BIOAUGMENTATION WITH BACTERIAL STRAINS FOR ENHANCED DEGRADATION OF ACETOCHLOR AND BENSULFURON-METHYL IN CONTAMINATED WATER AND SOIL

Nguyen Thi Thanh Chung[1], Tran Quang Dang[2], Tran Thi Cam Tu[3], Tran Dat Huy[3], and Ha Danh Duc[3*]

[1]Vinh Medical University, 161 Nguyen Phong Sac street, Vinh city, Nghe An province,Vietnam

[2]Mien Tay Construction University, 20B Pho Co Dieu street, Vinh Long city, Vinh Long province, Vietnam

[3]Dong Thap University, 783 Pham Huu Lau street, Dong Thap province, Cao Lanh 870000, Vietnam

[*]Corresponding author: Ha Danh Duc, Email: hadanhduc@gmail.com

History article

Received: 23/11/2024; Received in revised form: 10/12/2024; Accepted: 18/12/2024

Abstract

Acetochlor and bensulfuron-methyl are the main ingredients of herbicides used worldwide. This study evaluated the contamination of these compounds in water and soil samples collected from a paddy field, and their dissipation in the field and under a laboratory condition. The results showed that the concentrations of acetochlor and bensulfuron-methyl in water were 683.5±71.5 µg/L and 131.6±14.4 µg/L, respectively, while the soil data were 343.3±34.2 µg/L and 98.4±9.2 µg/L. The average concentrations of acetochlor and bensulfuron-methyl were dissipated by about 92.4% and 89.6% in water, and 86.8% and 91.0% in soil in the field after 30 days, respectively. These compound dissipations at the field site were higher compared to those under a laboratory condition. Fortunately, the inoculation of acetochlor degrading bacteria (Pseudomonas fluorescens KT3 and Bacillus subtilis 2M6E) and a bensulfuron-methyl degrading bacterial strain (Methylopila sp. DKT) increased the degradation process under the laboratory condition. This study, therefore, provides valuable information on the contamination of acetochlor and bensulfuron-methyl in water and soil, the dissipation of these compounds at the site and the roles of isolated bacteria in enhancing the degradation.

Keywords: *acetochlor, bensulfuron-methyl, contamination, degradation, inoculation.*

DOI: https://doi.org/10.52714/dthu.14.5.2025.1404

Cite: Nguyen, T. T. C., Tran, D. Q., Tran, T. C. T., Tran, D. H., & Ha, D. D. (2025). Bioaugmentation with bacterial strains for enhanced degradation of acetochlor and bensulfuron-methyl in contaminated water and soil. *Dong Thap University Journal of Science, 14*(5), 33-42. https://doi.org/10.52714/dthu.14.5.2025.1404

TĂNG CƯỜNG PHÂN HỦY ACETOCHLOR VÀ BENSULFURON-METHYL TRONG NƯỚC VÀ TRONG ĐẤT BẰNG VI KHUẨN PHÂN HUỶ

Nguyễn Thị Thanh Chung[1], Trần Quang Đăng[2], Trần Thị Cẩm Tú[3], Trần Đạt Huy[3] và Hà Danh Đức[3*]

[1]Trường Đại học Y khoa Vinh, 161 Nguyễn Sỹ Sách, thành phố Vinh, tỉnh Nghệ An, Việt Nam

[2]Trường Đại học Xây dựng Miền Tây, 20B Phố Cơ Điều, thành phố Vĩnh Long, tỉnh Vĩnh Long, Việt Nam

[3]Trường Đại học Đồng Tháp, 783 Phạm Hữu Lầu, thành phố Cao Lãnh, tỉnh Đồng Tháp, Việt Nam

[*]Tác giả liên hệ: Hà Danh Đức, Email: hadanhduc@gmail.com

Lịch sử bài báo

Ngày nhận: 23/11/2024; Ngày nhận chỉnh sửa: 10/12/2024; Ngày duyệt đăng: 18/12/2024

Tóm tắt

Acetochlor và bensulfuron-methyl là thành phần chính của thuốc diệt cỏ được sử dụng rộng rãi trên toàn thế giới. Nghiên cứu này đánh giá mức độ ô nhiễm của thuốc trừ cỏ trong các mẫu nước và đất thu thập được từ một ruộng lúa trên cánh đồng lúa, cũng như sự phân huỷ của chúng trên ruộng lúa và trong phòng thí nghiệm. Kết quả cho thấy nồng độ acetochlor và bensulfuron-methyl trong nước lần lượt là 683,5 ± 71,5 µg/L và 131,6 ± 14,4 µg/L, trong khi dữ liệu tương ứng trong đất là 343,3 ± 34,2 µg/L và 98,4 ± 9,2 µg/L. Nồng độ trung bình của acetochlor và bensulfuron-methyl bị phân huỷ khoảng 92,4% và 89,6% trong nước, và 86,8% và 91,0% trong đất tại cánh đồng sau 30 ngày. Sự phân huỷ của các hợp chất này tại ruộng lúa cao hơn so với trong phòng thí nghiệm. Việc bổ sung vi khuẩn phân hủy acetochlor (Pseudomonas fluorescens KT3 và Bacillus subtilis 2M6E) và vi khuẩn phân hủy bensulfuron-methyl (Methylopila sp. DKT) đã làm tăng quá trình phân hủy ở điều kiện phòng thí nghiệm. Nghiên cứu này cung cấp thông tin có giá trị về sự ô nhiễm acetochlor và bensulfuron-methyl trong nước và đất, sự phân huỷ của các hợp chất này và vai trò của vi khuẩn phân lập trong việc tăng cường quá trình phân hủy.

