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Effect of Temperature on the Biodegradation of Glyphosate by Soil Bacteria

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ABSTRACT

Glyphosate, a broad-spectrum systemic herbicide, is widely used in agriculture to control weeds. Its extensive use has raised concerns about its environmental impact and persistence in ecosystems. Understanding the biodegradation of glyphosate is crucial for evaluating its long-term effects and developing effective remediation strategies. Biodegradation is the process by which microorganisms break down substances and plays a vital role in mitigating the accumulation of harmful compounds in the environment. Therefore, this study investigates the effect of temperature on glyphosate degradation by soil bacteria, which is crucial for understanding its breakdown in soil ecosystems. Soil bacteria have shown potential in degrading glyphosate, but the impact of temperature remains understudied. The research aims to study the effect of temperature conditions on glyphosate degradation by soil bacteria. Soil samples were collected from Mardi Bachok, Kelantan, and their coordinates, pH, and temperature were recorded. Soil samples were incubated in Mineral Salts Medium (MSM) containing 100 mg/L glyphosate at 28°C and 37°C for seven days, with optical density measured every 24 hours. Both temperature treatments showed microbial communities capable of thriving in glyphosate-supplemented MSM, utilizing it as a sole carbon and phosphate source. Statistical analysis revealed no significant difference in microbial growth between the two temperatures. These results suggest that soil bacteria are capable of thriving in a range of temperature conditions while effectively degrading glyphosate. Understanding these dynamics is essential for developing effective bioremediation strategies and predicting the environmental fate of glyphosate in various climatic conditions. Future research should explore additional environmental factors and microbial interactions to further elucidate the complexities of glyphosate biodegradation in soil ecosystems.

INTRODUCTION

The glyphosate [N-(phosphonomethyl) glycine] $C_3H_8NO_5P$ is a synthetic pesticide used most often in horticulture, agriculture, and urban areas. It is the active ingredient in many popular weed killers and is commonly used to control unwanted plants and weeds in agriculture, forestry, gardening, and landscaping. Glyphosate was the second-most used herbicide in 2007, behind 2,4-D, in the commercial, government, and industrial and agricultural sectors in the United States of America. Globally, glyphosate-based herbicides (GBHs) have increased in usage 100-fold in terms of both frequency and volume from the year 1970 to 2016, with more growth expected in the future [1]. This helped GBHs become market highest in the global pesticide market. Thus, 600 000 to 750 000 tonnes of glyphosate are used per year, and by 2025, that number will rise between 740 000 to 920 000 tonnes [2].

Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum systemic pesticide and crop desiccant. As a systemic pesticide, glyphosate is absorbed by the foliage and then translocated throughout the plant, inhibiting the growth of grasses and weeds that threaten plants. The 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) enzyme in plants is inhibited by this organophosphorus compound, specifically a phosphonate. Considering that

aminomethylphosphonic acid (AMPA), the result of glyphosate's transformation is also extensively present in the environment and is currently classified as a possible carcinogen [1,3,4].

Glyphosate contamination is a potential issue in both agricultural and ecological environments. To decrease glyphosate contamination, several techniques and optimal strategies can be implemented, including the utilization of alternative pest management technologies and biological remediation. Alternative pest control approaches involve using alternative techniques for managing weeds and pests, including organic farming practices, crop rotation, and integrated pest management (IPM) [5]. Biological remediation studies applying biological techniques to address the issue of glyphosate-contaminated soil and water. Bioremediation is a process that involves employing living or dead biological systems, such as microalgae, fungi, bacteria, and plants, to remove pollutants from various environmental sources such as water, air, flue gases, soil, and industrial effluents [6]. Specific bacteria can decompose glyphosate gradually.

Microorganisms have challenges degrading glyphosate in the environment. Soil bacteria are known to degrade glyphosate, but the effect of temperature on this process has not been well studied. The interactions between glyphosate, soil bacteria, and temperature could be a key factor in determining how quickly the compound breaks down, potential metabolite formation, and the overall fate of the herbicide in soil ecosystems [7]. The study of the effect of temperature on glyphosate biodegradation by soil bacteria is significant because it identifies the optimal temperature for a higher biodegradation rate.

Studying the impact of temperature on biodegradation helps determine how quickly the compound breaks down in different temperature conditions, affecting its persistence and potential for accumulation in soil and water. Understanding the optimal temperature for higher glyphosate biodegradation rates holds several implications for farming practices and industry dynamics. Farmers can benefit from knowing the optimal temperature conditions for glyphosate biodegradation, as this knowledge aids in developing sustainable agricultural practices. By aligning herbicide applications with temperature conditions conducive to faster biodegradation, farmers can potentially reduce the environmental impact of glyphosate. Thus, this study aims to investigate how temperature influences the glyphosate biodegradation by soil bacteria.

MATERIALS AND METHODS

Sampling Site and Sampling Method

The soil sample was collected from Mardi Bachok, Kelantan **(Fig. 1).** The soil was randomly collected from three sampling stations with their coordinates for Location 1: 102.425227/E 102° 25' 30.817", Location 2: 102.426504/E 102° 25' 35.415" and Location 3: 102.417820 / E 102° 25' 4.15" that had been exposed to the pesticide. The temperature, and pH of the soil were recorded using a digital soil meter. The soil sample was taken from a surface layer and placed in sterile sample containers before being transported to the laboratory for further analysis.

