



Characterization of profenofos degradation by *Pseudomonas plecoglossicida* strain PF1 using surface response methodology

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ABSTRACT

Profenofos pesticide residue has been successfully removed via microbial degradation in laboratory experiment. Profenofos degradation by *Pseudomonas plecoglossicida* strain PF1 (PF1) under environmental influence was characterized using response surface methodology with central composite design method. The models were applied to investigate the effects of key environmental parameters including pH, temperature, and initial profenofos concentration on profenofos biodegradation performance based on the profenofos biodegradation kinetic rates. The result showed that profenofos removal percentages by PF1 at the different initial concentrations (5–20 mg L⁻¹) were 50%–90% with the utilization rates of 0.17–0.78 mg L⁻¹ h⁻¹. The biodegradation kinetic rates well fit the first-order kinetic equation. The kinetic rates increased with rising of pHs and profenofos concentrations. The optimum levels of the key parameters were pH of 5.89, temperature of 32.94°C, and profenofos concentration of 20.15 mg L⁻¹ with the highest profenofos biodegradation kinetic rates of 0.10 h⁻¹. The interaction between pH and profenofos concentration obviously impacted the profenofos biodegradation kinetic rates.

Keywords: Biodegradation; Central composite design; Organophosphorus pesticide; Profenofos

1. Introduction

Profenofos is one of the common organophosphorus pesticides applied for cotton, fruits, and vegetable production [1]. Extensive profenofos utilization caused contamination in the environment. For example, Harnpicharnchai et al. [2] reported the surface water contaminated profenofos

concentrations of 0.32–0.95 mg L⁻¹ in Khon Kaen, Thailand, which exceeded the concentration of 0.003 mg L⁻¹ for drinking water standard [3]. Profenofos is toxic to birds, mammals, and aquatic invertebrates leading to damage nervous system. It has been listed as a restricted used pesticide by US Environmental Protection Agency [4]. Based on its toxicity, the profenofos contamination in water is a problematic issue for environment and organisms.

Microbial degradation is an effective technique for pesticide removal in environment. The technique is efficient,

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cost-effective, and environmentally friendly. In addition, the biodegradation products (intermediate or end products) from microbial process are less toxic. Generally, for bioremediation practice, environmental conditions, such as pH, temperature, and substrate concentration influenced microbial activities [5–7]. For profenofos biodegradation, *Pseudomonas plecoglossicida* strain PF1 (PF1) was previously isolated from a profenofos-contaminated chili farm soil [8]. The strain successfully degraded profenofos in water at a wide range of concentrations (up to hundreds mg L⁻¹) under laboratory conditions. PF1 also well degraded other organophosphorus pesticides including chlorpyrifos and dicotophos. Thus far, information of profenofos degradation by PF1 was limited. There was no published study on characterization of the profenofos biodegradation influenced by environmental conditions.

Conventional method for characterization of the biodegradation was time and budget consuming. Recently, statistical method using response surface methodology (RSM) has been introduced for characterization and optimization of the biodegradation. The RSM method reduces the amount of experiments and errors resulting in less time and cost consuming. The RSM method applies to analyze and investigate the interaction effect among the tested parameters. In addition, the statistical experimental design as central composite design (CCD) was successfully used to find the key parameters influencing the biodegradation [9,10].

Aim of the present work was to characterize profenofos degradation by PF1 under influence of environmental factors. The removal of profenofos contaminated in water was emphasized. The RSM with CCD method was applied to investigate the effects of key environmental parameters including pH, temperature, and initial profenofos concentration on profenofos biodegradation performance. The interaction of each parameter was determined. The ranges of tested parameters (pHs of 5–8, temperatures of 15°C–45°C, and the profenofos concentrations of 5–20 mg L⁻¹) were chosen based on environmental values. The profenofos biodegradation kinetic experiment and primary degradation intermediate (4-bromo-2-chlorophenol [BCP]) monitoring also carried out. The biodegradation performance information for treating profenofos-contaminated water will be helpful for both academics and bioremediation practices in the future.

