Study on factors affecting the remediation of DDT-contaminated soil using the Biosoil bio-preparation

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ABSTRACT

This study evaluates the remediation potential of the bioproduct Biosoil containing Bacillus subtilis for DDT-contaminated soil and identifies factors affecting treatment efficiency. Two field soil samples with initial DDT concentrations of approximately 80 mg/kg (MĐ-TXL-01) and 120 mg/kg (MĐ-TXL-02), exceeding the limits specified in QCVN 03:2023/BTNMT, were investigated. Experiments were conducted with straw supplementation to improve soil porosity, while the effects of temperature, product dosage, and treatment time were assessed. Isothermal conditions showed higher efficiency; however, variable-temperature conditions were selected for practical application. A 3% product dosage was optimal in terms of both technical performance and cost. DDT concentrations decreased rapidly within the first 5÷20 days, followed by a slower degradation rate. After 20 days of treatment with 3% Biosoil under variable-temperature conditions, MĐ-TXL-01 achieved concentrations below the permissible limit, while MĐ-TXL-02 remained above the threshold. The results demonstrate the potential of Bacillus subtilis-based Biosoil for DDT soil remediation; however, additional measures such as nutrient supplementation, soil turning, or supporting technologies are needed for highly contaminated soils.

Keywords: DDT-contaminated soil; Biosoil; Bio-preparation.

1. INTRODUCTION

DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane], an organochlorine pesticide widely used for decades, is a highly toxic persistent organic pollutant (POP) with strong bioaccumulation potential and an environmental half-life of 20÷30 years [4]. DDT and its metabolites can persist in soil, water, and sediments, causing adverse effects on ecosystems and human health through the food chain [4, 7]. Studies have shown that DDT is associated with endocrine disruption, reproductive dysfunction, neurotoxicity, and carcinogenic risks [4]. Various treatment technologies, including secure landfilling, high-temperature incineration, advanced oxidation, UV photocatalysis, and chemical treatment, have been investigated and applied [2, 7, 14]. However, these methods often require high investment and operating costs, consume significant energy, and may generate toxic by-products leading to secondary pollution. Biological treatment using microorganisms has recently emerged as a promising alternative due to its environmental friendliness, low cost, and in situ applicability [2,5]. Several microbial groups, including *Pseudomonas*, *Sphingomonas*, *Alcaligenes*, *Bacillus*, and white-rot fungi, have been reported to participate in DDT degradation through dechlorination, oxidation, and aromatic ring cleavage reactions [1, 8].

Compared with conventional physicochemical methods, bioremediation minimizes secondary waste generation, causes less disturbance to soil properties, and supports the recovery of soil biological activity after treatment. However, DDT degradation efficiency remains limited because of its hydrophobicity, low solubility, and strong adsorption to soil organic matter, which restrict microbial access to the substrate [5]. Treatment performance is also strongly influenced by pH, temperature, moisture, organic matter content, and microbial density [7]. Moreover, many studies remain at the laboratory scale, while field applicability and long-term stability of microbial systems

require further evaluation [9]. This represents an important research gap in improving the practical application of biotechnology for DDT-contaminated soil remediation.

Recent research has focused on microbial consortia, in which multiple microbial species interact synergistically to enhance the degradation of persistent organic compounds [9]. Different microbial groups within consortia can complement each other through the production of enzymes such as dehalogenase, monooxygenase, lignin peroxidase, and laccase, which contribute to DDT dechlorination, oxidation, and aromatic structure cleavage [11]. Some strains can also produce biosurfactants that improve DDT solubility and bioavailability in soil [10].

Nevertheless, single-strain studies remain important for evaluating the degradation capability of specific microorganisms under contaminated soil conditions, clarifying their roles and mechanisms, and controlling factors affecting biodegradation efficiency. The selected microorganism in this study belongs to the genus *Bacillus*, which exhibits high adaptability under adverse conditions through spore formation and extracellular enzyme production, including dehalogenase, oxidase, and peroxidase, contributing to pollutant transformation [6]. Although single strains may show lower efficiency than microbial consortia due to a narrower enzymatic spectrum, they provide valuable insights into DDT degradation mechanisms and support the development of specialized bioproducts for sustainable soil remediation.

