CHAPTER 4: BIOREMEDIATION OF IMIDACLOPRID IN BATCH AND STIRRED TANK BIOREACTOR

4.1. General

Bioremediation is one of the most sustainable methods for environmental cleanup. In an attempt to solve the problem arising due to imidacloprid contamination, bacterial strains possessing the ability to degrade imidacloprid were isolated from contaminated agricultural soil samples.

The present work intends to investigate the effectiveness and applicability of acclimatized and isolated bacteria as well as consortia prepared to degrade imidacloprid. Isolation and identification of bacteria have been done and optimization of biodegradation conditions have been conducted. The capacity of bacteria to degrade imidacloprid in batch and stirred tank batch bioreactors has been studied, and biodegradation kinetic analyses have been done using various kinetic models. Mass balance and stoichiometric analysis have also been conducted.

4.2. Identification of bacteria

The primary objective of this study was to isolate and identify bacterial strains capable of degrading imidacloprid, a widely used neonicotinoid insecticide known for its persistence in the environment and potential impact on non-target organisms. To achieve this, soil samples contaminated with imidacloprid were collected as the source for bacterial isolation. Initially, eight bacterial cultures were isolated through culturing on nutrient agar plates. Sub-culturing was done, and pure bacterial strains, capable of degrading imidacloprid, were isolated. The ability of these isolates to degrade imidacloprid was assessed based on their growth response in Minimal Salt Medium (MSM) containing imidacloprid as the sole carbon source. Four isolates demonstrated significant growth in MSM, indicating their potential for imidacloprid degradation.

The four selected isolates were identified using 16S rRNA gene sequencing, followed by phylogenetic analysis to determine their taxonomic positions. The identified strains were assigned specific strain designations and corresponding accession numbers in the GenBank database. The strains were identified as *Tepidibacillus decaturensis* strain ST1 (Accession number MZ208922), *Klebsiella variicola* strain ST3 (Accession number MZ209047), *Brevibacillus borstelensis* strain ST4 (Accession number MZ220460), and *Bacillus licheniformis* strain ST5 (Accession number MZ220456). Phylogenetic trees for these species were represented in Figures 4.1, 4.2, 4.3, and 4.4, respectively.