Từ khóa: *acetochlor, bensulfuron-methyl, ô nhiễm, phân hủy, bổ sung vi khuẩn.*

1. Introduction

Acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)-acetamide) is a chloroacetamide herbicide frequently used for controlling grasses. Due to its low sorption, high water solubility and long persistence, the compound has a high potential to contaminate soil and water. For examples, acetochlor and its metabolites were detected in groundwater and surface water (Kolpin et al., 1996), in soil and sediment (Sun et al., 2011), in surface water, groundwater and soil (Wang et al., 2023), in drinking water (An et al., 2021) and in sediment (San Juan et al., 2023). In soil, the compound is absorbed in surface and deep soil (Janniche et al., 2010). Acetochlor is considered as an endocrine disruptor (Crump et al., 2002; Li et al., 2009), a genotoxic agent (Hill et al., 1997), a mutagenic compound of male rat germ cells (Ashby et al., 1997) and a carcinogen (Xiao et al., 2006).

Bensulfuron-methyl has been detected in water (Okamoto et al., 1998; Pareja et al., 2011). It also exists in soil for a long time due to nonvolatile, faintly acidic and limited photodegradation (Thompson et al., 1992; Delgado-Moreno et al., 2007). The compound causes serious damage to DNA of aquatic organisms and inhibits the growth of algae and pondweed (Coyner et al., 2001; Yang et al., 2023). Moreover, bensulfuron-methyl severely inhibits the growth of maize and wheat (Zhang et al., 2022; Wang et al., 2023). Therefore, the natural remediation of the compound in soil and water needs to be evaluated to ensure crop production and crop rotation for the next generation.

Acetochlor and bensulfuron-methyl are the main ingredients of widely used herbicides. Both compounds can be mixed to increase their efficiency. Acetochlor and bensulfuron-methyl are ingredients of 48 and 34 herbicides in the list of traded pesticides permitted for production and use in Viet Nam in 2023. Among them, 12 types of traded herbicides contain both of them, i.e., Beto 14WP, Afadax 170WP, Acenidax 17WP, Arorax 17WP, Bpanidat 170WP, Aloha 5GR, Aloha 25WP, Natos 15WP, Alphadax 250WP, Sarudo 18WP, Sarudo 500.5EC and Sun-like 18WP. Most of these herbicides are used for rice cultivation. Therefore, both acetochlor and bensulfuron-methyl have a high potential to co-contaminate water and soil in paddy fields.

Some bacterial strains showing degradability towards acetochlor and bensulfuron-methyl have been isolated. Two acetochlor degrading bacterial strains, *P. fluorescens* KT3 and *B. subtilis* 2M6E were isolated and determined their degradation ability of the compound (Duc & Oanh, 2019). Meanwhile, *Methylopila* sp. DKT showing effective degradation of bensulfuron-methyl and stimulating the growth of peanut was reported (Ha & Nguyen, 2020).

This study aimed to evaluate acetochlor and bensulfuron-methyl contaminating water and soil in a paddy field, where herbicides have been used extensively. Moreover, the degradation of these compounds at the field site and under a laboratory condition was compared. Besides, the inoculation of *P. fluorescens* KT3, *B. subtilis* 2M6E and *Methylopila* sp. DKT to augment the degradation was carried out. In addition, the degradation of pure acetochlor and bensulfuron-methyl and the compounds in water and a mineral medium was conducted.

2. Materials and methods

2.1. Collection of water and soil samples

Soil and site water were collected from a small unit area (about 120 m^2) in a large paddy field where farmers had sprayed an herbicide named Acenidax 17WP two days before. Acenidax 17WP is produced by CP Nicotex Company, which contains 14.6% acetochlor and 2.4% bensulfuron-methyl. Water and soil samples were collected from 10 field sites evenly distributed in the unit area. At each site, approximately 0.5 L of water and 0.5 kg of soil were collected. Soil was collected at the same sites with water using a spoon, at the soil surface, not over 5 cm in depth. In this field, rice had been cultivated for several days before water and soil collection. After collecting water and soil, the small unit area was embanked to prevent water from moving in and out. The water and soil samples were individually stored in plastic bags and bottles, transferred to a lab and removed large debris (> 1.0 cm in size). Water in soil samples was partially removed using blotting paper to achieve a mixture of 50% dry soil weight. The paddy field located in Cao Lanh district, Dong Thap province, Vietnam. The experiment was conducted in February and March, 2023.

2.2. Degradation of acetochlor and bensulfuron-methyl in water and soil

2.2.1. The compounds' degradation in water and soil in a paddy field

Water and soil samples were first analyzed for concentrations of acetochlor and bensulfuron-methyl. Moreover, physico-chemical characteristics of all samples were determined. The acetochlor and bensulfuron-methyl in the samples after collection were considered as the zero days. Subsequently, samples were collected from the field after 5, 10, 15, and 30 days from the initial collection time to evaluate the chemical concentrations. Each time, about 10 mL of water and 10 g of soil were collected.

2.2.2. The compounds' degradation in water and soil under laboratory condition

Water (500 mL) and soil (500 g, 50% moisture) and were individually transferred to plastic containers

(length×width×depth of 15×25×20 cm). The containers were capped with plastic covers and incubated at a static condition, in the dark at room temperature (~ 30 °C) in the lab for 30 days. The cover was opened for 10 min, once a day. Samples (5 mL or 5 g) were collected each time during the incubation to determine acetochlor and bensulfuron-methyl remaining.

