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ผลกระทบของเกลืออนินทรีย์ต่อการย่อยสลายสารกำจัดศัตรูพืชโพรฟีโนฟอสด้วยจุลินทรีย์สายพันธุ์ Pseudomonas Plecoglossicida Strain PF1 และ Acinetobacter Baylyi Strain GFJ2 ในรูปแบบเซลล์ตรึง

Effect of Inorganic Salts on Profenofos Pesticide Degradation of Immobilized Pseudomonas Plecoglossicida Strain PF1 and Acinetobacter Baylyi Strain GFJ2

ชุติมา พลอยจันทร์กุล อลิสา วังใน และ สุมนา ราษฎร์ภักดี

Chutima Ploychankul[1,2], Alisa S. Vangnai[1,3] and Sumana Ratpukdi[1,4*]

[1]ศูนย์ความเป็นเลิศด้านการจัดการสารและของเสียอันตราย จุฬาลงกรณ์มหาวิทยาลัย

[2] สาขาวิชาการจัดการสิ่งแวดล้อม (หลักสูตรนานาชาติ) บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

[3]ภาควิชาชีวเคมี คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

[4]ภาควิชาวิศวกรรมสิ่งแวดล้อม คณะวิศวกรรมศาสตร์ และกลุ่มวิจัยวิศวกรรมฟาร์มและเทคโนโลยีการควบคุมอัตโนมัติ มหาวิทยาลัยขอนแก่น อำเภอเมือง จังหวัดขอนแก่น 40002

[1] Center of Excellence for Environmental and Hazardous Waste Management, Chulalongkorn University

[2] International Postgraduate Programs in Environmental Management, Graduate School, Chulalongkorn University

[3] Department of Biochemistry, Faculty of Science, Chulalongkorn University

[4] Department of Environmental Engineering, Faculty of Engineering and Farm Engineering and Automatic Control Technology Research Group, Khon Kaen University, Khon Kaen 40002

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บทคัดย่อ

โพรฟีโนฟอสเป็นสารกำจัดศัตรูพืชในกลุ่มออร์แกโนฟอสเฟตที่ได้รับถูกใช้อย่างแพร่หลายซึ่งทำให้มีโอกาสตรวจพบการปนเปื้อนในสิ่งแวดล้อมในปริมาณที่สูง การย่อยสลายโฟรฟีโนฟอสด้วยวิธีทางชีวภาพภายในพื้นที่นั้นต้องอาศัยสภาวะที่เหมาะสมต่อการทำงานของจุลินทรีย์ในสิ่งแวดล้อม ซึ่งเทคโนโลยีการตรึงเซลล์นั้นช่วยปรับปรุงการทำงานของจุลินทรีย์ได้ ดังนั้นในงานวิจัยนี้จึงได้มีการศึกษาผลกระทบของเกลืออนินทรีย์ที่ปนเปื้อน

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ในสิ่งแวดล้อมที่มีผลต่อการย่อยสลายสารโพรฟีโนฟอส โดยจุลินทรีย์สายพันธุ์ Pseudomonas plecoglossicida strain PF1 (PF1) และ Acinetobacter baylyi strain GFJ2 (GFJ2) ในรูปแบบเซลล์ตรึง การศึกษานี้เลือกสารแคลเซียมแอลจิเนตเป็นวัสดุตรึง ส่วนเกลืออนินทรีย์ที่ศึกษา ได้แก่ โซเดียมคลอไรด์ แมกนีเซียมซัลเฟต และแคลเซียมคาร์บอเนต ที่ความเข้มข้น 0 - 1,000 มิลลิกรัมต่อลิตร จากผลการทดลองปรากฏว่าเซลล์ตรึงทั้ง 2 ชนิดสามารถย่อยสลายสารโพรฟีโนฟอสได้ดีกว่าเซลล์อิสระโดยมีประสิทธิภาพการย่อยสลายจาก 40-70% เป็น 50-90% ส่วนการทดลองการย่อยสลายสารโพรฟีโนฟอสของเซลล์ตรึงทั้งสองชนิดหลังจากที่มีการเติมเกลืออนินทรีย์ พบว่าเกลือมีส่วนทำให้ประสิทธิภาพการย่อยสลายนั้นลดลงในทุกการทดลอง

คำสำคัญ: โพรฟีโนฟอส สารเคมีกำจัดศัตรูพืชในกลุ่มออร์แกโนฟอสเฟต เซลล์ตรึง เกลืออนินทรีย์

