

BIODEGRADATION OF PROFENOFOS BY *Pseudomonas* STRAINS UNDER
PRESENCES OF OXYGEN AND NITRATE

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บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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การย่อยสลายสารโพรฟิโนฟอสทางชีวภาพโดยจุลินทรีย์ *Pseudomonas* ภายใต้สภาวะที่มี
ออกซิเจนและไนเตรท



วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ทิพย์สุดา ทรัพย์สงวน : การย่อยสลายสารโพรฟีโนฟอสทางชีวภาพโดยจุลินทรีย์ *Pseudomonas* ภายใต้สภาวะที่มีออกซิเจนและไนเตรท (BIODEGRADATION OF PROFENOFOS BY *Pseudomonas* STRAINS UNDER PRESENCES OF OXYGEN AND NITRATE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร. สุมณา ราษฎร์ภักดี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร. อติสา วัจโน, 145 หน้า.

การปนเปื้อนสารกำจัดศัตรูพืชโพรฟีโนฟอสภายใต้สภาวะที่มีไนเตรตในแหล่งน้ำใต้ดินจัดเป็นปัญหาที่สามารถพบได้ในประเทศเกษตรกรรม งานวิจัยนี้มีจุดมุ่งหมายเพื่อศึกษาการย่อยสลายสารโพรฟีโนฟอสภายใต้สภาวะที่มีไนเตรตเปรียบเทียบกับสภาวะที่มีออกซิเจน โดยจุลินทรีย์ที่ใช้ในการศึกษา ได้แก่ *Pseudomonas plecoglossicida* strain PF1 (PF1) *Pseudomonas aeruginosa* strain PF2 (PF2) และ *Pseudomonas aeruginosa* strain PF3 (PF3) ในงานวิจัยนี้ศึกษาที่ความเข้มข้นของสารโพรฟีโนฟอสเริ่มต้น 10 -150 มิลลิกรัมต่อลิตร และ ความเข้มข้นของไนเตรตเริ่มต้น 100 - 300 มิลลิกรัมต่อลิตร การทดลองศึกษาด้วยระบบแบบค้ำด้วยเครื่องเขย่าแบบบ่ม (ทำซ้ำ 2 รอบ) ที่ความเร็วรอบ 150 รอบต่อนาที และอุณหภูมิห้อง เป็นระยะเวลา 6-8 วัน จากผลการศึกษาพบว่าประสิทธิภาพการย่อยสลายสารโพรฟีโนฟอสภายใต้สภาวะที่มีออกซิเจนโดย PF1 PF2 และ PF3 อยู่ในช่วงร้อยละ 38.14-55.39 31.62-61.22 และ 34.72 63.81 และภายใต้สภาวะที่มีไนเตรตอยู่ในช่วงร้อยละ 27.50-45.33 39.09-75.36 และ 13.78-48.89 ตามลำดับ โดยการย่อยสลายสารโพรฟีโนฟอสเป็นไปตามปฏิกิริยาอันดับที่หนึ่ง จุลินทรีย์ PF1 และ PF3 สามารถย่อยสลายสารโพรฟีโนฟอสในสภาวะที่มีออกซิเจนได้ดีกว่าภายใต้สภาวะที่มีไนเตรตในขณะที่ PF2 ใช้สารโพรฟีโนฟอสภายใต้สภาวะที่มีไนเตรตได้ดีกว่า สำหรับการเจริญเติบโตของจุลินทรีย์พบว่าจุลินทรีย์ PF1 PF2 และ PF3 เจริญเติบโตเพิ่มขึ้นจาก 10^4 เป็น 10^8 ซีเอฟยูต่อมิลลิลิตร ตามปฏิกิริยาอันดับที่หนึ่งทั้งภายใต้สภาวะที่มีออกซิเจนและไนเตรต (แม้ไนเตรตสามารถใช้เป็นเป็นตัวรับอิเล็กตรอนในกระบวนการย่อยสลายสารโพรฟีโนฟอสได้ แต่ไนเตรตสามารถยับยั้งกระบวนการย่อยสลายโพรฟีโนฟอสได้เช่นกัน) สำหรับการติดตามสารมัธยันต์ภายใต้สภาวะที่มีออกซิเจนและไนเตรตตรวจพบ 4-bromo-2-chlorophenol (BCP) and 1,1-diethylethylphenol นอกจากนี้ยังตรวจพบ Triethyl phosphate ในการย่อยสลายสารโพรฟีโนฟอสสภาวะที่มีไนเตรต การศึกษานี้ชี้ให้เห็นว่าจุลินทรีย์ PF1 PF2 และ PF3 สามารถนำไปประยุกต์ใช้กับสิ่งแวดล้อมที่ปนเปื้อนสารโพรฟีโนฟอสรวมถึงแหล่งน้ำใต้ดินที่มีไนเตรตได้ จุลินทรีย์ดังกล่าวมีศักยภาพสำหรับการฟื้นฟูพื้นที่ปนเปื้อนสารโพรฟีโนฟอสในอนาคต

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Profenofos pesticide contaminated in groundwater with presence of nitrate was an problematic issue potentially found in agricultural countries. This study aimed to investigate profenofos biodegradation under presence of nitrate compared to the condition with oxygen. Three isolates, *Pseudomonas plecoglossicida* strain PF1 (PF1), *Pseudomonas aeruginosa* strain PF2 (PF2) and *Pseudomonas aeruginosa* strain PF3 (PF3), were used. In this research, concentrations of profenofos (10-150 mg/L) and nitrate (100-300 mg/L) were varied. Duplicate batch experiments were conducted using incubating shaker at 150 rpm and room temperature for 6-8 days. The results showed that removal efficiency percentages under presence of oxygen by PF1, PF2 and PF3 ranged from 38.14 to 55.39, 31.62 to 61.22 and 34.72-63.81%, respectively whereas PF1, PF2 and PF3 was removed profenofos from 27.50 to 45.33, 39.09-75.36 and 13.78 to 48.89% under presence of nitrate, respectively. The profenofos degradation kinetics followed the first order kinetic reaction. Strains PF1 and PF3 degraded profenofos under presence of oxygen better than presence of nitrate while PF2 utilized profenofos under presence of nitrate superior than presence of oxygen (For microbial growth, strains PF1, PF2 and PF3 grew from 10^4 to 10^8 CFU/ml followed the first kinetic order for both presence of oxygen and nitrate). Although nitrate was used as an electron acceptor during profenofos degradation, nitrate could inhibit the profenofos biodegradation as well. For intermediate metabolite monitoring, under presence oxygen and nitrate, 4-bromo-2-chlorophenol and 1,1-diethylethylphenol were found. Moreover, triethyl phosphate was also found as the metabolite on profenofos degradation under presence of nitrate. This study indicated that strains PF1, PF2 and PF3 could be applied in the profenofos-contaminated environment including groundwater containing nitrate. The cultures are promising for profenofos bioremediation in the future.