For the degradation with the inoculation of acetochlor and bensulfuron-methyl degrading bacteria, one colony of each bacterial strain was cultured in the mineral medium amended with 1.0 g/L yeast extract for 24 hours. Bacteria were collected after centrifuging at 10.000 rpm for 5 min, rinsed twice with the mineral medium and re-suspended in the same medium at 10^9 coliform forming unit (CFU)/mL. *P. fluorescens* KT3 and *B. subtilis* 2M6E were inoculated at 0.5×10^6 CFU/mL of each isolate for acetochlor degradation, and *Methylopila* sp. DKT was amended at 1.0×10^6 CFU/mL for bensulfuron-methyl degradation. The number of bacteria was enumerated based on CFU emerging in agar plates after diluting and spreading on agar plate and incubating for two days at 30 ^{o}C.

2.3. Mineral medium

A mineral medium consisted of 1.5 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.5 g/L NaCl, 0.2 g/L $MgSO_4$, 0.5 g/L $CaCl_2$, $(NH_4)_2SO_4$ 0.5 g/L and 1 mL of trace element solution (39.9 mg $MnSO_4{\cdot}H_2O$, 42.8 mg $ZnSO_4{\cdot}H_2O$, 3.8 mg $CuSO_4{\cdot}5H_2O$, 11.6 mg H_3BO_4 and 27.8 mg $FeSO_4{\cdot}7H_2O$ per liter). The pH was adjusted to 7.0 ± 0.1. The medium was sterilized at 121 °C for 15 min. Individual acetochlor and bensulfuron-methyl (>99.6% purity) and other chemicals were purchased from Sigma-Aldrich Chemical Co. (Singapore) and Merck (Germany).

2.4. Degradation of the compounds in mineral medium

The degradation of acetochlor and bensulfuron-methyl was conducted with the inoculation of them degrading bacteria. They were amended at equal concentrations to the concentrations in the water and soil collected from the paddy field. The compounds were used as pure substrates and the herbicide named Acenidax 17WP.

Their individual and mixed cultures degrading bacteria were inoculated into water and soil as described above. Water and soil samples were collected every one hour to determine the substrate concentrations.

2.5. Extraction of the compounds, and chemical analysis

Acetochlor and bensulfuron-methyl in water and soil were extracted with hexane three times as described in a previous study (Nguyen Thanh Hung et al., 2022). Soil (5 g) or water (5 mL) was added to a plastic tube, added with 100 mL of hexane (>99% purity), shaken at 250 rpm for 30 min. The above layer was collected, passed through a 0.22-µm syringe filter and concentrated. The extract solution was used to determine the two compounds contaminated the collected samples. The recoveries of acetochlor from water and soil were 95.3% and 93.7%, respectively, while those of bensulfuron-methyl were 95.4% and 96.4%.

The concentrations of these compounds were analyzed using high performance liquid chromatography (HPLC) with a Waters column (4.5 × 250 mm with a particle size of 5 µm). A mobile phase (acetonitrile/water: 75: 25, v/v) was run at a flow rate of 1.0 mL/min. A UV-900 wavelength absorbance detector was used to monitor the solution at 215 nm. Each experiment was conducted at least three replicates, and data were shown at figures as means and standard derivations.

3. Results and discussion

3.1. Physico-chemical characteristics of water and soil, acetochlor and bensulfuron-methyl concentration

Water and soil samples collected from the paddy field were analyzed for the compounds' concentrations. The ratio of soil and water were adjusted to 100% water, 80% water + 20% soil and 50% water + 50% soil. Acetochlor in water and soil samples from paddy fields were 683.5±71.5 µg/L and 343.3±34.2 µg/L, respectively. Meanwhile, the concentrations of bensulfuron-methyl in water and soil samples (50% dry weight) were 131.6±14.4 µg/L and 98.4±9.2 µg/L, respectively. In the medium with 80% water, bensulfuron-methyl and acetochlor concentrations were 547.4 and 118.3 2 µg/L, respectively. Moreover, the physico-chemical characteristics of soil and water at the first collection time are shown in Table 1. Water level was about 3-5 cm above the soil surface and did not significantly change for one month. Moreover, pH levels and other parameters of soil and water were stable during the experiment. The concentrations of the compounds were also monitored.

Table 1. Physico-chemical characteristics of water and soil

Water			Dry soil		
pH		7.1	pH		7.2
BOD_5	mg/L	14.1	Clay	%	25.6
COD	mg/L	16.6	Silt	%	38.6
DO	mg/L	5.7	Sand	%	37.8
	mg/L		Total organic carbon	g/kg	7.73
Turbidity		58.4			
TDS	mg/L	122.2			
TSS	mg/L	62.3			

Water			Dry soil		
PO_4^{3-}	mg/L	0.31	Total P	g/kg	1.02
SO_4^{2-}	mg/L	22.4	Total S	g/kg	0.77
Total K	mg/L	0.23	Total K	g/kg	7.2
Total N	mg/L	3.3	Total N	g/kg	1.12

3.2. Degradation of the compounds contaminating water and soil at field site

At the field site, the average concentrations of acetochlor and bensulfuron-methyl were dissipated about 92.4% and 89.6% in water (Figure 1a), and 86.8% and 91.0% in soil (Figure 1b) after 30 days, respectively. However, the degradation mostly occurred within 15 days in both water and soil. The results showed that the degradations at the field site were much higher than those under the laboratory condition. Indigenous microorganisms played an important role in the degradation of herbicides. Moreover, the large media in the field probably resulted in their higher reduction. Besides, physical and chemical processes in the site, the irreversible absorption of the substrates in soil, and the dispersing of the compounds resulted in higher dissipation.

