ภาคผนวก ค
ผลงานตีพิมพ์ภายใต้ทุนวิจัย

Siripattanakul-Ratpukdi, S., Vangnai, A.S., Pankaew, K., Patichot, W. Biodegradation of profenofos pesticide by novel isolated cultures and their characterization. The 4th KKU International Engineering Conference 2012 (KKU-IENC 2012), Khon Kaen, Thailand, May 10-12, 2012.

ผลงานดังกล่าวได้รับรางวัล KKU-IENC 2012 Best Paper Award

The 4th KKU International Engineering Conference

Organized by the Faculty of Engineering, Khon Kaen University

is proud to present

"KKU-IENC 2012 Best Paper Award"

on Topic F : Environmental Engineering and Management

to

Sumana Siripattanakul, Alisa S. Vangnai, Kanjana Pankaew and Warayut Patichot

for the paper

"Biodegradation of Profenofos Pesticide by Novel Isolated Cultures and Their Characterization"

May 10-12, 2012

Khon Kaen, Thailand

IEEE THAILAND SECTION

Assoc. Prof. Dr. Somnuk Theerakulpisut
General Chair
Dean, Faculty of Engineering,
Khon Kaen University

The 4th KKU International Engineering Conference 2012 (KKU-IENC 2012)
"Driving together towards ASEAN Economic Community"
Faculty of Engineering, Khon Kaen University, Thailand, May 10-12, 2012

Biodegradation of Profenofos Pesticide by Novel Isolated Cultures and Their Characterization

Sumana Siripattanakul-Ratpukdi[1,2*] Alisa S. Vangnai[3] Kanjana Pankaew[1] Warayut Patichot[1]
[1]Department of Chemical Engineering, Faculty of Engineering, Ubon Ratchathani University,
Ubon Ratchathani 34190, Thailand
*E-mail: ensumasi@ubu.ac.th, jeans_sumana@yahoo.com
[2] Center of Excellence for Environmental and Hazardous Waste Management,
Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand
[3] Department of Biochemistry, Faculty of Science, Chulalongkorn University,
Bangkok 10330, Thailand

***Abstract*—This study aims to isolate and characterize a profenofos pesticide (PF)–degrading bacterial consortium and pure cultures from chilli farm soil. The work also focused on bacterial growth and profenofos degradation. The experiment was performed at the initial profenofos concentration of 20 mg/L. The result showed that the enriched consortium comprised three predominant PF-degrading strains designated PF1, PF2, and PF3. The isolates (PF1, PF2, and PF3) were characterized as *Pseudomonas plecoglossicida*, *Pseudomonas aeruginosa*, and *Pseudomonas aeruginosa*, respectively. For the growth and PF degradation kinetic tests, a consortium and all isolates could utilize PF as a sole carbon source with PF removal of more than 90% within 6 d. A large number of cells increased during the first two days. The bacterial growth and PF degradation rates followed the first order kinetic reaction with the rates of 0.40 to 2.68 1/d and 0.34 to 1.96 1/d, respectively. The PF utilization rates of 3.0 to 19.6 mg/L/d were determined. The results indicated that the consortium and isolates are efficient for profenofos degradation and have potential for profenofos remediation.**

Keywords: degradation, insecticide, isolation, profenofos

I. INTRODUCTION

It is known that organophosphorus pesticides (OP) have been developed to replace organochlorine pesticides for agricultural purpose. The pesticides are efficient and inexpensive which result in extensive utilization [1,2]. This leads to OP contamination in either agricultural products or environmental media [1,3]. Organophosphorus pesticides do not only damage weeds and insects but also could cause inhibition of cholinesterase activity resulting in malfunction of nerve impulse transmission and toxic to invertebrates, fish, animal, and human [4,5]. Among OPs, profenofos (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate), $C_{11}H_{15}BrClO_3PS$, is one of OPs broadly used in many countries (Figure 1). Profenofos (PF) is normally applied for pest control in cotton, fruit, and vegetable cultivation. Intensive use of PF leads to its accumulation in environment and contamination as residues in foods, fruits, vegetables, and environment [5]. For example, it was found that profenofos was the highest applied pesticide for chilli cultivation in the north eastern area of Thailand. Profenofos residues in chilli soil, and farmers were also detected. As a result, there should be a technique to alleviate the problem.

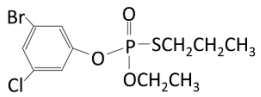


Figure 1. Profenofos

Bioremediation is one of the key attenuation processes of pesticide contamination in the environment. The technique has been successfully applied for OP remediation [2,5,6]. The technique involved the isolation of pesticide-degrading microbial cultures and the utilization of the isolated cultures for the pesticide removal later on. Thus far, there are only two publications on isolation and characterization of PF-degrading bacteria [5,7]. Three PF-degrading bacteria (*Pseudomonas putida* strain W, *Burkholderia gladioli* strain Y, and *Pseudomonas aeruginosa* strain OW) were previously isolated for China soil remediation site at PF concentration of 200 μg/g [5,7]. In the real practices, failure of bioremediation by exogenous cultures is considered because the cultures somehow do not well survive and work in different environmental media and conditions [8,9].

For successful PF bioremediation, indigenous PF-degrading cultures were enriched and isolated. Degradation kinetics of PF by an enriched consortium and novel isolated strains were conducted. The cultures were also identified and characterized. In the future, the isolated cultures could be applied for the PF-contaminated site remediation. Also, the kinetic result could be used as a basic knowledge for the future application.

II. Materials and Methods

A. Chemicals

Commercial grade PF was used in the experiment (Profenofos 500EC, Syngenta Crop Protection Co., Bangkok, Thailand). Profenofos (analytical grade, Sigma Chemical Co., Singapore) was used for PF analysis. All other chemicals for bacterial medium preparation and PF analysis were analytical and HPLC grades from local chemical distributers.

B. Enrichment and isolation of PF-degrading cultures

Profenofos-degrading species were isolated from chilli farm soil experienced PF exposure for years (Ubon Ratchathani, Thailand). A sterile basal salts medium containing KH_2PO_4 of 3.0 g/L, NH_4Cl of 1 g/L, NaCl of 0.5 g/L, $MgSO_4$ of 0.25 g/L, and PF of 20 mg/L (in 10 mM of phosphate buffer solution) was utilized. Agar of 2.0% (w/v) and yeast extract of 0.1% (w/v) were added in agar medium preparation.

The culture enrichment and isolation procedures were as follows. Air-dried soil sample (20 g) from the site were mixed in the PF-containing basal salt medium (100 mL), incubated on a rotary shaker at 100 rpm and 30±2°C for two weeks. The soil suspension (10 mL) was used as the inoculum and inoculated into the PF-containing fresh medium (100 mL). Then, the re-cultivation was conducted for 4 times consecutively. The enriched consortium was maintained for further investigation and was purified by spreading and streaking plate techniques. All plates were incubated at 30±2°C for 14 days. Single colonies were obtained after several subcultures. The consortium and isolated cultures were then characterized.