Abstract

Profenofos commonly used in organophosphate pesticides has been detected and contaminated in environment. The in situ biodegradation of profenofos required suitable environmental conditions to support microorganisms. The cell immobilization technique has been initiated to improve biodegradation. The study aimed to investigate on the influence of environmental conditions (inorganic salts) on profenofos biodegradation by immobilized microbial cells. Pseudomonas plecoglossicida strain PF1 (PF1) and Acinetobacter baylyi strain GFJ2 (GFJ2) in calcium alginate immobilization matrices were selected. The inorganic salts including NaCl, $MgSO_4$, and $CaCO_3$ with the concentrations of 0 to 1,000 mg/L were applied. The result showed that profenofos biodegradation by the immobilized PF1 and GFJ2 (50-90%) was better than that by the free cells (40-70%). Profenofos biodegradation by immobilized PF1 and GFJ2 under presence of inorganic salts obviously decreased.

Keywords: Profenofos Oganophosphate pesticide Immobilization technique Inorganic salt

*ติดต่อ: อีเมล: sumana.r@kku.ac.th, โทรศัพท์: 043-202571, โทรสาร: 043-202571

1. Introduction

Profenofos ($C_{11}H_{15}BrClO_3PS$), O-4-bromo-2-chlorophenyl O-ethyl S-propyl phophorothioate is one of the most widely used organophosphorus insecticides in many countries such as Thailand (3,892 t/y), Malaysia (2,826 t/y), Myanmar (476 t/y), and India (16,922 t/y) [1]. This situation led to profenofos contamination in environment and agricultural

products. For example, Harnpicharnchai et al. (2013) reported the profenofos-contaminated concentrations in water and soil during summer of up to 0.95 mg/L and 41.81 mg/kg, respectively [2]. Another example is profenofos of 0.5-183,000 µg/L contaminating in Chinese kale (Maximum residue limits by Codex Alimentarius Commission of 50 µg/L) [3]. This compound has been known as acetylcholinesterase inhibitors and toxic to organisms [4]. Based on this information, profenofos remediation technology is required.

It was reported environmental factors influencing the microbial degradation in the contaminated sites, such as pH, temperature, inorganic salts [5]. Previously, it was found that bacterial species including Pseudomonas plecoglossicida strain PF1 (PF1) and Acinetobacter baylyi strain GFJ2 (GFJ2) successfully degraded profenofos [6]. So far, there was no published report on influence of inorganic salts which typically found in groundwater on profenofos biodegradation.

Cell immobilization technique by entrapping microbial cells in polymeric materials could lessen the problem described previously. Advantages of the cell immobilization include the cell protection from toxic substances and environmental stresses, high cell density and retention, and ease of cell preparation and reuse [7].

This study aimed to investigate the influence of profenofos biodegradation by the immobilized PF1 and GFJ2 under different types and concentration of inorganic salts. Calcium alginate was selected as the immobilization material. It is noted that the types and concentrations of inorganic salts (NaCl, $MgSO_4$, and $CaCO_3$ at concentrations of 100-1000 mg/L) were chosen based on typical salt types and concentrations present in groundwater. The study by the free cells was performed along with the test by the immobilized cells for comparative purpose.

2. Methodology

2.1 Chemicals and bacterial cultivation

Commercial profenofos emulsifiable concentrate (50% w/v) applied in the experiments was purchased from a local distributer (Syngenta Crop Protection Co., Thailand). The chemicals used for bacterial medium and chemical analysis were laboratory and analytical grades obtained from Himedia (India), Ajax (Australia), and RCI Labscan (Australia). Alginic acid sodium salt (ACROS Organics, Singapore) was used for cell immobilization.

Pseudomonas plecoglossicida strain PF1 was previously isolated from profenofos-contaminated chili farm soil [6]. The strain was subcultured in minimal salt medium (MSM) every 4 days for 2 weeks before used. The formulation of MSM (pH of 7.0) was followed Siripattanakul-

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Ratpukdi et al. (2014) [6]. For biodegradation assay, the activated culture (10%) was enriched by transferring into 1 liter of MSM medium with 0.1% yeast extract (MSMY) with incubation time for 15h at shaking condition of 120 rpm and 30 °C. The PF1 suspended cells were used to prepare the immobilized cells.