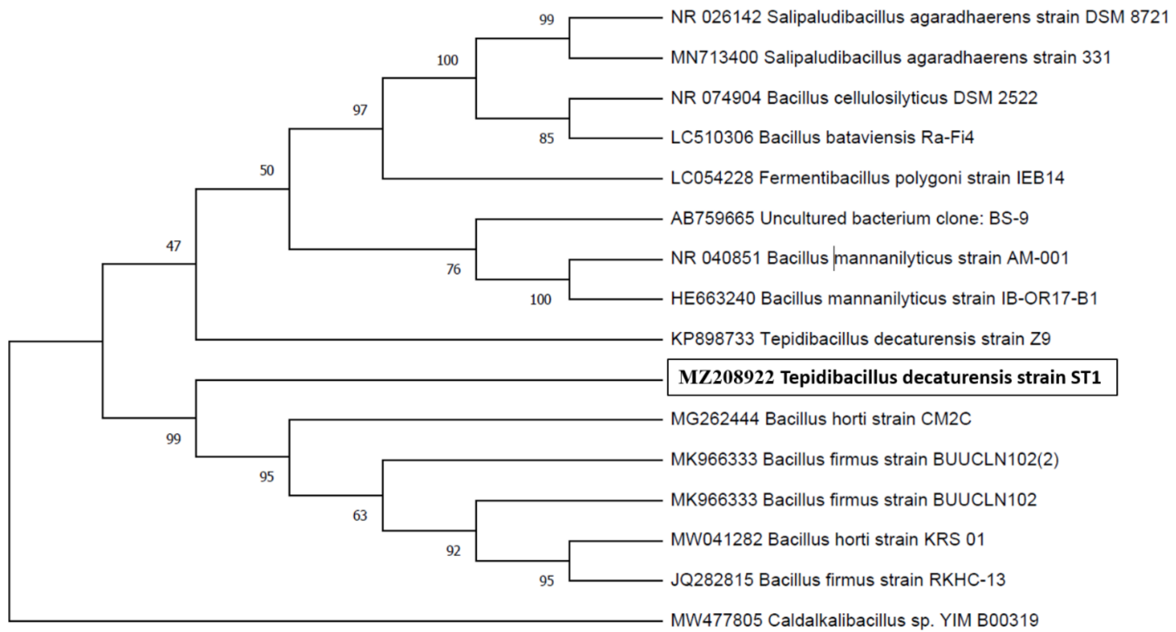


Figure 4.1: Phylogenetic tree of *Teipidibacillus decaturensis* strain ST1

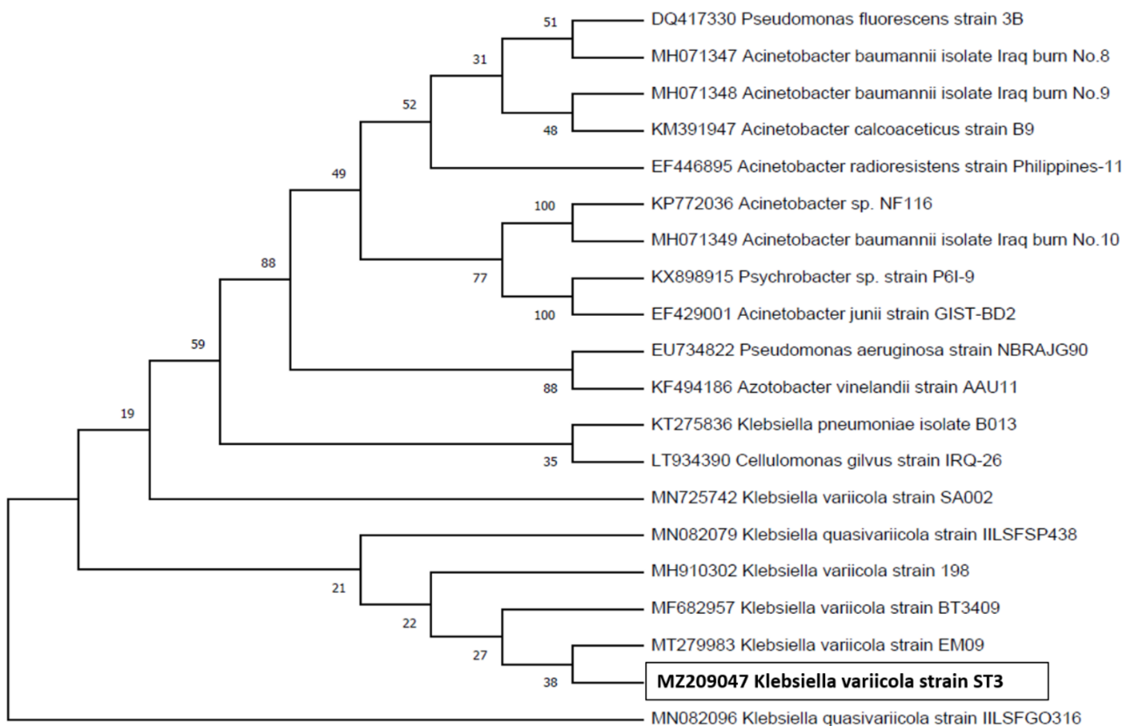


Figure 4.2: Phylogenetic tree of *Klebsiella variicola* strain ST3

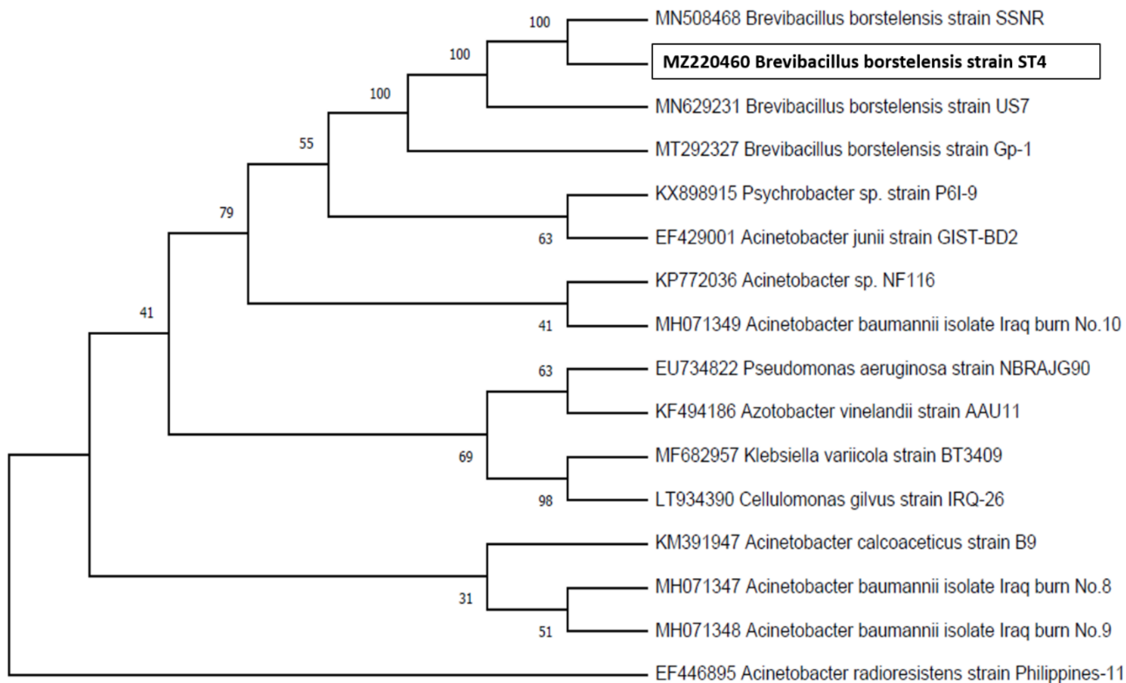


Figure 4.3: Phylogenetic tree of *Brevibacillus borstelensis* ST4

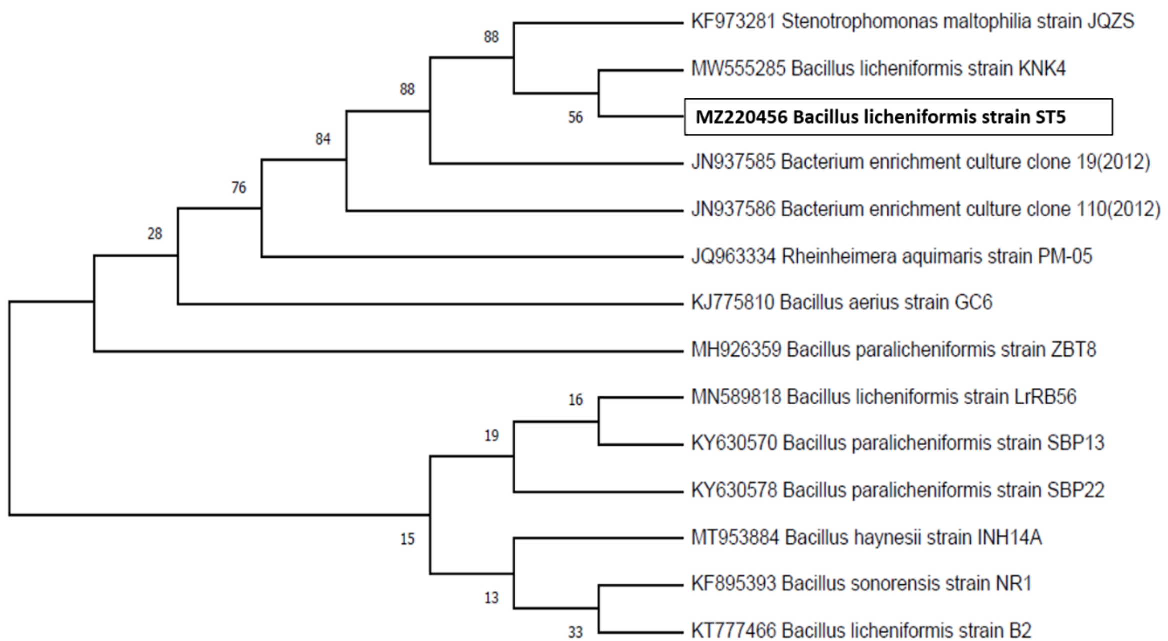


Figure 4.4: Phylogenetic tree of *Bacillus licheniformis* ST5

The individual species were found to be effective in imidacloprid degradation. Two different bacterial consortia were prepared (Consortium CS1 and Consortium CS2). In preparation of CS1, only two isolates, i.e., *Klebsiella variicola* strain ST3 and *Brevibacillus borstelensis* strain ST4 were used, while *Klebsiella variicola* strain ST3, *Brevibacillus borstelensis* strain ST4 and *Bacillus licheniformis* ST5 were used for the preparation of consortium CS2.

4.3. Batch study

The analysis for degradation of imidacloprid was conducted in 250 mL flasks containing 100 mL of MSM broth, supplemented with increasing concentration of imidacloprid (50-300 mg/L). The inoculation of flasks was done with all the individual bacteria isolates as well as the consortia. As a control, non-inoculated flasks were used. The flasks were incubated for 8 days in an incubator at 35±2°C. For analysis of bacterial biomass and imidacloprid concentration, samples were collected at regular intervals. Degradation of imidacloprid was quantified using HPLC. Each of the isolates and consortium were exposed to increasing concentrations of imidacloprid.

It was observed that with an increase in concentration beyond 150 ppm, the growth of bacteria slowed down. At higher concentrations, the bacteria took a longer time to acclimatize, and hence, the degradation time increased, and % degradation was found to decrease, which could be possibly due to the lesser number of viable bacteria with increasing imidacloprid concentration. The results of the degradation of each of the isolated bacteria as well as the consortia at different concentrations, have been depicted in Figures 4.5, 4.6, 4.7, 4.8, 4.9 and 4.10.

The enhanced degradation of imidacloprid at low concentrations is attributable to several key factors. At these lower levels, imidacloprid is less toxic, facilitating optimal bacterial growth and proliferation. This favorable environment induces the production of specific enzymes that efficiently degrade imidacloprid. Additionally, bacteria experience reduced metabolic stress, allowing them to allocate more resources to degradation processes. Nutrient availability is also improved at lower concentrations, further supporting bacterial growth and metabolic functions. The enzymes involved in the degradation are more effective when imidacloprid is present in smaller amounts. Furthermore, the microbial community remains more diverse and resilient, promoting beneficial interactions among different bacterial species that enhance degradation. These combined factors result in accelerated degradation rates of imidacloprid at low concentrations.

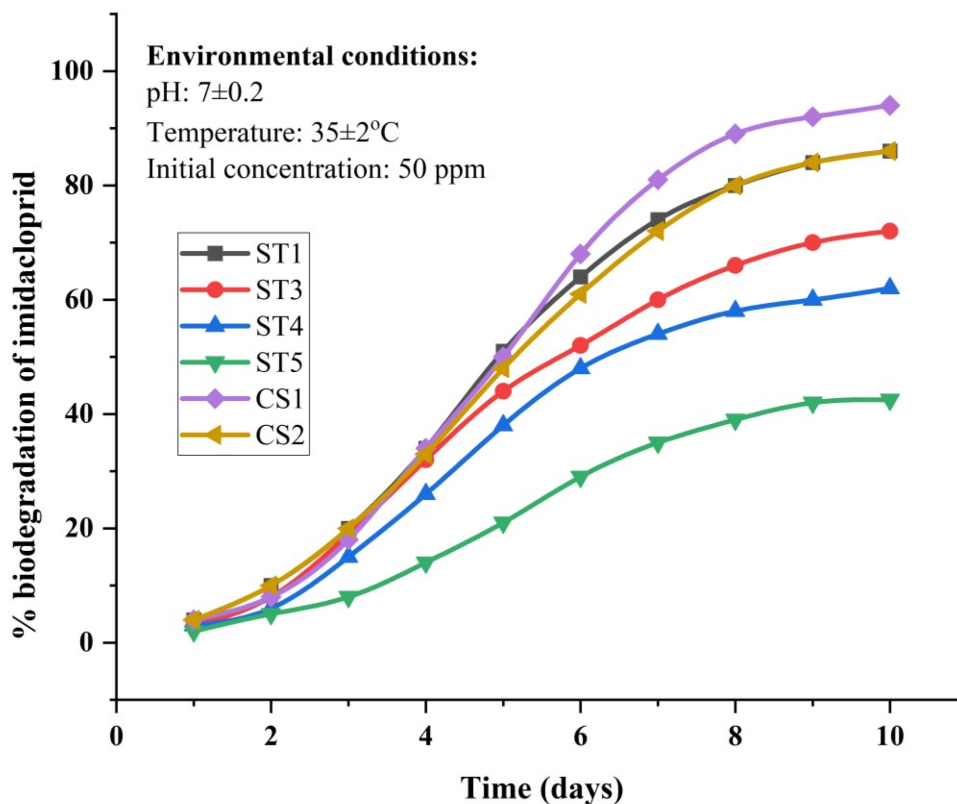


Figure 4.5: Imidacloprid biodegradation at 50 ppm

Figure 4.5 clearly demonstrates that when imidacloprid is present at low concentrations, the bacteria exhibit enormous growth, leading to a notably accelerated degradation rate. This phenomenon is highlighted by the substantial increase in bacterial biomass and activity. Notably, all the isolated bacterial strains display a remarkable ability to degrade imidacloprid, indicating a broad-spectrum biodegradation capability. This is reflected in the degradation profiles observed across different bacterial isolates, highlighting their potential for effective bioremediation of imidacloprid-contaminated environments.

At a concentration of 100 ppm, the bacteria were able to effectively degrade imidacloprid within 8 days, as shown in Figure 4.6. Notably, *Bacillus licheniformis* strain ST5 displayed the lowest percentage of degradation, while the consortium CS1 and CS2, as well as *Tepidibacillus*

decaturensis strain ST1, achieved degradation of over 80% of the imidacloprid within the same timeframe.