Field of Study: Environmental Management Student's Signature

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CONTENTS

	Page
THAI ABSTRACT.....	iv
ENGLISH ABSTRACT	v
ACKNOWLEDGEMENTS	vi
CONTENTS.....	vii
LIST OF TABLES	x
LIST OF FIGURES	xi
CHAPTER I.....	1
INTRODUCTION.....	1
1.1 Motivation	1
1.2 Objectives.....	3
1.3 Scopes of the Study	3
1.4 Hypotheses	4
CHAPTER II.....	5
LITERATURE REVIEW	5
2.1 Overview of profenofos	5
2.1.1 Physical and chemical properties	5
2.1.2 Utilization.....	6
2.1.3 Toxicity	6
2.1.4 Profenofos contamination and fate in environment.....	7
2.1.5 Profenofos degradation pathway	8
2.1.6 Microbial cultures involving in profenofos degradation.....	11
2.2 Biological treatment.....	11
2.2.1 Biotransformation	12
2.2.2 Biodegradation	12
2.2.3 Biodegradation under aerobic and anaerobic conditions	13
2.3 Microbial growth, degradation and degradation kinetics.....	15
2.3.1 Microbial growth	15
2.3.2 Degradation kinetics	16

	Page
CHAPTER III.....	19
MATERIALS AND METHODS	19
3.1 Experimental Framework and scheme.....	19
3.2 Materials and Methods.....	20
3.2.1 Bacteria cultivation and medium	20
3.2.2 Profenofos biodegradation by PF-degradaing cultures.....	21
3.2.3 Kinetic analyses	21
3.2.4 Chemical analysis	22
CHAPTER IV	24
RESULTS AND DISCUSSION	24
4.1 Profenofos degradation and degradation kinetics under presence of oxygen and nitrate.....	24
4.1.1 Profenofos degradation under presence of oxygen and nitrate	24
4.1.2 Profenofos degradation kinetics	34
4.2 Microbial growth under presence of oxygen and nitrate	40
4.3 Nitrate reduction under presence of nitrate.....	47
4.4 Profenofos intermediate metabolite under presence of oxygen and nitrate	52
CHAPTER V.....	59
CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK.....	59
5.1 Conclusion.....	59
5.1.1 Profenofos degradation and degradation kinetics under presence of oxygen and nitrate.....	59
5.1.2 Microbial growth under presence of oxygen and nitrate	59
5.1.3 Nitrate reduction (under presence of nitrate)	60
5.1.4 Profenofos intermediate metabolite monitoring under presence of oxygen and nitrate.....	60
5.2 Recommendations for future work	60
REFERENCES.....	61
APPENDIX A	66

	Page
Medium preparation and aseptic technique	66
APPENDIX B	72
Chemical Analysis.....	72
APPENDIX C	78
Raw data.....	78
VITA.....	145



LIST OF TABLES

	Page
Table 2.1 Physical and chemical properties of profenofos	5
Table 2.2 Microbial cultures of profenofos degradation	11
Table 4.1 Degradation kinetics of PF1 under presence of oxygen.....	25
Table 4.2 Degradation kinetics of PF1 under presence of nitrate	25
Table 4.3 The percentages of profenofos removal efficiency by PF1 at 5 days	27
Table 4.4 Degradation kinetics of PF2 under presence of oxygen.....	28
Table 4.5 Degradation kinetics of PF2 under presence of nitrate	28
Table 4.6 The percentages of profenofos removal efficiency by PF2 at 5 days	30
Table 4.7 Degradation kinetics of PF3 under presence of oxygen.....	31
Table 4.8 Degradation kinetics of PF3 under presence of nitrate	31
Table 4.9 The percentages of profenofos removal efficiency by PF3 at 5 days	33
Table 4.10 Degradation kinetic parameters	38
Table 4.11 Growth kinetics of PF1, PF2 and PF3 under presence of oxygen	41
Table 4.12 Growth kinetics of PF1 under presence of nitrate	41
Table 4.13 Growth kinetics of PF2 under presence of nitrate	42
Table 4.14 Growth kinetics of PF3 under presence of nitrate	42
Table 4.15 Nitrate reduction (%).....	51
Table 4.16 BCP-profenofos ratio on profenofos degradation.....	55

LIST OF FIGURES

	Page
Figure 2.1 Molecular structure of profenofos	6
Figure 2.2 Proposed metabolic pathway of profenofos in soil, plant and enzymatic reactions	9
Figure 2.3 Proposed pathways for profenofos degradation by <i>Pseudomonas aeruginosa</i> (strain OW)	10
Figure 2.4 Potential profenofos degradation pathway	10
Figure 2.5 Relationship between substrate degradation rate and substrate concentration	17
Figure 2.6 Relationship of substrate degradation rate and substrate concentration in Lineweaver-Burk model	17
Figure 3.1 Framework of the study	19
Figure 3.2 Experimental scheme	20
Figure 3.3 Degradation rate	22
Figure 4.1 Profenofos degradation under presence of oxygen and nitrate by PF1	24
Figure 4.2 Profenofos degradation under presence of oxygen and nitrate by PF2	27
Figure 4.3 Profenofos degradation under presence of oxygen and nitrate by PF3	30
Figure 4.4 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF1 (a) under presence of oxygen (b) under presence of nitrate	35
Figure 4.5 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF2 (a) under presence of oxygen (b) under presence of nitrate	36
Figure 4.6 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF3 (a) under presence of oxygen (b) under presence of nitrate	37
Figure 4.7 The relationship between V and S of profenofos degradation by PF1 under presence of oxygen and nitrate (100, 200 and 300 mg/L)	38
Figure 4.8 The relationship between V and S of profenofos degradation by PF2 under presence of oxygen and nitrate (100, 200 and 300 mg/L)	39
Figure 4.9 The relationship between V and S of profenofos degradation by PF3 under presence of oxygen and nitrate (100, 200 and 300 mg/L)	39
Figure 4.10 Microbial growth of PF1 under presence of oxygen and nitrate	43

Figure 4.11 Microbial growth of PF2 under presence of oxygen and nitrate	44
Figure 4.12 Microbial growth of PF3 under presence of oxygen and nitrate	45
Figure 4.13 The normalized nitrate concentration percentages of PF1 at profenofos 10,25,50,80,100 and 150 mg/L.....	48
Figure 4.14 The normalized nitrate concentration percentages of PF2 at profenofos 10,25,50,80,100 and 150 mg/L.....	49
Figure 4.15 The normalized nitrate concentration percentages of PF3 at profenofos 10,25,50,80,100 and 150 mg/L.....	50
Figure 4.16 Mass spectrum of 4-bromo-chlorophenol (BCP)	53
Figure 4.17 Mass spectrum of 1,1 diethylethylphenol	53
Figure 4.18 Mass spectrum of triethyl phosphate	54
Figure 4.19 Proposed commercial profenofos degradation pathway under presence of oxygen and nitrate	57

CHAPTER I

INTRODUCTION

1.1 Motivation

Pesticides play an important role in agriculture for controlling pests and improving agricultural productivity. However, the indiscriminate use of pesticides has inflicted serious environmental problems and human health because of pesticide contamination spread over the application area. Some Pesticides remain for a long time in environment including soil, sediment, and groundwater while others directly go to food chain. Therefore, the fate of pesticides is uncertain; it depends on several factors such as persistence and mobility (Gunawan et al., 2014).

Organophosphate compounds (OPs) are a group of compounds that have been used as pesticides in agricultural area; they also used as municipal hygiene, disease vector control and against household pest (Silva et al., 2013) OPs have been developed to replace the organochlorine pesticides which were banned in USA since 1970 (Jauregui et al., 2003). However, OPs are also high toxic since they are potent irreversible acetylcholinesterase inhibitors. They have a profound effect on the nervous system of exposed organisms (Edwards and Tchounwou, 2005) One of the organophosphorus pesticides is profenofos (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate) which is extensively used in agricultural and household applications causing environmental pollution with adverse effect on human, wildlife and environment (Kumar et al., 2014). In USA, profenofos is a restricted use as pesticide only on cotton crop. Residential uses of profenofos is not allowed (USEPA, 2006). In Brazil, this pesticide can be used for foliar application on cotton, peanuts, potatoes, coffee, onions, peas, beans, watermelon, corn, cucumber and cabbage (Silva et al., 2013) Furthermore, this chemical was applied the most for chili farms in the north eastern area of Thailand (Siripattanakul-Ratpukdi et al., 2015) Based on the utilization information, profenofos is potential to accumulate in soil and sediment and percolate into the groundwater (Ngan et al., 2005).

