EFFECTS OF NUTRIENTS AND ENVIRONMENTAL CONDITIONS ON THIOBENCARB DEGRADATION BY A MIXED CULTURE OF *PSEUDOMONAS* SP. TH1 AND *CUPRIAVIDUS OXALATICUS* Th2

ẢNH HƯỞNG CỦA CHẤT DINH DƯỠNG VÀ CÁC YẾU TỐ MÔI TRƯỜNG ĐẾN SỰ PHÂN HUỶ THIOBENCARB CỦA HỖN HỢP VI KHUẨN *PSEUDOMONAS* SP. TH1 VÀ *CUPRIAVIDUS OXALATICUS* Th2

Ha Danh Duc[1]*, Huynh Thi Thanh Thuy[2,3], Nguyen Thanh Hung[2,3], Tran Ngoc Chau[2,3], Tran Thi Cam Tu[1], Ha Huynh Hong Vu[1]

[1]Dong Thap University, Dong Thap, Vietnam
[2]An Giang University, An Giang, Vietnam
[3]Vietnam National University Ho Chi Minh City, Vietnam

*Corresponding author: hadanhduc@gmail.com

(Received: November 17, 2023; Revised: January 27, 2024; Accepted: February 01, 2024)

Abstract - Thiobencarb is an herbicide component extensively applied for weed control. In this study, the mixed culture of *Pseudomonas* sp. Th1 and *Cupriavidus oxalaticus* Th2 was determined for degradability towards thiobencarb at several conditions. The degradation of thiobencarb at 50 µM by the mixture of these strains in the medium without any co-substrate was 48.5±5.5% for 24 hours, increased to 78.0% with the addition of 0.5 mg/L ammonium sulfate and 0.5 mg/L succinate in to the medium. Moreover, the mixed culture could degrade the compound at quite ranges of NaCl concentrations, pH and temperatures. To increase the degradation of the compound in a commercial herbicide, bacteria were immobilized in a matrix consisting of polyvinyl alcohol and sodium alginate. The average degradation of thiobencarb in an herbicide named Satunil 60E by immobilized bacteria was 25.8% higher than that of non-immobilized bacteria at a cell density of 1.0×10^7 CFUs/mL.

Key words - Degradation; herbicide; immobilization; thiobencarb.

Tóm tắt - Thiobencarb là một hoạt chất của thuốc trừ cỏ được sử dụng rộng rãi. Trong nghiên cứu này, hỗn hợp hai chủng vi khuẩn *Pseudomonas* sp. Th1 và *Cupriavidus oxalaticus* Th2 được khảo sát về khả năng phân hủy thiobencarb ở các điều kiện nuôi cấy khác nhau. Hỗn hợp vi khuẩn trên phân huỷ 48,5±5,5% thiobencarb ở nồng độ 50 µM trong môi trường khoáng lỏng sau 24 giờ, tăng lên 78,0% khi có bổ sung thêm 0,5 mg/L ammonium sulfate và 0,5 mg/L succinate vào môi trường. Ngoài ra, sự phân huỷ thiobencarb bởi hỗn hợp vi khuẩn trên thay đổi ở các nồng độ NaCl, pH và nhiệt độ khác nhau. Để tăng khả năng phân huỷ thiobencarb trong thuốc trừ cỏ thương mại, vi khuẩn được cố định trong chất nền gồm polyvinyl alcohol và sodium alginate. Sự phân hủy thiobencarb trung bình trong thuốc trừ cỏ Satunil 60E bởi vi khuẩn cố định cao hơn so với vi khuẩn không cố định là 25,8% ở mật độ $1,0\times10^7$ CFUs/mL.

Từ khóa - Phân huỷ; thuốc trừ cỏ; cố định; thiobencarb.

1. Introduction

Thiobencarb is an active component of herbicides used to control weeds. The herbicide has been extensively applied causing severe environmental pollution. For example, thiobencarb has been detected in water collected from rice fields [1] and tap water [2]. In soil, the compound caused negative effects on soil microbial processes [3]. Therefore, the elimination of thiobencarb in contaminated environments is urgently needed. The compound is quite persistent in aquatic media [4], so the degradation should be augmented by microorganisms.