2. Materials and methods

2.1. Chemicals

Commercial grade profenofos (50%, w/v, Syngenta Crop Protection Co., Thailand) used for the experiment was obtained from a local pesticide distributor. Analytical grade profenofos (Supelco, Sigma Chemical, Singapore) and BCP (Dr. Ehrenstorfer GmbH, LGC Standards, UK) were obtained for chemical analysis. Other chemicals for experiment were purchased from Himedia (India), Ajax (Australia), and RCI Labscan (Australia) via local chemical suppliers.

2.2. Microorganism and cultivation

Pseudomonas plecoglossicida strain PF1 (GenBank accession number KJ620776), a previously isolated bacterium, was

chosen [8]. The bacterial strain was first activated in minimal salt medium (MSM) supplementing with profenofos of 20 mg L⁻¹ every 4 d for three times before used. Formulation of MSM included Na₂HPO₄·2H₂O 6.82 g, KH₂PO₄ 3 g, NaCl 0.5 g, NH₄Cl 2 g, and MgSO₄·7H₂O 0.51 g in 1,000 mL of phosphate buffer at pH 6.80 (NaH₂PO₄·2H₂O 0.083% (w/v) and Na₂HPO₄·2H₂O 0.17% (w/v)) [8].

For PF1 enrichment, the active culture (10% by volume) was inoculated into fresh MSM medium with 0.1% (w/v) yeast extract. The enrichment conditions were shaking at 150 rpm, 24 h, and 30°C. The enriched suspension (OD₆₀₀ = 1) was harvested by centrifugation (5,000 rpm, 20 min, and 15°C). The pellet was twice washed by a NaCl solution of 0.85% (w/v). Then, the washed pellet was resuspended in the MSM medium (no yeast extract) to obtain PF1 of approximately 10¹² CFU mL⁻¹ in the reactor.

2.3. Profenofos biodegradation experiment

For profenofos biodegradation experiment, three replicate tests with the 30 mL MSM medium supplemented with different initial profenofos concentrations and pHs were carried out in 125 mL serum bottles. It is noted that the pH adjustment was performed using hydrochloric acid and sodium hydroxide of 1 N. The experiment was operated under shaking conditions of 160 rpm and different incubation temperatures. The MSM medium without the cell suspension was conducted as a control. The profenofos biodegradation kinetic rate was calculated using Eq. (1):

$$C_t = C_0 \times e^{-kt} \quad (1)$$

where C_0 and C_t are the initial and final concentrations of profenofos (mg L⁻¹), respectively. The k value is the kinetic rate of profenofos biodegradation (h⁻¹) whereas t is time (h).

Based on literatures, the environmental conditions including pHs (6.50–8.50), temperatures (20.00°C–40.00°C), and profenofos concentrations (0.32–1.00 mg L⁻¹) were found in surface water of agricultural area [11–16]. The boarder ranges of pHs, temperatures, and profenofos concentrations as shown in Table 1 were selected because of potential for future applications in various contaminated sites. It is noted that the profenofos concentrations of up to 20 mg L⁻¹ (the water solubility) were selected. The interaction of each parameter to a response was simplified as variables in CCD employed using Minitab 16 statistical software (Minitab Inc., Pennsylvania, USA; Table 1). The profenofos biodegradation kinetic rate was chosen as the response for this study.

Table 1
Parameters and coded level used for the experimental design of profenofos biodegradation by PF1 ($\alpha = 1.5$)

Factors	Parameters	Level				
		- α	-1	0	+1	+ α
A	pH	4.64	5.30	6.25	7.20	7.87
B	Temperature, °C	14.70	21.00	30.00	39.00	45.30
C	Profenofos concentration, mg L ⁻¹	4.85	8.00	12.50	17.00	20.15

The kinetic rate of profenofos biodegradation was applied in the mathematical model designated by the full quadratic equation (Eq. (2)). Analysis of variance (ANOVA) was applied for calculating Fisher test (F), its associated probability (p) and the coefficient of determination (R^2) which indicated the goodness-of-fit to the regression model. The contour plots were constructed by fitting the quadratic equation from regression analysis, holding one parameter at the optimum value, and changing the other two parameters. The plots were used to analyze the interaction between the significant parameters.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i x_i + \sum_{i=1}^3 \beta_{ii} x_i^2 + \sum_{i=1}^3 \sum_{i \neq j}^3 \beta_{ij} x_{ij} \quad (2)$$

Y is the response of interest (profenofos biodegradation kinetic rate). x_i and x_j are the parameters (pH, temperature, and profenofos concentration). β is the regression coefficient values of the model.