One treatment method is bioremediation which is a technique applied to clean up organic substances by microorganisms. Bioremediation performance relies on biodegradation ability of microorganisms to remove pollutants from contaminated area. Bioremediation is effective, less hazardous, economical, versatile and environmental friendly (Finley et al., 2010). Previous studies successfully achieved biodegradation of organophosphorus (Yadav et al., 2014). The studies carried out under aerobic condition. However, in practice, the amount of oxygen in groundwater may not be enough for aerobic degradation leading to anoxic or anaerobic degradations which nitrate, manganese (IV) oxide, iron (III) hydroxides, sulfate and carbon dioxide are presented as terminal electron acceptor (Schink, 2005). In agricultural area, groundwater containing nitrate from fertilizers was reported (Sunitha, 2013). In addition, based on redox potential, nitrate was preferred electron acceptor in anaerobic condition. Therefore, biodegradation kinetics with nitrate as electron acceptor is also important.

Some literatures explained the biodegradation under aerobic and anaerobic conditions (Liu et al., 2011). Tiwari and Guha (2013) reported that biodegradation of endosulfan (ES), an Organochlorine, under aerobic and anaerobic conditions which showed the different pathways after degradation. The results showed the rates of degradation of endosulfan were higher in the aerobic environment compared with anaerobic environment. The chemical was oxidized to endosulfan sulfate (ESS) which was the rate limiting step in aerobic degradation then further degradation to endosulfan ether (ESE) and endosulfan lactone (ESL), respectively while ES was hydrolyzed to ESD which was the rate limiting step in anaerobic condition and further degradation to ES. Based on the example, it is obvious that the different degradation mechanism including reactions, kinetics, enzymes and reaction rates led to different intermediates in the pathways which affect to further degradation (Tiwari and Guha, 2013). To the best of our knowledge, all previous studies on profenofos biodegradation have been focused only the degradation under aerobic condition (Malghani et al., 2009; Silva et al., 2013).

Therefore, the thesis entitled “biodegradation of profenofos under presences of oxygen and nitrate” will be carried out. The aim of this study is to determine

profenofos degradation kinetics and potential pathway under presences of oxygen and nitrate. Additionally, the kinetics with different nitrate concentrations will be examined. The *Pseudomonas* strains previously isolated and characterized will be used in this study (Siripattanakul-Ratpukdi et al., 2015).

1.2 Objectives

Main objective

To investigate kinetics of profenofos biodegradation under presences of oxygen and nitrate

Specific objectives

1. To investigate profenofos biodegradation and microbial growth in presence of oxygen and nitrate at different profenofos concentrations
2. To investigate nitrate reduction during profenofos biodegradation under presence of nitrate
3. To estimate kinetics of profenofos biodegradation in presence of oxygen and nitrate
4. To determine potential pathways of profenofos biodegradation under presences of oxygen and nitrate degradations

1.3 Scopes of the Study

- 1.31 The isolates (*Pseudomonas plecoglossicida* (GenBank accession number KJ620776), *Pseudomonas aeruginosa* (GenBank accession number KJ143903) and *Pseudomonas aeruginosa* (GenBank accession number KJ143904) were taken from the previous study. The cultures were isolated from profenofos pesticide-contaminated soil in chili farm, Ubon Ratchathani, Thailand (Siripattanakul-Ratpukdi et al., 2015).
- 1.32 The study was carried out in the laboratory scale.

- 1.33 Commercial grade PF was used in the experiment (Guddacy (50% w/v), Thailand) while standard PF (analytical grade) was used for analysis (Dr.Ehrenstorfer GmbH, Germany).
- 1.34 Profenofos concentrations were varied at 10, 25, 50, 80, 100 and 150 mg/L under presences of oxygen and nitrate.
- 1.35 Profenofos concentration was measured by gas chromatograph with electron capture detector (GC-ECD).
- 1.36 Profenofos degradation pathway was examined by gas chromatography/mass spectrometry (GC/MS).
- 1.37 Nitrate was used as electron acceptor under anaerobic condition.
- 1.38 Influence of nitrate concentrations (100, 200 and 300 mg/L) on profenofos biodegradation was investigated.
- 1.39 Nitrate concentration was detected by colorimetric method followed standard method for the examination of water and wastewater (APHA, 1975).
- 1.40 Profenofos biodegradation kinetics under presences of oxygen and nitrate followed Michaelis-Menten model was performed.

1.4 Hypotheses

1. Degradation and degradation rate of profenofos under presence of oxygen are higher than those of presence of nitrate
2. Microbial growth and growth rate under presence of oxygen are higher than those of presence of nitrate
3. Higher concentration of nitrate can promote higher profenofos biodegradation.
4. Degradation kinetics under presences of oxygen and nitrate are different
5. Profenofos degradation pathways under presences of oxygen and nitrate are different

CHAPTER II

LITERATURE REVIEW

2.1 Overview of profenofos

2.1.1 Physical and chemical properties

Profenofos, a thiophosphate organophosphorus, have been developed to replace the organochlorine pesticides which banned in the United State since 1970 (Jauregui et al., 2003) and other higher toxic organophosphorus compounds (OPs) (Dadsona et al., 2013) It was first registered by the Agency in 1982 for using as an insecticide/miticide in agricultural and household purposes (USEPA, 2006). Physical and chemical properties of profenofos are presented in Table 2.1 and molecular structure in Figure 2.1.

Table 2.1 Physical and chemical properties of profenofos

Pesticide type	Insecticide
Chemical class	Organophosphate
Chemical formula	$C_{11}H_{15}BrClO_3PS$
Chemical name	<i>O</i> -(4-bromo-2-chlorophenyl) <i>O</i> -ethyl <i>S</i> -propyl phosphorothioate
Molecular mass	373.63 g/mol
Appearance	Slightly clear pale yellow to light brown liquid
Odor	Pungent, like garlic or cooked onions
pH value	4.2 at 25°C
Boiling point	110 °C at 1.8 Pa
Density	1.46 g/cm ³ at 20 °C
Half-life	About one week (7 days)
Henry's Constant	$1.65 \times 10^{-3} \text{ Pa m}^3 \text{ mol}^{-1}$
Vapor Pressure	$1.24 \times 10^{-1} \text{ mPa}$ at 25 °C
Log Kow	4.68
Solubility in water	20 mg/L
Solubility in other solvent	Soluble in several organic solvent such as ethanol, acetone, toluene, n-octanol, and n-hexan
Stability	Relatively stable under neutral and slightly acidic conditions. under alkaline conditions; on hydrolysis

Reference: (DowAgroSciences, 2002; USEPA, 2006)

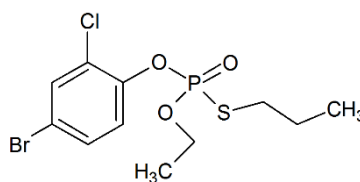


Figure 2.1 Molecular structure of profenofos

2.1.2 Utilization

Profenofos is widely used as pesticide in agriculture for pest control such as tobacco budworm, cotton bollworm, armyworm, cotton aphid, whiteflies, spider mites, plant bugs and fleahoppers. An estimated 85% of profenofos at varying rates is used to control lepidopteron species (worm complex). In addition, an approximately 775,000 lb/y of active ingredient profenofos are applied to cotton crops in the United States (USEPA, 2006). Otherwise, there are many countries which extensively used this chemical. In Pakistan, 80% of pesticide used for cotton crop was profenofos (Ismail et al., 2009). In Egypt, profenofos was applied for vegetable crop, especially field-grown pepper and eggplant (Radwan et al., 2005). In Korea, profenofos was used for pest control, such as white fly, rocket and plantlouse (Min and Cha, 2000). In the United State, Profenofos is a restricted use as pesticide only for cotton crop (USEPA, 2006). In Brazil, Profenofos can be used for foliar application for cotton, peanut, potato, coffee, onion, pea, bean, watermelon, corn, cucumber and cabbage (Silva et al., 2013). Profenofos was one of the most used pesticides for chili farm in the north eastern area of Thailand (Siripattanakul-Ratpukdi et al., 2015).