Thiobencarb can be removed using physical and chemical methods such as using zero valent iron [5], photocatalytic degradation over $BiVO_4$ driven [6] and photocatalytic degradation by a visible light-driven MoS_2 [7]. Biodegradation by microorganisms is considered as an environmentally friendly and effective method. In previous studies, some aerobic thiobencarb-degrading microbes, such as *Aspergillus niger* [8] and *Acidovorax* sp. strain T1 [9], have been isolated. Recently, two bacterial strains, *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 isolated from soil, showed effective degradation of thiobencarb [10]. *Acidovorax* sp. T1, *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 could utilize thiobencarb as a sole carbon, nitrogen and sulfur source for growth.

The degradation of toxic compounds may be conducted by free and immobilized microorganisms. Polyvinyl alcohol (PVA) and sodium alginate (SA) are suitable carriers for entrapping microorganisms used to remove pollutants due to their non-toxicity and biocompatibility [11]. PVA-SA also possessed high mass transfer efficiency and mechanical strength [12]. A commercial herbicide usually contains some active ingredients and adjuvants, which may inhibit the degradation of a target compound. Moreover, the degradation may be affected by environmental conditions such as pH, temperature and others.

In the previous study, *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 were determined for thiobencarb degradation at an optimal condition [10]. These bacteria transformed thiobencarb to S-4-chlorobenzyl ethylthiocarbamate and 4-chlorobenzyl mercaptan before complete degradation. The synergistic degradation of the of *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 in the mixed culture reduced the concentrations of the intermediate metabolites [10]. However, the degradation of the compound in a commercial herbicide by these bacteria

was inhibited even though they were immobilized in rice straw [10]. Besides, the activities of bacteria can be affected by various environmental conditions. Therefore, in this study, thiobencarb degradation by the mixed culture of *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 with the effects of environmental conditions was conducted. The degradation of pure thiobencarb and thiobencarb in an trade herbicide was also compared. Moreover, the immobilization of bacteria in PVA-SA to overcome the inhibition in degradation of the compound in a trade herbicide with the presence of adjuvants was conducted.

2. Material and methods

2.1. Chemicals and culture media

The mineral medium was prepared according to a previous study [10] and used to do all experiments. The components of the mineral medium included 2.79 g/L Na_2HPO_4, 1.00 g/L KH_2PO_4, 0.20 g/L $MgCl_2{\cdot}6H_2O$, and 1.0 mL of trace mineral solution. The trace mineral solution consisted of 0.30 g/L H_3BO_3, 0.20 g/L $FeCl_2{\cdot}6H_2O$, 0.10 g/L $ZnCl_2{\cdot}7H_2O$, 0.03 g/L $Na_2MoO_4{\cdot}2H_2O$, 0.03 g/L $MnCl_2{\cdot}4H_2O$, and 0.01 g/L $CuCl_2{\cdot}2H_2O$. The pH was adjusted to 7.0±0.1 using NaOH. The medium was autoclaved at 120 ºC for 15 min.

Thiobencarb (>98% purity, Sigma-Aldrich) was dissolved in absolute ethanol at 0.1 M (25.8 g/L) and used as a stock solution. This thiobencarb was considered as a pure substrate. Moreover, a commercial herbicide named Satunil 60EC (Summit Agro Company, Vietnam) containing adjuvants, 400 g/L thiobencarb and 200 g/L propanil was also used in degradation experiment. The stock solution and Satunil 60EC were added into liquid media at 50 µM thiobencarb.

2.2. Bacteria culture

Two bacterial strains, *Pseudomonas* sp. Th1 (GenBank under accession numbers OP980960) and *C. oxalaticus* Th2 (GenBank under accession numbers OP980961), isolated from soil [10] were used in this study. These bacteria were separately cultured in the mineral medium supplemented with 0.5 g/L ammonium sulfate, 0.5 g/L succinate and 50 µM thiobencarb. After 24 hours, bacteria were collected by centrifuging at 10,000 rpm for 5 min, rinsed twice with the mineral medium and then resuspended in a fresh mineral medium to 10^{10} CFU/mL. This condensed bacteria solution was used to inoculate.