2.4. Analytical methods

Profenofos and BCP concentrations were analyzed using a gas chromatography (GC) with electron capture detector (Agilent 4890, Agilent, USA). Liquid/liquid extraction technique was employed for sample preparation. *n*-Hexane with 0.01% (by volume) acetic acid of 500 μ L was added into 500 μ L of the samples from the experiment. The mixture was vigorously mixed for 10 min and centrifuged at 10,000 rpm for 5 min. The organic phase was selected and filtered by 0.22 μ m filter nylon.

The filtered sample (1 μ L) was injected into the GC with a HP-5 column (30 m length, 0.25 mm inner diameter, and 0.25 μ m film thickness). The GC condition was splitless mode, injection temperature of 240°C, and helium gas flow of 1.5 mL min⁻¹. The GC temperature program was 10 min period. The program started at 180°C and hold for 2.00 min, increased to 250°C with the rate of 40°C min⁻¹ and hold for 6.25 min. Profenofos and BCP peaks came out at 8.78 and 3.08 min, respectively.

3. Results and discussion

3.1. Profenofos biodegradation and identification of intermediate product

The profenofos biodegradation by PF1 at the different initial concentrations (5, 10, and 20 mg L⁻¹) is shown in Fig. 1. The biodegradation trends were similar for all tests. Profenofos concentration quickly decreased in the first 12 h and gradually reduced later on. The profenofos removal percentages were 50%–90% with the utilization rates of 0.17–0.78 mg L⁻¹ h⁻¹ as presented in Table 2. The profenofos degradation well fit the first-order kinetic model ($R^2 > 0.97$). This indicated that the initial profenofos concentrations influenced the degradation performance. The primary profenofos intermediate, BCP (<1 mg L⁻¹), was detected (Fig. 1). The detected BCP concentration was much lower than the removed profenofos concentration.

The profenofos biodegradation result showed that PF1 was an efficient profenofos-degrading microorganism. Previously, it was reported that typical primary intermediate

product of profenofos biodegradation is BCP [8,9]. It was also claimed that BCP was a toxic intermediate product. Siripattanakul-Ratpukdi et al. [8] found potential of profenofos and BCP removal by a microbial consortium containing PF1. The result from this study showed that at low profenofos concentration (5 mg L⁻¹), BCP decreased along with the time as shown in Fig. 1(a). During the experiments with higher profenofos concentrations (10–20 mg L⁻¹), it was found that slight BCP accumulation of <1 mg L⁻¹ (Figs. 1(b) and (c)). Based on the unbalance of profenofos removal and BCP accumulation concentrations and BCP (higher) toxicity, it might imply that profenofos and some portion of BCP might be degraded. However, the BCP biodegradation experiment should be further studied for the confirmation.

3.2. Response surface methodology

3.2.1. Statistical characterization of profenofos biodegradation

The profenofos biodegradation kinetic rates (k) depending on three main parameters (pH, temperature, and profenofos concentration) were calculated by Eq. (1). Twenty runs of the experiment were conducted and analyzed using CCD to evaluate the response (profenofos biodegradation kinetic rates; Table 2). From Fig. 2, the accuracy of data is confirmed