Acinetobacter baylyi strain GFJ2 was isolated from herbicide-contaminated soils (Hongsawat and Vangnai, 2011). The GFJ2 cells from Luria-Bertani (LB) agar plate was inoculated into LB broth with shaking condition at 200 rpm and 30 °C for overnight. To enrich GFJ2 for the experiment, the active GFJ2 culture of 1% was transferred into 1 liter of minimal medium (called MMSAY) with incubation time for 12 h at 30 °C and 120 rpm. Formulation of MMSAY was modified from Hongsawat and Vangnai (2011) by adding 4 mM of succinic acid, 1 mM of ammonium sulfate and 0.1% (w/v) of yeast extract.

The filtered sterile profenofos (the concentration of 20 mg/L in bacterial medium) was applied as the main carbon source on the enrichment process for both strains. After enrichment, the strains were harvested by centrifugation (5,000 rpm, 30 min, and 4 °C). The pellets were washed and resuspended with 0.85% NaCl. The cells of approximately 1.5×10^{12} CFU/mL were used for the profenofos biodegradation assay by the free and immobilized cells.

2.2 Cell immobilization procedure

The suspension of PF1 and GFJ2 were separately added and mixed into 90 mL of a sterile sodium alginate solution (3% (w/v)). The cell-alginate mixture was dropped by a peristaltic pump with tube at 2.76 mm into a $CaCl_2$ solution of 3.5% (w/v). The 4- mm immobilized PF1 and GFJ2 (based on biodegradation performance described later) were selected. The beads were hardened in the $CaCl_2$ solution for 2 h with gentle agitation. The hardened beads were washed before used. The washing solution containing Tris-HCl (10 mM, pH 7.0) (designated as TSM) was applied to avoid de-entrapment of the immobilized cells.

2.3 Profenofos biodegradation

The inorganic salt types and concentrations (NaCl, $MgSO_4$, and $CaCO_3$ at 100-1000 mg/L) were selected. The experiments by the free PF1 and GFJ2 cells were run along with ones by the immobilized cells. All experiments were carried out in duplicate.

The cells (PF1 and GFJ2) of 3 mL and 10 g for the free and immobilized cells, respectively was inoculated into 27 mL of TSM in 125 mL serum bottles. The filtered sterile profenofos was added to obtain the initial concentration of 20 mg/L in the serum bottle (except the tests with variation of profenofos concentrations). The batch tests were conducted on rotary shaker at 150 rpm and 30 °C for 4 days.

The profenofos concentration remaining in the reactors was monitored continuously.

2.4 Analysis

To analyze profenofos remaining, the sample was extracted using liquid/ liquid extraction technique. The samples from the batch reactors were added (ratio 1:1) with n-hexane plus 0.01% acetic acid. The mixtures was vigorously mixed by vortex mixer for 20 min and centrifuged at 10,000 rpm for 5 min. The organic phase (the extract) was filtered with a nylon filter (0.22 µm). A gas chromatography (GC) with electron captures detector (model GC-2014, Shimazu) with a DB-5 column (30-m length, 0.32-mm i.d., and 0.25-µm film thickness) was used. A microliter of the filtered extract was injected into GC with conditions of splitless injection, injection temperature of 240°C, and helium gas flow of 37.4 mL/min. The GC temperature program started at 180 °C and hold for 3 min, increased to 240 °C with the rate of 30 °C/min and hold for 4 min. The total run time was 9 min. The peak retention time of profenofos was 7.9 min.

3. Results and discussion

Inorganic salts as NaCl, $MgSO_4$, and $CaCO_3$ in range of 0 to 3,000 mg/L are mostly found in groundwater and surface water on agricultural area [9-11]. The existence of these inorganic salts might influence on profenofos degradation. In this study, types (NaCl, $MgSO_4$, and $CaCO_3$) and concentrations (0, 100, and 1,000 mg/L) of inorganic salt were varied.

3.1 Influence of inorganic salts on profenofos biodegradation by free cells

The biodegradation performance of the free PF1 under presence of low salt concentration (100 mg/L) was slight better (18-28%) than the test without salt (Table 1 and Figure 1). It indicated that the adaptation of the cell occurred under the low concentration of inorganics [12]. For the tests with salts at 1,000 mg/L, the biodegradation performance was depended on salt types. The tests under presence of high $MgSO_4$ and $CaCO_3$ still achieved good biodegradation performance. This could be because PF1 was used to the environment with these salts. This culture originated from northeastern area of Thailand where high hardness in groundwater was reported. Conversely, in the test with high NaCl, the utilization rate reduced.