2.1.3 Toxicity

World Health Organization (WHO) has been classified profenofos as a moderately hazardous (Toxicity class II) pesticide (Malghani et al., 2009; Silva et al., 2013). It has a moderate order of acute toxicity following oral and dermal administration (Pandey et al., 2011). Profenofos also has been examined to be high toxic substance affecting mammals (Dadsona et al., 2013) fish (Ismail et al., 2009; Pandey et al., 2011) and shrimp (Rao et al., 2007). For acute toxic action, profenofos

could inhibit acetylcholinesterase (AChE) and decrease the hydrolysis of acetylcholine in both central and peripheral cholinergic synapses which resulting in overstimulation of nicotinic and muscarinic receptors followed by receptor down-regulation on post-synaptic membranes (Dadsona et al., 2013). According to the toxicity of profenofos, it is extreme to aquatic invertebrates and vertebrates which continuously effect on animal through food and water (Rao et al., 2007). Furthermore, profenofos can stimulate the nervous system causing nausea, dizziness, confusion and respiratory paralysis and death at high concentration exposure (USEPA, 2006). Many studies of profenofos toxicity were done. In India, toxicity of organophosphate (acephate, chloropyrifos, monocrotophos and profenofos) in short-term bioassay using brine shrimp was studied (Rao et al., 2007). In China, the acute cytotoxic effect of the organophosphorus pesticide (profenofos) to gill of flounder was determined. The results show that the cell growth rate was reduced and the fine cell structures were changed by profenofos (Hong-yan et al., 2001). In Korea, bioconcentration of phosphamidon and profenofos in Zebrafish was examined. It was found that profenofos had effect on survival of vertebrate, tissue accumulation and physiological and reproductive process of organism especially aquatic organism (Min and Cha, 2000).

2.1.4 Profenofos contamination and fate in environment

From physical and chemical properties of profenofos mention earlier, profenofos directly releases and spread over to the environment including air, soil, water and sediment. Some literature reviews reported the profenofos contamination in environment (Ngan et al., 2005). Profenofos was detected in runoff water and soil range of < 0.01-0.08 ng/mL and < 0.01–0.02 mg/kg, respectively. Moreover, surface water in Khon Kaen, Thailand was found 1 mg/L of profenofos (Harnpicharnchai et al., 2013).

Hydrolysis is the important way of dissipation whereas photolysis is not a main route of profenofos degradation. If profenofos released to soil, it was slightly mobile based on K_{oc} value. However, it was rapidly degraded in alkaline under aerobic condition (half-life of 2 days) and was quickly utilized in anaerobic condition (half-life 3 days). The degradation rate of profenofos is influenced by chemical

hydrolysis. Some reports showed that profenofos may release in air through volatilization which half-life is approximately 8.6 hr. Based on water solubility (20 mg/L), it is moderately soluble in water. Profenofos can release into water; it can adsorb to suspended solid and sediment. Also, profenofos is potential groundwater contaminant. For surface water, profenofos was accumulated by spraying of agricultural application or runoff. It rapidly dissipated on surface water only a few days in alkaline condition whereas it likely to persist in acidic conditions. Dissolution and adsorption processes affected profenofos transport in runoff. The persistence of profenofos in water depended on pH, microbial population and hydrologic residence time of water body. For groundwater, profenofos was further reduced under alkaline condition because of rapid hydrolysis (USEPA, 1998).

2.1.5 Profenofos degradation pathway

1. Abiotic degradation

Profenofos, the compound is stable to photolysis in water and soil (USEPA, 2006). There are many studies on abiotic degradation of profenofos including advanced oxidation processes (the combination of the Fenton reaction, UV/H₂O₂ and the photo-Fenton process) (Badawy et al., 2006; Oller et al., 2011). This reaction combines with oxidants, ultraviolet irradiation and catalysts to generate hydroxyl radicals in solution. Hazardous organic compounds are oxidized by free radicals and mineralized to water, carbon dioxide and mineral salts. In addition, it was found profenofos abiotic hydrolysis reaction on profenofos degradation (Siripattanakul-Ratpukdi et al., 2015).

2. Biotic degradation

There were only a few reports in biotic degradation pathways of profenofos; however, some degradation pathways are described in the literature (Malghani et al., 2009). The metabolic pathway of profenofos in cotton crop involves the cleavage of the phosphorothioate ester bond to yield 4-bromo-2-chlorophenol (BCP), followed by conjugation with glucose (Silva et al., 2013). According to the Food and Agriculture Organization of the United States (FAO), profenofos was metabolized rapidly under aerobic (1.9 days) and anaerobic (2.9 days) (FAO, 2007).

In sterilized soil, cleavage of phenol phosphorous ester bond of profenofos proceeded via chemical hydrolysis, with accumulation of BCP and formation of unextracted residues. For metabolic biotransformation in plant and animal similar to soil, it occurred through hydrolysis which was formed 4-bromo-2-chlorophenol as the intermediate and conjugated by several enzymatic activities (Silva et al., 2013). The metabolic pathways of profenofos are showed in Figure 2.2

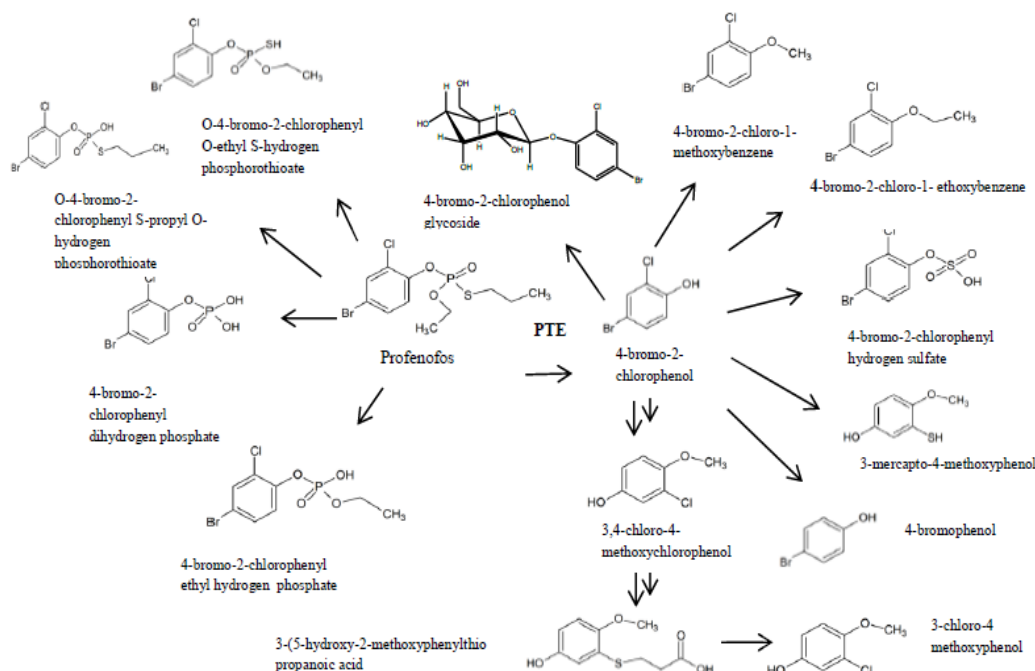


Figure 2.2 Proposed metabolic pathway of profenofos in soil, plant and enzymatic reactions

In addition, some literatures are explained profenofos degradation pathway by both bacteria and fungi (Malghani et al., 2009) For example, isolation and characterization of a profenofos degrading bacteria was performed by (Silva et al., 2013). In this study, 86.81% of pure profenofos disappeared after 48 hours followed by a decreasing of profenofos with a longer incubation time and 4-bromo-2-chlorophenol (BCP) was identified as a metabolite in profenofos degradation pathway via esterase enzymes profenofos biodegradation pathways are presented in Figure 2.3.