Fig. 1. The sampling area is in Mardi Bachok, Kelantan, Malaysia.

Preparation of Mineral Salts Medium (MSM)

For the preparation of modified Mineral Salts Medium (MSM), 10 g of sodium sulphate Na_2SO_4, 13.5 g of ammonium sulfate $(NH_4)_2SO_4$, 2.5 g ammonium chloride NH_4Cl, 70 g of potassium dihydrogen phosphate KH_2PO_4 and 18 g of sodium phosphate monobasic NaH_2PO_4 were dissolved in 5 liters distilled water [8]. The solutions were mixed well after addition. The pH of the mixture was measured using a pH meter. The mixture was poured into the 250 ml conical flask and was sterilized by autoclave for 15 minutes at 121°C to avoid contamination.

Enrichment Culture Preparation

For the enrichment culture preparation, 2 g of soil samples were added to MSM media containing 100 mg/L of glyphosate in a conical flask sealed with gauze and cotton. The enrichment culture was incubated at 28°C and 37°C using an incubator shaker, Innova 44 by New Brunswick, at 150 rpm (revolutions per minute) for 7 days [8].

Optical Density Measurement

For optical density (OD) measurement, a cuvette was filled with 1 ml of MSM media as a blank medium using a micropipette of 1000 µl and inserted into the spectrophotometer at 600 nm. Another cuvette was filled with 1 ml of sample solution using a micropipette 1000 µl and was inserted into the spectrophotometer at 600 nm. The OD data were recorded every 24 hours on days 0, 1, 2, 3, 4, 5, 6, and 7 to monitor the growth of the soil bacteria.

Statistical Analysis

The difference in absorbance of bacteria growth between treatments at 28°C and 37°C was analyzed statistically using IBM SPSS software. A t-test was conducted to identify significant differences in absorbance within two different culture mediums. A p-value of less than 0.05 was considered statistically significant, indicating notable differences in microbial growth under the specified temperature conditions.

RESULTS AND DISCUSSION

Effect of temperature on microbial growth at 28°C and 37°C for Location 1

At the initial incubation treatment at 28°C, which was on day 0, the growth pattern of bacteria for treatment at location 1 increased rapidly until day 3 **(Fig. 2).** It was identified as the log phase. From 3 until day 4, which is the stationary phase, the growth pattern of bacteria increased slowly and decreased until day seven which was identified as the death phase. At the start of incubation at 37°C, the growth pattern of bacteria for treatment at location 1 increased rapidly at the log phase, which is from day 0 until day 2. In the stationary phase, the microbial growth does not increase rapidly, which is from day 2 until day 3. The death phase was from day 3 until day 7 since the microbial growth decreased rapidly.

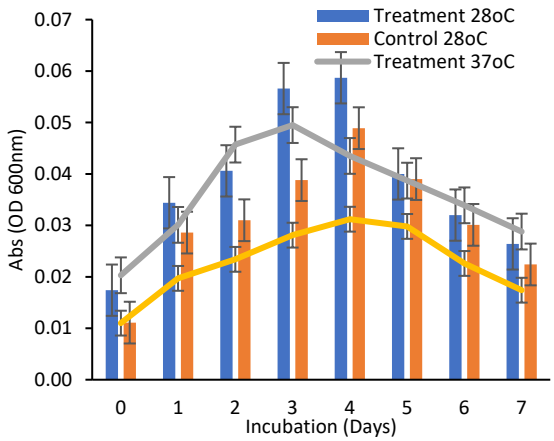

Fig. 2. Microbial growth during the treatment process at 28°C and 37°C for Location 1.

Effect of temperature on microbial growth at 28°C and 37°C for Location 2

For location 2, the growth pattern increased rapidly from day 0 until day 2 at 28°C **(Fig. 3)**. It was a log phase. The microbial growth showed a slight increase from day 2 until day 3 since it was a stationary phase, and it decreased rapidly from day 3 until day 7 since it was the death phase. For treatment at 37°C, the log phase started from day 0 until day 4 since the growth pattern of bacteria significantly increased. The stationary phase started from day 4 until day 5 and the death phase started from day 5 until day 7 since the number of bacteria decreased.