The bioproduct Biosoil was selected because it contains *Bacillus subtilis*, a strain capable of degrading complex organic compounds and adapting to contaminated soil environments. *Bacillus* strains contribute through extracellular enzyme production, spore formation under unfavorable conditions, and promotion of organic substrate transformation [6]. Although *Bacillus subtilis* is not considered a major microorganism for complete degradation of persistent organochlorine compounds due to its limited capacity for aromatic ring cleavage, it plays a supportive role by improving microbial system stability, enhancing adaptation under field conditions, and contributing to overall treatment performance [10]. The use of commercially available Biosoil in this study aimed not only to evaluate DDT removal efficiency but also to assess the practical potential of this bioproduct for sustainable and environmentally friendly soil restoration.

2. STUDY SUBJECTS AND METHODS

2.1. Study subjects

Soil samples were collected from the burial area of DDT-contaminated soil currently managed by Artillery Brigade 204, Artillery and Missile Corps. The samples were randomly taken from the 0-50 cm soil layer at the burial site. After mechanical impurities had been removed and the samples had been homogenized, they were placed in sealed PE bags and transported to the laboratory. The analytical results of the two selected samples, with concentrations of approximately 80 mg/kg and 120 mg/kg, were designated MĐ-TXL-01 and MĐ-TXL-02, respectively. The analysis of the soil parameters of sample showed that the moisture content was 18.02%, which corresponded to a moderate level for natural soil and did not affect the representativeness of the sample. The organic carbon content (OC = 0.93%) and total organic carbon content (TOC = 1.56%) were both low, reflecting soil poor in organic matter and having low fertility. The total nitrogen content was 0.08%, corresponding to a C/N ratio of approximately 11÷12, which was consistent with natural soil and did not indicate any abnormal nutrient condition or nitrogen contamination. The pH_{KCl} value of 6.56 indicated a slightly acidic to neutral soil environment, which was consistent with the common characteristics of soils in Northern Vietnam.

The commercial bio-preparation Biosoil is a ready-to-use product manufactured by Xingtai Sinobest Biotech Co., and supplied in the form of a brown powder with no unusual odor. The product contained *Bacillus subtilis* at a high microbial density (>20×10^9 CFU/g). This bacterial strain is well adapted to adverse environmental conditions owing to its spore-forming ability and

its capacity to secrete extracellular enzymes that support the degradation of organic matter. Its specifications are presented in Table 1.

Table 1. *Technical Specifications of the bio-preparation.*

No.	Technical Parameter	Unit	Standard	Result
1	*Bacillus subtilis*	CFU/g	$\geq 20\times10^9$	10.6×10^{10}
2	Physicochemical properties			
-	Moisture	%	≤ 8	4.20
-	Through rate of 40 mesh standard sieve	**mesh**	≥ 80	95
-	**Bacterial contaminant rate**	%	≤ 20.0	15
3	Heavy metals			
-	Arsenic (As)	mg/kg	≤ 30	0.3
-	Lead (Pb)	mg/kg	≤ 200	12
-	Cadmium (Cd)	mg/kg	≤ 15	Not detected
4	Pathogenic microorganisms			
-	*Salmonella*	**CFU/25g**	≤ 0	Not detected

2.2. Research methods

2.2.1. Experimental model

Figure 1. *Illustrates the experimental setup for the DDT-contaminated soil treatment using the Biosoil bio-preparation.*

The DDT-contaminated soil samples were sieved through a steel mesh to remove stones and gravel, then ground and thoroughly mixed to improve homogeneity. Three kilograms of prepared soil were placed into clean containers, and Biosoil was added at predetermined ratios. Straw pieces (2 ÷ 3 cm) were mixed into the samples at approximately 3% to increase porosity, improve aeration, and enhance microbial nutrient exchange and dispersion. The samples were incubated at 25 ÷ 30 °C, covered with plastic film with an air vent, for 5 ÷ 40 days. Soil samples were collected at different intervals to monitor changes in DDT concentration.