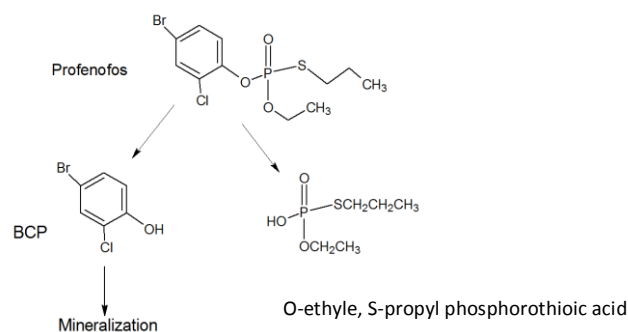


Figure 2.3 Proposed pathways for profenofos degradation by *Pseudomonas aeruginosa* (strain OW)

Another work on biodegradation of profenofos pesticide by novel isolated cultures and their characterization was performed by Siripattanakul-Ratpakdi et al. (2015). In this case, profenofos degradation continuously decreased over two days and the concentration dropped gradually thereafter and 4-bromo-2-chlorophenol (BCP) was detected during profenofos degradation via the organophosphorus hydrolase enzyme (Siripattanakul-Ratpakdi et al., 2015) and profenofos degradation pathway is demonstrated in Figure 2.4.

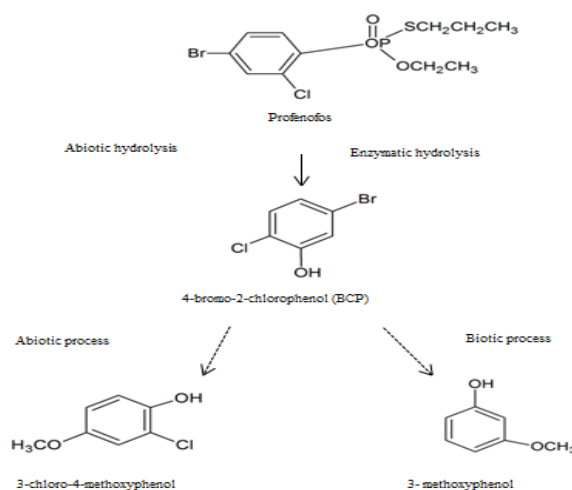


Figure 2.4 Potential profenofos degradation pathway

2.1.6 Microbial cultures involving in profenofos degradation

There were a few reports on microbial cultures which can degrade profenofos. the microbial cultures involving in profenofos degradation are presented in Table 2.2.

Table 2.2 Microbial cultures of profenofos degradation

Microbial cultures	Isolate	Strains	Results	Reference
Bacteria	<i>Pseudomonas plecoglossicida</i>	PF 1	After 6 d, profenofos was removed 95.0%	(Siripattanakul Ratpukdi et al. 2015)
	<i>Pseudomornas aeruginosa</i>	PF 2	Profenofos was degraded 93.1% within 6 d.	
		PF 3	Profenofos was degraded 95.0% within 6 d.	
	<i>Pseudomornas aeruginosa</i>	OW	86.81% of profenofos disappeared after 48 hr.	(Malghani et al., 2009)
	<i>Bacillus subtilis</i>	DR-39	89.4% of profenofos was degraded in 9 d.	(Salunkhe et al., 2013)
		CS-126	87.6% of profenofos was degraded in 9 d.	
		TL-171	74.2% of profenofos was degraded 9 d.	
		TS-204	72.8% of profenofos was degraded 9 d.	
Fungi	<i>Penicillium raistrickii</i>	CBMAI 931	This strain degraded approximately of 82% of profenofos.	(Silva et al., 2013)
	<i>Aspergillus sydowii</i>	CBMAI 935	It showed 71% of profenofos degradation	

2.2 Biological treatment

Biological treatment is a process which contaminants in environmental media are transformed or degraded to non-hazardous substances such as carbon dioxide, water, fatty acids and biomass through the action of microorganisms. The biological treatment process is low cost and no residual treatment requirement; however, it may be difficult and take longer time compared to chemical and physical processes. (EUGIRS, 2014).

2.2.1 Biotransformation

Biotransformation is the process which a substance is changed from one chemical structure to another by chemical reaction (USGS, 2014). Some literatures described the mechanism of biotransformation of organophosphorus compound (Jokanovic, 2001) Usually, biotransformation of xenobiotics are divided into 2 phases: (1) reaction which a polar group, such as hydroxyl (-OH), carboxyl (-COOH), thiol (-SH) and amino (-NH₂) groups is introduced through oxidation, reduction or hydrolysis. In this step, metabolites could be more or less toxic than that of parent compounds and (2) polar metabolites are conjugated with endogenous substrates such as sulfate, acetate and amino acid which hydrosoluble products are formed. For Organophosphorus compound biotransformation, the reaction is separated into (1) activation processes and (2) detoxification processes.

Since profenofos is a thiophosphate organophosphorus, the activation of this compound may occur through oxidative desulfuration of thiophosphate to form phosphorus compound. Based on the structure of profenofos which contained anhydride bond, it could be cleavage via oxidative dearylation or enzymatic hydrolysis (Jokanovic, 2001). Some previous work showed the biotransformation of organophosphorus pesticide (Lavado and Schlenk, 2011). It was reported chlorophrifos, parathion and fenthion biotransformation. During fenthion dedegradation, 3-methyl-4-methylphenol, fenoxon and fenthion sulfoxides were detected as the metabolites in liver and gills of rainbow trout. Chlorpyrifos was converted to chlorpyrifos oxon and 3,4,6-trichloro-2-pyridinol. Paraxon and p-nitrophenol were detected as the metabolites on parathion biotransformation.

2.2.2 Biodegradation

Biodegradation is a subset in biotransformation which the intramolecular bond of organic compound structure is broken down. It involves substitution of functional groups leading to mineralization (Madsen, 2008). Some prior works defined biodegradation. U.S. Geological Survey stated biodegradation as “transformation of a substance into new compound through biochemical reactions or the actions of microorganism such as bacteria” (USGS, 2007).

Pesticides have been extensively used in agricultural area which can accumulate and persist for a long time in the environment. Microbial degradation of hazardous substance such as pesticides is an important way to reduce or remove the concentration of these compounds to acceptable level. There are many organic compounds biodegradation; for example, More than 95 % removal efficiency of chloropyrifos by *Pseudomonas* sp. in a continuous packed bed bioreactor was observed (Yadav et al., 2014) Chloropyrifos, fenitrothion and parathion were degraded 58.9%, 70.5% and 82.5%, respectively within 14 days by diazinon-degrading *Serratia marcescens* (M. Cycon et al., 2013) Diazinon was degraded 80-92% by *Serratia* sp. and *Pseudomonas* sp. and the consortium. When MSM was supplemented with glucose, the degradation was accelerated (M. Cycon et al., 2009).