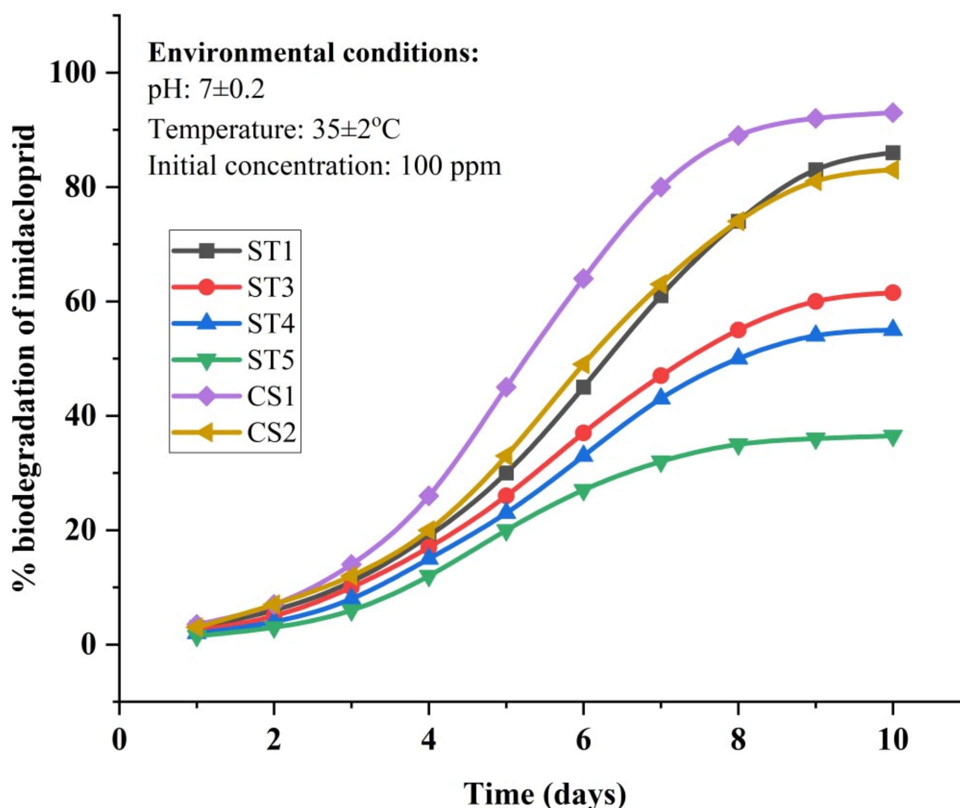


Figure 4.6: Imidacloprid biodegradation at 100 ppm

The observed degradation patterns of imidacloprid at 150 ppm indicate that *Tepidibacillus decaturensis* (ST1) and microbial consortia exhibit high degradation capacity due to their robust adaptive responses, efficient enzymes, and potential synergistic effects within the consortia. These mechanisms include the use of efflux pumps, activation of stress response pathways, and a diverse metabolic network that can handle high pollutant loads. In contrast, *Klebsiella variicola* (ST3), *Brevibacillus borstelensis* (ST4), and *Bacillus licheniformis* (ST5) show reduced degradation efficiency, likely due to higher sensitivity to imidacloprid toxicity, enzyme saturation or inhibition, and a greater metabolic burden at elevated concentrations. These findings highlight the importance

of selecting strains like *Tepidibacillus decaturensis* (ST1) and effective consortia for bioremediation in environments with high levels of imidacloprid contamination.

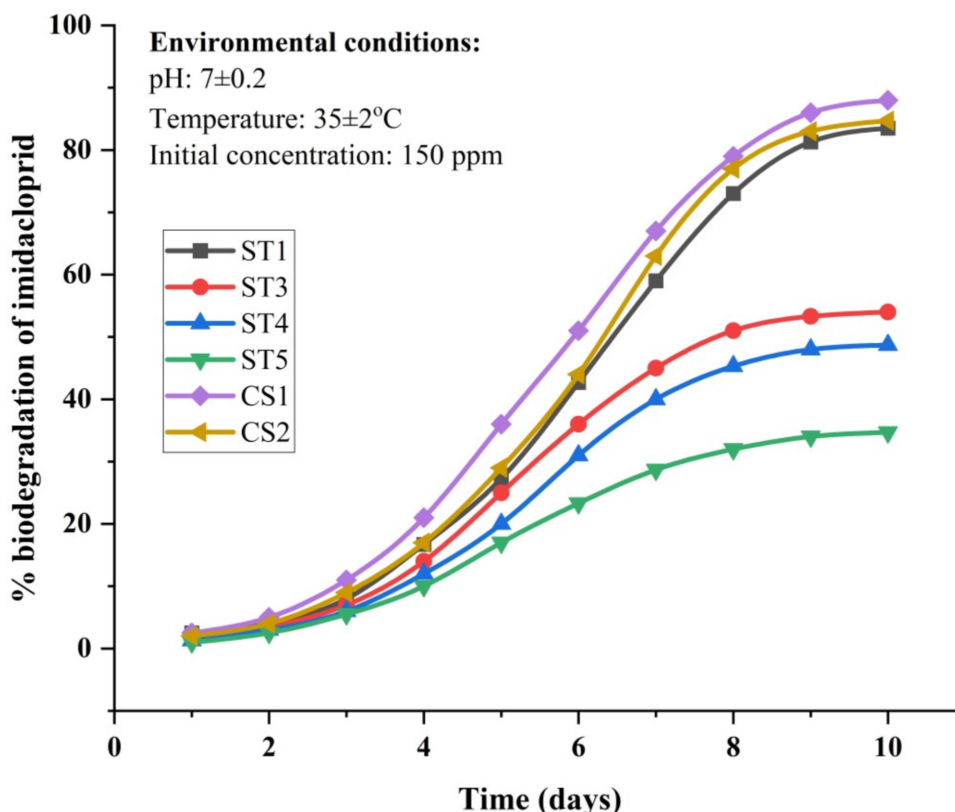


Figure 4.7: Imidacloprid biodegradation at 150 ppm

On further increase in imidacloprid concentration to 200 ppm, it was observed that there was a notable decrease in the efficiency of the isolates in metabolizing the pesticide. The consortia CS1, CS2, and *Tepidibacillus decaturensis* strain ST1 demonstrated a remarkable capacity to degrade approximately 80% of the imidacloprid. However, the strains ST3, ST4 and ST5 could degrade less than 50% of the insecticide. This indicates that CS1, CS2, and ST1 possess robust adaptive mechanisms, such as highly efficient enzymatic systems, effective stress response pathways, and possibly synergistic interactions within the consortia, which enable them to maintain high degradation efficiency even under increased pollutant stress. These strains and consortia are thus particularly promising for bioremediation efforts in environments with high concentrations of imidacloprid.

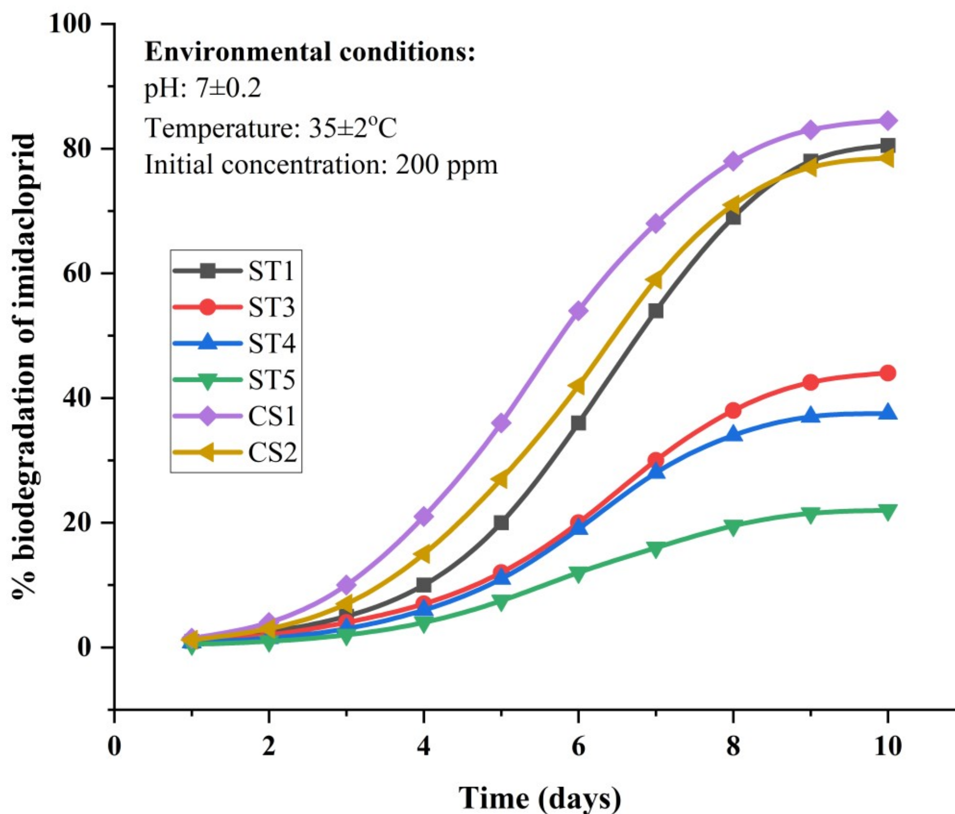


Figure 4.8: Imidacloprid biodegradation at 200 ppm

At 250 ppm imidacloprid, strain ST4 exhibited a significant decline in degradation efficiency, managing to degrade only about 10% of the pesticide in 10 days. Strain ST5 was entirely unable to metabolize imidacloprid at this concentration, leading to its exclusion from further study. In contrast, the consortium CS1 demonstrated robust degradation capability, effectively breaking down over 70% of the imidacloprid within 8 days. This highlights the potential of the consortium CS1 for bioremediation in environments with high levels of imidacloprid, likely due to the synergistic interactions and combined metabolic capabilities of the strains within the consortium.