C. Characterization of PF-degrading cultures

Colony and cell morphology of the isolated cultures were characterized. The isolates were identified by 16S ribosomal Ribonucleic acid (16S rRNA) sequence analysis. In brief, each bacterial culture was grown overnight. The genomic DNA from each culture was extracted using a standard boiling method. The 16S rRNA gene fragment was amplified from the genomic DNA by the polymerase chain reaction (PCR) using the bacterium-specific primers: a 63f-forward primer (5'CAGGCCTAACACATGCAAGTC3') and a 1387r-reverse primer (5'GGGCGGWGTGTACAAGGC3'). A 25-µl PCR reaction mixture was prepared according to the manufacturer's protocol (Fermentas, USA). The 16S rRNA amplification was performed in a thermal cycler (Perkin Elmer model-2400, USA) with the following conditions: 94ºC (3 min), followed by 30 cycles of 95ºC (1 min), 55ºC (1 min) and 72ºC (1.5 min), with a final extension of 72ºC (5 min). The PRC product (ca. 1,300 bps) was then cloned into pGEM-T Easy vector (Promega, USA) and transformed into competent Escherichia coli DH5a cells. The plasmid DNA was then isolated using the QIAprep Spin Miniprep kit (Qiagen, Netherlands) and sequenced. The partial 16S rRNA gene sequence of the isolates was analyzed using the nucleotide BLAST (BLASTN) algorithm of the National Center for Biotechnology Information (NCBI).

D. Profenofos biodegradation by PF-degrading cultures

Duplicate batch experiments of PF biodegradation by the consortium and the isolated cultures were conducted. The consortium and cultures were shaken in the 200-mL medium with the initial PF concentration of 20 mg/L on a rotary shaker at 100 rpm and 30±2°C for 6 days. Profenofos concentration and cell number (viable plate count) measurements were performed daily. Abiotic control test (no bacterial cultures) was also performed. The bacterial growth and PF degradation kinetic rates were then calculated.

E. Profenofos and intermediate metabolite analysis

Profenofos concentraion was measured using a QuEChERS extraction technique and a gas chromatograph with a mass selective detector (GC-MSD) and DB-5 column (30.0 m length, 0.25 mm i.d., 0.25 µm film thickness). The sample of 10 mL of was placed into a 50-mL disposable polypropylene centrifuge tube with 10-mL of acetonitrile and acetic acid of 0.1% mixture. The centrifuge tube was capped and shaken for 1 min. After that, NaCl of 1 g and anhydrous $MgSO_4$ of 4 g were added. The tube was then shaken vigorously for 1 min and centrifuged at 5,000 rpm for 5 min. The supernatant (acetonitrile extract) was transferred into a SPE tube (QuEChERS D-SPE, Agilent, USA) to clean up residue. The tube was capped and mixed in a vortex mixer for 1 min, and then centrifuged at 5,000 rpm for 3 min. The cleaned sample was transferred to a GC vial.

One-microliter of the cleaned sample was injected to the GC. The GC conditions were: splitless injection, injection port temperature of 220ºC, and helium gas (carrier gas) flow of 2 µL/min. The GC temperature program was started at 90ºC, increased to 220ºC at a rate of 20ºC/min and retained for 1 min, increased to 280ºC at a rate of 10ºC/min and retained for 3 min, and increased to 300ºC. Profenofos peak was detected at 9.07 min. Degradation intermediate product was monitored along with PF detection.

III. Results and Discussion

A. Enrichment, isolation, and characterization of PF-degrading cultures

The PF-degrading consortium (referred to as MIX hereafter) was enriched in aerobic condition at pH of 7 and temperature of 30°C. Based on the enrichment medium formulation, the consortium could utilize PF as a sole carbon source. This is similar to the previous studies which reported the OP utilization as a sole carbon source [2,5,7]. The consortium plated onto the medium agar comprised numerous types of bacterial colonies. This indicates that the soil used in this study was rich in PF-degrading cultures.

The PF-degrading cultures were then isolated from MIX. Three isolates designated PF1, PF2, and PF3 were observed morphological characteristics (Table I and Figure 2). All isolates were different in colony morphology. The isolates were observed by microscopic approach. It was found that the isolates were short rod with negative in gram staining.

The isolates, PF1, PF2, and PF3, were identified by 16S rRNA analysis as *Pseudomonas plecoglossicida*, *Pseudomonas aeruginosa*, and *Pseudomonas aeruginosa* with similarity of 100%, 98%, and 99%, respectively. Previously, *Pseudomonas aeruginosa* strain OW was isolated for PF degradation [5]. The isolate was enriched from the PF-contaminated soil in China. In this study, two more strains in the same species (*Pseudomonas aeruginosa* strain PF2 and *Pseudomonas aeruginosa* strain PF3) isolated from chilli farm soil in Thailand were proposed for PF degradation. There were prior studies reporting OP remediation by *Pseudomonas aeruginosa* strains, such as profenofos and chlorpyrifos degradations [5,10]. The result suggested that *Pseudomonas aeruginosa* might conserve OP-degrading microbial enzymes resulting widely discovered the *Pseudomonas aeruginosa* strains able to degrade OPs. For PF1, *Pseudomonas plecoglossicida* was reported pyrethroid degradability [11]. To the best of our knowledge, this is the first report on OP degradation by *Pseudomonas plecoglossicida*.

TABLE I. MORPHOLOGICAL CHARACTERISTICS OF PF-DEGRADING BACTERIA

Characteristic	Isolate		
	PF1	PF2	PF3
Colony color	Yellow	Yellow	Yellow
Colony form	Circular	Circular	Circular
Colony elevation	Raised	Raised	Raised
Colony margin	Entire	Undulate	Entire
Colony diameter (at 24 h)	2.0 mm	1.5 mm	1.0 mm
Gram staining	Negative	Negative	Negative
Cell shape	Short rod	Short rod	Short rod

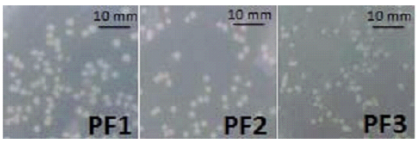


Figure 2. Colonies of PF-degrading bacteria isolated from chilli farm soil.

B. Kinetics of bactieral growth and PF degradation

The growth of MIX, PF1, PF2, and PF3 measured by viable cell counting is shown in Figure 3. The MIX consortium and the pure cultures (PF1, PF2, and PF3) provided the different results. The viable cell number of MIX grew from 7.1 to 7.6 logCFU/mL. The MIX cultures reached the stationary phase after 2 d. Cell numbers of all three isolates quickly increased more than 7 logCFU/mL within 4 d. This indicates that both consortium and isolated cultures were able to survive and reproduce in the medium containing PF but the pure isolates likely to endure in the PF-contaminated environment. Based on the growth kinetics, the pure isolates perforated 5 to 6 times higher than MIX (Table II). This may be due to the competition between the cultures in the consortium.

The reduction of PF concentration during the biodegradation test is shown in Figure 4. Similar to the bacterial growth result, MIX and the pure isolates performed differently. In the test by MIX, the PF concentration continuously decreased in the first two days and the concentration dropped gradually thereafter. For all pure isolates, the PF concentration dramatically decreased in the first three days. After the 6-d experiment, MIX, PF1, PF2, and PF3 removed PF for 90.0%, 95.0%, 93.1%, and 95.3%, respectively (Table III). In the abiotic control test (no cell), the PF concentration remained more than 95% (data not shown). The consortium and isolates performed the PF treatment (PF utilization) at the rate of 3.0 to 19.6 mg/L/d. The pure isolates performed better than the consortium. The treatment result well correlated to the bacterial growth result. The MIX consortium grew slower resulting in lower PF treatment rate compared to those by the pure isolates. The PF degradation by all cultures followed the first-order kinetics (Table IV).

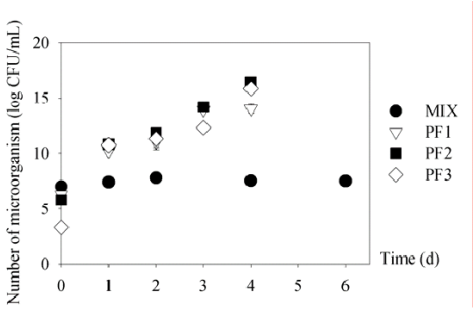


Figure 3. Bacterial growth by MIX, PF1, PF2, and PF3.

