CHAPTER 8: APPLICATION OF LYOPHILIZED AND ENCAPSULATED BACTERIA FOR IMIDACLOPRID BIOREMEDIATION

8.1. General

Pesticides like imidacloprid, which are widely used in agriculture to control pests, present significant environmental challenges due to their high toxicity and persistence. These compounds are difficult to degrade because their complex chemical structures resist breakdown by most organisms. Only certain species of microorganisms possess the necessary metabolic pathways to degrade such compounds effectively (Singh et al., 1999). The degradation of imidacloprid at a reasonable rate is critical to minimize its harmful impacts on the environment, such as contamination of soil and water bodies and potential harm to non-target organisms including beneficial insects and microorganisms.

Selecting a suitable microbial consortium is a pivotal step in addressing this issue. A microbial consortium is a specifically curated community of different microbial species that work synergistically to degrade complex compounds more efficiently than individual species alone. The selection process involves identifying and combining microorganisms that have complementary metabolic capabilities, ensuring a comprehensive breakdown of the pesticide into non-toxic byproducts. This approach not only accelerates the degradation process but also enhances the overall effectiveness of bioremediation efforts.

To ensure that these beneficial microbes remain viable and effective over time, their shelf-life must be extended. One of the most effective methods for achieving this is lyophilization, also known as freeze-drying. Lyophilization involves freezing the microbial cultures and then reducing the surrounding pressure to allow the frozen water in the cultures to sublimate directly from solid to gas. This process preserves the microorganisms in a dry state, which significantly extends their shelf-life by protecting them from environmental factors that could otherwise degrade their viability (Teixidó et al., 2022).

Lyophilization process drastically slows down the metabolic activities of the microbes, allowing them to remain viable for extended periods, often several years. This method is particularly valuable in bioremediation, where the long-term storage of bacterial strains is crucial. By lyophilizing these strains, they can be preserved until needed, then easily rehydrated and deployed to contaminated sites to break down pollutants. The flexibility offered by this technique facilitates more strategic planning and implementation of bioremediation projects.

However, ensuring the success of lyophilization requires optimization of the process and the use of suitable cryoprotectants to protect the bacteria during drying and storage (Strasser et al., 2008).

The effectiveness of the lyophilized bacteria in breaking down contaminants depends on various factors, including the type of bacteria used, the specific pollutants targeted, and the conditions under which the lyophilized bacteria are stored and handled (Kawasaki et al., 2019). Regular monitoring of these stored bacterial strains is essential to confirm their continued viability and suitability for bioremediation tasks. By leveraging the benefits of lyophilization, we can preserve crucial microbial consortia, ensuring they are ready for future environmental remediation efforts, thus enhancing the sustainability and effectiveness of our approach to managing pollution. Combining the strategic selection of robust microbial consortia with advanced preservation techniques like lyophilization makes it possible to create effective and sustainable solutions for bioremediation of toxic pesticides such as imidacloprid (Bhatt et al., 2021). This integrated approach addresses the immediate need for detoxification of contaminated sites and provides a practical means of maintaining and deploying these biological agents over the long term. Studies conducted by Kupletskaya and Netrusov (2011) reported a sustained viability for up to five decades post-storage after lyophilization.

Encapsulation of lyophilized bacteria is a technique used to further protect bacterial cells from environmental stress and to prolong their shelf-life. Encapsulation of bacteria is crucial because it offers several key benefits. It protects from environmental stressors such as UV radiation, desiccation, and temperature fluctuations, thereby enhancing bacterial survival and activity (John et al., 2011). The encapsulation process involves the formation of a protective coating around the lyophilized bacteria, which can provide additional stability and protection during storage and transportation (Liu et al., 2019). Encapsulation also creates a supportive microenvironment that maintains the metabolic activity and stability of the bacteria. This technique allows for the controlled and targeted release of bacteria, ensuring they are active where and when needed, which is particularly beneficial in bioremediation. Additionally, it reduces contamination risks and makes bacteria easier to handle and apply in various forms (de Oliveira et al., 2021). The versatility of encapsulated bacteria extends to applications in agriculture, wastewater treatment, and bioprocessing, improving the efficiency and cost-effectiveness of these processes. Encapsulation ensures the long-term viability and effectiveness of bacteria in a range of industrial and environmental applications.