2.3. Effects of nutrients, environmental conditions and bacterial numbers on thiobencarb degradation

The degradation was carried out in the mineral medium containing 50 µM thiobencarb at various conditions. For effects of nutrients on the degradation, bacteria were cultured in the medium without any co-substrate, added with ammonium sulfate or/and succinate. The degradation was conducted at 0.5 g/L succinate and from 0 to 1.5 g/L ammonium sulfate to determine the effects of ammonium sulfate, and 0.5 g/L ammonium sulfate and from 0 to 1.5 g/L succinate to determine the effects of succinate on thiobencarb degradation.

For the effects of salinity, pH and temperature on degradation, the process was conducted in the mineral medium supplemented with 0.5 g/L ammonium sulfate and 0.5 g/L succinate. The medium was supplemented with NaCl (1.0, 2.0, 3.0 and 4.0 g/L), or added with HCl/NaOH to the pH of 4.0, 5.0, 6.0, 7.0 and 8.0. For the effects of temperature on degradation, the incubation was controlled at 20, 30, 40 and 50ºC. For the degradation at different bacterial numbers, the experiment was conducted at from 0.01×10^7 to 10.0×10^7 CFUs/mL. Each strain was inoculated at the same bacteria number. The control was inoculated with 1.0×10^7 CFUs/mL and then autoclaved at 121°C for 20 min.

2.4. Immobilization of bacteria in PVA-SA bead

The immobilization was processed as a previous study [13]. The mineral medium was added with PVA (10 %, w/v) and alginate SA (2 %, w/v), then heated and stirred at 80°C for 1 hour. The mixture was cooled to ambient temperature before adding the condensed bacteria solution to 10^8 CFU/mL. The medium with bacteria was dropped using a syringe into a beaker containing a solution of saturated boric acid and $CaCl_2$ (4 %, w/v), stirred at 200 rpm using a magnet bar. All formed beads about 3 mm in diameter were stored for 12 hours for crosslinking, then collected, rinsed with mineral medium and kept at -20ºC before using. The control was autoclaved at 121°C for 20 min. For the degradation, bead numbers were transferred in flasks to 0.1×10^7 and 1.0×10^7 CFUs/mL.

2.5. Analytical methods

The concentrations of the thiobencarb were determined using HPLC. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of LC 20AD pumps, SIL-20A autosampler, an SPDM20A photodiode array (PDA) detector and Shimadzu Shim-Pack XR-ODS column (3.0×30 mm, 2.2 µm) was used to separate thiobencarb metabolites. The mobile phase including acetonitrile and water (30:70, v/v) was pumped isocratically at a rate of 0.5 mL/min, and a 5 µl sample was injected into the HPLC system. The column oven temperature was maintained at 40°C, and the detection was performed at a wavelength of 250 nm.

2.6. Incubation condition and statistical analysis

The degradation was conducted using 250 mL flasks. Each flask was added with 50 µM thiobencarb. The shaking speed was controlled at 150 rpm. The incubation was carried out at 30ºC except for the degradation at different temperatures. Moreover, the pH of the mineral medium was adjusted 7 except for the degradation at different pH levels. Each experiment was conducted at least three replicates. Data obtained from all replicates were shown as the means ± standard deviation. The variance and the significant differences ($p < 0.05$) were calculated using Duncan's test in SPSS software program version 22.0.