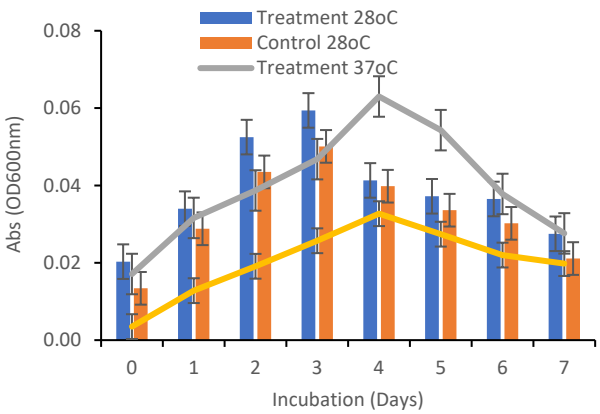

Fig. 3. Microbial growth during the treatment process at 28°C and 37°C for Location 2

Effect of temperature on microbial growth at 28°C and 37°C for Location 3

At the start of incubation, the growth pattern of bacteria for treatment at 28°C for location 3 increased rapidly at the log phase, which is from day 0 until day 2 **(Fig. 4)**. In the stationary phase, the microbial growth does not increase rapidly, which is from day 2 until day 3. The death phase was from day 3 until day 7 since the microbial growth decreased rapidly. For the treatment at 37°C, the microbial growth increased rapidly at the log phase from 0 until day 4. The growth pattern showed that the microbial growth was at the death phase since the growth of bacteria decreased from day 4 until day 7. The microbial growth for treatment for all locations was higher than the control since the treatment consisted of 100 mg/L of glyphosate.

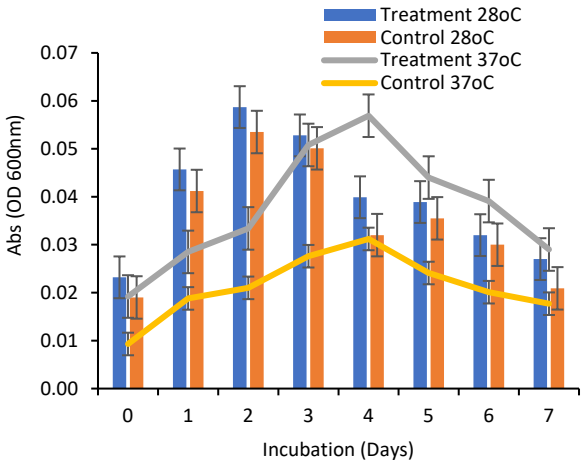

Fig. 4. Microbial growth during the treatment process at 28°C and 37°C for Location 3.

Based on the growth pattern of bacteria in both treatments showed they could grow and multiply in MSM supplied with 100 mg/L glyphosate and utilized glyphosate as the sole source of carbon and phosphate. The statistical analysis showed a non-significant difference (P-value ≥ 0.05) between treatment 28°C and 37°C. According to Manogaran et al. [9], the optimum glyphosate degradation occurred from 30°C to 35°C, illustrating at least 80% degradation. The bacterial community in locations 1, 2, and 3 can degrade glyphosate biologically by using it as a source of phosphorus [10]. Pesticides are typically broken down by microorganisms, which turns them into a food source for growth and energy [11]. Soil bacteria such as *Pseudomonas* sp. and *Bacillus* sp. are effective strategies to degrade glyphosate in contaminated soils and aquifers since these bacteria can use glyphosate as the sole source of phosphorus [12]. These findings indicated that the growth rates of the two strains reflected glyphosate degradation trends. This means that glyphosate supported the growth of the bacterial isolate since the growth was higher than the control [13].

The bacteria use pesticides as a primary source of microbial nutrients, and as a result, they break down into small, non-toxic molecules like CO_2 and H_2O and prevent secondary pollution. This is the mechanism behind the growth of these bacteria [14]. Several glyphosate degradation pathways have been identified in microorganisms, including oxidation, reduction, dehydrogenation, dehalogenation, and decarboxylation [15]. Meanwhile, mineralization and co-metabolism by microbes like bacteria are the main processes leading to the additional breakdown of pesticides and their intermediate products [16]. The degradation mechanism was broken down into three main

components. First, the target pesticides were adsorbed onto the bacterial cell membrane surface. Second, the target entered the cell through the cell membrane's surface, and the target's molecular structure determined the penetration rate and efficiency. Thirdly, enzymatic reactions occur in the membrane where pesticide targets are located [17].

The process of binary fission causes cells to divide regularly during the log (exponential) phase. The culture reaches the maximum growth rate, which could be estimated by generation time or doubling time [18]. As the microbial growth increased, the rate of glyphosate degradation also increased, indicating that the cells were in an exponential stage and were sustaining themselves with an alternate source of carbon, phosphate, and energy.

The bacteria use the carbon from glyphosate as an energy source for growth and cell division, which results in high metabolic activity. Degradation of pesticides is often related to the typical sigmoidal microbial growth curves with a significant lag, exponential increase, saturation phase, and death phase [19]. During the stationary phase, the number of cells that are dividing seems to be equal to the number of cells that have died from nutrient exhaustion [18]. The cell population remains constant at this stage. During the death phase, bacteria lose the ability to divide, and the number of dead cells exceeds that of live cells [20]. The growth rate of the bacteria cells is limited by the accumulation of toxic compounds and the depletion of nutrients in the media.

CONCLUSION

After the treatment using different temperatures, 28°C and 37°C, the microbial community grew higher than the control. The microbial community can utilize glyphosate as the sole source of nutrients and phosphorus. Both 28°C and 37°C show non-significant differences in the growth of the microbial community during the treatment process. A further recommendation of this study is to explore a broader range of temperatures to identify the optimal conditions for microbial growth. This can investigate a broader spectrum of temperatures to determine the ideal conditions for microbial development.

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