TABLE II. BACTERIAL GROWTH RATES OF PF-DEGRADING CULTURES

Culture	Growth kinetic equation*	R^2
MIX	y = 0.40x + 7.05	1.00
PF1	y = 1.91x + 7.28	0.90
PF2	y = 2.48x + 6.86	0.95
PF3	y = 2.68x + 5.36	0.85

* y = log (bacterial number)
x = time (hr)

The PF degradations by the consortium and cultures (more than 90%) were comparable to those by *Pseudomonas aeruginosa* strain OW, *Pseudomonas putida* strain W, *Burkholderia gladioli* strain Y, previously isolated [5,7]. The result indicated that the novel enriched consortium and three isolated cultures were potential for the PF bioremediation. Generally, the consortium has been shown to be more suitable for bioremediation compared to pure cultures in practice. This is because their biodiversity can enhance environmental survival and increase the number of catabolic pathways available for contaminant biodegradation [12,13]. In this case; however, the pure cultures were acclimated in the medium and

no environmental stresses in the tested condition leading to better growth and performance presented by the isolated cultures. Before the real practices, the influence of environmental conditions, such as pH and temperature on PF degradation should be conducted.

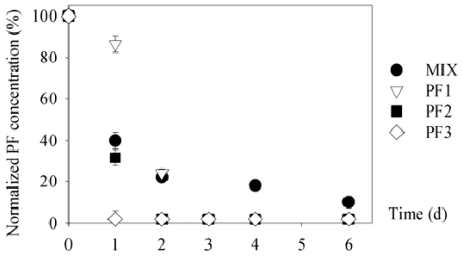


Figure 4. PF degradation by MIX, PF1, PF2, and PF3.

TABLE III. PROFENOFOS UTILIZATION RATES OF PF-DEGRADING CULTURES AND PF REMOVAL PERCENTAGE

Culture	Substrate utilization rate (mg/L/d)	PF removal (%)
MIX	3.0	90.0
PF1	7.6	95.0
PF2	9.8	93.1
PF3	19.6	95.3

TABLE IV. PROFENOFOS DEGRADATION RATES OF PF-DEGRADING CULTURES AND PF REMOVAL PERCENTAGE

Culture	PF degradation kinetic equation*	R^2
MIX	y = -0.15x + 1.12	0.87
PF1	y = -0.57x + 1.55	0.86
PF2	y = -0.85x + 1.42	0.95
PF3	y = -0.85x + 1.02	0.75

* y = log (PF concentration)
x = time (hr)

C. *Detection of profenofos intermediate metabilite*

During the profenofos degradation by the pure isolates and consortium, 4-bromo-2-chlorophenol (BCP) (a GC peak with m/z of 207, 281, and 73) was detected (data not shown). Similar result was also reported elsewhere [5]. The result suggested that PF was transformed *via* organophosphorus hydrolase enzyme, which was known as a typical bacterial enzyme for wide range of organophosphorus pesticide degradation [1]. The potential profenofos degradation pathway was proposed (Figure 5).

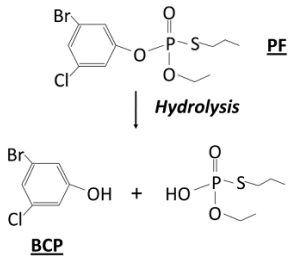


Figure 5. Potential profenofos degradation pathway.

IV. CONCLUSIONS

Novel PF-degrading consortium and three isolated cultures including *Pseudomonas plecoglossicida* strain PF1, *Pseudomonas aeruginosa* strain PF2, and *Pseudomonas aeruginosa* strain PF1 were isolated. All cultures were efficient in PF insecticide degradation (PF removal of more than 90% within 6 d). The novel cultures were promising for PF remediation. For further study, the influence of environmental conditions on PF degradation should be performed. Also, the full PF degradation pathway by the novel consortium and isolates should be conducted for insight investigation.

ACKNOWLEDGMENT

This material is based upon work supported by Ubon Ratchathani University and Office of the National Research Council of Thailand. This work is also conducted under the research cluster "Fate and Removal of Emerging Micropollutants in Environment" granted by Center of Excellence for Environmental and Hazardous Waste Management. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author and do not necessarily reflect the views of grant providers.

REFERENCES

[1] P.P. Kanekar, B.J. Bhadbhade, N.M. Deshpande and S.S. Sarnaik, "Biodegradation of organophosphorus pesticides." Proc. Indian Natn. Sci. Acad., vol. B70(1), pp.57-70, 2004.

[2] M. Cycon, M. Wojcik, Z. Piotrowska-Seget, "Biodegradation of the organophosphorus insecticde diazinon by *Serratia* sp. And *Pesudomonas* sp. And their use in bioremediation of contaminated soil." Chemsphere, vol. 76, pp.494-501, 2009.

[3] M.G. Roverdatti, "Monitoring of organochlrine and organophosphorus pesticdes in the water of the Reconquista River." Water Res., vol. 35(14), pp.3457-3461, 2001.

[4] M. Gotoh, M. Sakata, T. Endo, H. Hayashi, H. Seno, and O. Suzuki, "Profenofos metabolites in human poisoing." Forensic Sci. Int., vol.116, pp.221-226, 2001.

[5] S. Malghani and N. Chatterjee, "Isolation and characterization of a profenofos degrading bacterium," J. Environ. Sci., vol. 21, pp.1591-1597, 2009.

[6] G. Xu, W. Zheng, Y. Li, S. Wang, J. Zhang, Y. Yan, "Biodegradation of chlorpyrifos and 3, 5, 6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP." Int. Biodeter. Biodegr., vol. 62, pp.51-56, 2008.

[7] S. Malghani, N. Chatterjee, H.X. Yu, Z. Luo, "Isolation and identification of Profenofos degrading bacteria." Braz. J. Microbiol., vol.40(4), pp.893-900, 2009.

[8] J.K. Struthers, K. Jayachandran, and T.B. Moorman, "Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated Soil." *Appl. Environ. Microbiol.*, vol. 64, pp.3368-3375, 1998.

[9] S. Rousseaux, A. Hartmann, B. Lagacherie, S. Piutti, F. Andreux, and G. Soulas, "Inoculation of an atrazine-degrading strain, *Chelatobacter heintzii* Cit1, in four different soils: effects of different inoculum densities." *Chemsphere*, vol. 51, pp.569-576, 2003.

[10] M.H. Fulekar and M. Geetha, "Bioremediation of Chlorpyrifos by Pseudomonas aeruginosa using scale up technique," J. Appl. Biosci., vol. 12, pp. 657-660, 2008.

[11] H. Boricha and M.H. Fulekar, "*Pseudomonas plecoglossicida* as a novel organism for the bioremediation of cypermethrin," Biol. Med., vol. 1 (4), pp. 1-10, 2009.

[12] S. Alvey, and D.E. Crowley, "Suvival and activity of an atrazine-mineralizing bacterial consortium in rhizoshere soil." Environ. Sci. Technol. vol.30, pp.1596-1603, 1996.

[13] D.Smith, S. Alvey, and D.E. Crowley, " Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil." FEMS Microbiol. Ecol., vol.53, pp.265-273, 2005.