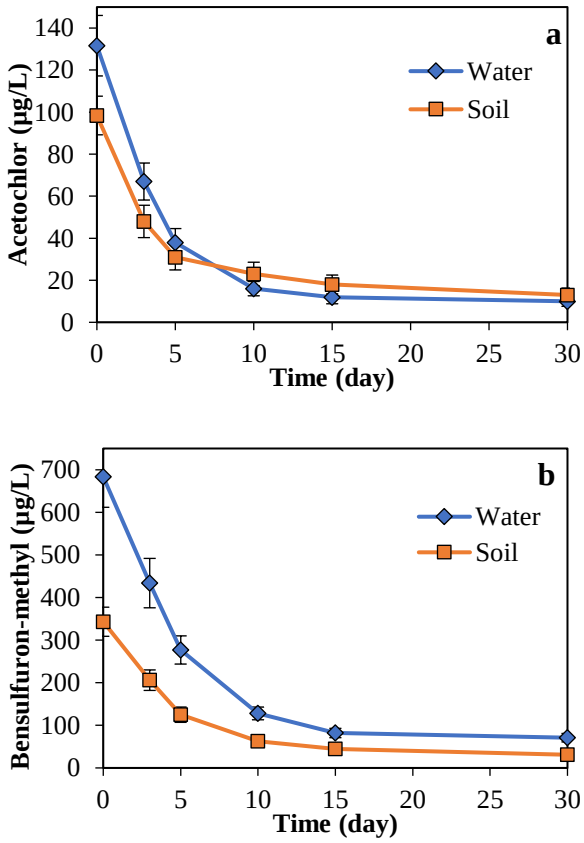

Figure 1. Degradation of (a) acetochlor and (b) bensulfuron-methyl in water and soil at the paddy field

The persistence of chloroacetamide herbicides in the field varies with soil type, soil-water content, temperature, and depth below the soil surface (Kotoula-Syka et al., 1997). The estimated half-lives of acetochlor were 6.3 days in a surface soil field study (Mueller et al., 1999), 6.9 days in an in vitro aerobic soil, and 1.1 days in anaerobic sewage sludge (Mueller & Buser, 1995). In flooded soils, half-lives of the compound were 15 days for unamended soil, and 10 days for iron and sulfate amendments under anaerobic conditions (Loor-Vela et al., 2003). For bensulfuron-methyl, the half-lives were depended on the chemical concentrations, 12.03 days at 0.0355 mg/kg dry soil and 57.27 days at 3.55 mg/kg dry soil (Lin et al., 2012). It is considered as immobile to moderately mobile depending on the soil organic matter and pH (Roberts et al., 1998). The compound may be degraded through chemical hydrolysis and microbial processes in soil (Roberts et al., 1998). Therefore, its degradation in the field site was higher than the degradation under the laboratory condition

3.3. Degradation of the compounds contaminating water and soil under lab condition

Water and soil samples were collected to assess their biodegradability in the laboratory. After 30 days, acetochlor concentrations were dissipated by 60.2%, 90.7% and 70.5% in the media of 100% water, 80% water + 20% soil and 50% water + 50% soil on average (Figure 2a), respectively. The corresponding data for bensulfuron-methyl were 63.1%, 92.7% and 79.3% (Figure 2b). Half-lives of acetochlor in the corresponding media were 22.6, 17.0 and 8.8 days, and the bensulfuron-methyl values were 20.8, 13.2 and 7.9 days in vitro aerobic condition.

The dissipation of both substrates in the media of 100% water and 80% water + 20% soil mostly occurred during the first 15 days, while their concentrations gradually decreased in the media of 50% water + 50% soil. The calculation of degradation during the first 15 days showed that the degradation rates of acetochlor in the media of 100% water, 80% water and 50% water were 7.9, 10.7 and 6.9 µg/days, respectively. The corresponding data for bensulfuron-methyl were 43.2, 50.7 and 27.2 µg/days. The low degradation observed in water was likely due to a low density of indigenous acetochlor-degrading microorganisms and a lack of necessary supportive components.

A previous study showed that acetochlor hydrolysis was extremely slow in river water (Ye, 2003). The low dispersing of the compounds in the media with 50% water resulted in lower degradation percentages compared to the degradation in the media with 80% water. Meanwhile, some components in soil might provide nutrients for microorganisms resulting in higher degradation in the media of 80% water + 20% soil.

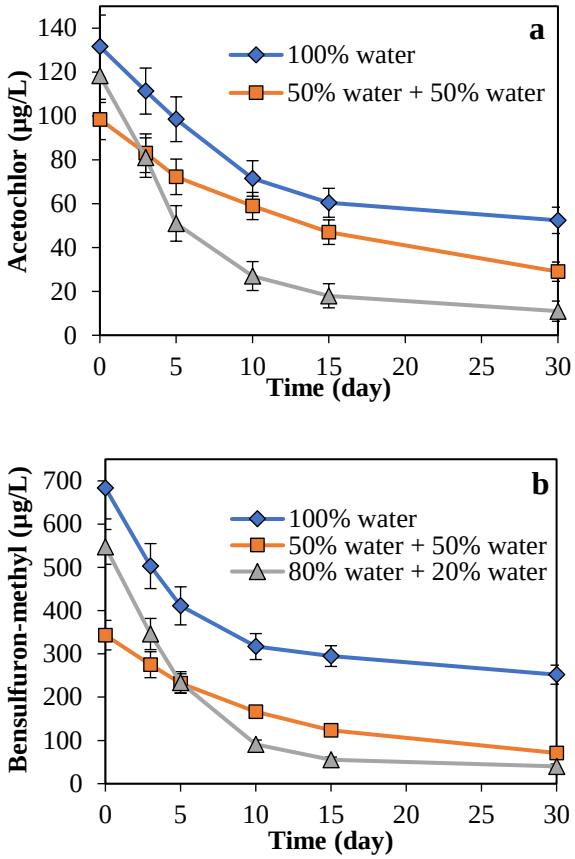

Figure 2. Degradation of (a) acetochlor and (b) bensulfuron-methyl in the media of 100% water and water plus soil under laboratory condition

