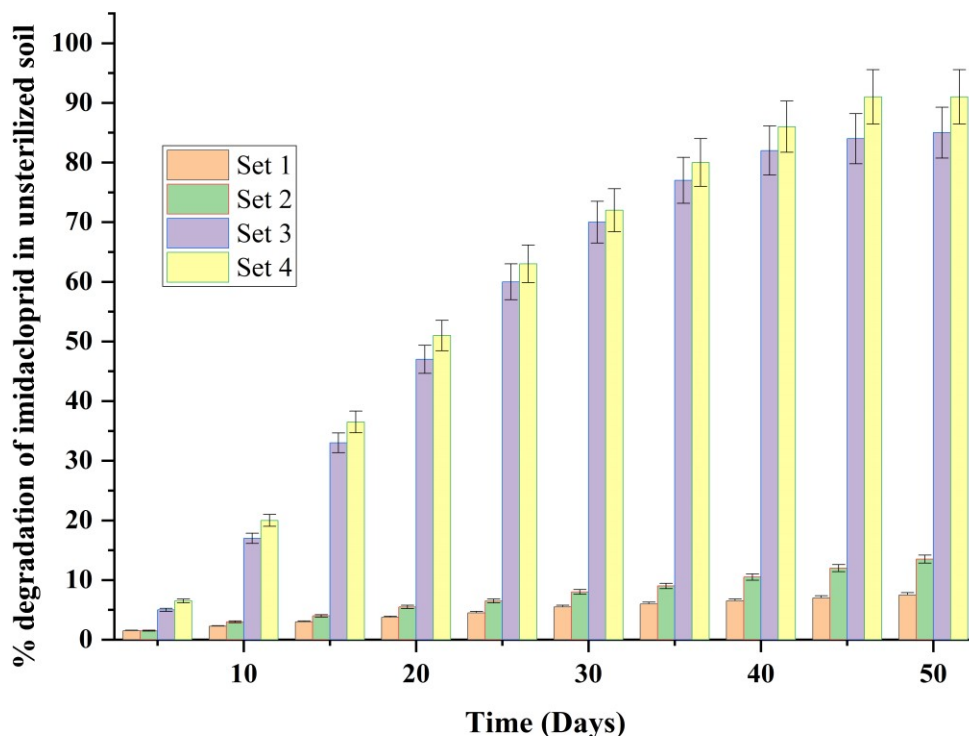


Figure 6.3: Degradation pattern of imidacloprid with time in unsterilized soil in Set 1, Set 2, Set 3, and Set 4