Siripattanakul-Ratpukdi, S., Vangnai, A.S. Sangthean, P., Singkibut, S. Profenofos insecticide degradation by novel microbial consortium and isolates enriched from contaminated chili farm soil. *Submitted to* ***Environmental Science and Pollution Research***

Profenofos Insecticide Degradation by Novel Microbial Consortium and Isolates Enriched from Contaminated Chili Farm Soil

Sumana Siripattanakul-Ratpukdi[1,2*], Alisa S. Vangnai[2,3], Puttaporn Sangthean[4], Sirinuttakan Singkibut[4]

[1]Department of Environmental Engineering, Faculty of Engineering, Khon Kaen University, Khon Kaen 40002, Thailand

[2] Center of Excellence on Hazardous Substance Management (HSM), Bangkok 10330, Thailand

[3] Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

[4]Department of Chemical Engineering, Faculty of Engineering, Ubon Ratchathani University, Ubon Ratchathani 34190, Thailand

***Corresponding author.** Department of Environmental Engineering, Faculty of Engineering, Khon Kaen University, 123 Mittapap Road, Muang District, Khon Kaen 40002, Thailand. Phone: 66-43-202572; Fax: 66-43-202571; E-mail: sumana.r@kku.ac.th; jeans_sumana@yahoo.com

Abstract

Profenofos (PF) is one of the heavily used organophosphorus pesticides (OPPs) of which its contamination is ubiquitous in agricultural area. This study aims to acquire and characterize PF-degrading bacterial cultures from contaminated soil. OPP degradation by the novel isolates was then investigated. The experiment was performed at the initial PF concentration of 20 mg L^{-1}. The result showed that the enriched consortium comprised three predominant PF-degrading strains designated PF1, PF2 and PF3. The isolates (PF1, PF2 and PF3) were characterized as *Pseudomonas plecoglossicida*, *Pseudomonas aeruginosa* and *Pseudomonas aeruginosa*, respectively. A consortium and all isolates could utilize PF as a sole carbon source with PF removal of more than 90% *via* hydrolysis process. The bacterial growth and PF degradation rates followed the first order kinetic reaction with the rates of 0.4 to 2.7 h^{-1} and 0.15 to 1.96 h^{-1}, respectively. Additional carbon supplement deteriorated PF biodegradation. The enriched cultures were also capable for degrading chlorpyrifos and dicrotophos pesticides (33-73% removal). The results indicated that the consortium and isolates are efficient for PF and other OPP degradation and have potential for PF remediation.

Keywords: biodegradation, insecticide, isolation, organophosphorus, profenofos, *Pseudomonas*.

39

40 **Introduction**

41 Organophosphorus pesticide (OPP) has been developed for agricultural purpose for years.
42 One-third of pesticides used globally belong to OPP because they are efficient and
43 inexpensive (Kanekar et al. 2004; Singh and Walker, 2006; Cycon et al. 2009). This
44 results in OPP contamination in environmental media including soil, groundwater and
45 surface water (Roverdatti, 2001; Kanekar et al. 2004). Among pesticides under OPP,
46 profenofos (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate),
47 $C_{11}H_{15}BrClO_3PS$, is one of OPPs broadly used in many countries, such as Thailand,
48 Vietnam and India (Swarnam and Velmurugan, 2013; Toan et al. 2013). Profenofos (PF)
49 is normally used for pest control in cotton, fruit, chili and vegetable cultivation. Intensive
50 use of PF leads to its contamination in environment.

51 Bioremediation is one of the effective environmental treatment technologies
52 (Siripattanakul et al. 2009). The technique has been successfully applied for OPP
53 remediation (Xu et al. 2008; Cycon et al. 2009). The technique involved enrichment of
54 pesticide-degrading microbial cultures and the utilization of the enriched cultures for
55 removing pesticide later on. So far, there are only a few publications on enrichment and
56 isolation of PF-degrading bacteria such as the enrichment of the degrading cultures
57 (Malghani and Chatterjee, 2009; Malghani et al. 2009; Salunkhe et al. 2013). Also, there
58 are limited information on PF biodegradation kinetics and potential biodegradation
59 pathway.

60 This study aimed to enrich and identify indigenous PF-degrading cultures from
61 heavily PF-contaminated soil. Degradation of PF by an enriched consortium and
62 predominant isolated strains were subsequently performed. The possibility of additional

63 carbon supplement on PF degradation was carried out. This work also determined
64 potential PF degradation pathway using detection of degradative intermediate
65 metabolites. The PF-enriched cultures could later on be applied for treatment of PF-
66 contaminated sites. The basic knowledge of the degradation kinetics and pathway could
67 be used to ensure the optimal treatment operation.
68
69 **Materials and Methods**
70
71 Chemicals
72 Commercial grade OPPs including Profenofos 500EC (50% W/V EC, Syngenta Crop
73 Protection Co., Bangkok, Thailand) were used in the degradation assay experiment. PF,
74 chlorpyrifos (CF) and dicrotophos (DP) (analytical standard grade, Supelco, Sigma
75 Chemical Co., Singapore) were used for OPP analysis. All other chemicals for bacterial
76 medium preparation and OPP analysis were analytical and HPLC grades, respectively
77 purchased from local chemical distributers.
78
79 Enrichment and isolation of PF-degrading cultures
80 PF-degrading bacteria were enriched from PF-contaminated chili farm soil (Ubon
81 Ratchathani, Thailand). The culture enrichment and isolation procedures were as follows.
82 Air-dried soil sample (20 g) from the site were mixed in the PF-containing basal salt
83 medium (100 mL), incubated on a rotary shaker at 100 rpm and 30 ± 2 °C for two weeks.
84 The soil suspension (10 mL) was used as the inoculum and inoculated into the PF-
85 containing fresh medium (100 mL). Then, the re-cultivation was conducted for 4 times
86 repeatedly. The enriched consortium was subcultured into the PF-containing medium for

87 every two weeks to obtain a stable PF-degrading consortium. The enriched consortium
88 was purified by spreading and streaking plate techniques. All plates were incubated at 30
89 ± 2 °C for 14 d. Single colonies were obtained after several streaking-plate cultivations.
90 Formulation of PF-containing basal salts medium included KH_2PO_4 of 3.0 g L^{-1},
91 NH_4Cl of 1 g L^{-1}, NaCl of 0.5 g L^{-1}, $MgSO_4$ of 0.25 g L^{-1} and PF of 20 mg L^{-1} (in 10 mM
92 of phosphate buffer solution, pH 7.0). All chemicals except PF were sterilized by
93 autoclave while PF were filtered sterile. Agar of 2.0% (w/v) was added in agar medium
94 preparation.
95
96 Identification of PF-degrading isolates
97 Colony and cell morphology of the isolated cultures were preliminarily observed using
98 traditional microbiological methods. The isolates were classified by 16S rRNA sequence
99 analysis. Each bacterial culture was cultivated overnight. The genomic DNA from each
100 culture was extracted using a standard boiling method. The 16S rRNA gene fragment was
101 amplified from the genomic DNA by the polymerase chain reaction (PCR) using the
102 bacterium-specific primers: a 63f-forward primer
103 (5'CAGGCCTAACACATGCAAGTC3') and a 1387r-reverse primer
104 (5'GGGCGGWGTGTACAAGGC3') (Marchesi *et al.* 1998). A 25-µl PCR reaction
105 mixture was prepared according to the manufacturer's protocol (Fermentas, USA).
106 The 16S rRNA amplification was performed in a thermal cycler (Perkin Elmer
107 model-2400, USA) with the following conditions: 94°C (3 min), followed by 30 cycles of
108 95°C (1 min), 55°C (1 min) and 72°C (1.5 min), with a final extension of 72°C (5 min).
109 The PRC product was then cloned into pGEM-T Easy vector (Promega, USA) and
110 transformed into competent *Escherichia coli* DH5α cells. The plasmid DNA was then

isolated using the QIAprep Spin Miniprep kit (Qiagen, Netherlands) and sequenced (Macrogen, Korea). The partial 16S rRNA gene sequence of the isolates was analyzed using the nucleotide BLAST (BLASTN) algorithm of the National Center for Biotechnology Information (NCBI).