The effects of key factors were evaluated, including temperature (20 days, 3% Biosoil dosage under variable environmental temperature (22 ÷ 28 °C in October 2025) and controlled temperature (26 °C)), Biosoil dosage (2%, 3%, and 4% for 20 days under variable-temperature conditions), and treatment duration (up to 40 days with 3% Biosoil under variable-temperature conditions).

2.2.2. DDT analysis method

Soil samples were analyzed for DDT content following the USEPA 8280B method using gas chromatography-mass spectrometry (GC-MS) with an Agilent 6890 gas chromatograph coupled to an Agilent 5975 mass spectrometer (Agilent, USA).

The chromatographic program was as follows: initial temperature of 80 °C; ramped at 20

°C/min to 130 °C, then at 5 °C/min to 210 °C, followed by 15 °C/min to 300 °C and held for 10 minutes. The analysis was conducted under isocratic flow conditions, with helium as the carrier gas at a flow rate of 1.0 mL/min; injection volume of 1 µL in splitless mode. Mass spectrometry utilized electron impact (EI) ionization, with an ion source temperature of 220 °C.

DDT treatment efficiency was calculated using formula:

$$CH = \frac{C_O - C_t}{C_O} \times 100\% \quad (1)$$

Where: CH is the treatment efficiency (%); Co and Ct are the concentrations of TNT at the initial time and time t, respectively, in mg/L (mg/L).

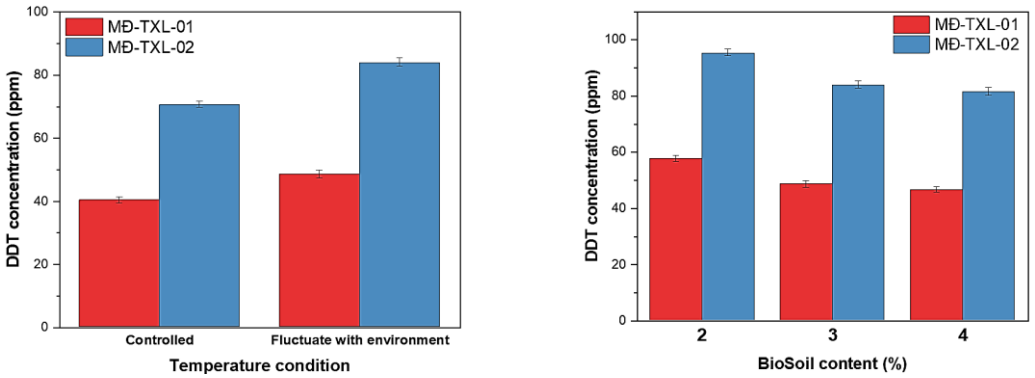
3. RESULTS AND DISCUSSION

3.1. Assessment of the characteristics of DDT-contaminated soil

The analytical results of the two selected soil samples showed that the DDT concentrations in samples MĐ-TXL-01 and MĐ-TXL-02 were 81.8 mg/kg and 121.5 mg/kg, respectively. Analysis of DDT content indicated that the measured values were 1.64 ÷ 2.43 times higher than the regulatory limit (50 mg/kg) for total DDT content specified in QCVN 03:2023/BTNMT - the National Technical Regulation on Soil Quality, which applies to the soil samples under study.

3.2. Study on the biodegradation of DDT using the bio-preparation

3.2.1. Effect of temperature on the DDT degradation ability of bacillus strains



a) *b)*

Figure 2. *a) Evaluation of DDT degradation by bacillus strains environment-dependent temperature and controlled temperature conditions;*
b) Effect of bio-preparation dosage on DDT degradation efficiency.

Experiments were conducted to evaluate the DDT degradation capability of Bacillus subtilis strains over 20 days, using a Biosoil dosage of 3% under two temperature conditions: Environment-dependent temperature (October 2025, ranging from 22 ÷ 28 °C) and Controlled temperature (26 °C).