This chapter of the thesis presents the studies related to lyophilization and encapsulation of bacteria for application in imidacloprid biodegradation. Soil microcosms were prepared, and imidacloprid degradation in soil was evaluated. Soil samples were collected and microcosms were prepared. Biostimulation with urea, bioaugmentation with the lyophilized and encapsulated bacteria, as well as combined biostimulation and bioaugmentation studies, were conducted in the microcosms.

8.2. Imidacloprid degradation using lyophilized bacteria

The research project sought to comprehensively evaluate the potential of lyophilized bacteria in degrading imidacloprid and to assess their long-term viability. This involved conducting a series of meticulous laboratory experiments to examine the cell viability and imidacloprid degradation capabilities of the lyophilized bacteria. These experiments included initial efficiency tests conducted immediately after lyophilization, as well as subsequent evaluations after storing the bacteria for various durations ranging from 30 days to 1 year. The primary objective was to gain a deep understanding of the impact of long-term storage on the effectiveness of the lyophilized bacteria in degrading imidacloprid.

The findings indicated that although the lyophilized bacteria displayed noteworthy efficacy in degrading imidacloprid, a gradual decrease in efficiency was observed after 6 months of storage. Despite this decline, it was noted that the bacteria remained viable enough for subculturing purposes, providing valuable insights into their long-term viability and potential applications.

The results, as presented in Figure 8.1, indicate that both lyophilized and encapsulated bacteria were highly effective in breaking down imidacloprid. The application of isolated bacteria after lyophilization and encapsulation resulted in about 80% of the insecticide within 8 days. This highlights the strong ability of the preservation method to maintain the bacterial activity for environmental cleanup. The similar performance of both lyophilized and encapsulated bacteria suggests that encapsulation could be a practical way to keep bacteria working effectively over time. These findings contribute valuable insights into the application of bacterial biodegradation for addressing pesticide pollution in the environment. These findings underscore the potential of both preservation techniques in maintaining the efficacy of bacteria for imidacloprid degradation, with encapsulation showing promise in enhancing their performance over extended periods. It was observed that the encapsulated bacteria could degrade more imidacloprid as compared to the lyophilized bacteria. The general concept of encapsulation is that bacterial cells can be stabilized by encapsulating and protected against harsh environmental conditions. Compared with free bacteria, encapsulated bacteria have some benefits, including greater stability, continuous application, better control, higher efficiency, reduced environmental pollution, and economic efficiency.