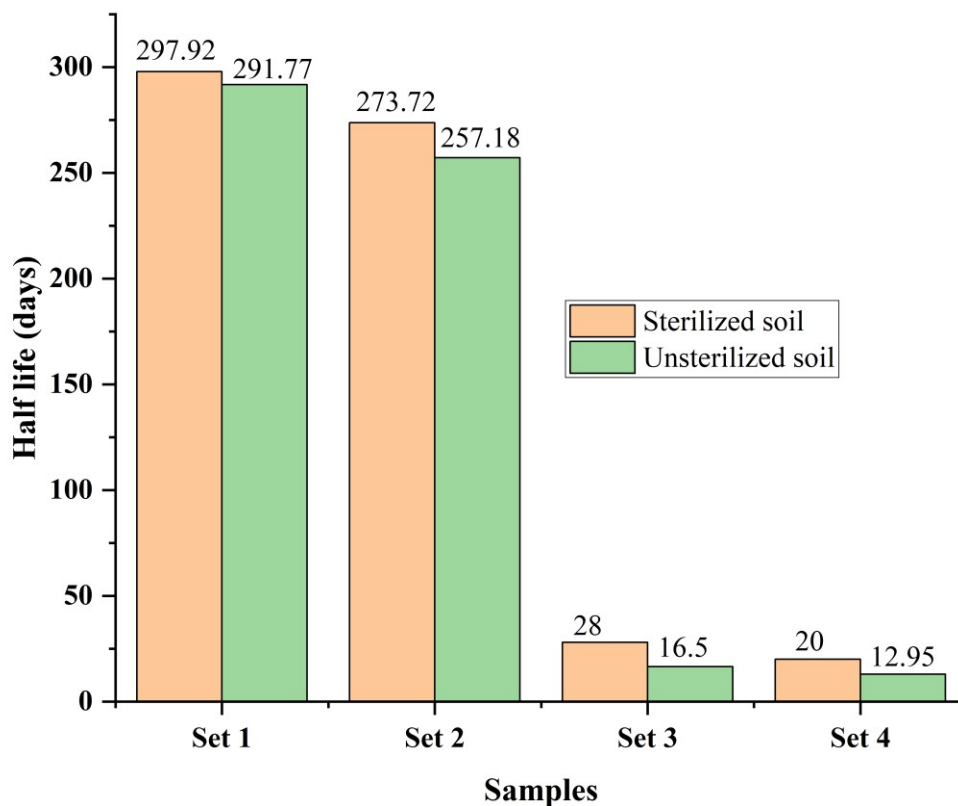


Figure 6.4: Half-life of imidacloprid in different soil samples

Pesticides are degraded in the soil by physical, chemical, and biological processes. The availability of pesticides and the ability of microorganisms to use them are the major factors that influence the degradation of pesticides in soil. The efficacy of pesticide biodegradation could definitely be influenced by the physicochemical characteristics of soil. The natural loss of imidacloprid could be due to volatilization, photocatalysis, or deep percolation. The uptake of imidacloprid by plants remains constant in inoculated as well as uninoculated soils. The degradation in the sterile soil was always found lower than unsterile soil under same condition. This clearly indicates that the natural environment present in the soil favors the degradation.

6.4. Metabolites identified after GCMS analysis

GC-MS analysis of the degraded imidacloprid samples led to the identification of two significant metabolites: Hydrazinecarboxamide and Hydroxyurea. The mass spectral profiles of these

metabolites are presented in Figure 6.5, showcasing the detailed fragmentation patterns and peak intensities. Hydrazinecarboxamide was identified with a retention time of 14.2 minutes, exhibiting major mass spectral peaks at m/z 60, 43, and 30. Similarly, hydroxyurea was detected at a retention time of 12.5 minutes, with significant peaks at m/z 76, 59, and 43. These findings were summarized in Table 6.2, which provides a clear overview of the retention times and mass spectral characteristics of each metabolite.

Table 6.2: Retention times and major mass spectral peaks of identified metabolites

Metabolite	Retention time (min)	Major peaks (m/z)
Hydrazinecarboxamide	14.2	32, 44
Hydroxyurea	12.5	31, 33, 44

The identification of hydrazinecarboxamide and hydroxyurea as metabolites of imidacloprid through GC-MS analysis offers significant insights into the degradation pathways of this neonicotinoid insecticide. Hydrazinecarboxamide, detected at a retention time of 14.2 minutes with major peaks at m/z 32 and 44 suggests a degradation pathway involving the breakdown of imidacloprid into simpler nitrogen-containing compounds. This indicates the occurrence of reductive processes that lead to the formation of hydrazine derivatives.

Hydroxyurea, identified at a retention time of 12.5 minutes with major peaks at m/z 31, 33 and 44, points to oxidative processes in the degradation pathway of imidacloprid. The presence of hydroxyurea indicates that the urea moiety of imidacloprid undergoes oxidation, resulting in the formation of this metabolite. These findings align with the known environmental fate of imidacloprid, where both reductive and oxidative degradation processes play a role in breaking down the parent compound.

The detection of these metabolites is crucial for understanding the environmental impact of imidacloprid. Hydrazinecarboxamide and hydroxyurea, despite being simpler molecules compared to the parent compound, may still possess biological activity, thus influencing non-target organisms and environmental health. The identification and quantification of these metabolites help in assessing the persistence and potential risks associated with imidacloprid use.