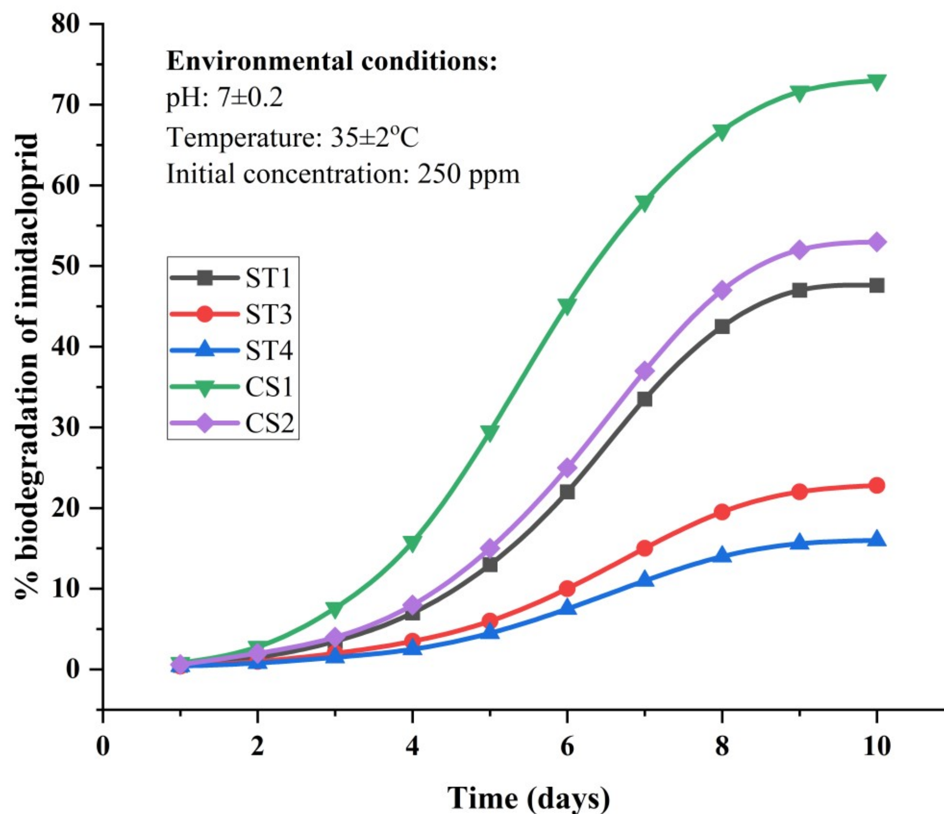


Figure 4.9: Imidacloprid biodegradation at 250 ppm

It can be inferred from the results that an increase in concentration of imidacloprid creates a toxic environment for the bacteria. It can be noted that the bacteria *Bacillus licheniformis* strain ST5 could not degrade imidacloprid at all at 250 ppm. With a further increase in imidacloprid concentration to 300 ppm, the bacteria *Brevibacillus borstelensis* strain ST4 could not degrade imidacloprid. The bacteria need more to adapt to higher insecticide concentrations before the biodegradation process begins. Therefore, biodegradation is time-dependent as well as dose-dependent (Saied et al., 2021). The degradation of carbofuran by *Pichia anomala* strain HQ-C-01 was concentration-dependent; at low insecticide concentrations, higher degradation was observed (Yang et al., 2011). The degradation efficiency of all the strains decreased with an increase in concentration. Hence, imidacloprid concentration of 150 ppm and 200 ppm was considered in studies conducted further. From the results, it can be concluded that *Tepidibacillus decaturensis*

strain ST1 and consortium CS1 were the most effective and therefore, have been taken into consideration to carry out further studies in the present work.

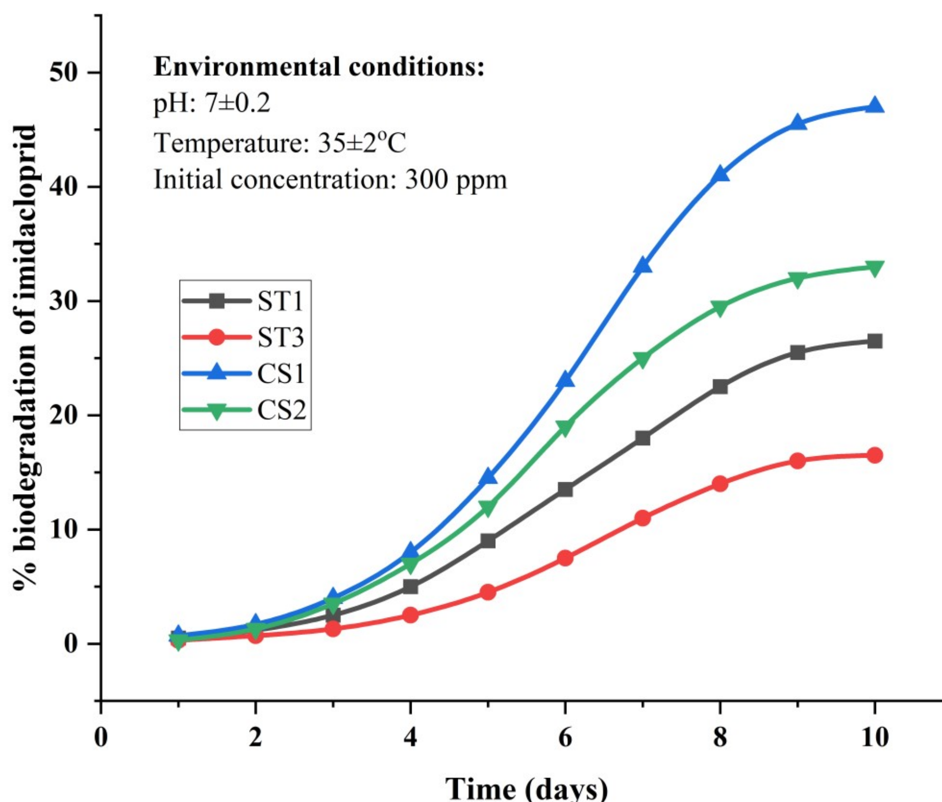


Figure 4.10: Imidacloprid biodegradation at 300 ppm

4.4. Optimization of degradation conditions

The degradation of contaminants is affected by numerous biotic as well as abiotic factors. The most effective imidacloprid-degrading isolated bacteria (*Tepidibacillus decaturensis*) and consortium CS1 (consisting of *Klebsiella variicola* strain ST3 and *Brevibacillus borstelensis* strain ST4) were used to examine the parameters affecting the biodegradation of imidacloprid. In the current investigation, various parameters, including pH, incubation temperature, and incubation condition (static or shaking), were considered and have been found to have an impact on the growth and degradation potential of bacteria. The biodegradation percentages were calculated for each parameter in the case of the isolated bacteria as well as the consortium.

4.4.1. Effect of pH

The pH of the environment has a significant impact on insecticide biodegradation. It affects microbial growth and activity, enzyme functionality, chemical stability of the insecticide, solubility, microbial community composition, and interactions with soil and sediment particles. Optimal pH conditions, which are typically neutral to slightly alkaline, can enhance the biodegradation process, while extreme pH levels may hinder it. Therefore, understanding and controlling pH is crucial in bioremediation efforts to effectively mitigate insecticide contamination. The growth of bacterial cells and imidacloprid biodegradation are significantly affected by changes in pH. In this study, pH was varied from 5 to 9 (5, 6, 7, 8 and 9).

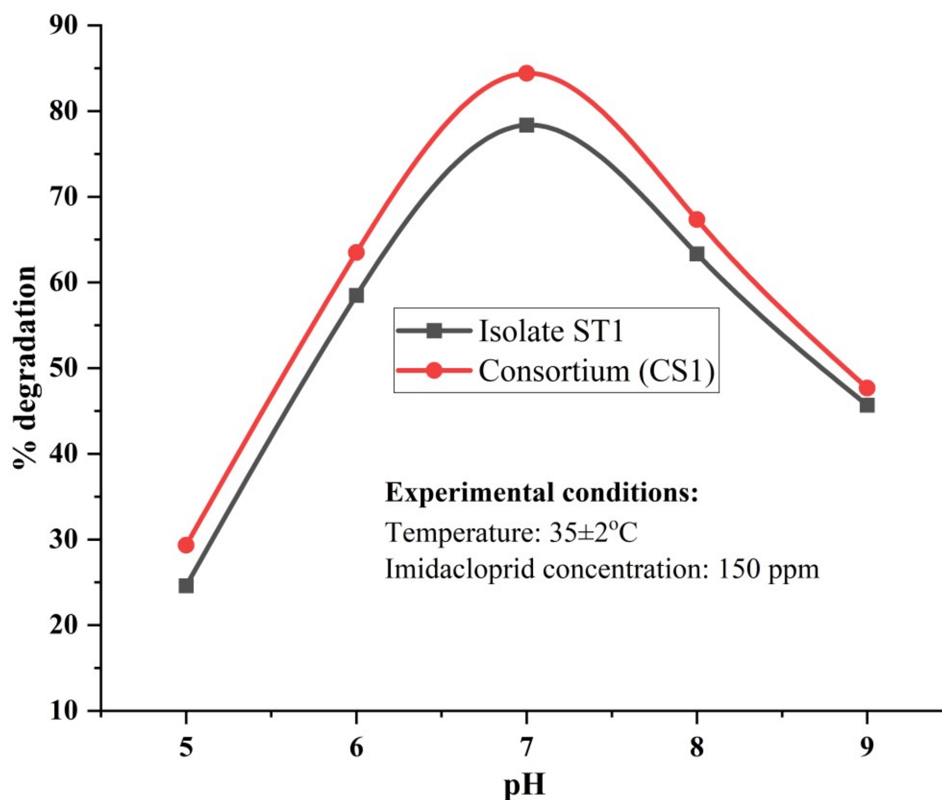


Figure 4.11: Effect of pH on bacteria

Bacterial strain *Tepedibacillus decaturensis* strain ST1 as well as consortium CS1 showed maximum degradation at pH 7 (Figure 4.11). Comparable conclusions were made by Sharma and

Singh (2014) for the degradation of imidacloprid. The bacteria are most effective at neutral pH. With the change in pH to acidic and alkaline, the efficiency of bacteria decreases due to the low activity of the bacterial enzymes involved in biodegradation.