3. Results

3.1. Effects of nutrients on thiobencarb degradation by suspended bacteria

Before determining the degradation by the mixture of *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 in the presence of ammonium sulfate and succinate, the utilization of thiobencarb as a sole carbon, nitrogen and

sulfur source was carried out. The result showed that utilization of thiobencarb at 50 µM in the medium without any co-substrate was 48.5±5.5% for 24 hours. The average degradation percentage was 64.8% with the addition of only 0.5 g/L succinate (Figure 1a1), 60.5% with the supplementation with only 0.5 g/L ammonium sulfate (Figure 1b1), and 78.0% with the addition of the both co-substrates (0.5 g/L succinate and 0.5 g/L ammonium sulfate). However, the ammonium sulfate addition at higher 0.5 g/L did not statistically alter the degradation performances. In the medium supplemented 0.5 g/L ammonium, thiobencarb degradation rates at different succinate concentrations were in the order: 0 m/L ≈ 1.5 g/L < 0.5 m/L ≈ 1.0 m/L succinate (Figure 1b1). However, the degradation by bacteria was reduced at 1.5 g/L succinate. The growth rates of bacteria increased at the higher co-substrate concentrations (Figure 1a2 and 1b2). Meanwhile, the degradation and bacterial growth of dead cells (control) were not found. The addition of succinate showed higher stimulating effects on bacterial growth than that of ammonium sulfate. For example, the bacterial numbers in the mineral medium with the addition of ammonium sulfate and thiobencarb were $(2.8\pm0.41)\times10^7$ CFUs/mL, while data for medium supplemented with succinate and thiobencarb were $(4.2\pm0.45)\times10^7$ CFUs/mL after 30 days.