There were a few works on biodegradation of profenofos. Profenofos was removed 86.81% within 48 hours incubation by *Pseudomonas aeruginosa* and the metabolite was identified as 4-bromo-2-chlorophenol (BCP) which occurred through hydrolysis reaction (Malghani et al, 2009). Another study reported that more than 90% of profenofos was degraded within 6 days by the isolates, *Pseudomonas plecoglossicida* (PF1), *Pseudomonas aeruginosa* (PF2) and *Pseudomonas aeruginosa* (PF3) which BCP was detected as the intermediate through organophosphorus hydrolase enzyme (Siripattanakul-Ratpukdi et al., 2015). Recently, the profenofos removal of more than 70% by *Bacillus subtilis* strains was found (Salunk et al., 2013). Not only bacteria can degrade profenofos but fungi can remove profenofos also. *Penicillium raistrickii* strain CBMAI 931 and *Aspergillus sydowii* strain CBMAI 935 showed profenofos degradation of 82.0% and 71.0%, respectively (Silva et al., 2013).

2.2.3 Biodegradation under aerobic and anaerobic conditions

Metabolism is the biochemical reaction which carries out by living organism. Metabolism consists of two types of processes including catabolism and anabolism. Catabolic and anabolism reaction is very important for biodegradation which involves electron transport and redox reaction (oxidation-reduction reaction). For aerobic degradation, enzymatic key reactions of aerobic biodegradation are oxidations catalyzed by oxygenases and peroxidases which oxygenases are oxidoreduatases that

used O_2 to incorporate with oxygen into the substrate. Microbial degradation needs oxygen in two metabolic sites 1) the initial attack of substrate 2) the end of respiratory chain. Xenobiotic compound is classified as carbon source and electron donor for generation of energy and oxygen as electron acceptor. In some case, though a single bacterium is able to metabolize organic pollutant, mixed culture communities are higher degradation. This is because the mixed cultures containing more various genetic information resulting in better degradation of the xenobiotic compounds. Therefore, the genetic potential and environmental factors such as temperature, pH, available nitrogen and phosphorus sources are important influences on the degradation rate and further degradation (Fritsche and Hofrichter, 2008).

In general, a wealth of aromatic compound is degraded by a limited number of reaction: hydroxylation, oxygenolytic ring cleavage, isomerization and hydrolysis. Some report described that profenofos was degraded by *Pseudomonas aeruginosa* under aerobic degradation which oxygen as an electron acceptor through hydrolysis reaction and BCP was detected as the intermediate. The isolated bacteria may contain hydrolase enzyme (Siripattanakul-Ratpukdi et al., 2015) or esterase enzyme (Malghani et al., 2009).

Anaerobic degradation processes has been examined inferior to aerobic degradation both kinetic and degradation capacities. The processes are slow and insufficient compared to aerobic degradation. Degradation of organic compounds in the absence of oxygen can be coupled to the reduction of alternative electron acceptors. Higher redox potentials lead to more preference of electron acceptors affecting anaerobic degradation ability (Schink, 2005). Oxygen (O_2/H_2O $E_h = +810$ mV) followed by nitrate (NO_3^-/NO_2^- $E_h = +430$ mV), manganese (IV) oxide (MnO_2/Mn^{2+} $E_h = +400$ mV), iron (III) hydroxides ($FeOOH/Fe^{2+}$ $E_h = +150$ mV), sulfate (SO_4^{2-}/HS^- $E_h = -218$ mV) and finally CO_2 (CO_2/CH_4 $E_h = -244$ mV). Alternative electron acceptors may release nitrite, ammonia, dinitrogen, manganese (II), iron (II) carbonates, sulfides and methane as products. For degradation of aromatic compounds in anaerobic condition, the concept is “destabilization of the aromatic nucleus in the absence of oxygen could proceed through a reductive rather than an oxidative reaction” (Evans, 1997) The important intermediate of aromatic hydrocarbon under anaerobic degradation is benzyl-CoA which further degradation to acid and

β -oxidation respectively. There was no report on profenofos biodegradation under anaerobic condition; however, some research showed biodegradation of organophosphorus pesticide under anaerobic condition. Parathion which is an organophosphorus compounds disappeared at a faster rate under anaerobic (presence of nitrate) compared with aerobic. (Reddy and Sethunathan, 1893). Moreover, it has been reported on organophosphohate compound degradation pathways under anaerobic conditions. Parathion was reduced to aminoparathion and further hydrolyzed to p-aminophenol and diethylthiophosphoric acid (Munnecke and Hsieh, 1976). Chlorpyrifos was converted to 3,5,6-chloro-2-pyridinol an diethyl thiophosphate via hydrolysis under anaerobic condition (Yadav et al., 2014).

2.3 Microbial growth, degradation and degradation kinetics

2.3.1 Microbial growth

Microorganism needs energy and raw material which essential for the building of cell component. Microorganisms require one or more factors for growth. All microbes require carbon, oxygen, nitrogen, sulfur and phosphorus sources. Microbial growth usually depends on two categories of influences including physical (temperature, pH and osmotic pressure) and chemical (source of carbon, nitrogen, sulfur and phosphorus, trace element and oxygen factors (Mckane and Kandel, 1985). Microbial growth is defined as an increase the cell number in a population when microorganisms reproduce by process like binary fission and budding (Waites, 2001). The growth curve consists of 4 phases: lag phase, log phase, stationary phase, and death phase.

For lag phase, when microbes are inoculated in the fresh medium, the cell number or cell mass does not increase immediately. It changes very little because the cells do not reproduce in the fresh medium right away; however, the cells are not dormant and still onto a period intense metabolic activity. Microorganisms start to proliferate at the maximum rate in exponential or log phase. This relies on the genetic potential, the nature of medium and the growing condition. During this phase, cellular reproduction is the most active and generation time is constant so that the rate of growth is constant. During stationary phase, the population growth curve turns to horizontal line and the growth cell rate is very slow. The total number of

microorganism remains constant because of the balance between the number of microbial deaths and the number of new cells. The microbial population reaching the stationary phase depends on nutrient limitation, insufficient oxygen, accumulation of toxic products and harmful changes in pH. When the death cell is higher than the new cells, the cell population enters to death phase. It may occur by detrimental environment changes like deficiency of nutrient or accumulation of toxic products.