4.4.2. Effect of temperature

Temperature has a significant impact on the biodegradation of insecticides. It affects microbial growth and activity, enzyme functionality, chemical stability of the insecticide, microbial community composition, solubility, and diffusion rates, as well as interactions with soil and sediment particles. Optimal temperatures, generally between 20°C and 40°C, promote maximal microbial activity and biodegradation efficiency, while temperatures outside this range can slow down or inhibit these processes. It is crucial to understand the role of temperature in order to optimize bioremediation strategies for effectively managing insecticide contamination. In the case of imidacloprid, the temperature was varied from 15°C to 45°C (15°C, 25°C, 35°C and 45°C). The results in Figure 4.12 show that biodegradation percentages gradually increased up to 35°C and then decreased.

These findings suggested that 35°C was the ideal temperature for bacterial consortium growth. A number of studies conducted by various researchers report the maximum degradation of organic pollutants in mesophilic temperature range. Temperature also plays an important role in imidacloprid biodegradation, with optimal degradation rates occurring within a specific temperature range. The exact temperature range can vary depending on the microorganisms involved in the degradation process, but generally falls within the mesophilic range (20-45 °C). Lower temperatures can slow down the rate of degradation, while higher temperatures can inhibit microbial activity or lead to the volatilization of the chemical, reducing the potential for biodegradation (Maqbool et al., 2016). Although biodegradation can occur over a wide pH range, in terrestrial and aquatic ecosystems, a pH range of 6.5–8.5 is considered as the optimal for imidacloprid biodegradation (Phugare et al., 2013; Kennedy et al., 2023).

Extreme pH values outside of this optimal range can inhibit the activity of microorganisms, significantly reducing the rate of degradation. Microorganisms involved in bioremediation processes, such as those capable of degrading imidacloprid, often have specific pH ranges within which they exhibit optimal enzymatic activity and growth. When the pH of the environment deviates significantly from this range, it can lead to denaturation of enzymes, disruption of cell

membrane integrity, and overall metabolic stress on the microorganisms. Consequently, maintaining an appropriate pH is crucial for sustaining the microbial activity necessary for effective degradation of imidacloprid.

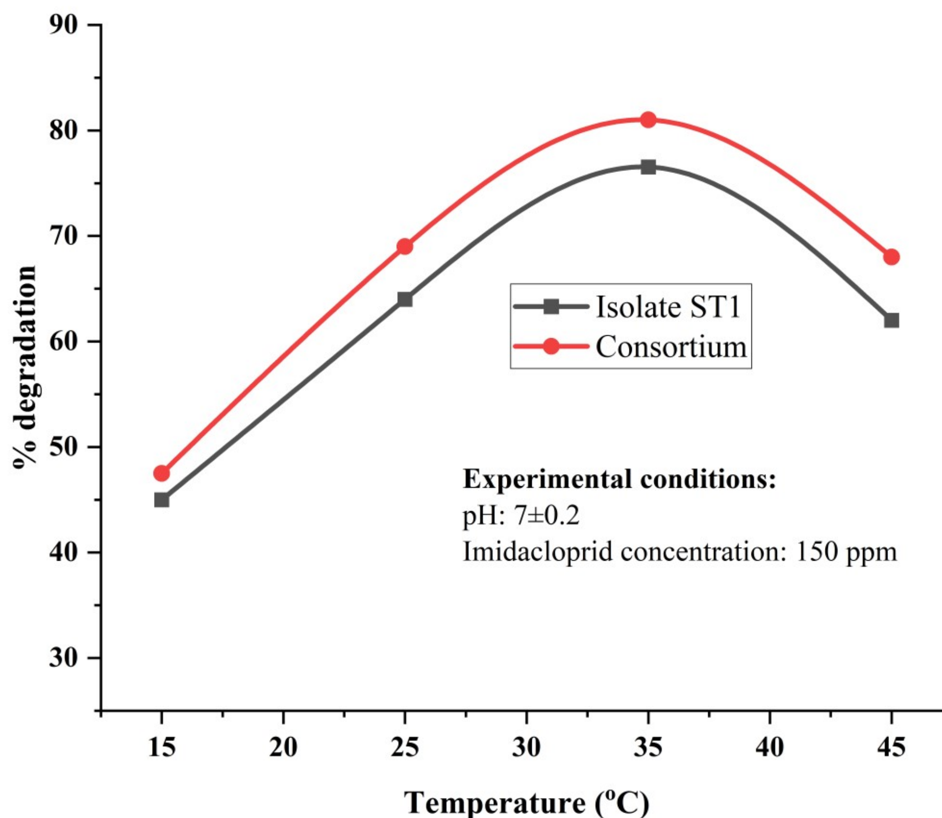


Figure 4.12: Effect of temperature on bacteria

4.4.3. Effect of shaking speed

Shaking speed significantly influences insecticide biodegradation by affecting oxygen availability, nutrient distribution, suspension of microbial cells, homogenization of the medium, and the reduction of inhibitory compounds. Optimal shaking speeds enhance microbial growth and activity, promoting efficient biodegradation of insecticides. However, excessively high shaking speeds may impose shear stress on microorganisms, reducing biodegradation efficiency. Therefore, determining the appropriate shaking speed is essential for optimizing biodegradation processes in both laboratory and field settings. The bacterial isolates and consortium were incubated in MS

broth containing 150 mg/L imidacloprid and incubated under static and shaking conditions (50-250 rpm). The maximum degradation of imidacloprid degradation could be achieved under shaking conditions after 8 days of incubation (Figure 4.13).

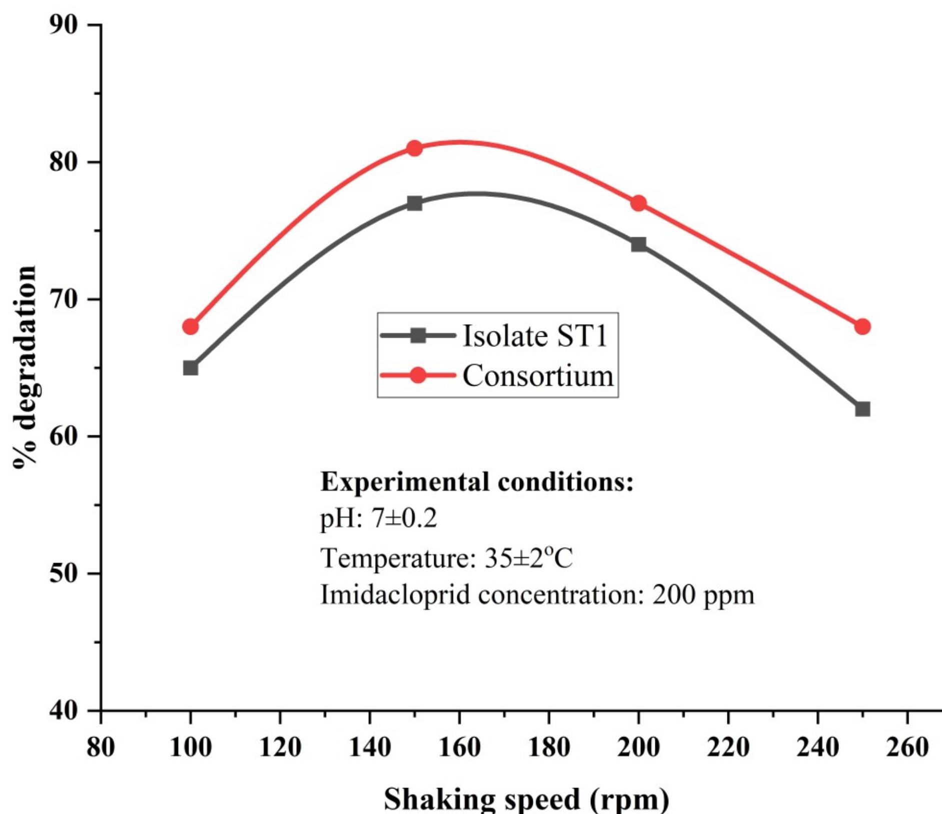


Figure 4.13: Effect of shaking speed on bacteria

After optimization of environmental parameters, a considerable increase in biodegradation of imidacloprid was observed. Table 4.1 shows the degradation of imidacloprid by bacteria *Tepidibacillus decaturensis* strain ST1 as well the consortium CS1, before and after optimization of environmental parameters. The data obtained are in agreement with the studies previously conducted on the optimization of parameters (Odukkathil and Vasudevan, 2013). It has been observed that microbial consortia are more efficient in the degradation of pollutants as compared to isolated pure strains (Castro-Gutiérrez et al., 2016). The successful employment of consortia in bioremediation, rather than pure strains, is supported by the findings in the present study.

