

ENHANCING BUTACHLOR DEGRADATION IN SOIL BY BIOAUGMENTATION OF BUTACHLOR-DEGRADING BACTERIA AND MUNG BEAN CULTIVATION

Nguyen Thi Oanh*, Hoang Thi Nghiep, and Nguyen Kim Bup

Faculty of Natural Sciences Teacher Education, Dong Thap University, Vietnam

*Corresponding author: Nguyen Thi Oanh, Email: noanh@dthu.edu.vn

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Abstract

*Butachlor has been extensively used to control weeds globally. In this study, the degradation performances of butachlor in soil by attenuation, augmentation with *Pseudomonas sp. But1* and *Pseudomonas sp. But2*, and mung bean (*Vigna radiata L.*) cultivation were determined. The results showed that the overall degradation rates in soil over 30 days were as follows: sterilized soil < non-sterilized soil < mung bean cultivation < bioaugmentation. The dissipation of the herbicide in non-sterilized soil was observed to be $58.3 \pm 5.5\%$, while butachlor was completely removed from soil with both bioaugmentation and mung bean cultivation after 30 days. Moreover, mung bean cultivation stimulated rhizospheric bacteria growth and enhanced the degradation. These results showed that both bioaugmentation and mung bean cultivation significantly increased the butachlor degradation in soil.*

Keywords: Attenuation, augmentation, butachlor, degradation, mung bean.

TĂNG CƯỜNG SỰ PHÂN HUỖ BUTACHLOR TRONG ĐẤT BẰNG CÁC CHỦNG VI KHUẨN PHÂN HUỖ VÀ TRỒNG ĐẬU XANH

Nguyễn Thị Oanh*, Hoàng Thị Nghiệp và Nguyễn Kim Búp

Khoa Sư phạm Khoa học tự nhiên, Trường Đại học Đồng Tháp, Việt Nam

*Tác giả liên hệ: Nguyễn Thị Oanh, Email: ntoanh@dthu.edu.vn

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Tóm tắt

*Butachlor được sử dụng rộng rãi để kiểm soát cỏ dại trên toàn thế giới. Trong bài báo này, khả năng phân huỷ của butachlor trong đất bởi quá trình tự nhiên, chủng vi khuẩn phân huỷ *Pseudomonas sp. But1* và *Pseudomonas sp. But2* và trồng đậu xanh (*Vigna radiata L.*) đã được khảo sát. Kết quả cho thấy tốc độ phân huỷ butachlor trong đất trong 30 ngày là: đất khử trùng < đất không khử trùng < đất trồng đậu xanh < đất có chủng vi khuẩn phân huỷ. Sự phân huỷ của butachlor trong đất không khử trùng là $58,3 \pm 5,5\%$, trong khi thuốc trừ cỏ này bị loại bỏ hoàn toàn khỏi đất khi có sự kết hợp giữa chủng vi khuẩn và trồng đậu xanh sau 30 ngày. Ngoài ra, việc trồng đậu xanh đã kích thích sự phát triển của hệ vi khuẩn vùng rễ, qua đó tăng cường sự phân huỷ. Những kết quả này cho thấy sự kết hợp giữa chủng vi khuẩn và trồng đậu xanh làm tăng nhanh sự phân huỷ butachlor trong đất.*

Từ khóa: Phân huỷ tự nhiên, chủng vi khuẩn, butachlor, phân huỷ, đậu xanh.

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1. Introduction

The overuse of pesticides causes serious environmental pollution globally. Toxic chemicals accumulation in soil and water is harmful to microorganisms, plants, animals and humans. Overall pesticide degradation in soil is mostly undertaken by indigenous microorganisms affected by the chemical and physical properties of pesticides and how they interact with biotic and abiotic soil components (Sannino et al., 1999). The size and the activity of soil microbial biomass also affect pesticide degradation rate.