The results in Figure 2a show that after 20 days of treatment under variable-temperature conditions, DDT concentrations in samples MĐ-TXL-01 and MĐ-TXL-02 decreased to 48.5 mg/kg and 83.8 mg/kg, corresponding to degradation efficiencies of 40.71% and 31.03%, respectively. Under controlled temperature conditions, DDT concentrations decreased further to 40.25 mg/kg and 70.46 mg/kg, with efficiencies of 50.97% and 42.01%. Thus, controlled temperature improved degradation efficiency by approximately 10÷11% compared with variable-temperature conditions. This improvement was attributed to the more favorable conditions for microbial growth and enzyme activity under stable temperatures, whereas temperature fluctuations could inhibit microbial performance and reduce DDT degradation efficiency.

These findings agree with Pan et al. (2016), who reported enhanced DDT degradation by *Stenotrophomonas* sp. DDT-1 when temperature increased from 15 °C to 35 °C, with DDT half-life decreasing from 17.7 to approximately 11.5 days under optimal conditions [12]. Similarly, Baczynski et al. (2010) observed increased degradation rates of organochlorine compounds under stable neutral temperature conditions due to enhanced microbial metabolism and enzymatic activity [13].

However, maintaining controlled temperature is impractical and costly for field-scale soil remediation. Variable-temperature conditions better represent natural environmental fluctuations and require no additional temperature-control systems. Although degradation efficiency was lower, the results remained favorable (approximately 30÷40% after 20 days), indicating that Biosoil maintained effective activity under realistic conditions. Therefore, variable-temperature conditions were selected for subsequent experiments.

3.2.2. Effect of bio-preparation dosage on DDT degradation efficiency

To investigate the effect of bio-preparation dosage on the DDT degradation process, experiments were conducted at three dosages (2.0%, 3.0%, and 4.0% by weight) over 20 days under fluctuating temperature conditions with two soil samples, MĐ-TXL-01 and MĐ-TXL-02.

The results in Figure 2b show that DDT concentration decreased with increasing bio-preparation dosage. At a 2% dosage, DDT concentrations in MĐ-TXL-01 and MĐ-TXL-02 decreased to 57.6 mg/kg and 95.2 mg/kg, corresponding to degradation efficiencies of 29.58% and 21.65%, respectively. Increasing the dosage to 3% improved degradation significantly, reducing DDT concentrations to 48.5 mg/kg and 83.8 mg/kg, with efficiencies of 40.71% and 31.03%. However, increasing the dosage further to 4% resulted in only slight improvements, with efficiencies of 43.35% and 33.05%. Thus, increasing the dosage from 2% to 3% enhanced degradation efficiency by approximately 10%, whereas increasing from 3% to 4% improved efficiency by only about 3%. This trend is consistent with previous studies on microbial treatment of persistent organic pollutants [5].

The results indicate that DDT degradation does not increase proportionally with bio-preparation dosage beyond an optimal level. At low dosage (2%), insufficient microbial density limits enzyme production and degradation capacity. At 3%, increased microbial populations enhance enzyme secretion and pollutant degradation. However, excessive dosage (4%) may lead to nutrient competition, oxygen limitations, spatial constraints, and possible inhibition by accumulated degradation intermediates [10].

Therefore, a 3% bio-preparation dosage was considered optimal, providing a balance between degradation efficiency, cost-effectiveness, and practical applicability. Further dosage increases offer limited technical and economic benefits.

3.2.3. Effect of treatment duration on DDT degradation

To investigate the effect of treatment duration on DDT degradation efficiency, experiments were conducted over 40 days under fluctuating ambient temperature conditions, with a bio-preparation dosage of 3%, using two soil samples, MĐ-TXL-01 and MĐ-TXL-02. Soil samples were collected at 5, 10, 15, 20, 30, and 40 days to analyze the remaining DDT content.

The results indicate that treatment duration significantly affects DDT degradation in soil (Figure 3). During the first 5 ÷ 20 days, DDT concentrations decreased rapidly: in MĐ-TXL-01, from 81.8 ppm to 48.5 ppm, corresponding to a degradation efficiency of 40.71%, and in MĐ-TXL-02, from 121.5 ppm to 83.8 ppm, with an efficiency of 31.0%. Extending the treatment period to 40 days resulted in a slower reduction rate, indicating that the degradation process gradually approached a steady state. This behavior is related to biodegradation kinetics: during the initial stage, microorganisms adapt to the environment, utilize DDT as a substrate, and increase in

population, leading to higher degradation rates. In the later stage, reduced DDT availability, accumulation of intermediate metabolites such as DDD, DDE, and DDMU [7], nutrient depletion, oxygen limitations, and microbial competition may restrict further degradation.