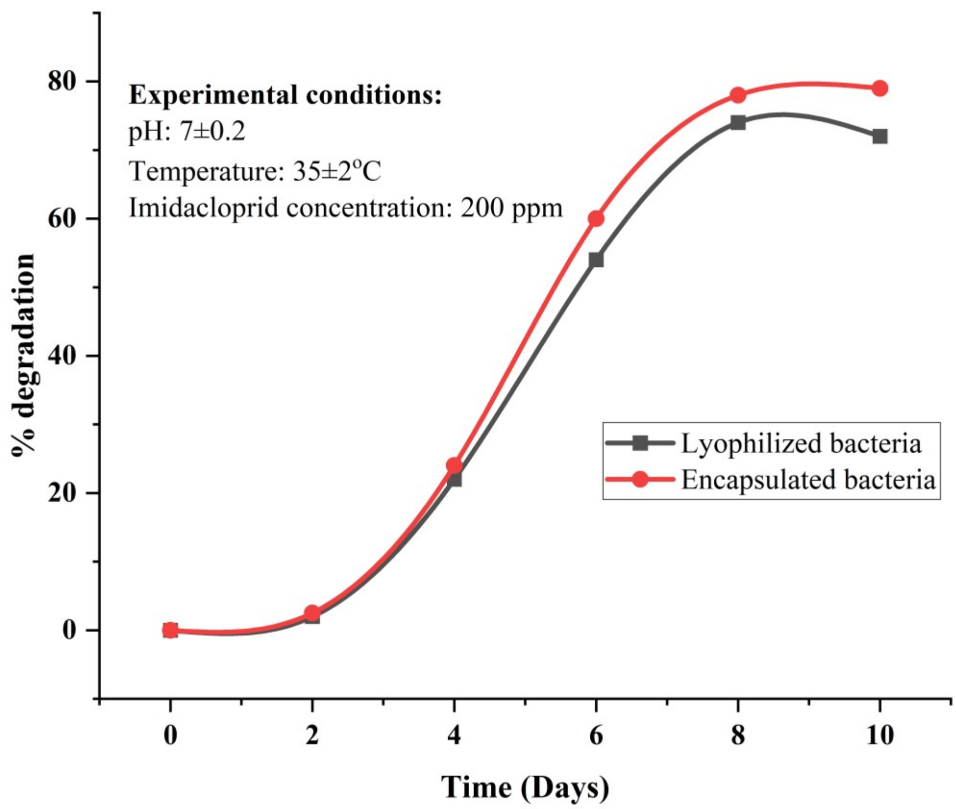

Figure 8.1: Imidacloprid degradation by lyophilized and encapsulated bacteria

To address the decline in efficiency over prolonged storage periods, the bacteria were encapsulated and subsequently employed in biodegradation experiments. It can be noted that the bacteria were initially able to degrade around 80% of the insecticide; however, on storage, it decreased gradually to 63%. The effect of long-term storage on bacteria has been presented in Figure 8.2. It can be observed that the lyophilized cells could effectively degrade imidacloprid for 200 days without any reduction in the degradation percentage. However, the degrading efficiency was found to drop marginally after 250 days. Further, after 300 days and 350 days, there was a further reduction in degradation. The biodegradation percentage decreased from about 80% to approximately 73%.