From Table 1, the different types of inorganic salts influenced on the biodegradation of free GFJ2 in similar manner. Profenofos removal from the tests with salts (both low and high concentrations) was lower (almost 19%). The result showed different trend of influence by salts for PF1 and GFJ2. This may be because GFJ2 which was isolated from soil in central of Thailand. Most location in the area did not report about hardness and salt in groundwater.

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The culture may not be able or take longer time for adaptation leading to lower biodegradation performance.

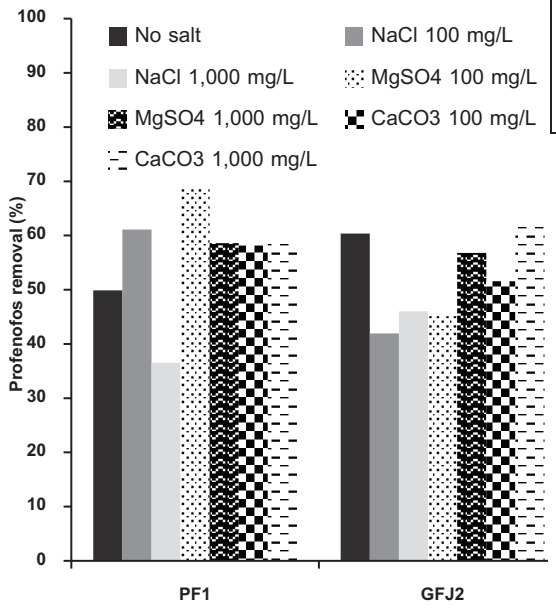

Figure 1 Profenofos biodegradation of free cells at the different types and concentrations of inorganic salts

Table 1 Profenofos biodegradation of free cells

Strains	Salt Types	Concentration of salt (mg/L)	Profenofos utilization rate (mg/L/h)
PF1	No salt		0.08
	NaCl	100	0.15
		1,000	0.06
	$MgSO_4$	100	0.12
		1,000	0.10
	$CaCO_3$	100	0.10
		1,000	0.11
GFJ2	No salt		0.10
	NaCl	100	0.07
		1,000	0.07
	$MgSO_4$	100	0.08
		1,000	0.07
	$CaCO_3$	100	0.08
		1,000	0.11

3.2 Influence of inorganic salts on profenofos biodegradation by immobilized cells

From Figure 2, the biodegradation trend of immobilized cell by PF1 and GFJ2 were similar. Under absence of salt, the immobilization material could accelerate biodegradation ability. It was found that the free PF1 and GFJ2 removed profenofos for 50 and 60% while profenofos of 88 and 71% was reduced by the immobilized PF1 and GFJ2, respectively. It could say that the immobilization material provided better environment (slow releasing toxic substance) leading to higher biodegradation ability. However, under presence of salt, profenofosremoval by the immobilized PF1 and GFJ2 were much lower in all tests. This may be about the substrate diffusion. However, the clear mechanism of salt influence was inconclusive. The continued work on substrate diffusion in porous material under presence of salt should be performed. The immobilized cells are able to apply to the profenofos-contaminated area by cost effective production.

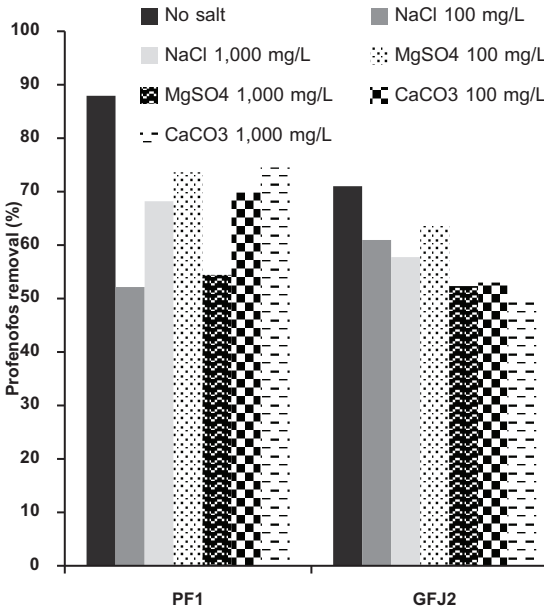

Figure 2 Profenofos biodegradation of immobilized cells at the different types and concentrations of inorganic salt