2.3.2 Degradation kinetics

The biodegradation kinetic study is to forecast the remaining substrate concentrations at given time during biodegradation. Chemical concentrations measurement is the way to ensure the efficiency biodegradation, e.g. disappearance of parent molecule, appearance of mineralization products and disappearance of other compounds used stoichiometrically during compound biodegradation. There are several scenarios which compound can be transformed in biological process such as carbon and energy sources, electron acceptor and source of other component. Other scenarios are the transformation of a compound by non-growing cell and co-metabolism (Okpokwasili and Nweke, 2005). The simple case is the transformation of compound as carbon source and energy source for microbial growth and other compounds is limiting. The Michaelis–Menten kinetics, a widely used model for biodegradation, was described in equation 3.1. The relationship between substrate degradation rate and substrate concentration is shown in Figure 2.5.

$$V = \frac{V_{\max} \times S}{K_m + S} \quad (3.1)$$

Where,

- V = Substrate degradation rate (mg/L/day)
- V_{\max} = Maximum substrate degradation rate (mg/L/day)
- K_m = Michaelis constant (mg/L)
- S = Substrate concentration (mg/L)

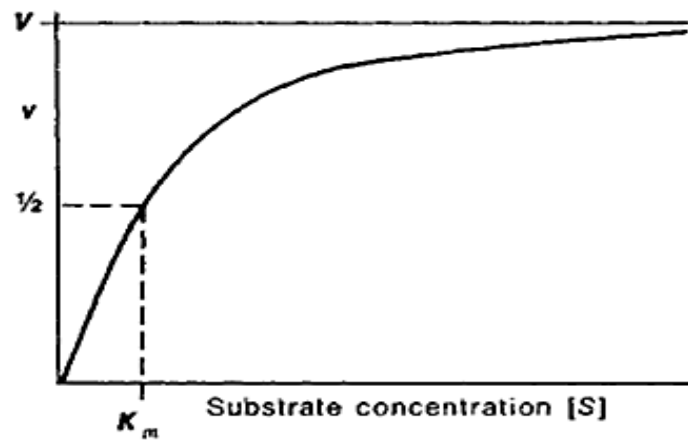


Figure 2.5 Relationship between substrate degradation rate and substrate concentration

The Lineweaver-Burk plot is a straight line which was described in equation 3.2 and the relationship of substrate degradation rate and substrate concentration was showed by plotting of $1/v$ versus $1/[S]$ in Figure 2.6.

$$\frac{1}{V} = \frac{K_m}{V_{\max}} \times \frac{1}{S} + \frac{1}{V_{\max}} \quad (3.2)$$

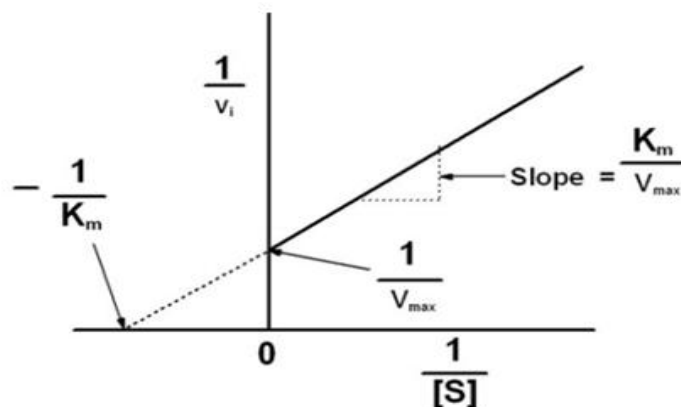


Figure 2.6 Relationship of substrate degradation rate and substrate concentration in Lineweaver-Burk model

The kinetic study is the important for bioremediation which can predict the velocity of compound in transformation or degradation under different conditions. It provides the data to design the appropriate conditions for the contaminated sites.



CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Framework and scheme

The framework of study is showed in Figure 3.1. This study aimed to investigate kinetics of profenofos biodegradation under presences of oxygen and nitrate. There are two main parts in the experiment including degradation kinetics under presences of oxygen and nitrate and biodegradation pathway of profenofos. Based on the framework, experimental descriptions and tasks are as shown in Figure 3.2.

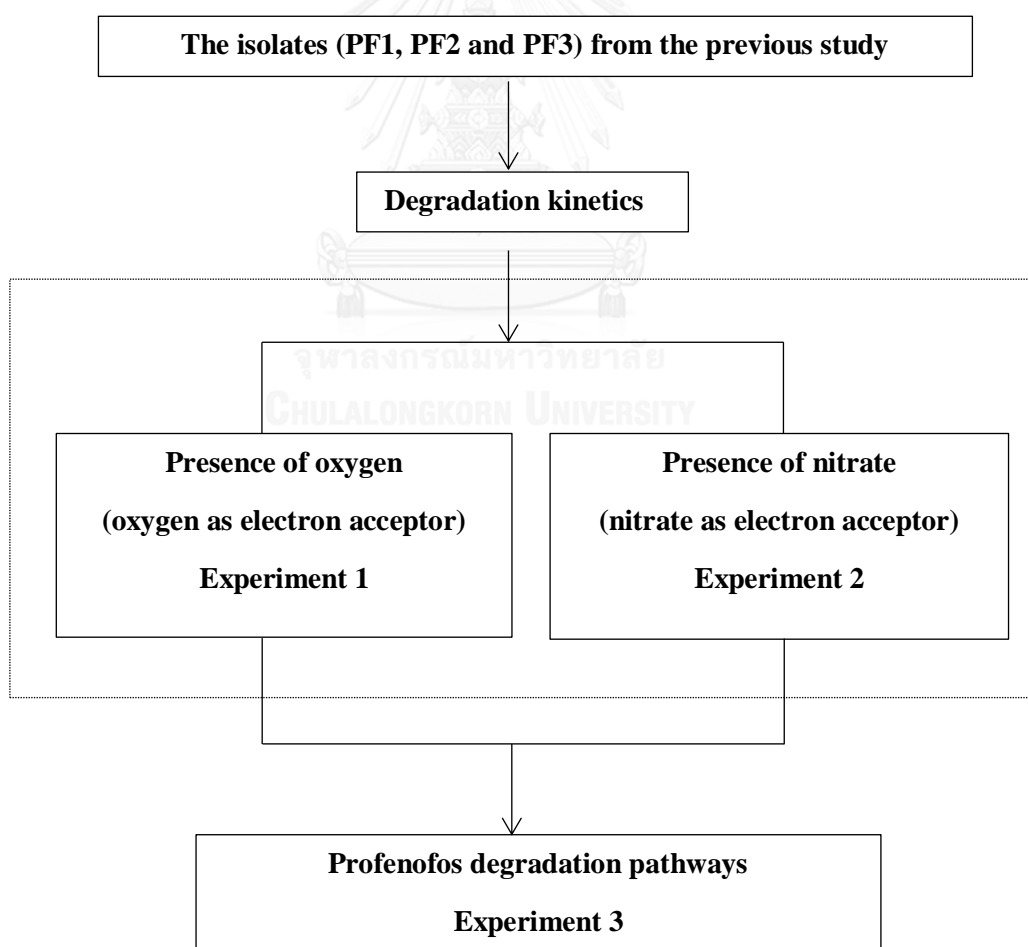


Figure 3.1 Framework of the study

Experiment 1: Microbial growth, degradation and degradation kinetics under presence of oxygen

- Oxygen was used as electron acceptor
- Profenofos concentrations was varied at 10, 25, 50, 80, 100 and 150 mg/L
- Degradation kinetic values (apparent K_s and V_{max}) were determined.

Experiment 2: Microbial growth, degradation and degradation kinetics under presence of nitrate

- Nitrate was used as electron acceptor
- Profenofos concentrations were varied at 10, 25, 50, 80, 100 and 150 mg/L
- Nitrate concentrations were investigated at 100, 200 and 300 mg/L.
- Degradation kinetics values (apparent K_s and V_{max}) were determined.

Experiment 3: Profenofos degradation pathway under presences of oxygen and nitrate

- Profenofos intermediates under presences of oxygen and nitrate degradations were investigated using GC/MS.