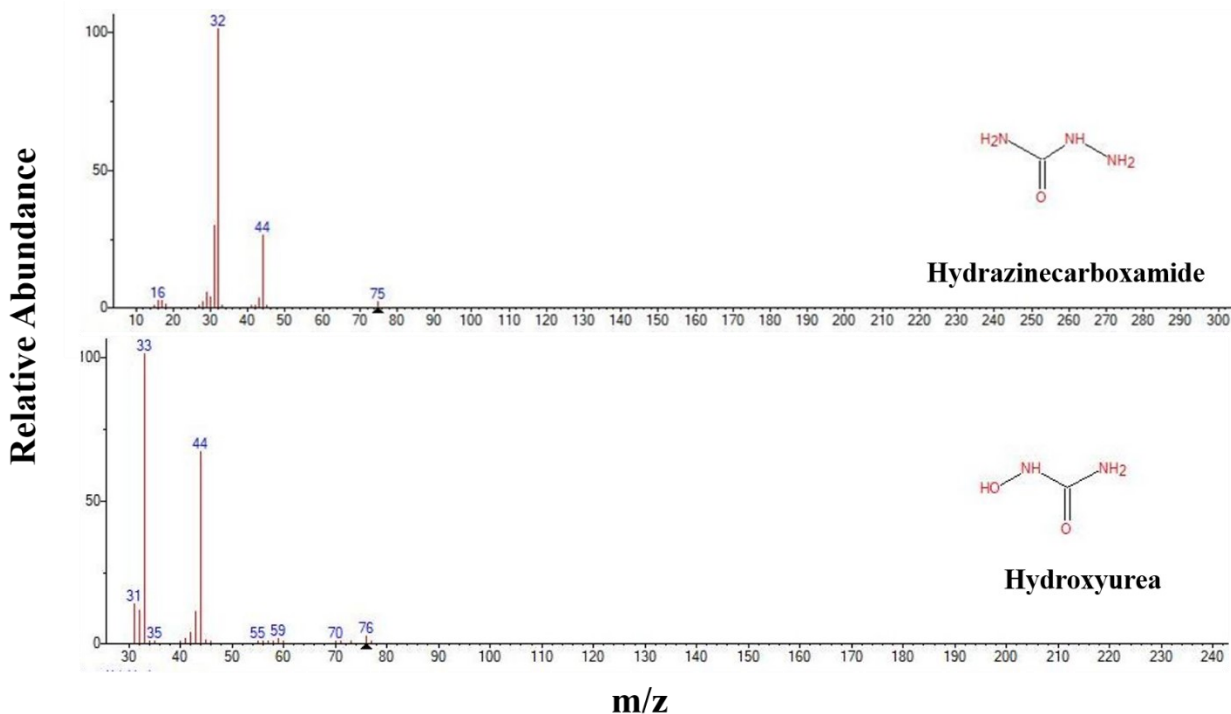


Figure 6.5: Mass spectral profile of metabolites formed during the degradation of imidacloprid

Numerous possible imidacloprid metabolites have been identified by the researchers in soil and water. *Leifsonia* sp. could degrade imidacloprid to 6-CNA in three weeks (Anhalt et al. 2007). Similarly, imidacloprid can be transformed to desnitro and urea metabolites by *Pseudomonas* sp. (Pandey et al. 2009). Imidacloprid can be hydroxylated to olefin-imidacloprid by *Stenotrophomonas maltophilia* (Dai et al. 2006).

6.5. Kinetic analysis

Using the exponential formula ($C_t = C_o \cdot e^{-kt}$), the kinetics parameters (k and $T_{1/2}$) of imidacloprid degradation were calculated and are presented in Table 6.3. The degradation of imidacloprid followed a first-order decay reaction at the above-described growth conditions in a soil microcosm. Figures 6.6 and 6.7 depict the linear plot of first and second-order kinetics, respectively, indicating that the degradation in the present study follows first-order kinetics.

The initial phase of imidacloprid degradation included a lag period of 5 days, during which only approximately 1% of initial imidacloprid was degraded. Bacterial strains exhibited higher imidacloprid degradation potential in non-sterilized soils as compared to sterilized soil. *Tepidibacillus decaturensis* strain ST1 degraded 91% of the applied imidacloprid within a time

frame of 45 days with an average rate of 4.56 and 5.04 mg/kg.d and rate constant of 0.039 and 0.048/d in sterile and non-sterile soils, respectively. The half-life ($T_{1/2}$) of imidacloprid was found to be 12.95 days in non-sterile soil and 18.77 days in sterile soil after bacterial treatment.