Plants sustain large microbial populations in the rhizosphere by secreting substances, such as carbohydrates and amino acids, through root cells and by sloughing root epidermal cells (Turpault et al., 2007). Larger microbial populations can exist in rhizosphere soil than in bulk soil, which increases organic chemicals degradation including pesticides (Joergensen, 2000; Molina et al., 2000; Yang et al., 2011). In a previous study, the plantation reduced butachlor attenuation in soil (Yang et al., 2011).

Butachlor (N-butoxymethyl-2-chloro-2',6'-diethylacetanilide) is a pre-emergence herbicide, widely used in rice fields. This herbicide is sometimes used to control weeds for other crops such as corn and soybean in the Mekong Delta. It is one of the most commonly used to control a wide range of annual grass and broad leaf weeds (Wang et al., 2013). Butachlor has been shown to affect microbial populations and enzyme activities (Chen et al., 1981; Min et al., 2001). Environmental degradation and dissipation studies showed that butachlor is a persistent pollutant in agricultural soil (Pal et al., 2006). In soil, butachlor influences microbial community and enzyme activities (Min et al., 2002). Some butachlor-degrading bacteria have been isolated from soil. Recently, *Pseudomonas* sp. But1 and *Pseudomonas* sp. But2 showed effective butachlor degradation in liquid media (Duc et al., 2020).

In Vietnam, mung bean (*Vigna radiata*) is widely cultivated. In the Mekong Delta, mung bean is usually cultivated in rotation with rice. A previous study showed that butachlor inhibited bacterial

community in soil, and caused negative effects of the plant in the rainy season (Tran et al., 2021). In this study, butachlor degradation in soil by *Pseudomonas* sp. But1 and *Pseudomonas* sp. But2 was conducted. The roles of mung bean cultivation in butachlor degradation were also determined.

2. Materials and methods

2.1. Soil collection, fertilizing and herbicide amendment

The soil collection and fertilizing were conducted as described in a previous study (Tran et al., 2021). Soil samples were collected from a mango garden, at a depth of 0-30 cm, in Cao Lanh city, Dong Thap Province. The soil components were shown in the previous report (Tran et al., 2021). Soil was transferred into thermocol boxes up to the depth of 15 cm. The dimension of each thermocol box was 41×60×15 cm (width×length×depth). Four holes were separately punched through the bottom of each box so that redundant water could be removed. NPK fertilizer (Binh Bien Company, Ninh Binh) containing 20% total N, 10% P₂O₅ and 5% K₂O was used at 200 kg/ha.

Butachlor (purity > 98%, Sigma-Aldrich) was diluted in absolute ethanol at 0.1 mM and used as a stock solution. The stock was added into water and sprayed on soil to give an equivalence of 6 mg/kg dry soil. The herbicide was sprayed before sowing one day. Soil was thoroughly mixed before seed sowing. All experiments were conducted four replicates. Soil sterilized at 121°C for 15 minutes served as a control.

2.2. Seed sowing and mung bean growth condition

The seeds of mung bean (Vinaseed Company, Dong Thap) were pregerminated by placing them in petri dishes on wet paper towels for 24 hours at room temperature (~30°C). Tap water was sprayed to obtain soil moisture content of 40% of field capacity. Thereafter, twelve seeds were sown in each box at equidistant positions to give a density of 0.02 m² per seed.

The thermocol boxes cultivated with mung bean were placed in a greenhouse. The experiment was

carried out from 15th November to 15th December, 2020. Weather conditions during the experiment was provided by the Center of Meteorological and Hydrological Administration, Dong Thap. The temperatures were from 24.3 to 33.1 °C (28.5 °C on average). Relative humidity and sunshine-hour were 81% and 9.8 hours/day on average, respectively. There was no rain during the experiment. The cultivated plants were also watered regularly from seed sowing to harvest.

2.3. Soil collection, enumeration of soil bacteria and butachlor analysis

Soil samples were collected immediately after herbicide amendment and every 5-day period. Soil was collected by a sterilized spoon at a depth of 1.0-3.0 cm. Soil was transferred to a lab within several hours. The soil samples were mixed, pulverized, and sieved through a 2 mm mesh to eliminate large debris. Bacterial density and butachlor remaining in the soil samples were determined.