PF biodegradation assay

Duplicate batch experiments of PF biodegradation by the consortium and the isolated cultures were conducted. The experiments with and without additional carbon supplement were performed. The consortium and isolates were shaken in the 200-mL medium with the initial PF concentration of 20 mg L^{-1} on a rotary shaker at 100 rpm and 30 ± 2°C for 4 d. For the experiment with additional carbon sources, sodium succinate ($C_4H_4O_4Na_2 \cdot 6H_2O$), sodium acetate ($C_2H_2O_2Na$) and glucose ($C_6H_{12}O_6 \cdot H_2O$) of 500 mg-carbon L^{-1} were added. PF concentration and cell number (viable plate count) measurements were performed daily. Abiotic control test (without bacterial cultures) was also performed. The PF residual was analyzed using a gas chromatograph with a mass selective detector (GC-MSD). The bacterial growth and PF degradation kinetic rates were then calculated.

The PF biodegradation assays at PF concentrations of 20-120 mg L^{-1} were conducted to identify PF intermediates and potential PF degradation pathway. The experiment was performed similar to earlier assay with different PF concentrations. The PF degradation intermediate peak was monitored using GC-MSD along with PF detection.

133 Biodegradation assay of selected OPPs

134 Degradation assay of other selected OPPs were performed. CF and DP which were the
135 insecticides widely used in vegetable cultivation were chosen. This is because these
136 pesticides possibly co-contaminate in the same area. The isolates were inoculated in a
137 liquid medium at 20 mg L^{-1} of the insecticides on a rotary shaker at 100 rpm and 30 ± 2
138 °C for 6 d.

139

140 PF, PF intermediate metabolite and other OPP analysis

141 PF concentration was extracted using a QuEChERS extraction technique. The sample of
142 10 mL of was placed into a 50-mL disposable polypropylene centrifuge tube with 10-mL
143 of acetonitrile and acetic acid of 0.1% mixture. The centrifuge tube was capped and
144 shaken for 1 min. After that, NaCl of 1 g and anhydrous $MgSO_4$ of 4 g were added. The
145 tube was then shaken vigorously for 1 min and centrifuged at 5,000 rpm for 5 min. The
146 supernatant (acetonitrile extract) was transferred into a SPE tube (QuEChERS D-SPE,
147 Agilent, USA) to clean up the residue. The tube was capped and mixed in a vortex mixer
148 for 1 min and then centrifuged at 5,000 rpm for 3 min. The cleaned sample was
149 transferred to a GC vial.

150 PF concentration was measured using a GC-MSD (Agilent 6890N, Agilent, USA)
151 with a DB-5 column (30.0 m length, 0.25 mm i.d., 0.25 μm film thickness). One-
152 microliter of the cleaned sample was injected into GC-MSD. The GC conditions were:
153 splitless injection, injection port temperature of 220°C and helium gas (carrier gas) flow
154 of 2 mL min^{-1}. The GC temperature program was started at 90°C, increased to 220°C at a
155 rate of 20°C min^{-1} and retained for 1 min, increased to 280°C at a rate of 10°C min^{-1} and

156 retained for 3 min and increased to 300°C. PF peak was detected at 9.07 min. Degradation
157 intermediate peak was monitored along with PF detection.
158 For CF and DP analysis, the samples were extracted using the method for PF analysis
159 as mentioned in earlier paragraph. The samples were measured using a gas
160 chromatograph (Agilent 6890N series) with a flame photometric detector and a DB-
161 1701column (30.0 m length, 0.25 mm i.d., 0.25 μm film thickness). One-microliter of the
162 cleaned sample was injected to the GC. The GC conditions were: splitless injection,
163 injection port temperature of 220°C and helium gas (carrier gas) flow of 0.75 mL min^{-1}.
164 The GC temperature program was started at 80°C and retained for 1 min, increased to
165 195°C at a rate of 12°C min^{-1}, increased to 210°C at a rate of 2°C min^{-1} and retained for 3
166 min, increased to 225°C at a rate of 15°C min^{-1} and retained for 2 min and increased to
167 275°C at a rate of 40°C min^{-1} and retained for 10 min. The peak retention times of DP and
168 CF were 11.7 and 18.0 min, respectively.
169
170 **Results and discussion**
171
172 Enrichment, isolation and identification of PF-degrading bacteria
173 The PF-degrading consortium (referred to as MIX hereafter) was enriched in aerobic
174 condition at pH of 7 and temperature of 30°C. The consortium plated onto the medium
175 agar comprised numerous types of bacterial colonies. The soil used in this study was rich
176 in PF-tolerant cultures. During the isolation process, only three isolates were well and
177 continuously grown in PF-containing medium. This indicates that the pure isolates were
178 PF-degrading bacteria (namely PF1, PF2 and PF3).

179 The isolates, PF1, PF2 and PF3, were then identified by 16S rRNA analysis as
180 *Pseudomonas plecoglossicida* (GenBank accession number KJ143902), *Pseudomonas*
181 *aeruginosa* (GenBank accession number KJ143903) and *Pseudomonas aeruginosa*
182 (GenBank accession number KJ143904) with similarity of 98% to *Pseudomonas*
183 *plecoglossicida* strain SR7 (GenBank accession number KC634234.1), 100% to
184 *Pseudomonas aeruginosa* strain PGPR 10 (GenBank accession number KF640236.1)
185 and 99% to *Pseudomonas aeruginosa* strain QHFQZ1 (GenBank accession number
186 HQ844495.1), respectively.

187 The consortium and three isolates reported in this work could utilize PF as a sole
188 carbon source. This is similar to the previous studies which reported the OP utilization as
189 a sole carbon source (Cycon et al. 2009). Previously, Malghani and Chatterjee (2009)
190 isolated *Pseudomonas aeruginosa* strain OW from the PF-contaminated soil in China.
191 Also, Fulekar and Geetha (2008) reported that *Pseudomonas aeruginosa* could remove
192 CF. In present study, two more strains belong to *Pseudomonas aeruginosa* (PF2 and PF3)
193 isolated from the contaminated chili farm soil in Thailand were proposed for PF
194 degradation. The result suggested that *Pseudomonas aeruginosa* may conserve OPP-
195 degrading microbial enzymes resulting widely discovered the *Pseudomonas aeruginosa*
196 strains able to degrade OPPs. On the other hand, although role of *Pseudomonas*
197 *plecoglossicida* as pyrethroid pesticide-degrading bacterium has been previously reported
198 (Boricha and Fulekar, 2009), there is no published report on OPP biodegradation by this
199 microbial species. To the best of our knowledge, this is the first finding on OPP
200 degradation by *Pseudomonas plecoglossicida* (strain PF1).