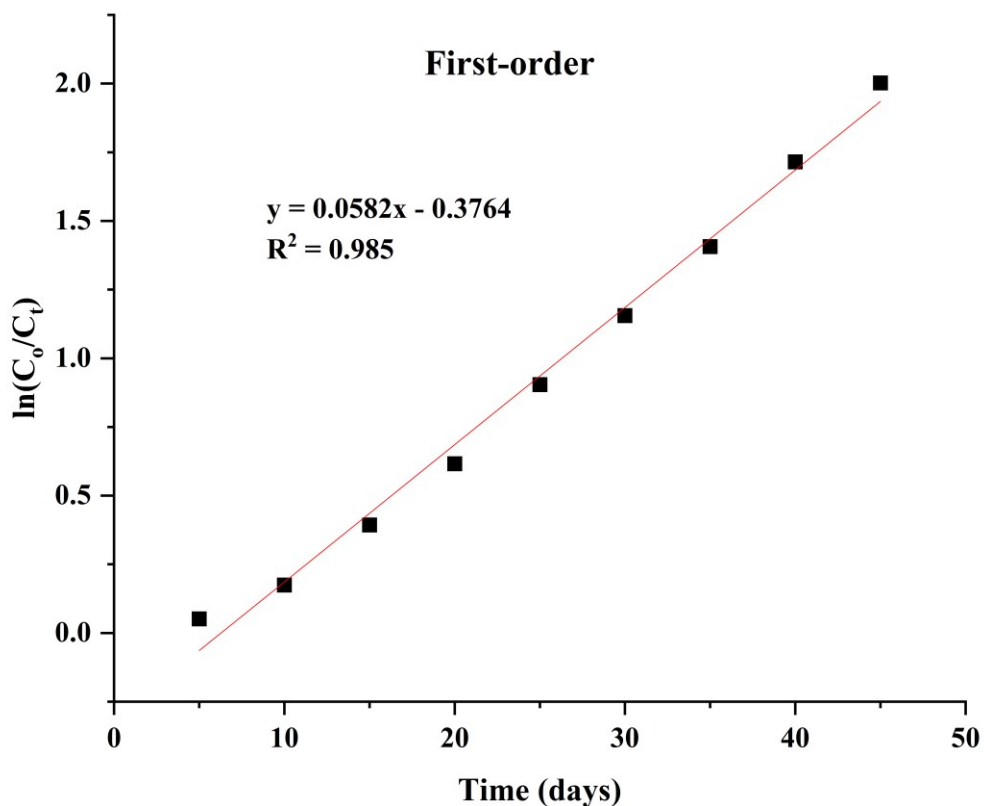


Figure 6.6: First-order kinetics plot of imidacloprid bioremediation

Under optimum conditions, in the case of unsterile case, biodegradation using the isolates followed first-order kinetics. Imidacloprid was degraded in uninoculated soils at the rate of 0.36 mg/kg.d and a rate constant of 0.0015/day, with a half-life of 291.77 days. The half-life of imidacloprid in un-inoculated planted soils was calculated to be 257.18 days, at a rate of 0.52 mg/kg.d and a rate constant of 0.0027/day. In inoculated soils, 85% of applied imidacloprid was degraded within 45 days with an average rate of 4.76 mg/kg.d and a rate constant of 0.042/day. Similarly, the value of k , $T_{1/2}$, and reduction per day was calculated for all the samples of set 1, set 2, set 3, and set 4 in sterile and unsterile soils.

