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Profenofos pesticide biodegradation under presence of natural organic carbon

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Abstract

This study was aimed to investigate influence of natural organic carbon on profenofos (PF) pesticide removal contaminated in environment. Microorganism used in this study was *Pseudomonas aeruginosa* PF2 (PF2) and humic acid was chosen as a model of the natural organic carbon. Initial profenofos concentrations of 20-120 mg/L and initial microbial cell of 10^4 CFU/mL were applied. Each experiment was performed in a batch test for 7 days. The result showed that PF2 grew from 10^4 to 10^8 CFU/mL. Profenofos removed for 23-45% and 30-58% during the tests with humic acid of 0 (control) and 10 mg/L, respectively. The profenofos removal was the integration of adsorption and biodegradation processes. Based on the result, it was found that natural organic carbon reduced the profenofos biodegradation. Nonetheless, overall profenofos removal increased due to adsorption by organic matter.

Keywords: Biodegradation, Humic acid, Natural organic carbon, Profenofos

1. Introduction

Profenofos (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate), $C_{11}H_{15}BrClO_3PS$, one of widely used organophosphorus pesticides, is normally applied for pest control in cotton, fruit, chili and vegetable cultivation. Intensive use of pesticides including profenofos leads to its contamination in environment [1-2]. For example, in the north-eastern region of Thailand, profenofos contamination of 1 mg/L in water was reported [3]. This chemical has been classified as a moderately hazardous pesticide (Toxicity class II) by the World Health Organization (WHO). Consequently, the contamination of profenofos could be harmful to water ecosystems.

Microbial remediation, contaminant degradation by isolated microbes, is one of effective environmental treatment techniques. The technique has been successfully investigated for pesticide remediation [4-5]. However, in practice, environmental factors such as pH, clay content, and organic content of soil might affect contaminant removal performance by the isolated microbes. Earlier, influence of natural organic carbon as either metabolism accelerant or inhibitor was reported depending on contaminants and isolated cultures [6].

For profenofos pesticide remediation, *Pseudomonas aeruginosa* strain PF2 (PF2), an efficient profenofos-degrading bacterium, was previously isolated [5]. It was found that the culture successfully removed profenofos of more than 90% in 3 days. However, as stated, in the real situation, the removal performance may be interfered by environmental factors. Thus far, there was no study on influence of natural organic carbon to profenofos degradation. Consequently, this study aims to investigate effect of natural organic carbon to profenofos degradation by PF2. In this study, humic acid was chosen as a model natural organic carbon. Influence of initial profenofos concentrations was examined. The result from this work could be used as basic information for profenofos-contaminated site remediation in the future.

2. Materials and methods

2.1 Chemicals

Commercial grade Profenofos (50% w/v EC, Syngenta Crop Protection Co., Switzerland) and humic acid (Sigma Chemical Co., Singapore) were used in the experiment. Profenofos (analytical grade, Sigma Chemical Co., Singapore) was obtained for analytical task. Other chemicals were laboratory grade obtained from local chemical suppliers.

2.2 Microbial cultivation

Pseudomonas aeruginosa strain PF2 (GenBank accession number KJ143903), a previously isolated profenofos-degrading culture, was applied. Minimal salt medium containing PF of 20 mg/L was prepared followed Siripattanakul-Ratpukdi et al. [5]. The bacterium was cultivated in the medium by shaking at 150 rpm under room

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temperature until reaching the late exponential phase (3 days).

2.3 Profenofos removal assay

Duplicate experiment of profenofos degradation by PF2 was conducted. The comparative experiment of the profenofos removal under presence and absence of organic matter was performed. It is noted that humic acid of 10 mg/L was applied since this is the typical concentration of organic matter in environment [7]. The experiment was performed by shaking the batch reactors (in the 200-mL medium) at 150 rpm with the initial profenofos concentrations of 20-120 mg/L, humic acid concentrations of 0 or 10 mg/L, and initial cell concentration of 10^4 CFU/mL under room temperature for 7 days. The control test (no microbial cell) was conducted along with the biodegradation assay. Profenofos remaining and cell number were measured consecutively (once a day).

2.4 Analytical methods

The cell number was measured using viable plate count technique. For profenofos measurement, liquid-liqiud extraction technique was applied for sample preparation. The extraction procedure using mixture of n-hexane and acetic acid of 0.1% was previously described in Siripattanakul-Ratpukdi et al. [5]. A gas chromatography (GC) with electron capture detector (SPB-608 fused silica capillary column, 4890D, Agilent Technologies, USA) was used for profenofos analysis.

3. Results

3.1 Microbial growth during profenofos degradation

The growth of PF2 during the profenofos degradation experiment under presence (HA10) and absence (HA0) of humic acid was shown in Figure 1. For all tests with the initial profenofos concentrations of 20 to 120 mg/L, PF2 grew from 4 to 8 logCFU/mL within 4 days. It was noticed that higher initial profenofos concentrations resulted in faster cell proliferation.