201

202 Bacterial growth and PF degradation

203 The growth of MIX, PF1, PF2 and PF3 measured by viable cell counting on basal salt
204 medium agar containing PF is shown in Figure 1. While growth of MIX reached the
205 stationary phase after 2 d and increased from 7.1 to 7.6 log CFU mL^{-1} after 4 d, cell
206 number of each isolate could quickly increase to 14.0-16.5 log CFU mL^{-1} within 4 d.
207 Based on the growth kinetic rates presented in Table 1, the pure isolates (at the growth
208 rates of 1.9-2.7 1 h^{-1}) perforated approximately 5 to 6 times higher than MIX (0.4 1 h^{-1}).
209 This result indicates that even though both consortium and isolated cultures were able
210 to survive and reproduce in the medium containing PF, growth of each isolate was
211 enhanced when grown independently the pure isolates likely to better survive in the PF-
212 contaminated environment. This may be due to the competition between the cultures in
213 the consortium. This is similar to the work by Salunkhe et al. (2013). It was reported the
214 rapid growth rate of pure cultures (*Bacillus subtilis* strains) isolated from grapevines or
215 grape rhizosphere during profenofos biodegradation.
216 The reduction of PF concentration during the biodegradation test is shown in Figure
217 1. For all tests, the PF concentration continuously decreased in the first three days and the
218 concentration dropped gradually thereafter. After the 4-d experiment, MIX, PF1, PF2 and
219 PF3 removed PF for 90.0-95.3% (Table 1). In the abiotic control test (no cell), the PF
220 concentration decreased less than 5.0% suggesting that PF removal by physical or
221 chemical reactions was minimal. The PF degradation by all cultures followed the first-
222 order kinetics (Table 1). The consortium and isolates utilized PF at the rate of 3.0-19.6
223 mg L^{-1} d^{-1} (Table 1). Apparently, the pure isolates performed better than the consortium.
224 The treatment result well correlated to the bacterial growth result. MIX grew slower
225 resulting in lower PF treatment rate compared to those by the pure isolates.

226 The PF degradations by the consortium and cultures (more than 90%) were
227 comparable to those by *Pseudomonas aeruginosa* strain OW, *Pseudomonas putida* strain
228 W, *Burkholderia gladioli* strain Y, previously isolated (Malghani and Chatterjee, 2009;
229 Malghani et al. 2009). The result indicated that the newly enriched consortium and three
230 isolated cultures have potential application for the PF bioremediation. Generally, the
231 consortium has been shown to be more suitable for bioremediation compared to pure
232 cultures in practice. This is because their biodiversity can enhance environmental survival
233 and increase the number of catabolic pathways available for contaminant biodegradation
234 (Alvey and Crowley, 1996; Smith et al. 2005). In this case; however, the pure cultures
235 were acclimated in the medium and no environmental stresses in the tested condition
236 leading to better growth and performance presented by the isolated cultures.
237
238 Bacterial growth and PF degradation with additional carbon supplement
239 The experiment in this section was to determine the potential of additional carbon sources
240 for enhancement of PF biodegradation. Three additional carbon sources including sodium
241 succinate, sodium acetate and glucose were chosen (Xie et al. 2008).
242 Microbial cell numbers quickly rose from 6.8 to 18.7 log CFU mL^{-1} after 48 hr.
243 Microbial cell growth followed the first kinetic reaction at the rates of 5.97, 4.38 and 5.26
244 h^{-1} with the supplementation of sodium succinate, sodium acetate and glucose,
245 respectively. The result suggested that the additional carbon source obviously accelerated
246 the growth rate (10-14 times). This result well related to previous work (Jianlong et al.
247 2002; Grant and Betts; 2004; Xia et al. 2009). The test with sodium succinate and glucose
248 supplements had higher growth rate than that of the test with sodium acetate. Additional

249 carbon sources affected the growth rates to different extents since carbon structure was
250 specific to each microorganism (Xie et al. 2009).
251
252 PF intermediate metabolite detection
253 In the control (no cell) test, it was detected an intermediate product of PF, 4-bromo-2-
254 chlorophenol (BCP) (a GC peak with m/z of 207, 281, and 73) (Figure 3) while PF
255 concentration slightly decreased (less than 5%). This indicated that PF was reacted with
256 water and produced BCP *via* abiotic hydrolysis process. After continued monitoring for
257 14 d, BCP concentration detected was quite stable (data not shown).
258 For PF biodegradation assay, the PF intermediate metabolite detection from tests at
259 the initial PF concentrations of 20-120 mg L^{-1} was performed. From the test with the
260 initial PF concentration of 20 mg L^{-1}, BCP (which was presented in the microbial
261 medium as mentioned in earlier section) disappeared along with PF biodegradation. This
262 revealed that the enriched cultures could simultaneously degrade PF and BCP.
263 During the PF degradation at the concentration of 40 mg L^{-1} and higher, BCP was
264 accumulated in early stage of the test (the first 3 hr). In later period (after 6 hr), it was
265 noticed that 3-methoxy phenol (3-MP) appeared and replaced BCP (Figure 3). This
266 potentially be that BCP degraded to 3-MP (Figure 4). Additionally, it was found 2,4-di-
267 tert-butyl phenol (2,4-DTBP) during the degradation test. It was known that 2,4-DTBP
268 could be used for pesticide production (Schwetlick et al. 1991).
269 For PF degradation intermediate detection, BCP production *via* abiotic hydrolysis
270 process well correlated to the study by Zamy et al. (2004). It was reported that OPPs
271 including PF could hydrolyze in dilute OPP solution. The result suggested that abiotic
272 hydrolysis slightly contributes PF degradation (less than 5%). Since BCP is more stable

than PF, BCP concentration remained constant for longer period. During the PF degradation, BCP was accumulated similar to the result described elsewhere (Malgani et al. 2009). The result suggested that PF degraded via enzymatic organophosphorus hydrolysis. Organophosphorus hydrolase has been known as a typical bacterial enzyme for wide range of organophosphorus pesticide degradation (Kanekar et al. 2004). The appearance of 3-MP is different from proposed PF degradation in soil by Food and Agriculture Organization of the United Nations (FAO) (FAO 2012). FAO expected that BCP potentially degraded to 3-chloro-4-methoxyphenol in a sterile soil (Figure 4). This could be state that abiotic and biotic processes may give different PF degradation pathway. The cultures enriched in this study were promising for PF degradation since they could degrade both PF and its intermediate product (BCP). The potential PF degradation pathway was proposed (Figure 4). The complete PF biodegradation pathway is recommended for future investigation.

Selected OPP degradation

In practice, farmers apply a couple types of pesticides simultaneously for better pest control. For instance, in Thailand, farmers concurrently apply PF, CF and DP for vegetable cultivation. Based on this information, degradation of CF and DP by the enriched cultures was performed for investigating the treatment potential. The degradation result is presented in Figure 5. After the 4-d experiment, the degradation performance by PF1, PF2 and PF3 was similar. When the microbial cells of 10^6 CFU mL^{-1} and the initial pesticide concentration of 20 mg L^{-1} were tested, PF, CF and DP reduced for 75-82%, 57-73% and 33-47%, respectively (Figure 5).