Table 4.1: Degradation of imidacloprid before and after optimization of environmental conditions

Bacteria	% degradation			
	Before optimization	After optimization		
		pH	Temperature	Shaking speed
Tepidibacillus decaturensis strain ST1	74	78	79	80
Consortium ST1	78	82	84	84

4.5. Imidacloprid degradation in stirred tank batch bioreactor

Imidacloprid degradation was carried out in a 3-L stirred tank reactor operated in batch mode. In addition, adsorption losses were insignificant, accounting for 4-5% removal of imidacloprid, indicating that degradation can be attributed to the influence of microbial biomass. No appreciable abiotic removal (3-4%) of imidacloprid was found in the reactors during the treatment. Imidacloprid was degraded to a large extent from a first batch assay after 8 days (~90%). The lag period in biomass growth increased with an increase in imidacloprid concentration, as demonstrated by biomass growth (Figure 4.14). A total initial imidacloprid concentration of 150 mg/L resulted in the greatest biomass growth. Imidacloprid biodegradation and biomass growth are both dependent on the initial concentration of imidacloprid.

The reduced rate of biomass growth can be attributed to the inhibitory effect of toxic pollutants on microbial proliferation. When exposed to an environment contaminated with imidacloprid, microorganisms require a prolonged period to acclimate, thereby hampering rapid biomass accumulation. Furthermore, the energy-intensive process of breaking down complex pollutants, such as imidacloprid contributes to the reduction of microbial growth.

Imidacloprid degradation in a stirred tank reactor reveals that imidacloprid is biodegraded efficiently but not completely. The lag phase in biomass growth is shorter in a stirred tank reactor as compared to the batch set-up. Degradation efficiency of 90% was attained at 150 mg/L initial concentrations, whereas in the batch study, the value was about 78%. The biodegradation of imidacloprid was higher in the reactor in comparison to the batch study due to higher nutrient and oxygen transfer, which are essentially required for the simultaneous biodegradation of pollutants

and biomass growth. Recently, Rodríguez-Castillo et al. (2019) used a stirred tank bioreactor for biodegradation of binary and ternary mixture of neonicotinoids using bacterial consortium and observed more than 95% degradation efficiency after 10 days.

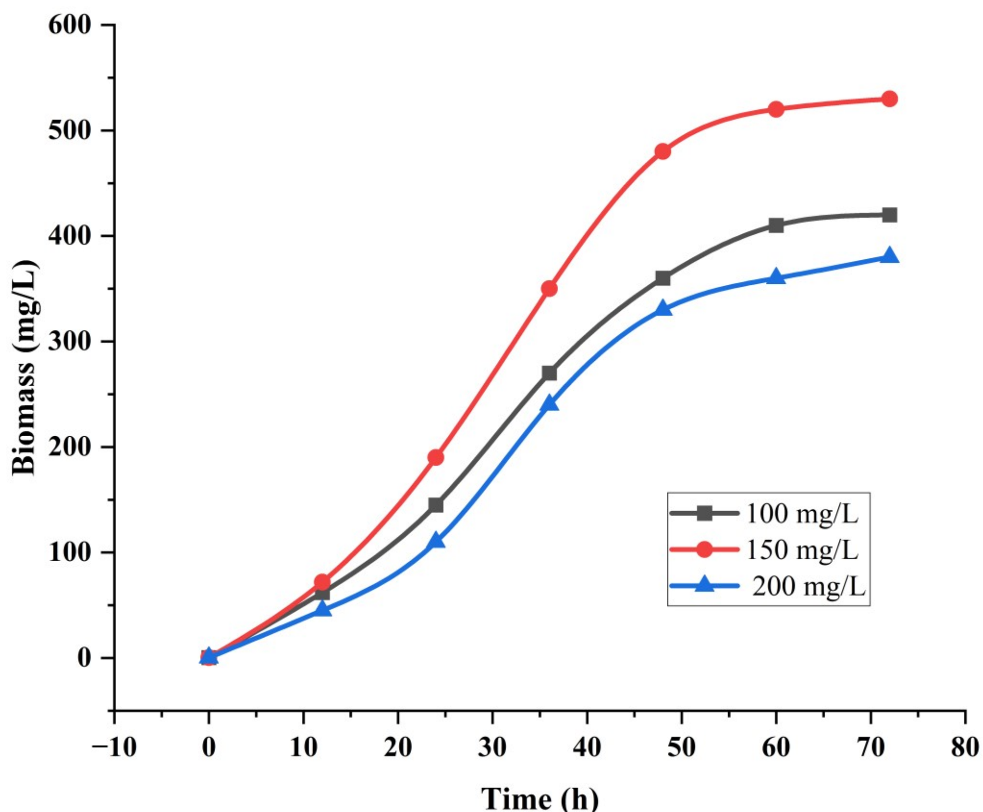


Figure 4.14: Growth of biomass with time

The present study shows that bacterial consortium can be effectively used in stirred tank batch reactor for degradation of imidacloprid. However, agitation adds to the cost in application of stirred tank reactor, therefore other bioreactor systems, with low or negligible agitation costs, could be investigated for wastewater treatment. Furthermore, cost-benefit analysis of treatment system would be beneficial in determining the large-scale industrial applications.

4.6. Kinetic analysis

The initial imidacloprid concentrations of 50, 100, 150, 200, 250, 300, 350, and 400 mg/L were used to analyze the biodegradation kinetics. The specific degradation rate for all the initial

concentrations was determined. To estimate the biokinetic parameters involved in imidacloprid degradation, the experimental data was subsequently fitted into various kinetic models as presented in Table 4.2.

Table 4.2: Kinetic models to illustrate imidacloprid biodegradation kinetics using the consortium

Model	Equation	Reference
Monod	$\mu = \frac{\mu_{max}.S}{K_s + S}$	Monod, 1949
Andrew	$\mu = \frac{\mu_{max}.S}{K_s + S + \frac{S^2}{K_i}}$	Andrews, 1968
Aiba	$\mu = \frac{\mu_{max}.S}{K_s + S}.exp\left(\frac{-S}{K_i}\right)$	Aiba, 1968
Webb	$\mu = \frac{\mu_{max}.S.\left(1 + \frac{S}{K}\right)}{K_s + S + \frac{S^2}{K_i}}$	Nemati and Webb, 1997
Edward	$\mu = \frac{\mu_{max}.S}{K_s + S + \left(\frac{S^2}{K_i}\right).\left(1 + \frac{S}{K}\right)}$	Edwards, 1970
Teisser	$\mu = \mu_{max}.\left[exp\left(\frac{-S}{K_i}\right) - exp\left(\frac{-S}{K_s}\right)\right]$	Edwards, 1970

μ: Specific growth rate (h^{-1})**; S:** substrate concentration (mg/L)**;** μ_{max}**:** Maximum specific growth rate (h^{-1})**;** K_i **:** Imidacloprid inhibition constant (mg/L)**;** K_s**:** half saturation constant (mg/L)**; K:** a constant in Edward and Webb model

The kinetics of imidacloprid biodegradation has been studied under non-inhibition condition using the Monod model and substrate inhibition condition using Aiba, Andrew, Webb, Teissier and Edward models. The experimental data profile and predicted models have been represented in Figure 4.15. In the case of the Teissier and Edward models, regression values (R^2) greater than 0.98 and 0.94 respectively were obtained and were well-fitted with the experimental data.

The ratio of μ_{max}/K_s is known as specific affinity and used as the convenient index to show the efficacy of microbes in the biodegradation of substrate[39]. In the present study, the value of μ_{max}/K_s calculated for all the models and was found to maximum in the case of Teisser model. The values of μ_{max}, K_s, and K_i have been presented in Table 4.3.