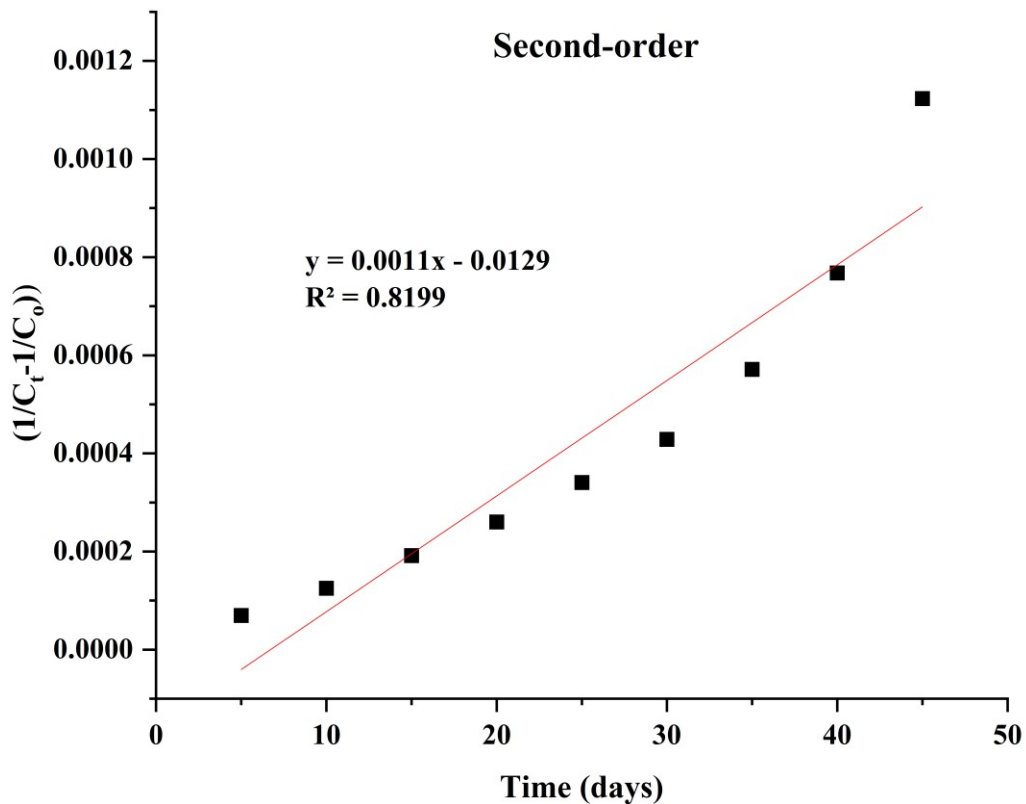


Figure 6.7: Second-order kinetics plot of imidacloprid bioremediation

The degradation kinetics were calculated using the first-order rate equation $C_t = C_0 \times e^{-kt}$, since the removal of imidacloprid was found to be time-dependent. The rate constants for imidacloprid degradation in soil by bacterial isolates ranged from 0.015/d to 0.048/d with a $T_{1/2}$ decrease from 291.77 (~300 days) in uninoculated soil set 1 to 12.95 (~13) days in set 4 and rate constant 0.0419/d with $T_{1/2}$ of 16.50 days in Set 3. A more than 20 times reduction in the half-life period clearly indicates the potential of bacterial species for real-time applications. With proper application of this technique, soil can be made imidacloprid-free before the start of the next cultivation. In a similar study, Cycoń et al. (2013) noted that *Serratia marcescens* was capable of degrading imidacloprid at a rate constant ranging from 0.017 to 0.052/d with $T_{1/2}$ of 13.6–37 days in different types of soils.

Table 6.2: First-order kinetic parameters

Treatment	Regression equation	First-order k (day ⁻¹)	R ²	T _{1/2} (days)
Sterile soil (A)				
Set 1	y= 0.0011x-0.0047	0.0011	0.99	297.92
Set 2	y= 0.0022+0.0008	0.0022	0.997	273.72
Set 3	y= 0.0336-0.1677	0.0336	0.98	23.29
Set 4	y= 0.0412x-0.2084	0.0384	0.987	18.77
Unsterile soil (B)				
Set 1	Y= 0.0014x+0.0096	0.0015	0.991	291.77
Set 2	Y= 0.0028x-0.0005	0.0027	0.996	257.18
Set 3	Y= 0.0455x-0.2209	0.0419	0.0985	16.50
Set 4	Y= 0.0568-0.03513	0.048	0.987	12.95

6.6. Summary of the chapter

Imidacloprid-degrading isolate, identified as *Tepidibacillus decaturensis* strain ST1, could effectively degrade imidacloprid in liquid media, slurry, and soil microcosms. The microcosm studies using the isolate resulted in the degradation of around 77.5% and 85 % of imidacloprid (200 ppm) in sterile and unsterile soils within 45 days. In addition to biodegradation, the sorption of insecticide by plants and the natural reduction of insecticide over time have also been reported. The degradation in soil follows first-order kinetics. Hydrazinecarboxamide and hydroxyurea were identified as metabolites on conducting GC-MS analysis of the degraded samples.

The overall findings reported here demonstrate the significant contribution of microorganisms in the degradation of imidacloprid. The presence and action of indigenous microorganisms are essential for the effective remediation of contaminants from the environment. The bacterial isolate, *Tepidibacillus decaturensis* strain ST1, used in the present study could effectively degrade imidacloprid up to 200 ppm in slurry as well as in soil microcosms.

However, it was observed that imidacloprid residues still remain in the soil at low concentrations along with the metabolites, which could not be achieved through bacterial means and a number of

metabolites have been formed, which could be a possible factor leading to ultimately lead to the toxicity of soil due to the effect of imidacloprid and its metabolites. Some of the insecticide is absorbed by the plant, while a portion of the insecticide is lost through natural processes like volatilization.