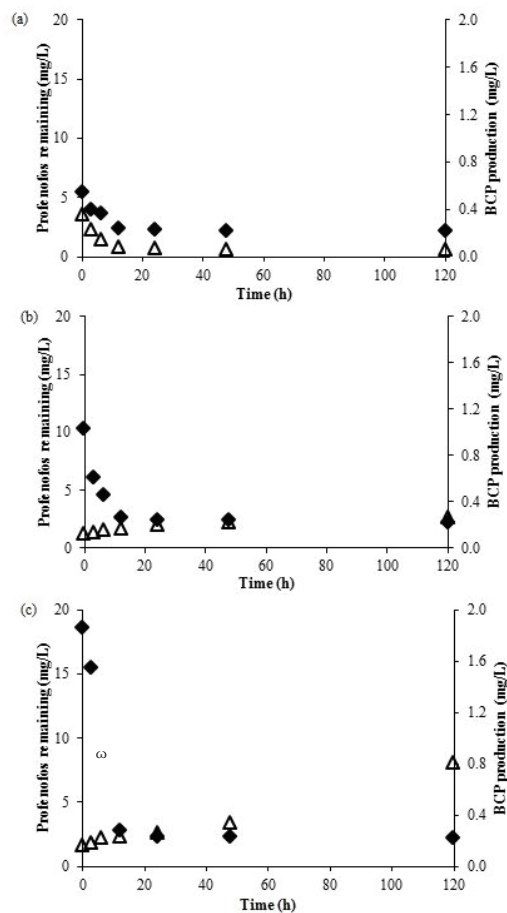


Fig. 1. Profenofos biodegradation (○) and BCP production (Δ): profenofos of (a) 5 mg L⁻¹, (b) 10 mg L⁻¹, and (c) 20 mg L⁻¹.

Table 2
Profenofos biodegradation kinetic rates and removal efficiencies

Run	pH	Temperature (°C)	Profenofos concentration (mg L ⁻¹)	<i>k</i> (h ⁻¹ × 10 ⁻²)	Profenofos removal efficiency (%)	Profenofos utilization rate (mg L ⁻¹ h ⁻¹)
1	7.20	39.00	17.00	9.10	84.25	0.69
2	6.25	30.00	12.50	9.30	81.89	0.69
3	6.25	14.70	12.50	1.00	71.81	0.38
4	7.20	21.00	8.00	6.70	70.76	0.28
5	5.30	21.00	8.00	4.30	57.53	0.18
6	5.30	39.00	8.00	4.00	54.83	0.17
7	4.64	30.00	12.50	6.30	63.26	0.38
8	7.87	30.00	12.50	8.60	78.23	0.48
9	6.25	30.00	12.50	9.50	84.36	0.76
10	7.20	39.00	8.00	7.00	64.57	0.32
11	6.25	30.00	12.50	9.30	84.77	0.72
12	6.25	30.00	12.50	9.80	83.81	0.74
13	6.25	30.00	20.15	9.40	85.20	0.78
14	7.20	21.00	17.00	7.80	77.27	0.69
15	6.25	30.00	12.50	8.90	83.14	0.75
16	6.25	30.00	12.50	8.20	81.01	0.66
17	6.25	30.00	4.85	4.80	55.91	0.17
18	6.25	45.30	12.50	5.20	63.64	0.33
19	5.30	39.00	17.00	8.90	83.07	0.91
20	5.30	21.00	17.00	7.60	76.22	0.65

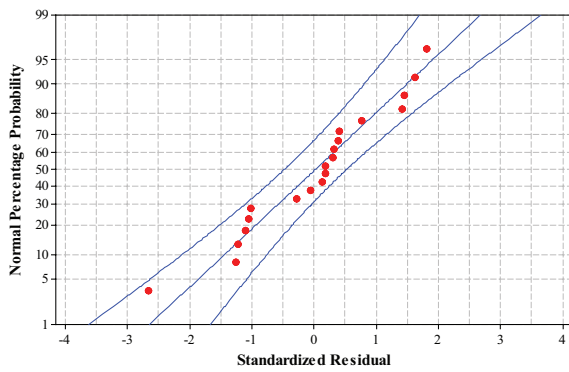


Fig. 2. Normal probability of standardized residual for profenofos degradation kinetic rates.

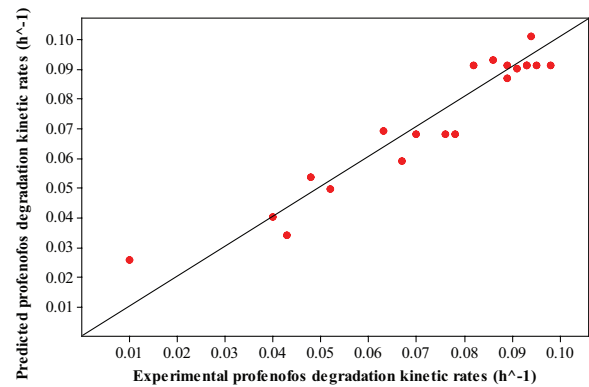


Fig. 3. Graphical plot of profenofos degradation kinetic rates (h⁻¹) between experimental and predicted values.