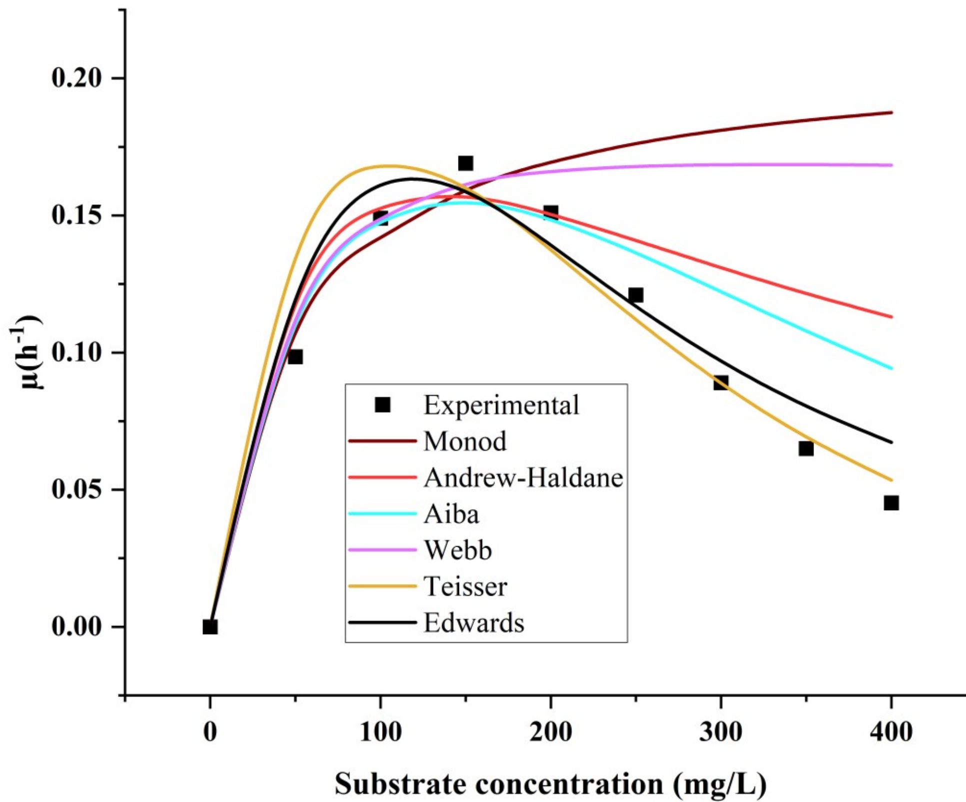


Figure 4.15: Experimental and predicted data plot fitted by various models

The Teissier model is a mathematical model used to describe bacterial growth, specifically focusing on the relationship between growth rate and substrate concentration. It is characterized by the equation:

$$\mu = \mu_{max}.\left[exp\left(\frac{-S}{K_i}\right) - exp\left(\frac{-S}{K_s}\right)\right]$$

where, μ is the specific growth rate (h^{-1}), S is the substrate concentration (mg/L), μ_{max} is the maximum specific growth rate (h^{-1}), K_i is imidacloprid inhibition constant (mg/L) and K_s is half saturation constant (mg/L).

This model is one of several that aim to characterize microbial growth kinetics, and it builds upon earlier models like the Monod model by introducing a more flexible mathematical form. The Teissier model produces a sigmoidal (S-shaped) curve when plotting the growth rate against

substrate concentration. This differs from the Monod model, which typically shows a hyperbolic relationship. The model provides greater flexibility in describing different phases of bacterial growth, especially at low substrate concentrations where other models might not fit well. It's particularly useful in describing growth in environments where substrate inhibition or other complex interactions affect bacterial proliferation.

Table 4.3: Biokinetic parameters for imidacloprid biodegradation by bacterial consortium

Models	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	K	μ_{max}/K_s (L/mg.h)	R^2
Monod	0.21	48	-	-	0.004375	0.462
Andrew	0.41	110	170	-	0.003727	0.672
Aiba	0.51	145	290		0.003517	0.757
Webb	0.24	71	80	120	0.00338	0.496
Edwards	0.51	205	250	125	0.002537	0.943
Teisser	0.51	74	180	-	0.006892	0.985

It was noticed that with an increase in imidacloprid concentration up to 150 mg/L, the value of μ increased. Further increase the concentration of imidacloprid resulted in decreased value of μ. The decrease in μ may be due to substrate inhibition above 150 mg/L. The previous researchers observed a comparable effect on specific growth rates with respect to different concentrations of substrates (Kureel et al., 2017; Geed et al., 2017)

4.7. Stoichiometric analysis and mass balance

Mass balance can simplify quantitative estimation of a substrate that enters and leaves a system/stream of known composition. Mass balance can be used to define the metabolic response of a bacterial cultures for the treatment of wastewater contaminated with organic contaminants. During the biological conversion process, mass balance and stoichiometric calculations were performed, assuming that only H_2O and CO_2 were produced and no extracellular product was formed (Shuler and Kargi, 2001). The following reaction was considered for the mass balance and stoichiometric calculations:

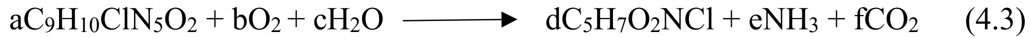
$$aCH_mO_n + bO_2 + cH_2O \longrightarrow dC_\alpha H_\beta O_\gamma N_\delta + eNH_3 + fCO_2 \qquad (4.1)$$

The biomass yield from imidacloprid biodegradation was determined based on the aforementioned equation and the results acquired from the experimental study.

The law of conservation of mass is strictly followed during the mass balance, which consists of a substrate of known composition that enters and exits a system. The transformation of substrates from one form to another describes the metabolic response of bacteria to treat contaminated wastewater. The following equation is the fundamental of mass balance:

$$X_{input} - X_{output} = X_{acc/deg} \qquad (4.2)$$

where X_{input} is the input concentration, X_{output} is the output concentration, and $X_{acc/deg}$ is the concentration after accumulation or degradation. A critical step in the biological treatment of organic substrate-containing wastewater is the mass balance of input and output concentrations of substrate, biomass, and dissolved oxygen (Kanaujiya et al., 2022). In the present study, concentration of biomass, dissolved oxygen (DO) and imidacloprid were considered for mass balance analysis. The dry weight of biomass was used to conduct a stoichiometry analysis of imidacloprid biodegradation and biomass accumulation. For this stoichiometric analysis, the elemental composition of biomass was assumed to be $C_5H_7O_2NCl$. The stoichiometry equations are as follows:



$$aC_9H_{10}ClN_5O_2 + bO_2 + cH_2O \longrightarrow dC_5H_7O_2NCl + eNH_3 + fCO_2 \qquad (4.3)$$

Since NH_4NO_3 was used in the MSM as a source of nitrogen, NH_3 was taken into account while calculating the nitrogen mass balance. The following five equations were derived from the stoichiometric equations above to balance the C, H, N, and O elements in this study.

Carbon balance: $9a = 5d + f$ (A)

Hydrogen balance: $10a + 2c = 7d + 3e$ (B)

Oxygen balance: $2a + 2b + c = 2d + 2f$ (C)

Nitrogen balance: $5a = d + e$ (D)

Chlorine balance: $c = d$ (E)

The following stoichiometrically balanced equation was obtained on solving the equations.

$$4C_9H_{10}ClN_5O_2 + 7O_2 + 18H_2O \longrightarrow 4C_5H_7O_2NCl + CO_2 + 16NH_3 \quad (4.4)$$

The stoichiometric and mass balance calculations in the study evidently demonstrated the efficacy of the stirred tank bioreactor-based biodegradation process. The following stoichiometric formula provides the yield of the biodegradation process.

$$Biomass\ yield = \frac{Molecular\ weight\ of\ Biomass}{Molecular\ weught\ of\ substrate}$$

Based on the above equations, the biomass yield value for imidacloprid was calculated to be 0.597. Biomass yield indicates the amount of biomass that can be produced on consumption of a particular amount of substrate. The estimation of biomass yield is essential in biological processes since it describes the growth efficiency of the strain in that substrate. Optimization of biomass yield can lead to higher degradation or conversion of substrate, thus enhancing the economic viability of the biological process. The substantial biomass output attained in the current investigation shows that the bacterial consortium is capable of effectively using imidacloprid. Theoretical oxygen demand was calculated using the above equation and found to be 224 for oxidizing 4 moles of imidacloprid, i.e., 56 for oxidizing 1 mole of imidacloprid.

4.8. Analysis of BOD, COD and TOC

The amount of oxygen required for the biological oxidation of organic materials in the effluent is BOD. It is determined by measuring the difference in dissolved oxygen over five days in a sample. The initial DO content of a given sample volume is recorded, and the sample is removed from the incubator after a five-day incubation period at 20°C to get the final DO content. A higher BOD means more oxygen is required, implying that the water quality is poor. Low BOD indicates that less oxygen is taken from the water, suggesting that the water is typically purer.

The COD measures how much oxygen is required to chemically oxidize the organic and inorganic components in the effluent. COD is calculated because, given acidic conditions, a powerful oxidizing agent may completely oxidize almost all organic ingredients to carbon dioxide. Under acidic conditions, COD is frequently measured using a strong oxidant (e.g., potassium dichromate or potassium permanganate). The oxidant is introduced to the sample in a concentration that is known to be in excess. After the oxidation process is complete, the amount of oxidant left in the solution is measured to determine the concentration of organics in the sample. Titration with an indicator solution is commonly used to accomplish this. The mass of oxygen used per liter of

solution is measured in milligrams per liter of COD. The sample oxidation was done using potassium dichromate in a digestor. The absorbance is measured for the Cr^{3+} ions generated in the COD analyzer.

The TOC determines the total amount of organic carbon in an effluent, including that which cannot be oxidized. The TOC analysis was done by performing combustion of the sample at 850ºC in a TOC analyzer (Model No.: Multi N/C 2100, Analytic Jena, Germany).