3.2 Profenofos removal

Profenofos remaining in the experiment under presence and absence of humic acid was shown in Figure 1. Profenofos continuously decreased for 7 days. At the end of the test, profenofos removal percentages were presented in Table 1. The control test (no microbial cell) was conducted along with the biodegradation assay. Based on Table 1, the control tests could remove profenofos for 10-35% while profenofos was decreased for 23-45% and 30-58% from the biodegradation tests without and with humic acid, respectively.

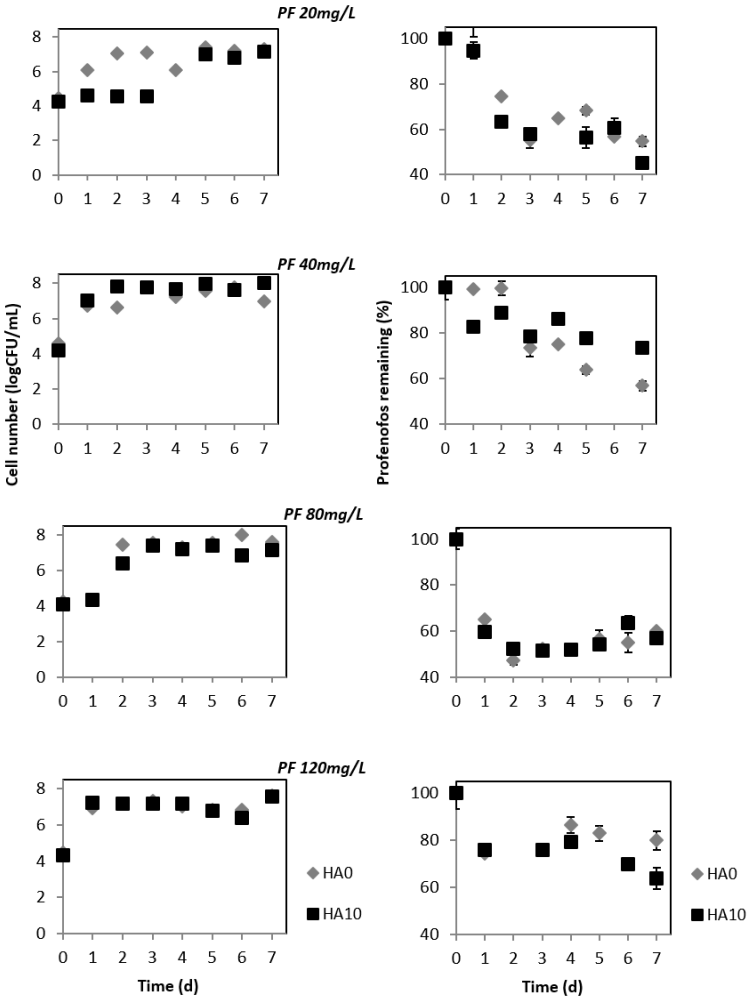

Figure 1 Microbial growth and profenofos remaining during profenofos degradation

Table 1 Profenofos removal percentage at the end of the test (7 days)

Profenofos concentration (mg/L)	Removal percentage by			
	Biodegradation (test without humic acid)	Biodegradation plus adsorption (test with humic acid)	Adsorption alone (control test)	Biodegradation alone in presence of humic acid[1]
20	44.70	58.10	23.22	34.88
40	45.27	29.75	10.06	19.69
80	41.81	45.79	26.84	18.95
120	22.55	39.28	34.54	4.74

[1] Removal percentage by biodegradation alone in presence of humic acid was calculated by subtracting "Biodegradation plus adsorption (test with humic acid)" with "Adsorption alone (control test)".

4. Discussion

The result clearly indicated that the initial profenofos concentrations influenced growth of PF2 because profenofos was the major substrate for the culture [5]. Previously, humic acid was reported as either growth-promoting or inhibiting substance. In this case, humic acid at 10 mg/L did not obviously play an important role on microbial growth.

For profenofos removal result, it is clearly that profenofos concentrations decreased in the control tests (Table 1). This is a typical situation which has been reported for the degradation under presence of natural organic carbon. This is because humic acid could adsorb contaminants including pesticides [8]. For the biodegradation test under absence of humic acid, it is obvious that PF2 well degraded profenofos at 20-80 mg/L. The test with too high concentration (120 mg/L), phenomenon of self-substrate inhibition took place.

When comparing profenofos removal from the tests with and without humic acid (30-58% and 23-45%, respectively), the removal percentages from the tests with humic acid were higher than those without humic acid because of the integration of biodegradation and adsorption processes. For biodegradation process only (based on the calculation shown in Table 1), in the tests with humic acid, it is apparent that humic acid inhibited biodegradation. This might be from competition of profenofos and humic acid as the main carbon source resulting in less profenofos biodegradation. Otherwise, it could be inhibition of enzymatic reactions involved in contaminant degradation [9]. The result proposed in this study should be continued the effect of humic acid with various concentrations for better clarification.

5. Conclusions

Humic acid (as a model of the natural organic carbon) did not influence microbial growth during profenofos degradation while it considerably affected profenofos removal performance. Profenofos degraded for 30-58% and 23-45% under presence and absence of humic acid, respectively. Natural organic carbon could either promote the profenofos removal *via* adsorption process or inhibit the biodegradation. The further work on the inhibition mechanism of humic acid to profenofos degradation should be performed for clearer identification.

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