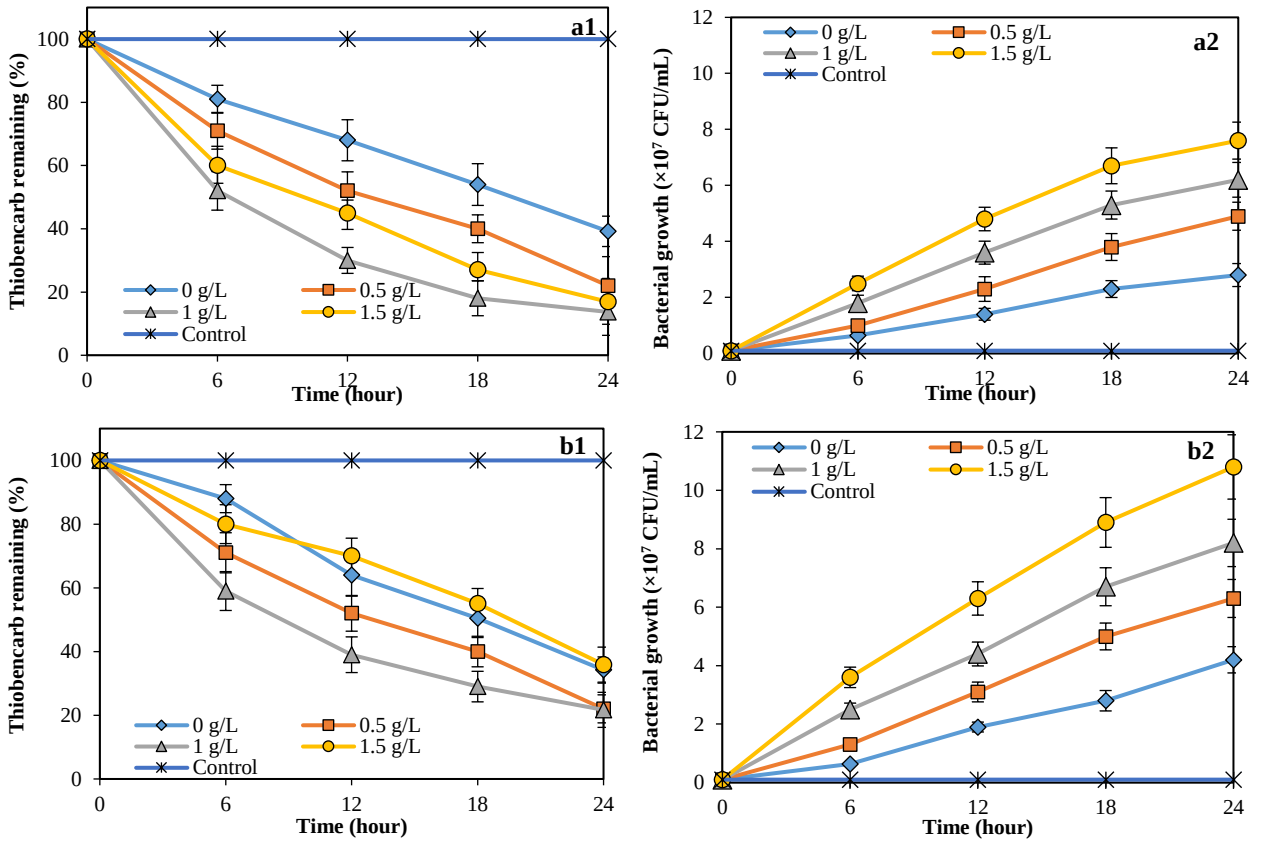

Figure 1. *Effects of (a) ammonium sulfate and (b) succinate on (1) thiobencarb degradation and (2) bacterial growth of the mixed bacterial culture. The experiments were conducted in mineral medium supplemented with 50 µM thiobencarb. The control was carried out using dead bacteria in the medium supplemented with 0.5 g/L ammonium sulfate and 0.5 g/L succinate*

3.2. Effects of sodium chloride, pH and temperature on thiobencarb degradation by suspended bacteria

Sodium chloride, pH and temperature are environmental conditions directly affecting biological processes. For sodium chloride, the degradation percentages were decreased at higher salt concentrations in general. The degradation percentages at 0 and 1.0 g/L NaCl were not statistically different (Figure 2). The degradation at higher these salt concentrations was inhibited.

For the effects of pH, the increase in pH levels enhanced the degradation until pH = 7. However, the degradation was significantly inhibited at pH = 8 (Figure 2). For the effects of temperature, the degradation rates were highest at 30 and 40°C. The lower or higher than these temperatures reduced the degradation by bacteria (Figure 2).

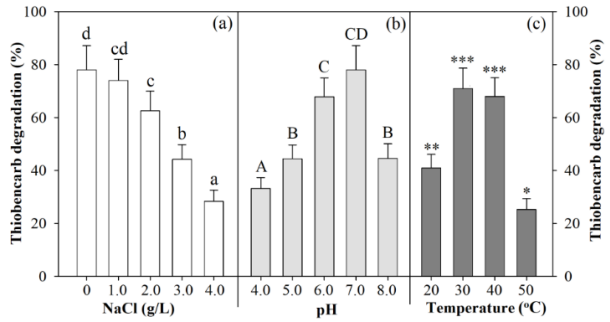

Figure 2. *Effects of (a) NaCl, (b) pH and (c) temperature on thiobencarb degradation*

The experiments were conducted in mineral medium supplemented with 0.5 g/L ammonium sulfate, 0.5 g/L succinate, 50 µM thiobencarb and incubated for 24 hours. The different lowercase and capitalized letters show statistically significant differences

within the treatments of NaCl and pH, respectively. Besides, the different numbers of asterisk symbol indicate statistically significant differences among treatments of temperature ($p<0.05$)

3.3. Effects of number of suspended bacteria on thiobencarb degradation

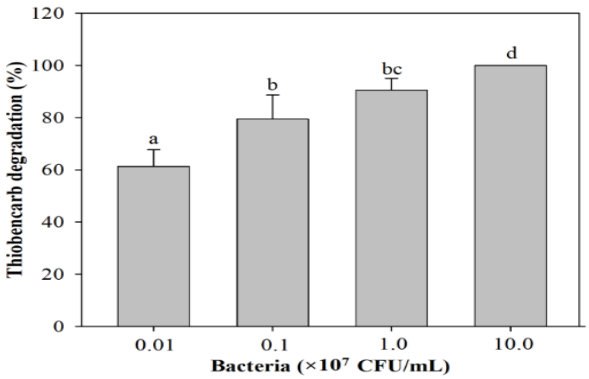

Figure 3. *Effects of initial bacterial numbers on thiobencarb degradation and bacterial growth.*