After 20 days of treatment, DDT concentration in MĐ-TXL-01 decreased below 50 mg/kg, meeting the Category 3 soil limit specified in QCVN 03:2023/BTNMT. However, MĐ-TXL-02 remained above the regulatory threshold, indicating insufficient removal efficiency for higher contamination levels. Additional measures, including increased bio-preparation dosage, nutrient supplementation, soil mixing, or integration with other remediation methods, are therefore required to improve treatment performance.

The results suggest that 5 ÷ 20 days is the most effective treatment period. In practical applications, enhancement measures introduced after this period may help maintain degradation efficiency and shorten the overall remediation time.

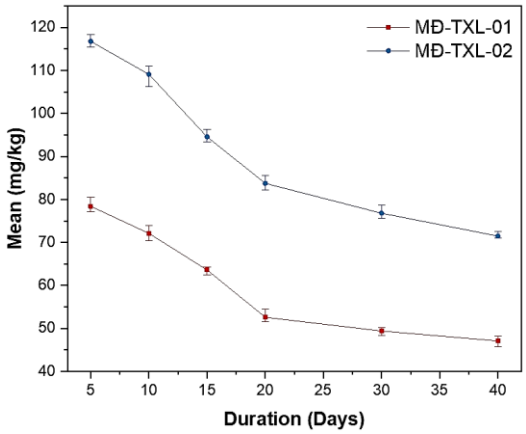


Figure 3. *Effect of treatment duration on DDT degradation using the Biosoil bio-preparation.*

4. CONCLUSIONS

The study demonstrated that the efficiency of DDT removal from contaminated soil by the Biosoil bioproduct containing *Bacillus* strains was significantly influenced by temperature conditions, product dosage, and treatment time. Higher degradation efficiency was obtained under constant-temperature conditions; however, variable-temperature conditions were selected to ensure practical applicability. A bioproduct dosage of 3% was determined to be optimal in terms of both technical performance and economic efficiency. DDT degradation occurred rapidly during the first 5–20 days and gradually slowed during the later stage because of substrate depletion and environmental limitations. The laboratory results confirmed that the application of the Biosoil bioproduct for the treatment of DDT-contaminated soil was feasible and showed considerable potential for large-scale implementation. Further studies on enhancement measures are still required to maintain long-term treatment efficiency.

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TÓM TẮT

Nghiên cứu một số yếu tố ảnh hưởng tới quá trình xử lý đất nhiễm DDT sử dụng chế phẩm sinh học Biosoil

Nghiên cứu này đánh giá hiệu quả của chế phẩm sinh học Biosoil chứa chủng Bacillus trong xử lý đất nhiễm DDT. Thí nghiệm được tiến hành tại các điều kiện nhiệt độ, tỉ lệ chế phẩm, thời gian xử lý khác nhau, kết quả như sau: Ảnh hưởng của nhiệt độ, điều kiện ổn nhiệt (26 ^oC) cho hiệu suất xử lý cao hơn so với điều kiện biến nhiệt (22 ÷ 28 ^oC). Tuy nhiên, lựa chọn chế độ biến nhiệt để nghiên cứu nhằm đảm bảo tính khả thi, ứng dụng vào thực tế. Tỷ lệ chế phẩm sinh học: 3% được xác định là phù hợp nhất về cả hiệu quả kỹ thuật và kinh tế. Thời gian xử lý, quá trình phân hủy DDT diễn ra mạnh trong giai đoạn đầu (5 ÷ 20 ngày) và chậm lại ở giai đoạn sau (20 ÷ 40 ngày). Những kết quả này minh chứng cho tính khả thi của phương pháp xử lý DDT trong đất ô nhiễm bằng chế phẩm Biosoil chứa chủng vi sinh vật Bacillus là hoàn toàn khả thi và có tiềm năng để triển khai quy mô lớn.

Từ khóa: Đất nhiễm DDT; Biosoil; Chế phẩm sinh học.