3.4. Bioaugmentation of acetochlor and bensulfuron-methyl degradation in water and soil

In this experiment, two mixed cultures of acetochlor-degrading bacteria (*P. fluorescens* KT3 and *B. subtilis* 2M6E) and bensulfuron-methyl degrading bacteria (*Methylopila* sp. DKT) were inoculated into water and soil collected from the paddy field. The results showed that the inoculation of these bacterial strains significantly increased the degradation of both compounds. After 60 hours, acetochlor was degraded by 86.3%, 95.8% and 69.5% in the media of 100% water, 80% water + 20% soil and 50% water + 50% soil, respectively (Figure 3a). The corresponding data for bensulfuron-methyl were 96.8%, 99.1% and 83.7% (Figure 3b). The highest degradation was observed within the first 15 hours. During the first 15 hours, the degradation rates for acetochlor were 171.0, 178.5 and 63.6 µg/day on average in the corresponding media. Data for bensulfuron-methyl were 783.5, 696.7 and 276.1 µg/day.

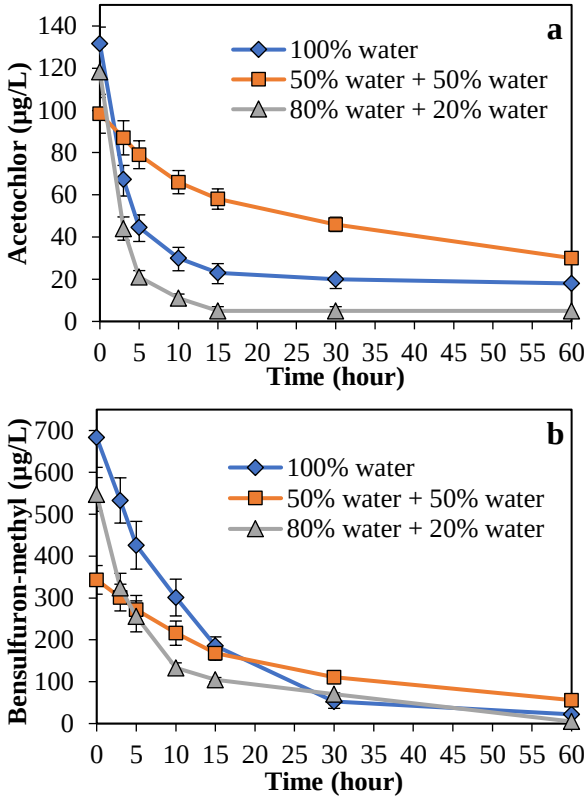

Figure 3. Degradation of (a) acetochlor and (b) bensulfuron-methyl in different media inoculated with degrading bacteria, under laboratory condition

The inoculation of degrading bacteria significantly enhanced the degradation compared to the natural degradation. With inoculation, the degradation was carried out by not only introduced bacteria but also by native microorganisms. The results in this study indicated that the isolated bacteria could adapt well to new media and other indigenous microorganisms in water and soil. The inoculation of *Methylopila* sp. DKT in soil in combination with peanut cultivation increased the bensulfuron-methyl degradation by about 57.7% (Ha and Nguyen, 2020). The degradation in the medium with 50% water + 50% soil was gradual and slower than that in other media. The limitation of bacteria to move and contact the substrates the media with high soil content was probably the reason to reduce the degradation process.

3.5. Degradation of acetochlor and bensulfuron-methyl in mineral medium

The degradation was first determined with a mixture of pure substrates by individual acetochlor and bensulfuron-methyl degrading bacteria. Figure 4a shows that acetochlor was completely degraded within 3 hours in the mineral medium inoculated with *P. fluorescens* KT3 and *B. subtilis* 2M6E. Meanwhile, the inoculation with *Methylopila* sp. DKT resulting in 93.7±3.2% of bensulfuron-methyl was degraded after 5 hours (Figure 4b).