by the parity plot between normal percentage probability and internally standardized residuals. The data in 95% confidence range indicated that the profenofos biodegradation kinetic rates from the experiment were reliable. In Fig. 3, a graphical plot of profenofos degradation kinetic rates between experimental and predicted values confirmed the accuracy of data [17,18]. The model for profenofos biodegradation kinetic rates (*Y*) is shown in Eq. (3).

$$\begin{aligned}
 Y = & 0.091 + 0.007A + 0.007B + 0.14C - 0.004A^2 \\
 & - 0.019B^2 - 0.005C^2 + 0.001AB - 0.006AC \\
 & + 0.003BC
 \end{aligned}
 \tag{3}$$

where *A*, *B*, and *C* coded for pH, temperature (°C), and profenofos concentration (mg L⁻¹), respectively.

From Table 3, regression coefficients of the main parameters (*A*, *B*, and *C*) are positive (*p* < 0.05). This indicated that all main parameters significantly influenced the response. The quadratic coefficient of *B* (*B*²) was the largest value comparing with the others (*p* < 0.05). This indicated that *B*² (temperature) was the most significant parameters influencing on the response while the *p* values of *A*², *C*², and interaction of all parameters were insignificant (*p* > 0.05).

From Fig. 3 and Table 4, *R*² of 91.61% indicates a goodness of the model. This revealed that the profenofos biodegradation

kinetic rates could be predicted from the model. The model showed $p < 0.05$ (0.00), $F_{\text{value}} > F_{\text{critical}}$ ($F_{\text{value}} = 12.21$ and $F_{\text{critical}} = 10.16$), and lack-of-fit with $p > 0.05$ (0.06). This could state that the regression model was accepted with high precision [19,20]. The model was useful to identify the significance of parameters on the profenofos biodegradation. The terms of A , B , C , and B^2 were the significant terms and influenced response ($p < 0.05$). The result was agreeable with a previous study using different culture [9]. It was found that the significant influence of pH, temperature, and inoculum size on the profenofos removal efficiency by a microbial consortium. This can imply that different profenofos-degrading cultures from the previous and

this works were influenced by pH and temperature. This study was the first report on significant effect of the initial profenofos concentration to the biodegradation kinetics.

3.2.2. Effects of pHs, temperatures, and profenofos concentrations on profenofos biodegradation

The effects of pH, temperature, and profenofos concentration on the profenofos degradation kinetics are illustrated in Fig. 4. Trends of pH and concentration on profenofos degradation kinetic rates were similar. The kinetic rates increased with rising of pHs and profenofos concentrations. It has been known that profenofos easily breakdowns at alkaline condition leading to increasing of the degradation kinetic rates [4]. The maximum profenofos biodegradation kinetic rates were obtained from the test with pH of 7.87. However, the rates from the tests with pHs of 6.25–7.87 were closed (0.076–0.086 h⁻¹). In practice, the profenofos removal by PF1 would successfully achieve in pH range of 6.25–7.87.

For profenofos concentration, the biodegradation kinetic rates increased along with concentrations followed the first-order kinetic model as discussed earlier. This indicated that PF1 is the efficient bacterial strain for profenofos degradation at a wide range of up to 20 mg L⁻¹ (water solubility value). This could state that PF1 possibly applies for agricultural, industrial, or spill cases. Additionally, according to Siripattanakul-Ratpukdi et al. [8], PF1 well degraded other organophosphorus pesticides including chlorpyrifos and dicotophos with up to 70% removal. In practice, it is likely that numerous pesticides contaminated in the same agricultural and agro-industrial contaminated areas; therefore, PF1 is promising for remediating the contaminated sites in the future.

Table 3

Regression coefficients for profenofos biodegradation by PF1 under influence of pH, temperature, and initial profenofos concentration

Terms	Coefficient	p Value
Constant	0.091	0.00
A	0.007	0.02
B	0.007	0.02
C	0.140	0.00
A^2	-0.004	0.18
B^2	-0.019	0.00
C^2	-0.005	0.08
AB	0.001	0.83
AC	-0.006	0.09
BC	0.003	0.35

Table 4

Analysis of variance for profenofos degradation by PF1 under influence of pH, temperature, and initial profenofos concentration