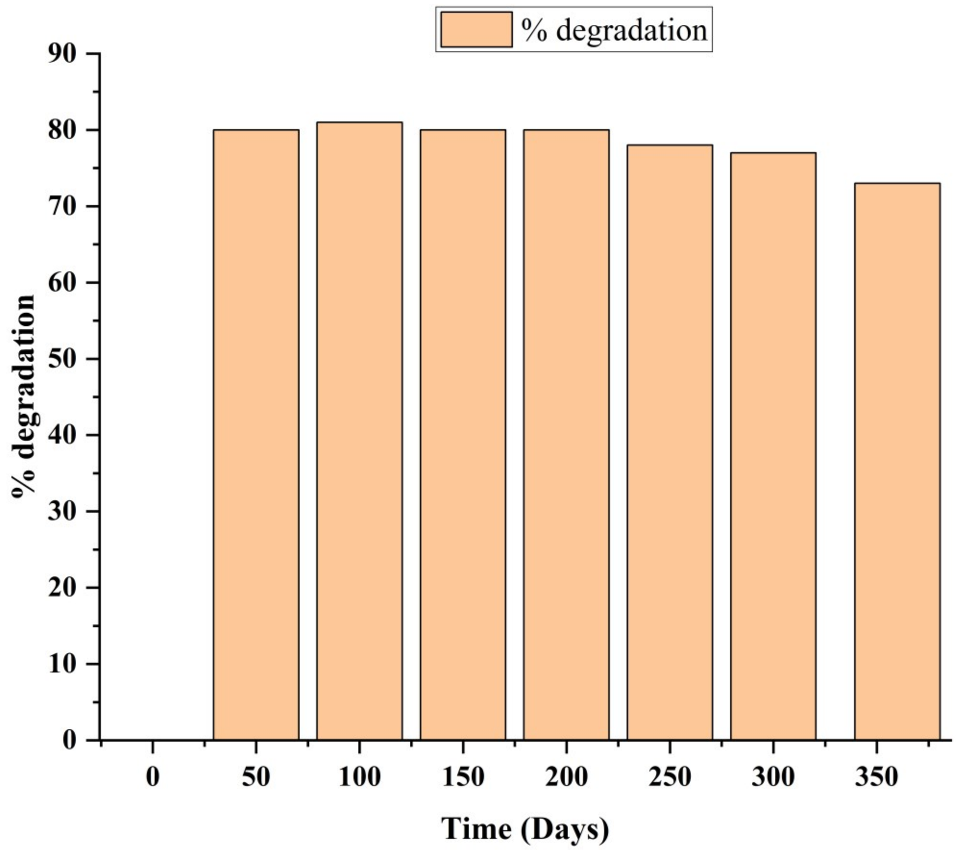

Figure 8.2: Imidacloprid degradation by lyophilized bacteria with time

After the process of lyophilization, which is also known as freeze-drying, the viability of cells tends to decrease gradually over time due to a variety of factors. These factors include the stress caused by rehydration, the formation of ice crystals within the cells, oxidative stress, the loss of protective compounds that are crucial for cell survival, the slow recovery of cellular functions, potential genetic mutations, the formation of ice within the cells, external environmental factors, and the possibility of microbial contamination. These combined factors contribute to the gradual decline in cell viability post-lyophilization.

8.3. Imidacloprid degradation by lyophilized and encapsulated bacteria in soil microcosm

In an effort to investigate the practical application of bacteria in the removal of residual insecticides from agricultural fields, a series of soil microcosm experiments were conducted. The study involved a comparative analysis where biostimulation, the process of stimulating the growth and activity of microorganisms to enhance biodegradation, was carried out using urea. Additionally, we delved into the process of biodegradation using both lyophilized (freeze-dried) and encapsulated bacteria.

Furthermore, the combined approach of biostimulation and bioaugmentation was explored for the biodegradation of imidacloprid using the encapsulated bacteria. As a control, natural attenuation (M1) was used, where no bacterial inoculation or biostimulation was applied. The findings of the study, including the results of the various treatments and their impact on the remediation of residual insecticides, are thoroughly detailed in Figure 8.3.

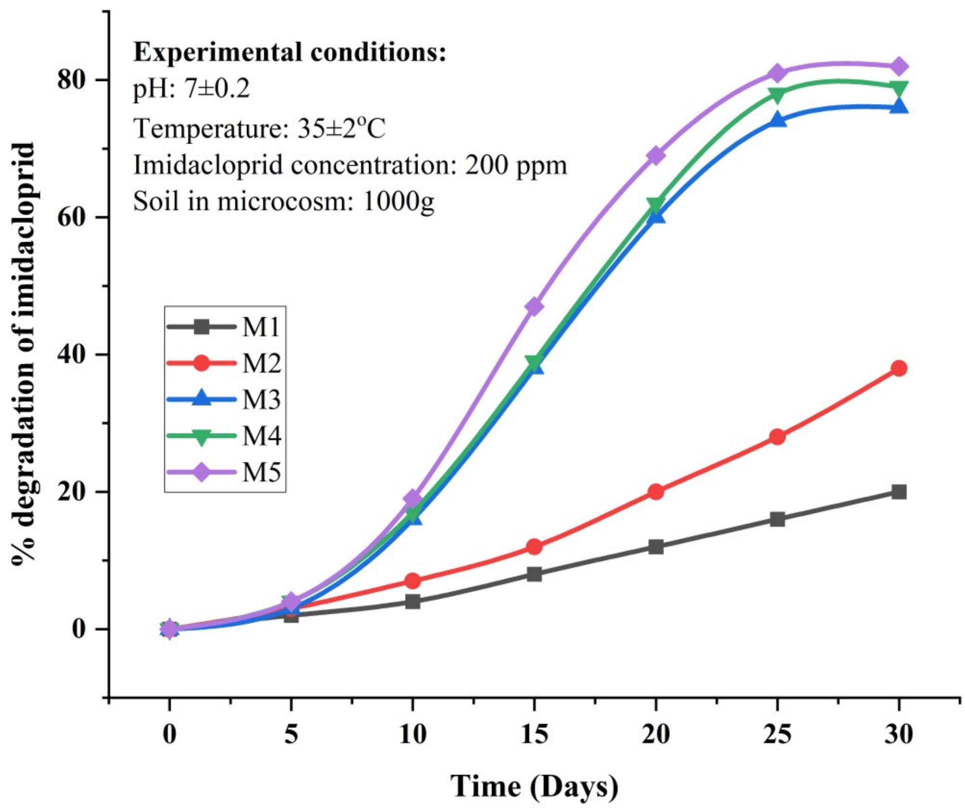

Figure 8.3: Biodegradation of imidacloprid in soil microcosm using encapsulated bacteria