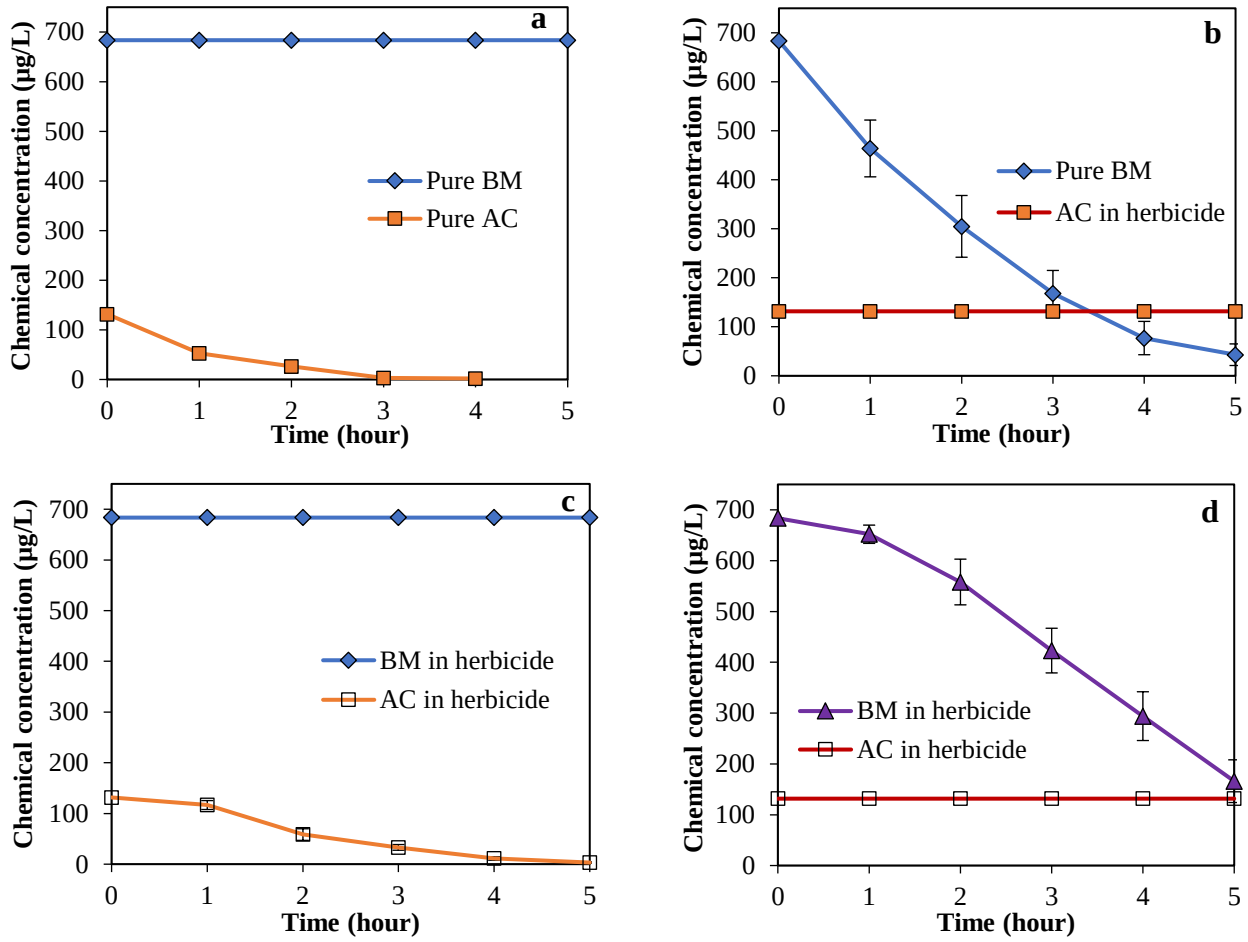

Figure 4. Degradation of (a) pure acetochlor (AC) and (b) acetochlor in herbicide by *P. fluorescens* KT3 and *B. subtilis* 2M6E, (c) pure bensulfuron-methyl (BM) and (d) bensulfuron-methyl in herbicide by *Methylopila* sp. DKT. The degradation was conducted in the mineral medium supplemented with mixed pure substrates or substrates in herbicide Acenidax 17WP, inoculation with either acetochlor or bensulfuron-methyl degrading bacteria.

The degradation processes of pure acetochlor and bensulfuron-methyl in the mineral medium with the inoculation of both degrading bacteria are shown in Figure 5a. Pure acetochlor and bensulfuron-methyl were completely degraded after 2 and 4 days, respectively. These data were higher than the degradation by individual acetochlor and bensulfuron-methyl degrading bacterial strains. The presence of acetochlor might cause toxicity for bensulfuron-methyl degrading bacteria and vice versa. However, the inoculation of both degrading bacteria of both compounds degraded both substrates reducing the toxicity. Moreover, the degradation in mineral medium was much higher than the degradation in water as described above. The inoculated bacteria did not compete with indigenous microorganisms and available nutrients in the mineral medium supported the activities of acetochlor and bensulfuron-methyl degrading bacteria.

The degradation of both substrates in the herbicide Acenidax 17WP required a one hour of lag phase. For acetochlor degradation by *P. fluorescens* KT3 and *B. subtilis* 2M6E, the substrate was completely removed after 5 hours (Figure 4c). Meanwhile, *Methylopila* sp. DKT degraded 75.7 ± 6.1% of bensulfuron-methyl in the herbicide for 5 hours (Figure 4d). Acetochlor degrading bacteria could not degrade bensulfuron-methyl and vice versa. Therefore, bensulfuron-methyl concentration was not changed in Figure 4a and 4c, and acetochlor was not reduced in Figure 4b and 4d.

Meanwhile, acetochlor and bensulfuron-methyl were degraded nearly completely after 4 hours, and 88.9 ± 11.2% after 5 hours (Figure 5b). The degradation processes of both acetochlor and bensulfuron-methyl in herbicide were slower than those of pure substrates. Adjuvants in the herbicide caused the degradation reduction at high concentrations. In previous studies on other herbicides, adjuvants caused the reduction of prometryn degradation by free cells (Pérez-Bárcena et al., 2014) and suppressed the degradation of butachlor and propanil by *Pseudomonas* sp. But2 and *Acinetobacter baumannii* DT in liquid media, but not in

soil (Duc et al., 2020), pymetrozine degradation by enrichment culture (Duc & Oanh, 2024).