296 Among three OPPs tested, PF and CF structure are similar. They are chemicals under
297 phosphorothioate group while DP is a chemical belong to phosphate group (Figure 6). PF
298 is *S*-alkyl phosphorothioate sub-group whereas CF is *O*-alkyl phosphorothioate sub-
299 group. It was observed that the pesticide degradation performances were different. This
300 may be from the pesticide structure. PF degradation performance is highest because the
301 cultures were acclimated for PF. The chemical structure of CF is more similar to PF
302 compared to that of DP resulting in high CF performance.
303 For enzymatic degradation point of view, four enzymes including phosphatase,
304 esterase, hydrolase and oxygenese involved in OPP biodegradation pathway (Kanekar et
305 al. 2004). Singh and Walker (2006) summarized CF and DP biodegradation pathways.
306 Like PF, CF primarily degrades *via* hydrolysis process. DP degradation is more
307 complicated. DP transforms to monocrotophos *via* methylation and continuously
308 degrades *via* hydrolysis process. This could be the reason why DP was degraded less than
309 PF and CF. However, based on the result in this section, the enriched cultures were
310 potential for PF and other OPP remediation. For future study, the influence of
311 environmental conditions on PF degradation should be performed. Also, the full PF
312 degradation pathway by isolates should be conducted for insight investigation.
313
314 **Conclusions**
315 Novel PF-degrading consortium and three isolated cultures including *Pseudomonas*
316 *plecoglossicida* strain PF1, *Pseudomonas aeruginosa* strain PF2 and *Pseudomonas*
317 *aeruginosa* strain PF3 were enriched. The novel cultures used PF as a sole carbon source.
318 All cultures were capable in PF insecticide degradation (PF removal of more than 90%
319 within 4 d). The cultures could degrade PF, PF intermediate and other OPPs. For future

320 study, the influence of environmental conditions on PF degradation should be performed.
321 Also, the full PF degradation pathway by the novel consortium and isolates should be
322 conducted for insight investigation.
323
324 **Acknowledgments**
325 This material is based upon work supported by Ubon Ratchathani University and Office
326 of the National Research Council of Thailand. This work is also conducted under a
327 research program granted by Center of Excellence on Hazardous Substance Management.
328 Any opinions, findings and conclusions or recommendations expressed in this material
329 are those of the author and do not necessarily reflect the views of grant providers.
330
331 **References**
332 Alvey S, Crowley DE (1996) Suvival and activity of an atrazine-mineralizing bacterial
333 consortium in rhizoshere soil. Environ Sci Technol 30: 1596-1603
334 Boricha H, Fulekar MH (2009) *Pseudomonas plecoglossicida* as a novel organism for the
335 bioremediation of cypermethrin. Biol Med 1: 1-10
336 Cycon M, Wojcik M, Piotrowska-Seget Z (2009) Biodegradation of the
337 organophosphorus insecticde diazinon by *Serratia* sp. and *Pesudomonas* sp. and
338 their use in bioremediation of contaminated soil. Chemosphere 76: 494-501
339 FAO (2012) *Profenofos 171.* http://www.fao.org/fileadmin/templates/agphome/
340 documents/ Pests_Pesticides/JMPR/Evaluation08/Profenofos.pdf
341 Fulekar MH, Geetha M (2008) Bioremediation of chlorpyrifos by *Pseudomonas*
342 *aeruginosa* using scale up technique. *J Appl Biosci* **12**: 657-660

343 Grant RJ, Betts WB (2004) Mineral and carbon usage of two synthetic pyrethroid
344 degrading bacterial isolates. J Appl Microbiol 97: 656-662
345 Kanekar PP, Bhadbhade BJ, Deshpande NM, Sarnaik SS (2004) Biodegradation of
346 organophosphorus pesticides. Proc Indian Natn Sci Acad B70: 57-70
347 Jianlong W, Xiangchun Q, Liping H, Yi Q, Hegemann W (2002) Kinetics of co-
348 metabolism of quinoline and glucose by *Burkolderia pickettii*. Process Biochem 37:
349 831-836
350 Malghani S, Chatterjee N (2009) Isolation and characterization of a PF degrading
351 bacterium. J Environ Sci 21: 1591-1597
352 Malghani S, Chatterjee N, Yu HX, Luo Z (2009) Isolation and identification of
353 profenofos degrading bacteria. Braz J Microbiol 40: 893-900
354 Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Dymock D, Wade
355 WG (1998) Design and evaluation of useful bacterium-specific PCR primers that
356 amplify genes coding for bacterial 16S rRNA. Appl Environ Microbiol 64: 795-799
357 Roverdatti MG (2001) Monitoring of organochlrine and organophosphorus pesticdes in
358 the water of the Reconquista River. Water Res 35: 3457-3461
359 Salunkhe VP, Sawant IS, Banerjee K, Rajguru YR, Wadkar PN, Oulkar DP, Naik DG,
360 Sawant SD (2013) Biodegradation of profenofos by *Bacillus subtilis* isolated from
361 grapevines (*Vitis vinifera*). J Agric Food Chem 61: 7195-7202
362 Schwetlick K, Pionteck J, Winkler A, Hfihner U, Kroschwitz H, Habicher WD (1991)
363 Organophosphorus antioxidants: part X mechanism of antioxidant action of aryl
364 phosphites and phosphonites at higher temperatures. Polym Degrad Stabil 31: 219-
365 228

366 Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds.
367 FEMS Microbiol Rev 30: 428-471
368 Siripattanakul S, Wirojanagud W, McEvoy JM, Limpiyakorn T and Khan E (2009)
369 Atrazine degradation by stable mixed cultures enriched from agricultural soil and
370 their characterization. J Appl Microbiol 106: 986-992
371 Smith D, Alvey S, Crowley DE (2005) Cooperative catabolic pathways within an
372 atrazine-degrading enrichment culture isolated from soil. FEMS Microbiol Ecol 53:
373 265-273
374 Swarnam TP, Velmurugan A (2013) Pesticide residues in vegetable samples from the
375 Andaman Islands, India. Environ Monit Assess 185: 6119-6127
376 Toan PV, Sebesvari Z, Bläsing M, Rosendahl I, Renaud FG (2013) Pesticide
377 management and their residues in sediments and surface and drinking water in the
378 Mekong Delta, Vietnam. Sci Total Environ 452-453: 28-39
379 Xie S, Liu J, Li L, Qiao C (2009) Biodegradation of malathion by *Acinetobacter*
380 *johnsonii* MA19 and optimization of cometabolism substrates. J Environ Sci 21:
381 76-82
382 Xu G, Zheng W, Li Y, Wang S, Zhang J, Yan Y (2008). Biodegradation of chlorpyrifos
383 and 3, 5, 6-trichloro-2-pyridinol by a newly isolated *Paracoccus* sp. strain TRP. Int
384 Biodeter Biodegr 62: 51-56
385
386

387 **Table and figure captions**

388

389 **Table 1** Microbial growth and PF degradation kinetic rates

390

391 **Figure 1** Bacterial growth and PF degradation by the enriched consortium and isolates

392

393 **Figure 2** Bacterial growth and PF degradation by MIX under presence of additional

394 carbon source conditions

395

396 **Figure 3** BCP (a) and 3-MP (b) chromatograms and mass spectra

397

398 **Figure 4** Potential pathway of PF microbial degradation by the consortium and isolates

399

400 **Figure 5** Comparison of PF, CF and DP degradation performance by the isolates