Butachlor in soil was extracted with hexane/ethyl acetate (1:1) twice according to Duc et al. (2020). Soil (5 g) was added to 10 mL of the extraction solution. The mixtures were vigorously shaken by hand for 10 minutes and then using a shaker at 250 rpm for 30 minutes. The extract was filtered using a 0.22 µm syringe filter and condensed. The mean recovery values of butachlor from the soil were 91.3%. The extract was the suspended in acetonitrile used for concentration determination by high-performance liquid chromatography (HPLC). The HPLC analysis was described by Duc (2016).

The half-lives ($t_{1/2}$) butachlor and degradation constant (λ) in soil were calculated based on the following equation: $t_{1/2} = \frac{\ln(2)}{\ln(\frac{N_0}{N_t})}$, $\lambda = \frac{\ln(2)}{t_{1/2}}$, where

N_0 and N_t are concentrations of butachlor at the initial and time t .

Populations of bacteria were enumerated and expressed as a number of CFUs/g soil. Soil samples were serially diluted and placed on agar plates containing mineral salt medium supplemented with glucose (1.0 g/L) and ammonium sulfate (1.0 g/L).

2.4. Statistical analysis

All obtained data from at least three experiment replicates are shown as the mean \pm standard deviation. Significant differences among means were statistically analyzed using one-way Duncan's test ($p < 0.05$) in SPSS program version 22.0.

3. Results and discussion

3.1. Butachlor degradation in soil by *Pseudomonas* sp. But1 and *Pseudomonas* sp. But2

The dissipation rates of butachlor in sterilized soil, non-sterilized soil, soil with and without bioaugmentation, with and without mung bean cultivation were compared. About 10% of butachlor was dissipated in sterilized soil after 30 days, indicating the herbicide was absorbed into soil components or degraded by physical and chemical processes. The dissipation of the herbicide in non-sterilized soil was observed to be $58.3 \pm 5.5\%$ after 30 days (Figure 1). The augmentation with *Pseudomonas* sp. But1 and *Pseudomonas* sp. But2 increased the butachlor degradation by about 35.0% and 40.0%, compared with the degradation by native microorganisms, respectively. This result showed that indigenous soil microorganisms could degrade the herbicide.

In soil samples augmented with *Pseudomonas* sp. But1, the degradation performance in non-sterilized soil was slightly higher than that in sterilized soil after 30 days (Figure 1A). Meanwhile, the degradation in non-sterilized soil with the augmentation with *Pseudomonas* sp. But2 was significantly higher compared with the sterilized counterpart (Figure 1B). These results showed that both bacterial strains could well adapt to the soil and cooperate with indigenous microorganisms.

The half-life values of butachlor degradation highly depended on soil microorganisms and mung bean cultivation. The half-life was the longest in sterilized soil, followed by attenuation. The attenuation is the degradation by native microorganisms, physical and chemical processes. The augmentation with strains But1 and But2 significantly reduced the half-life values of the butachlor decomposition (Table 1).