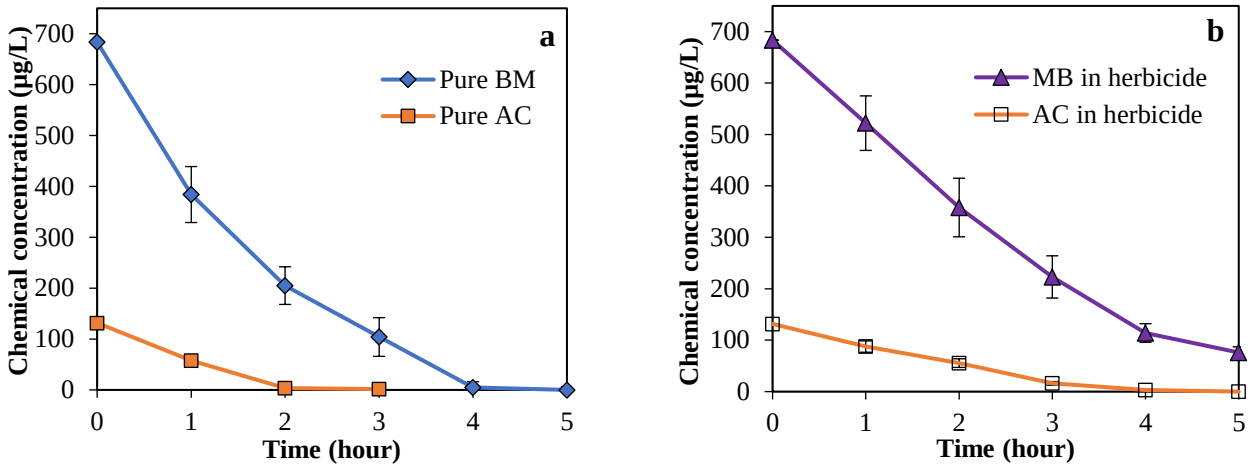

Figure 5. Degradation of (a) pure acetochlor (AC) and pure bensulfuron-methyl (BM), and acetochlor bensulfuron-methyl in herbicide Acenidax 17WP and by *P. fluorescens* KT3, *B. subtilis* 2M6E and *Methylopila* sp. DKT. The degradation was conducted in the mineral medium inoculated with both acetochlor and bensulfuron-methyl degrading bacteria.

4. Conclusion

This study showed that the application of herbicides caused contamination due to acetochlor and bensulfuron-methyl in water and soil. In a paddy field, acetochlor and bensulfuron-methyl were dissipated quite quickly within 10 days after use, but they were remained in water and soil after 30 days. The determination under lab conditions without inoculation showed that the degradation percentages of both substrates were highest in the media with 80% soil + 20% site water, following by 50% site water + 50% soil and lowest in 100% site water. The inoculation of *P. fluorescens* KT3 and *B.subtilis* 2M6E (acetochlor degrading bacterial strains) and *Methylopila* sp. DKT (bensulfuron-methyl degrading bacterial strain) increased the degradation process under the laboratory condition. This study provides information on the contamination of acetochlor and bensulfuron-methyl in water and soil at a paddy field, the natural dissipation of these compounds and the augmentation of the degradation by inoculation of degrading bacteria. However, this study only applied isolated bacteria to degrade the herbicides under lab conditions. Therefore, further study using bacteria to remediate the compounds at contaminated sites should be carried out.

References

An, W., Sang, C., Jensen, K. M., Sørensen, P. B., Zhang, B., & Yang M. (2021). Application of the health risk assessment of acetochlor in the development of water quality criteria. *J Environ Sci*, 110, 48-54.

Ashby J., Tinwell H., Lefevre P. A., Williams J., Kier L., Adler I. D., & Clapp M. J. L. (1997). Evaluation of the mutagenicity of acetochlor to male rat germ cells. *Mutat Res, 393*, 263-281.

Coyner, A., Gupta, G., & Jones, T. (2001). Effect of chlorsulfuron on growth of submerged aquatic macrophyte *Potamogeton pectinatus* (sago pondweed). *Environ Pollut, 111*(3), 453-455. https://doi.org/10.1016/S0269-7491(00)00084-1

Crump, D., Werry, K., Veldhoen, N., Van Aggelen, G., & Helbing, C. C. (2002). Exposure to the herbicide acetochlor alters thyroid hormone-dependent gene expression and metamorphosis in *Xenopus laevis*. *Environ Health Perspect, 110*, 1199-1205.

Delgado-Moreno, L., Sanchez, L., Castillo, A., Pot, V., & Pena, A. (2007). Behavior of bensulfuron-methyl in an agricultural alkaline soil. *J Environ Sci Health B, 42*(3), 241-248.

Duc, H. D., & Oanh, N. T. (2019). Biodegradation of acetochlor and 2-methyl-6-ethylaniline by *Bacillus subtilis* and *Pseudomonas fluorescens*. *Microbiology, 88*, 729-738. https://doi.org/10.1134/S0026261719060031

Duc, H. D., & Oanh, N. T. (2024). Pymetrozine degradation by an enrichment culture from paddy soil. *Microbiology, 93*, 324-332. https://doi.org/10.1134/S002626172360249X

Duc, H. D., Thuy, N. T. D., Truc, H. T. T., Nhu, N. T. H., & Oanh, N. T. (2020). Degradation of butachlor and propanil by *Pseudomonas* sp. strain But2 and *Acinetobacter baumannii* strain DT. *FEMS Microbiol Lett, 367*(18), fnaa151. https://doi.org/10.1093/femsle/fnaa151

Ha, D.D., & Nguyen, T.O. (2020). Application of *Methylopila* sp. DKT for Bensulfuron-methyl degradation and peanut crowth promotion. *Curr*

Microbiol, 77, 1466-1475. https://doi.org/10.1007/s00284-020-01953-0

Hill, A. B., Jefferies, P. R., Quistad, G. B., & Casida, J. E. (1997). Dialkylquinoneimine metabolites of chloroacetanilide herbicides induce sister chromatid exchanges in cultured human lymphocytes. *Mutat Res, 395*, 159-171.

Janniche, G. S., Mouvet, C., & Albrechtsen, H. J. (2010). Acetochlor sorption and degradation in limestone subsurface and aquifers. *Pest Manage Sci, 66*, 1287-1297.

Kolpin, D.W., Goolsby, D.A., & Thurman, E.M. (1996). Acetochlor in the hydrologic system in the midwestern United States 1994. *Environ Sci Technol, 30*, 459-464

Kotoula-Syka, E., Hatzios, K.K., Berry, D.F., & Wilson, H. P. (1997). Degradation of acetanilide herbicides in history and nonhistory soils from Eastern Virginia. *Weed Technology, 11*(3), 403-409.