Source	Degrees of freedom	F Value	p Value	Significant
Regression	9	12.21	0.00	Significant
Linear	3	15.23	0.00	Significant
A	1	7.70	0.02	Significant
B	1	7.75	0.02	Significant
C	1	30.24	0.00	Significant
Square	3	19.88	0.00	Significant
A^2	1	2.13	0.18	Insignificant
B^2	1	58.06	0.00	Significant
C^2	1	3.84	0.08	Insignificant
Interaction	3	1.51	0.27	Insignificant
AB	1	0.05	0.83	Insignificant
AC	1	3.52	0.09	Insignificant
BC	1	0.95	0.35	Insignificant
Residual error	10			
Lack-of-fit	5	4.72	0.06	Insignificant
Pure error	5			
Total	19			

Note: $R^2 = 91.66\%$, $R^2_{\text{(adj)}} = 84.15\%$.

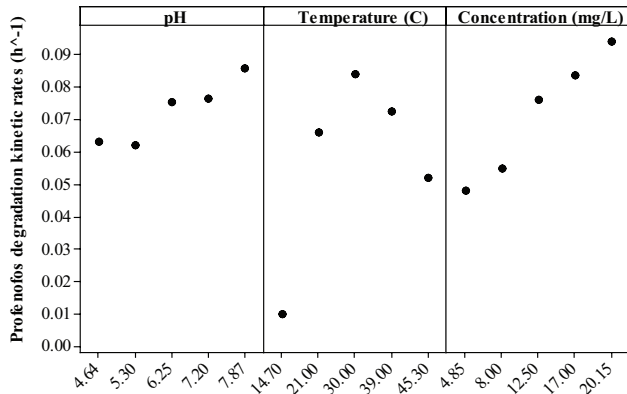


Fig. 4. Main effects for profenofos biodegradation kinetic rates.

The temperature as a main effect provided the different result from the other effects. The optimum temperature was approximately 30°C (Fig. 4). The reason for this situation could be explained by the activity of enzyme that was responsible for profenofos biodegradation. The organophosphorus hydrolase may lose its catalytic activity in the environment at inappropriate temperature [21,22]. Too high temperature caused protein oligomerization while too low temperature resulted in substrate specificity of enzyme [23]. The continued work on enzymatic inhibition mechanism should be performed. In overall, the ranges of tested parameters including pH, temperature, and profenofos concentration were selected based on conditions in environment. High profenofos degradation rates at wide ranges of tested conditions indicated that PF1 well degraded profenofos and was applicable for real site remediation practice.

3.2.3. Interactions between pH, temperature, and profenofos concentration

The interaction between main effects on response (profenofos biodegradation kinetic rates) was demonstrated by the contour plots (Figs. 5(a)–(c)). The figures are based on the regression model from Eq. (3) with one variable held constant at its optimum level and varied the other two variables. From Fig. 5(a), the interaction between pH and profenofos concentration on the response was investigated by keeping temperature constant at 30°C. The result showed the elongated diagonal pattern. Fig. 5(a) suggests that the interaction between pH and profenofos concentration was significant for profenofos biodegradation kinetic rates. Even though the F_{value} (3.52) and $p > 0.05$ from ANOVA was insignificant, F_{value} was just slightly different from F_{critical} (3.62). The interaction of pH and profenofos concentration should be considered.

From Figs. 5(b) and (c), the interaction between temperature and profenofos concentration (kept pH constant at 6.25) and the interaction between pH and temperature (held profenofos concentration constant at 12.5 mg L⁻¹) suggest that there were less significant interactions on the response. The optimum levels from the experimental data were attained: pH 5.89, temperature 32.94°C, and profenofos concentration 20.15 mg L⁻¹. Comparing with the study by Jabeen et al. [9], the consortium from the previous work and PF1 worked well