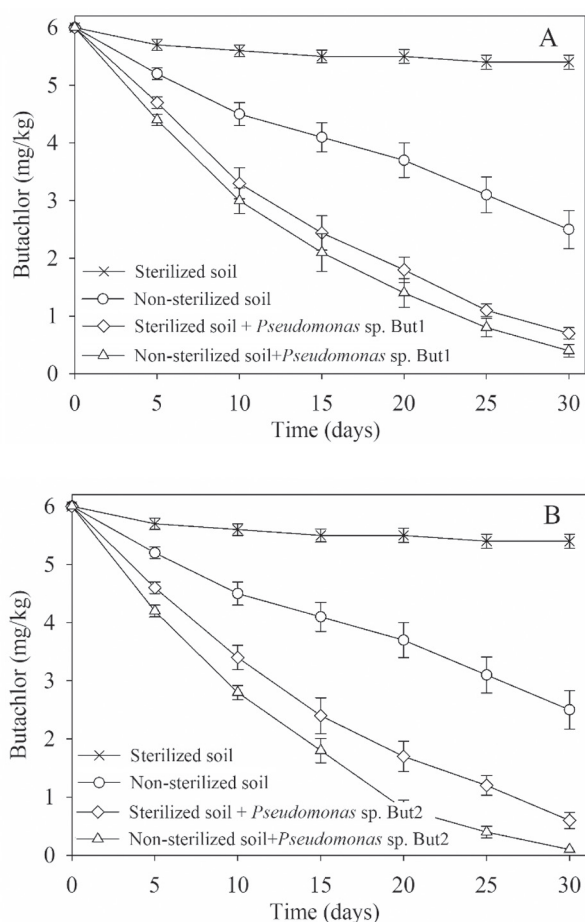


Figure 1. Butachlor dissipation in soil without and with augmentation with (A) *Pseudomonas* sp. But1 and (B) *Pseudomonas* sp. But2

The degradation rates of butachlor depended on soil components. The degradation due to the microbial activity was 49.9-63.9% in alluvial soil, and 48.2-62.3% in coastal saline soil over 90 days at 0.67 mg/kg dry soil (Pal et al., 2006). The half-life values ranged from 8.7-103.8 days in alluvial soil, and 9.7-107.5 in days coastal saline soil depending on the butachlor application rates (Pal et al., 2006). Butachlor degradation rates in soil also depended on herbicide concentrations. The higher concentration resulted in higher half-life values (Yu et al., 2003). The half-life values of butachlor in non-rhizosphere, wheat rhizosphere and inoculated rhizosphere soils at 10 mg/kg soil were 19.9, 11.0 and 2.9 days, respectively (Yu et al., 2003).

A previous study showed that *Stenotrophomonas acidaminiphila* strain JS-1 exhibited complete butachlor degradation in bioaugmented soil within 20

days, giving a half-life of 4.0 days at 6 mg/L (Dwivedi et al., 2010). The inoculation of *Catellibacterium caeni* sp. DCA-1 significantly accelerated butachlor degradation in both sterile and non-sterile soils, with 57.2%-90.4% of 50 mg/kg butachlor removed in 5 days compared to 5.4%-36% in the controls (Zheng et al., 2012). Enhanced degradation by augmentation with *Acinetobacter* sp. LYC-1 resulted in the reduction by 61.53% butachlor in soil contaminated with 10 mg/kg over 35 days (Feng et al., 2013).

Table 1. Half-life and degradation constant values (on average) of butachlor degradation in soil

Treatments	Half-life (days)	Degradation constant (λ)
Sterilized soil	167.63	0.004
Non-sterilized soil	23.75	0.029
Sterilized soil + <i>Pseudomonas</i> sp. But1	9.67	0.072
Non-sterilized soil + <i>Pseudomonas</i> sp. But1	7.68	0.090
Sterilized soil + <i>Pseudomonas</i> sp. But2	9.03	0.077
Non-sterilized soil + <i>Pseudomonas</i> sp. But2	5.08	0.136
Non-sterilized soil + mung bean	16.48	0.042
Non-sterilized soil + mung bean + <i>Pseudomonas</i> sp. But1	1.89	0.336
Non-sterilized soil + mung bean + <i>Pseudomonas</i> sp. But2	1.89	0.336

3.2. Butachlor degradation in soil cultivated with mung bean

The degradation performances in soil with and without mung bean cultivation were not statistically different before 25 days. However, degradation rates in soil cultivated with mung bean were higher than those in the soil without cultivation at the 25th and 30th days. Mung bean and its root development probably favored the soil microorganisms to increase the degradation. Organic carbon exudation from plant roots into the rhizosphere supports the increased microbial population in the soil. Mung bean cultivation resulted

in complete removal of the herbicide in the soil after 30 days, and reduced half-life overall (Table 2). In non-sterilized soil, mung bean cultivation reduced the half-life by nearly 7 days. The augmentation with But1 and But2 in the soil increased the degradation compared to non-augmented treatment (Figure 2). Both strains did not show the different degradation over the experiment (Figure 2). Overall degradation

rates were as follows: sterilized soil < non-sterilized soil < non-sterilized soil cultivated mung bean < sterilized soil with bioaugmentation < non-sterilized soil with bioaugmentation < non-sterilized soil with bioaugmentation and mung bean cultivation. These results indicated that both inoculated bacteria and mung bean cultivation play an important role on enhancement of the herbicide degradation.