401

402 **Figure 6** PF, CF and DP and their OPP chemical structures

403

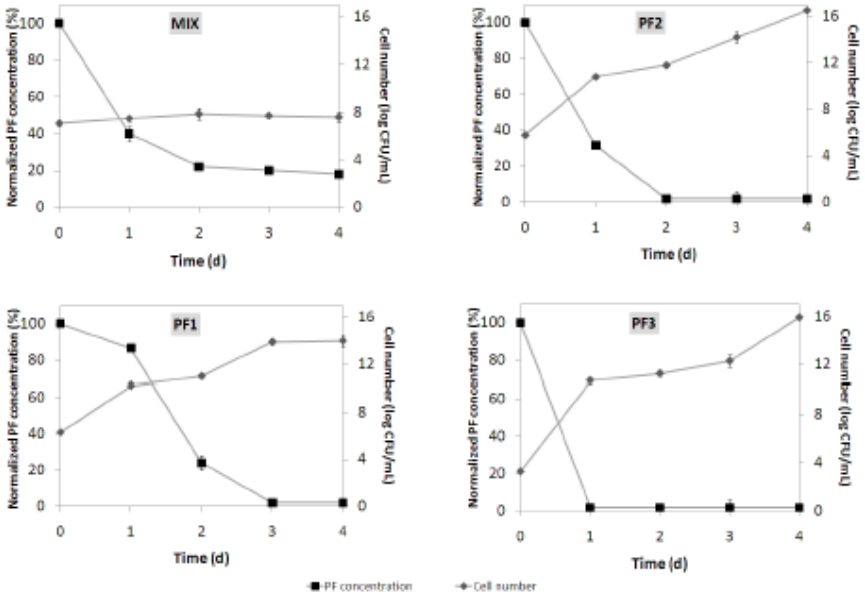
404 **Table 1**

Culture	Growth kinetics		PF degradation kinetics		Substrate utilization rate ($mg\ L^{-1}\ d^{-1}$)	PF removal (%)
	Equation[1]	R^2	Equation[2]	R^2		
MIX	y = 0.40x + 7.05	1.00	y = -0.15x + 1.12	0.87	3.0	90.0
PF1	y = 1.91x + 7.28	0.90	y = -0.57x + 1.55	0.86	7.6	95.0
PF2	y = 2.48x + 6.86	0.95	y = -0.85x + 1.42	0.95	9.8	93.1
PF3	y = 2.68x + 5.36	0.85	y = -0.85x + 1.02	0.75	19.6	95.3

405 [1] x = time (h) y = log (bacterial number)

406 [2] x = time (h) y = log (log (PF concentration)

407

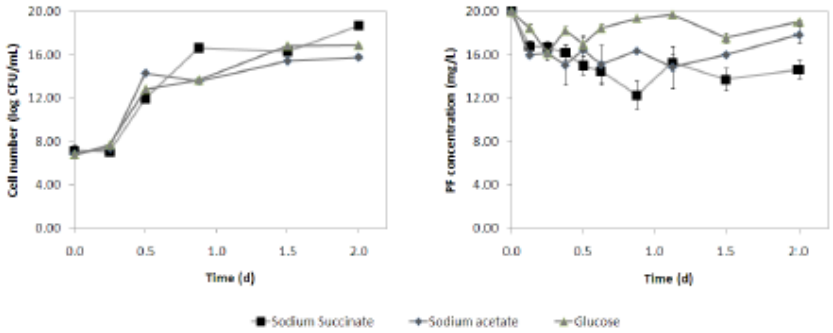


408

409 **Figure 1**

410

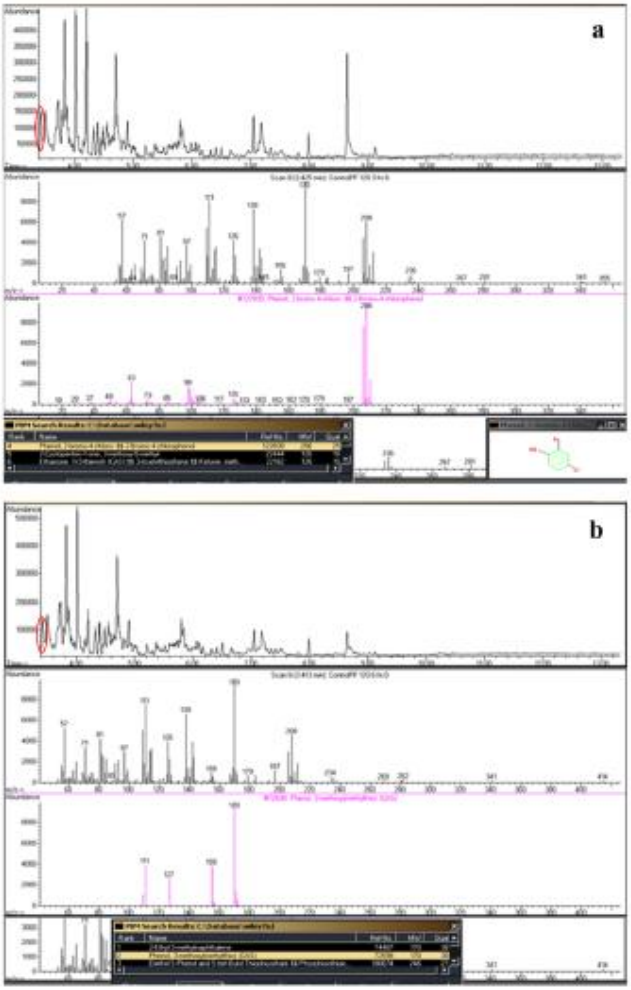
411



412

413 **Figure 2**

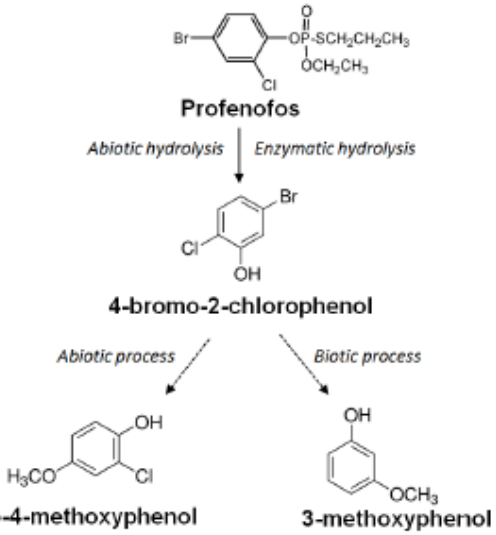
414



415

416 **Figure 3**

417

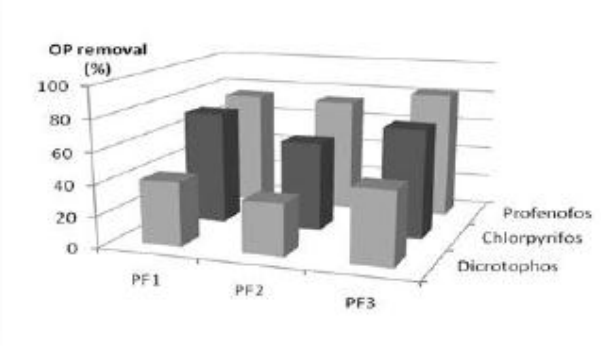


418

419 **Figure 4**

420

421

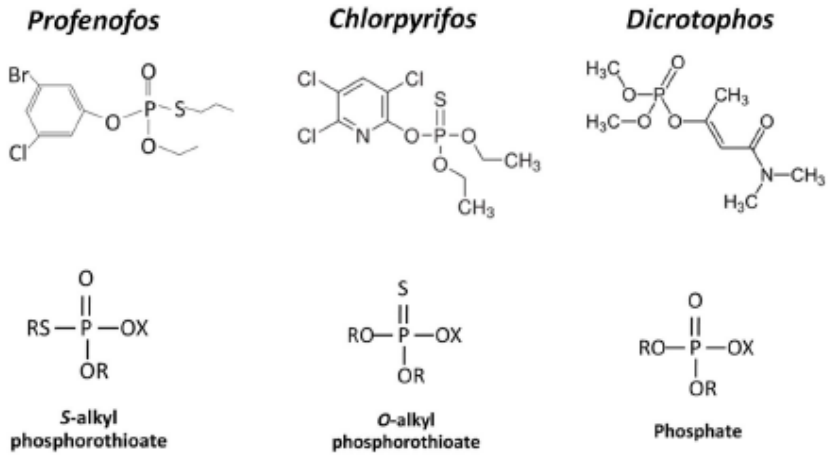


422

423 **Figure 5**

424

425



426

427 **Figure 6**

428