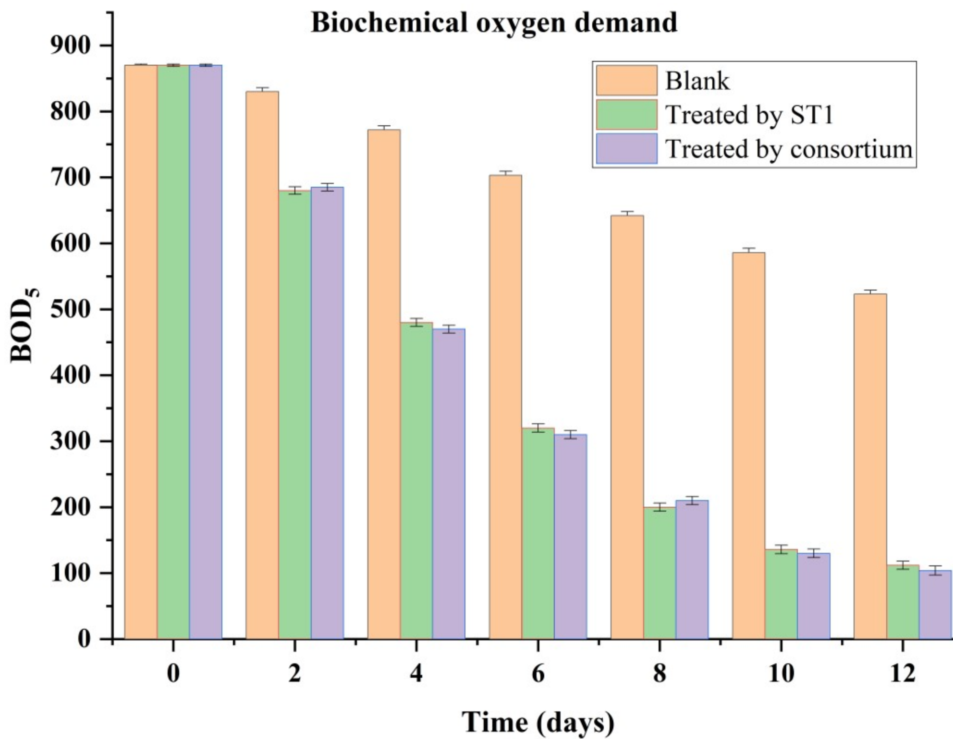


Figure 4.16: Reduction in BOD_5 after biodegradation

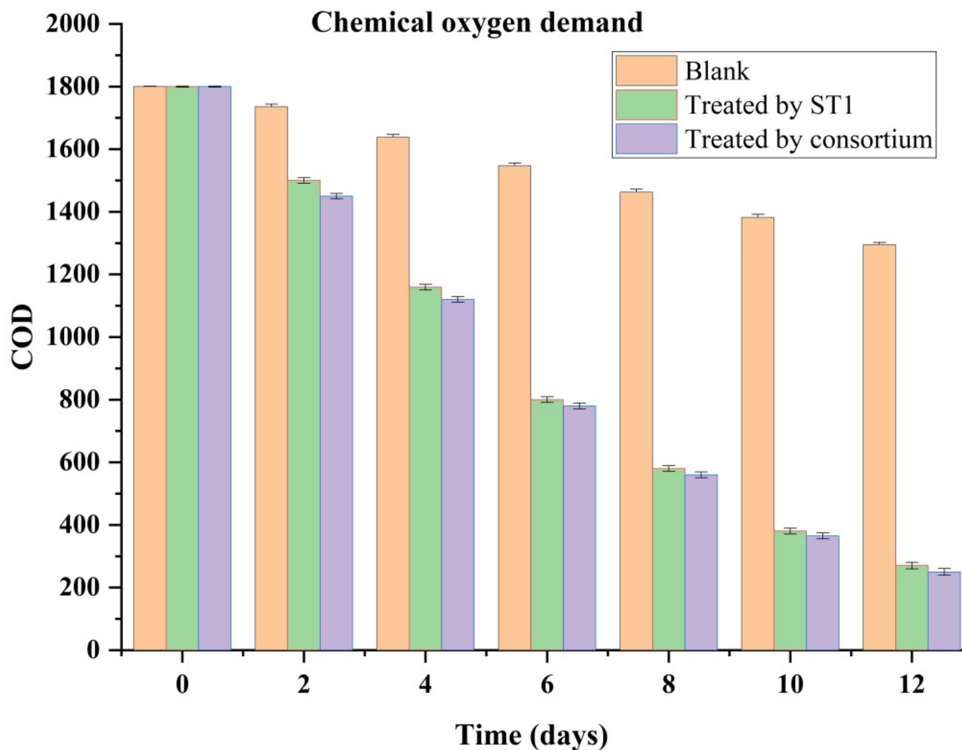


Figure 4.17: Reduction in COD after biodegradation

In the study, important environmental engineering parameters such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), and Total Organic Carbon (TOC) were included to evaluate their effects on the microbial breakdown of imidacloprid. Monitoring these parameters over a 12-day period revealed significant decreases: TOC levels dropped by 90% and 92.3%, COD by 85% and 86.1%, and BOD_5 by 87.13% and 88%. These results indicate the effective operation of the microbial degradation system. Such reductions in TOC, COD, and BOD_5 highlight the substantial breakdown of organic materials, confirming the efficiency of the microbial processes at degrading imidacloprid.

Similar outcomes have been reported by numerous researchers studying bacterial degradation of various pesticides, reinforcing the findings of this study. The data emphasize the importance of COD, BOD_5, and TOC as key environmental indicators for understanding the extent and efficiency of microbial degradation of imidacloprid. By tracking these parameters, researchers can assess the

progress and effectiveness of bioremediation efforts, ensuring that the conditions remain conducive to optimal microbial activity and pesticide degradation.

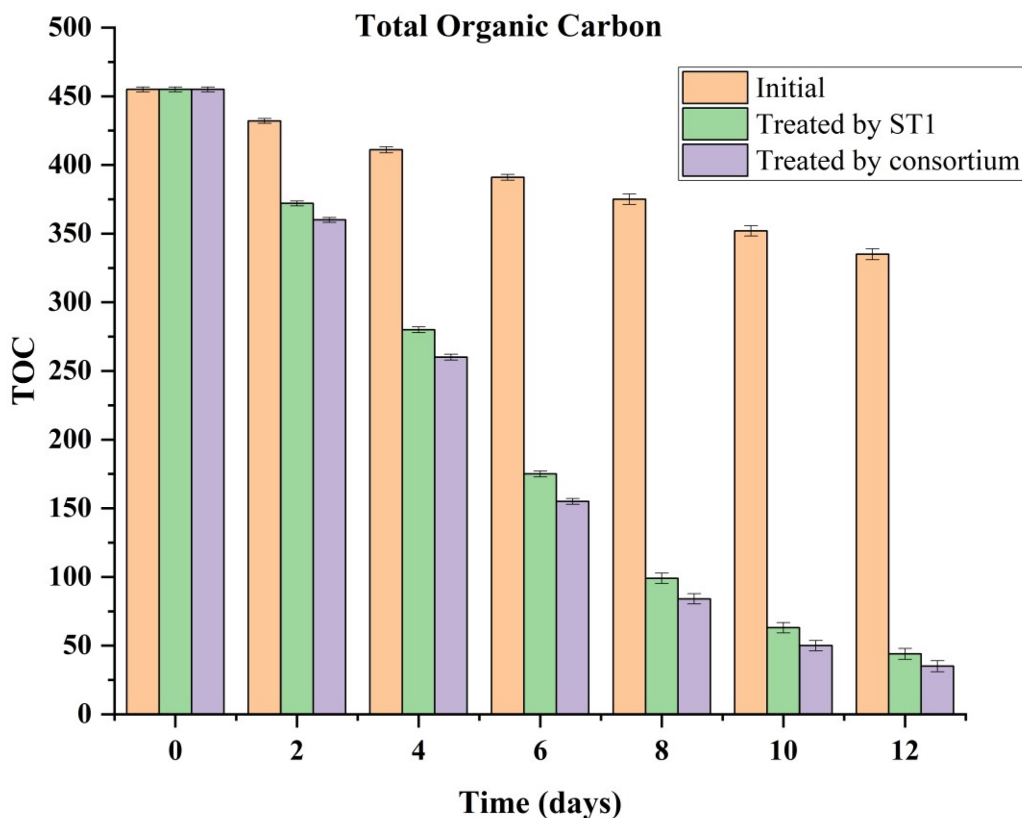


Figure 4.18: Reduction in TOC after biodegradation

4.9. Summary of the chapter

In this study, the efficacy of different bacterial species isolated from a contaminated soil sample to degrade the neonicotinoid insecticide imidacloprid was investigated. Data showed that the most potent bacterial isolate showed high efficiency in degrading imidacloprid insecticide and was identified as *Tepidibacillus decaturensis* based on a morphological and physiological test as well as amplification and sequencing of 16S rRNA. The application of a bacterial consortium for the remediation of imidacloprid was found to be promising. Environmental parameters such as pH and temperature have a significant effect on bioremediation. Data analysis revealed that the degradation percentages were increased after optimizing the environmental factors of incubation

condition (150 rpm), incubation period (8 days), incubation temperature (35°C), pH (7) and 200 ppm of imidacloprid.

Biomass growth has been found to be dose-dependent. The present study suggests greater imidacloprid reduction in the reactor, compared to the batch study, due to higher nutrient and oxygen transfer in the reactor. Kinetic analysis reveals that substrate inhibition takes place, and experimental data fit well in the Edwards and Teisser kinetic models. Environmental engineering parameters such as BOD, COD, and TOC provide a useful and alternative approach to calculating pesticide reduction rates, which may be advantageous to the scientific community as well as agriculturalists.