Table 2. Bacterial number in soil ($\times 10^7$ CFU/g dry soil) over 30 days

Treatments	Bacterial number ($\times 10^7$ CFU/g dry soil) at various collection times						
	0 days	5 days	10 days	15 days	20 days	25 days	30 days
Sterilized soil	0 ^a	0.004 \pm 0 ^a	0.05 \pm 0 ^a	0.15 \pm 0.01 ^a	0.42 \pm 0.05 ^a	0.95 \pm 0.08 ^a	1.58 \pm 0.10 ^a
Non-sterilized soil	0.4 \pm 0.03 ^c	0.51 \pm 0.06 ^b	1.42 \pm 0.18 ^c	1.92 \pm 0.16 ^{bc}	2.17 \pm 0.12 ^b	2.11 \pm 0.14 ^b	1.98 \pm 0.15 ^b
Non-sterilized soil + mung bean	0.4 \pm 0.03 ^c	0.62 \pm 0.06 ^b	1.54 \pm 0.12 ^c	2.08 \pm 0.14 ^{bc}	2.61 \pm 0.23 ^c	2.71 \pm 0.16 ^c	2.85 \pm 0.19 ^c
Sterilized soil + <i>Pseudomonas</i> sp. But1	0.1 \pm 0.02 ^b	0.62 \pm 0.06 ^b	1.12 \pm 0.11 ^b	1.72 \pm 0.16 ^b	2.42 \pm 0.21 ^{bc}	2.44 \pm 0.22 ^{bc}	2.42 \pm 0.24 ^{bc}
Sterilized soil + <i>Pseudomonas</i> sp. But2	0.1 \pm 0.02 ^b	0.62 \pm 0.06 ^b	1.02 \pm 0.12 ^b	1.62 \pm 0.16 ^b	2.42 \pm 0.28 ^{bc}	2.45 \pm 0.21 ^{bc}	2.41 \pm 0.23 ^{bc}
Non-sterilized soil + <i>Pseudomonas</i> sp. But1	0.5 \pm 0.03 ^c	1.22 \pm 0.07 ^c	1.68 \pm 0.16 ^c	2.14 \pm 0.26 ^{bc}	2.72 \pm 0.26 ^c	2.78 \pm 0.28 ^c	2.72 \pm 0.27 ^c
Non-sterilized soil + <i>Pseudomonas</i> sp. But2	0.5 \pm 0.03 ^c	1.18 \pm 0.07 ^c	1.68 \pm 0.18 ^c	2.34 \pm 0.21 ^c	2.62 \pm 0.15 ^c	2.68 \pm 0.21 ^c	2.62 \pm 0.24 ^c
Non-sterilized soil + mung bean + <i>Pseudomonas</i> sp. But1	0.5 \pm 0.03 ^c	1.21 \pm 0.10 ^c	1.78 \pm 0.18 ^{cd}	2.58 \pm 0.28 ^c	2.84 \pm 0.18 ^{cd}	2.98 \pm 0.24 ^{cd}	3.42 \pm 0.31 ^d
Non-sterilized soil + mung bean + <i>Pseudomonas</i> sp. But2	0.5 \pm 0.03 ^c	1.28 \pm 0.08 ^b	1.77 \pm 0.20 ^{cd}	2.46 \pm 0.25 ^c	2.72 \pm 0.18 ^c	2.81 \pm 0.22 ^{cd}	3.37 \pm 0.28 ^d