In Figure 8.3, the data illustrates that over a period of 25-30 days, only around 20% of the insecticide underwent degradation. In the absence of any enhancements or interventions, imidacloprid naturally degrades to a limited extent. This baseline degradation rate indicates the inherent capacity of the environment to break down the insecticide, although slowly. Factors such as microbial activity, sunlight, and chemical reactions in the soil contribute to this natural degradation.

When biostimulation was employed, the degradation increased to approximately 38%. This treatment nearly doubles the degradation rate compared to natural conditions. The increased microbial activity accelerates the breakdown of imidacloprid, demonstrating the potential of biostimulation to enhance natural bioremediation processes effectively.

However, the introduction of imidacloprid-degrading isolated bacteria after lyophilization (bioaugmentation) resulted in a significant 78% degradation of imidacloprid within the same 30-day period. The introduction of these specialized bacteria resulted in a significant increase in degradation efficiency, with nearly four times the amount of imidacloprid being broken down compared to the natural degradation process. This result underscores the effectiveness of bioaugmentation in enhancing the bioremediation of specific contaminants.

Additionally, a combined approach utilizing both biostimulation and bioaugmentation was implemented. The results clearly indicate that employing a combination of lyophilization and encapsulation, along with biostimulation, leads to the most significant degradation of imidacloprid, achieving approximately an 84% reduction within a span of 25-30 days. Furthermore, when urea was added, a marginal enhancement in imidacloprid degradation was observed. This synergistic approach yields the highest level of degradation.

The biostimulation prepares the soil environment by enriching it with nutrients, while the bioaugmentation introduces a potent population of imidacloprid-degrading bacteria. This synergistic approach leverages the strengths of both methods, resulting in the highest observed degradation rate in the study. The use of lyophilized and encapsulated bacteria ensures that the introduced microorganisms are viable and active, contributing to the enhanced degradation process.

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Expanding this research, it would be valuable to conduct similar experiments in mesocosms and macrocosms to analyze degradation trends on a larger scale. Such studies are crucial for extrapolating these findings to real-world agricultural settings, thereby facilitating the practical implementation of these methodologies in the field.

8.4. Summary of the chapter

The experimental investigation explicitly suggests lyophilization as a dependable method for the prolonged preservation and application of bacteria isolated in degrading imidacloprid. This innovative technique not only safeguards the integrity of these bacteria but also ensures their availability for future applications aimed at combatting a wide range of organic pollutants. However, the study reveals a gradual decline in cell viability with time. Encapsulation emerges as a pivotal strategy in this regard, shielding bacterial cells from mechanical and environmental stresses, thereby supporting their longevity during storage.

Moreover, the practical application of lyophilized and encapsulated bacterial cells within soil microcosms has yielded promising results. These controlled laboratory experiments provide compelling evidence of the efficacy of this approach in degrading imidacloprid residues. Extrapolating these findings to real-world agricultural settings presents an appealing prospect for farmers and agronomists alike. Similar experiments can be replicated on a large-scale in mesocosms and macrocosms, which can be helpful for agriculturalists and facilitate the successful remediation of recalcitrant insecticide residues from agricultural fields that could not be naturally degraded. This proactive approach not only promises to address the persistent challenge posed by recalcitrant insecticide residues but also paves the way for a paradigm shift in agricultural remediation strategies. As such, the integration of lyophilized and encapsulated bacterial cells holds immense potential in fostering the remediation of pesticide contamination and fostering environmentally viable agricultural practices.