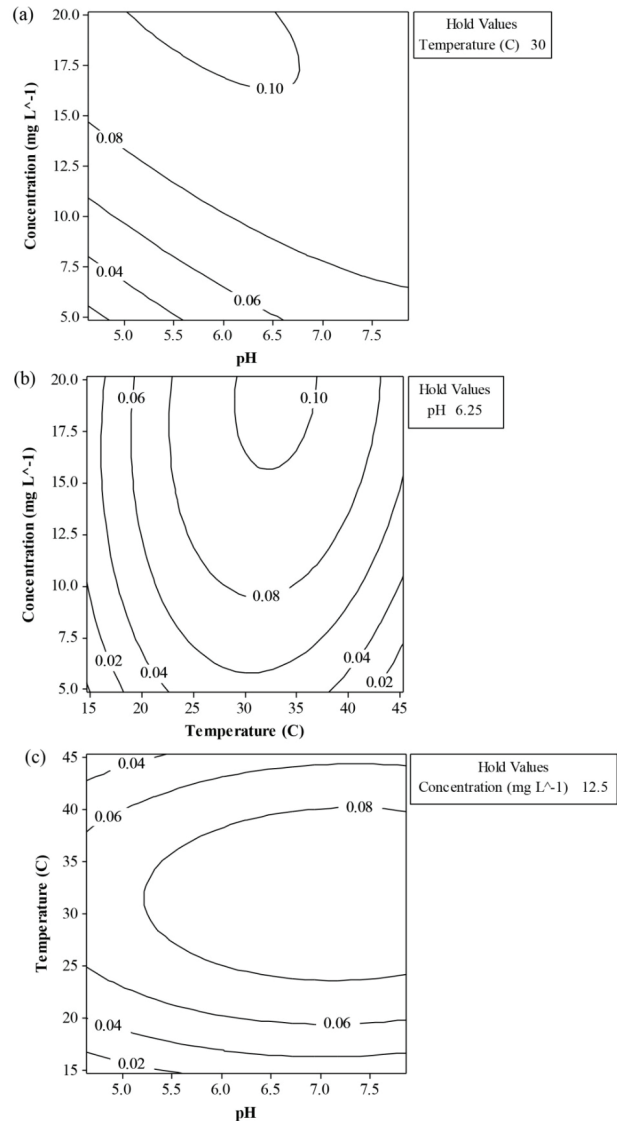


Fig. 5. Contour plot for profenofos degradation kinetic rates (h^{-1}) resulting from interaction of initial profenofos concentration (mg L^{-1}), pH, and temperature ($^{\circ}\text{C}$): (a) interaction between pH and initial profenofos concentration, (b) interaction between temperature and initial profenofos concentration, and (c) interaction between pH and temperature.

in similar range of temperatures (32°C–35°C) which was typical optimum temperature range for mesophilic microbial cultures [9].

PF1 worked well in all tested pHs (the profenofos degradation kinetic rates of 0.06–0.09 h^{-1}). Interestingly, based on the optimum pH found, PF1 preferred slightly acidic pH while the previous consortium worked well at approximately neutral pH (6.8). This could be because PF1 was originated from soil in the north-eastern region of Thailand. The soil pHs from most area in this region is in slightly acidic range of 4.5–6.5. Therefore, the isolated microbial culture favored the slightly acidic pH. This is the first report determining the bacterial culture which could successfully degrade profenofos at acidic pH. For future application, PF1 would be the

best candidate for remediating the contaminated area with broad range of pH (acidic to neutral pHs).

The interaction between pH vs. concentration to response was obviously significant. At low pH and low profenofos concentration, the profenofos biodegradation kinetic rates were relatively low compared with those from other conditions. Profenofos is likely to exist longer at acidic condition which introduced less abiotic degradation [24]. In addition, there was less biotic activity at low concentration of profenofos due to lower substrate (profenofos) for the bacterium. For the environment with neutral and basic pH, abiotic profenofos hydrolysis may occur [25]. It also increased bioavailability of organophosphorus pesticides and survival of microorganisms leading to higher profenofos biodegradation activity [26].

4. Conclusions

This study aimed to characterize profenofos degradation by PF1 under the influence of pH, temperature, and initial profenofos concentration. It was found that PF1 was an efficient profenofos-degrading microorganism. The profenofos removal by PF1 was up to 90%. The culture also well degraded BCP which was known as a toxic intermediate. Result from RSM analysis showed that pH, temperature, and profenofos concentration significantly affected profenofos degradation kinetic rates. The pH of 5.89, temperature of 32.94°C, and profenofos concentration of 20.15 mg L⁻¹ were optimum levels for profenofos degradation. Interaction between pH and concentration influenced the profenofos biodegradation kinetic rate. PF1 will be promising for site remediation practice in the future. Effect of other environmental factors, such as organic carbon and nutrient contents should be performed for further characterization.

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