Different small letters indicate statistically significant differences ($p < 0.05$) among treatments within a column. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

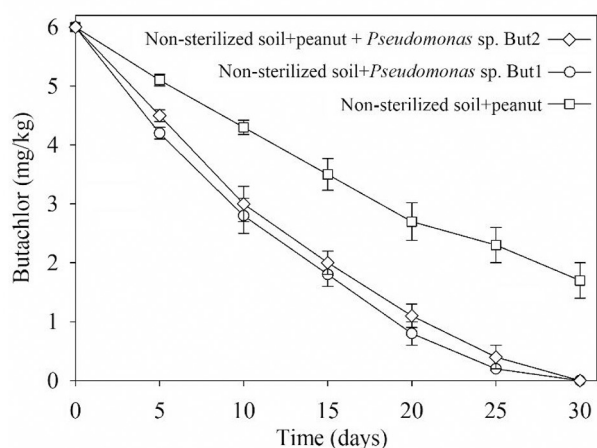


Figure 2. Attenuation and augmentation of butachlor degradation in soil cultured with mung bean (*Vigna radiata*)

In a previous report, the cultivation of some plants reduced butachlor attenuation in soil (Yang et al., 2011). Half-life time of butachlor degradation at 6 mg/kg in the rhizospheric soils of *Phragmites australis*, *Zizania aquatica* and *Acorus calamus* were 7.5, 9.8 and 5.4 days, respectively (Yang et al., 2011). In our study, the attenuation in soil cultivated with mung bean was 9.03 days, indicating the herbicide removal from soil depends on soil and plant cultivation.

3.3. Soil bacterial dynamics as affected by augmentation and mung bean cultivation

The densities of soil bacteria increased in all experiments. Bacteria appeared in the sterilized soil, and the numbers after 20 days were similar to non-sterilized soil at the beginning (Table 2). The appearance of bacteria in this soil probably due to the survival of bacterial spores, bacteria from air and water. Bacterial numbers in soil without mung bean sharply increased to 20th day, while bacteria densities in the soil with mung bean cultivation continuously increased until the 30th day. Bacterial numbers in soil with and without mung bean cultivation were not statistically different until 20 days; however, bacterial density in cultivated soil was higher in the following time (Table 2). In non-cultivated soil, bacterial numbers were higher in non-sterilized soil from the beginning to 15th day, but the numbers

were not statistically different in the following time. The more root development in soil during the plant growth increased microbial activities. Moreover, the inoculation into soil resulted in higher bacterial numbers compared to non-inoculated soil.

There were significant positive correlations between bacterial numbers in soil and the degradation rates. Significant negative relationships were detected between bacterial numbers and the half-life of the butachlor in the soils. These results indicated that native microorganisms, *Pseudomonas sp. But1*, *Pseudomonas sp. But2* and mung bean contributed to degrade butachlor.

In a previous study, microbial biomass and biochemical activities in rhizosphere soils were depressed by butachlor addition (Yang et al., 2011). The plant cultivation increased microbial numbers, enzyme activities, soil respiration rates and butachlor degradation in the rhizosphere soils (Yang et al., 2011). Fang et al. (2009) concluded that butachlor might cause adversely affect the growth and activities of beneficial microorganisms in soils. Butachlor inhibited bacterial growth in soil even after two months (Tran et al., 2021).

4. Conclusion

Overall herbicide degradation rates for 30 days were as follows: sterilized soil < non-sterile soil < green beans < bioaugmentation. The augmentation with two butachlor-degrading bacterial strains *Pseudomonas sp. But1* and *Pseudomonas sp. But2*, resulted in higher herbicide degradation in soil by about 35.0% and 40.0% than the degradation by native microorganisms, respectively. Moreover, the plantation of mung bean favored the growth of soil bacteria stimulating butachlor degradation. This study indicated that *Pseudomonas sp. But1* and *Pseudomonas sp. But2* well adapted to soil and cooperated with native microorganisms, increasing the butachlor removal process./.

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