The experiment was conducted in mineral medium supplemented with 0.5 g/L ammonium sulfate, 0.5 g/L succinate, 50 µM thiobencarb and incubated for 24 hours. The different lowercase letters show statistically significant differences among treatments of bacterial numbers ($p<0.05$)

The treatments at higher initial bacterial numbers showed significantly higher thiobencarb removal (Figure 3). After 24 hours, thiobencarb was degraded just over 60% at 0.01×10^7 CFU/mL, increased by nearly 20% at 0.1×10^7 CFU/mL, and completely degraded at 10.0×10^7 CFU/mL. Meanwhile, all abiotic controls showed no thiobencarb removal (data not shown).

3.4. Thiobencarb degradation by immobilized bacteria

Before the degradation by immobilized bacteria, the degradation of thiobencarb in the herbicide Satunil 60E by free cells was conducted at 50 µM in mineral medium supplemented with 0.5 g/L ammonium sulfate and 0.5 g/L succinate. The result showed that 51.4±4.6% and 62.4±6.8% of thiobencarb in Satunil 60E were degraded for 24 hours at initial bacteria of 0.1×10^7 and 1.0×10^7CFU/mL, respectively. This result indicated that the degradation of thiobencarb in the herbicide was significantly slower than the degradation of the pure substrate shown in Figure 3.

For the degradation of pure thiobencarb, the degradation rates of immobilized bacteria (Table 3) were significantly higher than those freely suspended bacteria (Figure 3) at the same bacteria numbers. However, thiobencarb was also decreased in the treatment using alginate beads without bacteria, probably the substrate was absorbed into the beads. Therefore, the degradation percentages of the pure compound by immobilized and freely suspended bacteria were not statistically different.

For the degradation of thiobencarb in the herbicide Satunil 60E by immobilized bacteria, the degradation rates were significantly slower than those of the pure substrate (Table 1). However, the rates of immobilized cells were significantly higher than those of free counterparts. Indeed, the average degradation percentages of the compound in Satunil 60E by bacteria immobilized in PVA-SA bead shown in Table 1 were 20.4% and 25.8% higher than those by freely suspended cells at 0.1×10^7 and 1.0×10^7 CFUs/mL, respectively.

Table 1. *Degradation of pure thiobencarb and thiobencarb in Satunil 60E by the mixed culture immobilized in PVA-SA bead*

Bacteria (CFU/mL)	Degradation of pure thiobencarb (%)		Degradation of thiobencarb in Satunil 60E (%)	
	Control (PVA-SA bead without bacteria)	PVA-SA bead with bacteria	Control (PVA-SA bead without bacteria)	PVA-SA bead with bacteria
0.1×10^7	13.4±3.3^a	90.2±4.4^c	14.5±3.1^a	71.8±6.9^b
1.0×10^7	17.4±4.1^a	100^c	18.2±3.5^a	88.2±4.0^b

The different lowercase letters show statistically significant differences among treatments within a line ($p<0.05$)

4. Discussion

Pseudomonas sp. Th1 and *C. oxalaticus* Th2 could utilize thiobencarb as a sole carbon and nitrogen source. The addition of co-substrates enhanced the degradation rates because bacteria used supplemental nutrients which stimulate bacteria growth resulting in higher degradation. Nitrogen content in thiobecarb molecule was small (5.42%); therefore, ammonium sulfate was an important supplemented nitrogen source. A previous study also showed that the supplementation with ammonium sulfate increased thiobencarb degradation by *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 [10]. The degradation in the media with the presence of 0.5 g/L ammonium sulfate was similar to the previous report [10]. In this study, succinate was used as an additional carbon source to increase the bacterial growth rates. Succinate was a carbon source which is an important compound in the Krebs cycle to provide energy for cells. However, a high succinate concentration (1.5 g/L) somewhat reduced the degradation performance, probably because bacteria preferred to use it to grow. Similarly, the supplementation with succinate and sodium nitrate enhanced the degradation rate of *Aspergillus niger* [14]. However, *Aspergillus niger* removed less than 90% pure thiobencarb at 20 mg/L in the Czapek-Dox medium containing co-substrate after 20 days [14].