Table 2 Profenofos biodegradation of immobilized cells

Strains	Salt Types	Concentration of salt (mg/L)	Profenofos utilization rate (mg/L/h)
PF1	No salt		0.26
	NaCl	100	0.04
		1,000	0.06
	$MgSO_4$	100	0.15
		1,000	0.12
	$CaCO_3$	100	0.07
		1,000	0.07
GFJ2	No salt		0.13
	NaCl	100	0.08
		1,000	0.06
	$MgSO_4$	100	0.12
		1,000	0.06
	$CaCO_3$	100	0.05
		1,000	0.05

4. Conclusion

The free and immobilized PF1 and GFJ2 well removed profenofos (40-90%). Profenofos biodegradation in free PF1 increased the performance after adding the inorganic salts ($MgSO_4$ and $CaCO_3$). While the performance of GFJ2 was reduced in all tests under presence of inorganic salts. For the immobilized cell, trend of the biodegradation by PF1 and GFJ2 were similar. The inorganic salts reduced the biodegradation performance in all tests.

However, the clear mechanism of salt influence was inconclusive. The continued work on substrate diffusion in porous material under presence of salt should be performed.

5. Reference

[1] Food and Agriculture Organization of The United Nations FAOSTAT. Statistics Division, Organophosphates Use. [Internet] 2013. Available from: URL: http://faostat3.fao.org/search/*/E

[2] Harnpicharnchai K, Chaiear N, Charerntanyarak L. Residues of Organophosphate Pesticides Used in Vegetable Cultivation in Ambient Air, Surface Water and Soil in Bueng Niam Subdistrict, Khon Kaen, Thailand, Southeast Asian. Journal of Tropical Medicine and Public Health. 2013; 44(6): 1088-97.

[3] Sapbamrer R, Hongsibsong S. Organophosphorus Pesticide Residues in Vegetables from Farms, Markets, and a Supermarket around Kwan Phayao Lake of Northern Thailand. Archives of Environmental Contamination and Toxicology. 2014; 67(1), 60-7.

[4] Santos da Silva S, Chiavone-Filho O, de Barros Neto EL, Foletto EL, Mota ALN. Effect of Inorganic Salt Mixtures on Phenol Mineralization by Photo-Fenton - Analysis via an Experimental Design. Water, Air, & Soil Pollution. 2013; 225(1): 1-10.

[5] Karpouzas DG, Walker A. Factors Influencing the Ability of Pseudomonas Putida Strains EpI and II to degrade the Organophosphate Ethoprophos. Journal of Applied Microbiology. 2000; 89(1): 40-48.

[6] Siripattanakul-Ratpukdi S, Vangnai AS, Sangthean P, Singkibut S. Profenofos Insecticide Degradation by Novel Microbial Consortium and Isolates Enriched from Contaminated Chili Farm Soil. Environmental Science and Pollution Research. 2014; 22(1): 320-328.

[7] Siripattanakul S, Khan E. Undamentals and Applications of Entrapped Cell Bioaugmentation for Contaminant Removal. Emerging Environmental Technologies. 2010; 2: 147-69.

[8] Hongsawat P, Vangnai AS.Biodegradation Pathways of Chloroanilines by Acinetobacter Baylyi Strain GFJ2. Journal of Hazardous Materials. 2011; 186(2-3): 1300-7.

[9] Chandrasekar N, Selvakumar S, Srinivas Y, John Wilson JS, Simon Pete T, Magesh NS. Hydrogeochemical Assessment of Groundwater Quality Along the Coastal Aquifers of Southern Tamil Nadu, India. Environmental Earth Sciences. 2014; 71(11): 4739-50.

[10] Chung SY, Venkatramanan S, Kim TH, Kim DS, Ramkumar T. Influence of Hydrogeochemical Processes and Assessment of Suitability for Groundwater uses in Busan City, Korea. Environment, Development and Sustainability. 2015; 17(3): 423-441.

[11] Singaraja C, Chidambaram S, Anandhan P, Prasanna MV, Thivya C, Thilagavathi R. A Study on the Status of Saltwater Intrusion in the Coastal Hard Rock Aquifer of South India. Environment, Development and Sustainability. 2015; 17(3); 443-75.

[12] Ebrahimi S, Nguyen TH, Roberts DJ. Effect of Temperature & Amp: Salt Concentration on Salt Tolerant Nitrate-Perchlorate Reducing Bacteria: Nitrate Degradation Kinetics. Water Research. 2015; 83: 345-53.