Li, W., Zha, J. M., Li, Z. L., Yang, L. H., & Wang, Z. J. (2009). Effects of exposure to acetochlor on the expression of thyroid hormone related genes in larval and adult rare minnow (*Gobiocypris rarus*). *Aquat Toxicol, 94*, 87-93.

Lin, X. Y., Yang, Y. Y., Zhao, Y. H., & Fu, Q. L. (2012). Biodegradation of bensulfuron-methyl and its effect on bacterial community in paddy soils. *Ecotoxicology, 21*(5), 1281-1290. https://doi.org/10.1007/s10646-012-0882-7.

Loor-Vela, S. X., Crawford Simmons, J. J., Simmons, F. W., & Raskin, L. (2003). Dissipation of [14C]acetochlor herbicide under anaerobic aquatic conditions in flooded soil microcosms. *J Agric Food Chem, 51*(23), 6767-6773.

Mueller, M.D., & Buser, H-R. (1995). Environmental behavior of acetamide pesticide stereoisomers. 2. stereo- and enantioselective degradation in sewage sludge and soil. *Environ Sci Technol, 29*(8), 2031-2037.

Mueller, T.C., Shaw, D.R., & Witt, W.W. (1999). Relative dissipation of acetochlor, alachlor, metolachlor, and SAN 582 from three surface soils. *Weed Technology, 13*(2), 341-346.

Nguyen Thanh Hung, Tran Ngoc Chau, Nguyen Thi Thuy, & Ha Danh Duc. (2022). Increased degradation of acetochlor in soil by mixed culture of *P. fluorescens* KT3 and *B. subtilis* 2M6E. *Dong Thap University, Journal of Science, Natural Sciences issue*, *11*(5), 60-67.

Okamoto, Y., Fisher, R. L., Armbrust, K. L., & Peter, C. J. (1998). Surface water monitoring survey for bensulfuron methyl applied in paddy fields. *J Pestic Sci, 23*(3), 235–240.

Pareja, L., Martinez-Bueno, M. J., Cesio, V., Heinzen, H., & Fernandez-Alba, A. R. (2011). Trace analysis of pesticides in paddy field water by direct injection using liquid chromatography-quadrupole-linear ion trap-mass spectrometry. *J Chromatogr A, 1218*(30), 4790-4798.

Pérez-Bárcena, J.F., Ahuatzi-Chacón, D., CastilloMartínez, K.L., Ruiz-Ordaz, N., Galíndez-Mayer, J., Juárez-Ramírez, C., & Ramos-Monroy, O. (2014). Effect of herbicide adjuvants on the biodegradation rate of the methylthiotriazine herbicide prometryn. *Biodegradation, 25*, 405-415. https://doi.org/10.1007/s10532-013-9669-7

Roberts, T.R., Hutson, D.H., Lee, P.W., Nicholls, P.H., & Plimmer J.R. (1998). Metabolic pathways of agrochemicals: Part 1: Herbicides and Plant Growth Regulators. Cambridge, Royal Society of Chemistry, Thomas Graham House, 483-487.

San Juan, M.F., Lavarías, S., Aparicio, V., Larsen, K.E., Colman Lerner, J.E., & Cortelezzi, A. (2023). Ecological risk assessment of pesticides in sediments of Pampean streams, Argentina. *Chemosphere, 313*, 137598.

Sun, X., Zhou, Q., Ren, W., Li, X., & Ren, L. (2011). Spatial and temporal distribution of acetochlor in sediments and riparian soils of the Songhua River Basin in northeastern China. *J Environ Sci, 23*, 1684-1690.

Thompson, D.G., Macdonald, L.M., & Staznik, B. (1992). Persistence of hexazinone and metsulfuron-methyl in a mixed-wood/boreal forest lake. *J Agric Food Chem, 40*(8), 1444-1449.

Wang, W., Man, Y., Xie, Zhang, J.Z., Wang, P., & Liu X. (2023). Occurrence and risk assessment of three chloroamide herbicides in water and soil environment in northeastern, eastern and southern China. *Environ Res, 219*, 115104

Wang, X., Tang, L., Yang, T., Shi, Y., Liu, F., & Jiang, H. (2023). Remediation of bensulfuron methyl polluted water and soil by Fe_2O_3/Fe_3O_4@C activating peroxymonosulfate: chloride enhancement effect and phytotoxicity assessment. *Chem Eng J, 474*, 145439. https://doi.org/10.1016/j.cej.2023.145439.

Xiao, N., Jing, B., Ge, F., & Liu, X. (2006). The fate of herbicide acetochlor and its toxicity to *Eisenia fetida* under laboratory conditions. *Chemosphere, 62*, 1366-1373.

Yang, D., Huo, J., Zhang, Z., An, Z., Dong, H., Wang, Y., Duan, W., Chen, L., He, M., Gao, S., & Zhang, J. (2023). Citric acid modified ultrasmall copper peroxide nanozyme for in situ remediation of environmental sulfonylurea herbicide contamination. *J Hazard Mater*, *443*, 130265. https://doi.org/10.1016/j.jhazmat.2022.130265.

Ye, C. (2003). Environmental behavior of the herbicide acetochlor in soil. *Bull Environ Contam Toxicol*, *71*, 919-923.

Zhang, H., Qian, Y., Fan, D., Tian, Y., & Huang, X. (2022). Biofilm formed by *Hansschlegelia zhihuaiae* S113 on root surface mitigates the toxicity of bensulfuronmethyl residues to maize. *Environ Pollut*, *292*, 118366. https://doi.org/10.1016/j.envpol.2021.118366