Biodegradation is always affected by environmental condition. Thiobencarb may contaminate in saline media, at various pH levels and different ambient temperature. In this study, the mixed culture of *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 showed the highest effective degradation at low salt concentrations, neutral pH and normal temperature. The degradation rates were decreased at high acidic and alkaline conditions, high salinity and high temperatures. Such severe conditions inhibited the activities of isolated bacteria. However, bacteria could degrade the compound at quite high ranges of described environmental conditions. The degradation rates of pure

thiobencarb were significantly higher than those of the compound in the herbicide Satunil 60E, which was described in a previous study [10]. Propanil and other adjuvants in the herbicide caused negative effects on the bacterial mixture. In a previous study, adjuvants in herbicides caused the reduction of prometryn degradation by free cells, but immobilized microorganisms [15].

In the previous report, degradation of pure thiobencarb by immobilized bacteria in rice straw was significantly higher than those of their free counterparts in liquid media, but in soil [10]. However, the immobilization in rice straw did not overcome the negative effects of herbicide adjuvants on thiobencarb degradation in a commercial herbicide [10]. In this study, the immobilized bacteria in PVA-SA showed higher effective degradation thiobencarb in the commercial herbicide than freely suspended bacteria probably because the alginate matrix reduced the direct contact between bacteria and herbicide components. Polyvinyl alcohol and alginate are a cheap material which has been applied to immobilize microorganisms. The application of alginate to enhance the degradation of other herbicide was also described in previous studies [13, 16].

5. Conclusion

The mixed culture of *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 could degrade thiobencarb at extreme conditions, but it showed the most effective degradation at low salt concentrations, the neutral pH and normal temperature. The degradation rates of the pure substrate were significantly higher than those of thiobencarb in Satunil 60E. The average degradation percentages of the compound in Satunil 60E by bacteria immobilized in PVA-SA bead were 20.4% and 25.8% higher than those by freely suspended cells at 0.1×10^7 and 1.0×10^7 CFUs/mL, respectively. This study provides information on the roles of nutrients, the effects of some environmental conditions on thiobencarb degradation, and degradation enhancement of the compound in an herbicide by using PVA-SA to immobilize bacteria.

Funding: This study was supported by the Vietnamese Ministry of Education and Training for the scientific theme (Code: B2022.SPD.04). The authors would like to thank them for all supports.

REFERENCES

[1] P. Sapari and B. S. Ismail, "Pollution levels of thiobencarb, propanil, and pretilachlor in rice fields of the muda irrigation scheme, Kedah, Malaysia", *Environ Monit Assess*, vol. 184, no. 10, pp. 6347-6356, 2012. https://doi.org/10.1007/s10661-011-2424-9

[2] M. N. Amin, S. Kaneco, T. Kato, H. Katsumata, T. Suzuki, and K. Ohta, "Removal of thiobencarb in aqueous solution by zero valent iron", *Chemosphere*, vol. 70, pp. 511-515, 2008. https://doi.org/10.1016/j.chemosphere.2007.09.017

[3] C. Saison, N. J. Waller, A. Kumar, and R. S. Kookana, "Effects of thiobencarb in combinations with molinate and chlorpyrifos on selected soil microbial processes", *J Environ Sci Health B*, vol. 44, no. 3, pp. 226-234, 2009. https://doi.org/10.1080/03601230902728195

[4] W. C. Quayle, D. P. Oliver, and S. Zrna, "Field dissipation and environmental hazard assessment of clomazone, molinate, and thiobencarb in Australian rice culture", *J Agric Food Chem*, vol. 54. pp. 213-220, 2006. https://doi.org/10.1021/jf061107u

[5] M. N. Amin, S. Kaneco, T. Kato, H. Katsumata, T. Suzuki, and K. Ohta, "Removal of thiobencarb in aqueous solution by zero valent iron", *Chemosphere*, vol. 70, pp. 511-515, 2008. https://doi.org/10.1016/j.chemosphere.2007.09.017

[6] H-F. Lai, C-C.Chen, Y-K.Chang, C-S. Lu, and R-J. Wu, "Efficient photocatalytic degradation of thiobencarb over $BiVO_4$ driven by visible light: parameter and reaction pathway investigations", *Sep Purif Technol*, vol. 122, pp. 78-86, 2014. https://doi.org/10.1016/j.seppur.2013.10.049

[7] S. Huang, C. Chen, H. Tsai, J. Shaya, and C. Lu, "Photocatalytic degradation of thiobencarb by a visible light-driven MoS_2 photocatalyst", *Sep Purif Technol*, vol. 197, pp. 147-155, 2018. https://doi.org/10.1016/j.seppur.2018.01.009

[8] M Torra-Reventós, M Yajima, S Yamanaka, and T Kodama, "Degradation of the herbicides thiobencarb, butachlor and molinate by a newly isolated *Aspergillus niger*", *J Pestic Sci*, vol. 29, pp. 214-216, 2004. https://doi.org/10.1584/jpestics.29.214

[9] C. W. Chu *et al.*, "A novel aerobic degradation pathway for thiobencarb is initiated by the TmoAB two-component flavin mononucleotide-dependent monooxygenase system in *Acidovorax* sp. strain T1", *Appl Environ Microbiol*, vol. 83, pp. e01490-17, 2017. https://doi.org/10.1128/AEM.01490-17

[10] H. D. Duc, N.T. Oanh, and N. T. M. Khanh, "Thiobencarb degradation by *Pseudomonas* sp. Th1 and *C. oxalaticus* Th2 isolated from soil, *Curr Microbiol*, vol. 80, pp. 342, 2023. https://doi.org/10.1007/s00284-023-03456-0

[11] X. Xu, H. Zhou, X. Chen, B. Wang, Z. Jin, and F. Ji, "Biodegradation potential of polycyclic aromatic hydrocarbons by immobilized *Klebsiella* sp. in soil washing effluent", *Chemosphere*, vol. 223, pp. 140-147, 2019. https://doi.org/10.1016/j.chemosphere.2019.01.196

[12] S. H. Liu, Z. T. Zeng, Q. Y. Niu, R. Xiao, G. M. Zeng, and Y Liu, "Influence of immobilization on phenanthrene degradation by *Bacillus* sp. P1 in the presence of Cd(II) ", *Sci Total Environ*, vol. 655, pp. 1279-1287, 2019. https://doi.org/10.1016/j.scitotenv.2018.11.272

[13] J. Liu, D. Pan, X. Wu, H. Chen, H. Cao, X. L. Qing, and H. Rimao, "Enhanced degradation of prometryn and other s-triazine herbicides in pure cultures and wastewater by polyvinyl alcohol-sodium alginate immobilized *Leucobacter* sp. JW-1", *Sci Total Environ*, vol. 615, pp. 78-86, 2018. https://doi.org/10.1016/j.scitotenv.2017.09.208

[14] M. Torra-Reventós, M. Yajima, S. Yamanaka, and T. Kodama, "Degradation of the herbicides thiobencarb, butachlor and molinate by a newly isolated *Aspergillus niger*", *J Pestic Sci* vol. 29, pp. 214-216, 2004. https://doi.org/10.1584/jpestics.29.214

[15] J. F. Pérez-Bárcena *et al.*, "Effect of herbicide adjuvants on the biodegradation rate of the methylthiotriazine herbicide prometryn", *Biodegradation*, vol. 25, pp. 405-415, 2014. https://doi.org/10.1007/s10532-013-9669-7

[16] B. Zhang *et al.*, "Bead-immobilized *Pseudomonas stutzeri* Y2 prolongs functions to degrade s-triazine herbicides in industrial wastewater and maize fields", *Sci Total Environ*, vol. 731, pp. 139183, 2020. https://doi.org/10.1016/j.scitotenv.2020.139183