Figure 3.2 Experimental scheme

3.2 Materials and Methods

3.2.1 Bacteria cultivation and medium

Profenofos degrading bacteria were isolated from chili farm soil, Ubon Ratchathani, Thailand in the previous study (Siripattanakul-Ratpukdi et al., 2015). Three isolates, PF1, PF2 and PF3, were identified as *Pseudomonas plecoglossicida* (GenBank accession number KJ620776), *Pseudomornas aeruginosa* (GenBank accession number KJ143903) and *Pseudomornas aeruginosa* (GenBank accession number KJ143904), respectively. Minimal salt medium (MSM) for bacteria cultivation was contained NaCl 0.5g/L, NH_4Cl 2g/L, KH_2PO_4 3g/L, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 6.816 g/L and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.513 g/L in 10 mM of phosphate buffer solution (pH of 6.8). The medium was autoclaved at 121°C for 15 min and then supplemented with 20 mg/L of filtered sterile profenofos. For solid medium, 1.5% of Agar and 0.1% of yeast

extract were added. Nutrient Agar (NA) with 20 mg/L of filtered sterile profenofos was prepared only for bacterial plate count.

3.2.2 Profenofos biodegradation by PF-degrading cultures

The profenofos biodegradation experiment including performed the presence of oxygen and nitrate degradation tests by PF1, PF2 and PF3 was. For the presence of oxygen degradation test, 10% (v/v) of the isolated bacteria (approximately 2.2×10^4 CFU/mL) were added in 70-mL medium supplemented with different initial PF concentrations (10, 25, 50, 80, 100 and 150 mg/L). All test flasks were shaken on a rotary shaker at 150 rpm, $30 \pm 2^\circ\text{C}$ for 6 days.

For the presence of nitrate degradation test, the medium preparation method was modified from (Gunawan et al., 2014). The experiment was carried out using 100 mL of serum bottles containing 70-mL medium (without profenofos and nitrate). The serum bottles were sealed with silicone rubber and then autoclaved at 121°C for 15 min. After sterilization, the bottles were purged with filter sterilized nitrogen gas for 15 min to ensure the absence of oxygen and capped with aluminium crimps (Figures 21 and 22). Then, profenofos, nitrate and the isolated bacteria (approximately 2.5×10^4 CFU/mL) were injected into the bottles, respectively. Six different initial profenofos concentrations (10, 25, 50, 80, 100 and 150 mg/L) and three nitrate concentrations (100, 200, 300 mg/L) were examined. After that, the bottles were shaken on a rotary shaker at 100 rpm and $30 \pm 2^\circ\text{C}$ for 8 days. Profenofos concentration, cell number and nitrate concentration (for anaerobic degradation test) were measured daily. All the batch experiments were conducted in duplicates. Abiotic control (the test without the isolated cultures) was also determined along with the experiment. Some selected samples under aerobic and anaerobic degradation tests were taken to identify the potential intermediate metabolites by GC/MS. The microbial growth and profenofos degradation kinetics were calculated. The medium preparation on profenofos degradation under presence of nitrate was shown in Appendix A.

3.2.3 Kinetic analyses

The profenofos degradation results under presences of oxygen and nitrate were plotted. Data of profenofos remaining concentrations and times was used to calculate

the profenofos degradation rate (Equation 3.3 and Figure 3.3) After that the degradation kinetics followed by the Michaelis-Menten Model (Equation 3.4) was estimated.

$$\text{Degradation rate} = \frac{\text{Concentration}_2 - \text{Concentration}_1}{\text{Time}_1 - \text{Time}_2} \quad (3.3)$$

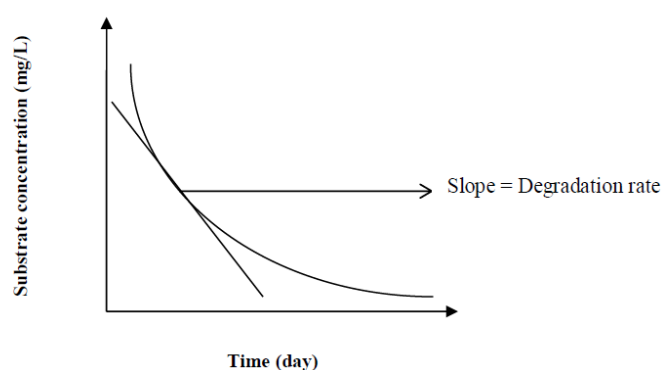


Figure 3.3 Degradation rate

$$V = \frac{V_{\max} \times S}{K_s + S} \quad (3.4)$$

V is substrate degradation rate (mg/L/day), V_{\max} is the maximum substrate degradation rate (mg/L/day), Apparent K_s replaced K_m is Michaelis constant (mg/L) and substrate concentration (mg/L), respectively. V_{\max} and apparent K_s were examined in linear plot by Lineweaver-Burk plot which showed by plotting of $1/v$ versus $1/[S]$ (detail of Lineweaver-Burk plot was described in chapter 2).

3.2.4 Chemical analysis

For profenofos analysis, the sample was extracted using liquid-liquid extraction technique and analyzed using gas chromatography. The sample (0.5 mL) was added in 1.5 mL of microcentrifuge tube with 0.5 mL hexane and 0.01% acetic acid then mixed on vortex mixer at 2,500 rpm for 10 min. Upper solution (hexane) was transferred to 2-mL GC vial.

Profenofos Extracted sample was analyzed by GC (Agilent 4890D, USA) equipped with Ni⁶³ electron capture detector. The column was a SPBTM-608 fused silica capillary column (15 m × 0.53 mm × 0.25 μm film thickness). One microliter of sample was injected into the inject port. The temperatures of injection port and detector were set at 240 °C and 250 °C, respectively. The temperature condition was started at 120 °C, hold at 120 °C for 2 min, ramped up 40 °C/min to final temperature 240°C and then hold at 240 °C for 5 min. Total retention time was 10 min. Helium and nitrogen gas were used as the carrier gas with flow rate 8 mL/min and 47 mL/min, respectively. Profenofos peak was detected at 3.1 min in chromatogram which showed in Appendix B.

Nitrate (NaNO₃, RCI Labscan, Thailand) was analyzed by Colorimetric Method (Brucine) according to standard method for the examination of water and wastewater 14th Edition (APHA, 1975). The 10 mL sample was placed in test tube (Pyrex, Maxigo) then added 2 mL of sodium chlorine solution (Analytical grade, Ajax Finechem Pty Ltd, Thailand), 10 mL of 4+1 Sulfuric acid (AR grade, RCI Labscan, Thailand) and 0.5 mL of brucine-sulfanic solution (Himedia, India and Loba chemie, India, respectively). The tubes were soaked into the water bath at 95 °C for 20 min. After cooling the sample to room temperature, the sample was measured the absorbance using spectrophotometer at wave length of 410 nanometer.

During profenofos degradation under presence of oxygen and nitrate, some selected sample at high profenofos concentrations were analyzed to identify the profenofos metabolites by GC/MS. Three microliter of extracted sample was injected into the inject port. The temperature condition was started at 100 °C, hold 100 °C at 5 min, ramped up 5 °C/min to final temperature 250°C and then hold at 250 °C for 10 min. Total retention time was 45 min. All chemical analysis was presented in Appendix B.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Profenofos degradation and degradation kinetics under presence of oxygen and nitrate

4.1.1 Profenofos degradation under presence of oxygen and nitrate

This study investigated the profenofos degradation under presence of oxygen and nitrate by PF1, PF2 and PF3. The degradation at six profenofos concentrations (10, 25, 50, 80, 100 and 150 mg/L) and three nitrate concentrations (100, 200 and 300 mg/L) were examined. The results of profenofos degradation by PF1 under the presence of oxygen and nitrate with various initial profenofos and nitrate concentrations were shown in Figure 4.1. The degradation kinetics under presence of oxygen and nitrate was shown in Table 4.1 and 4.2.

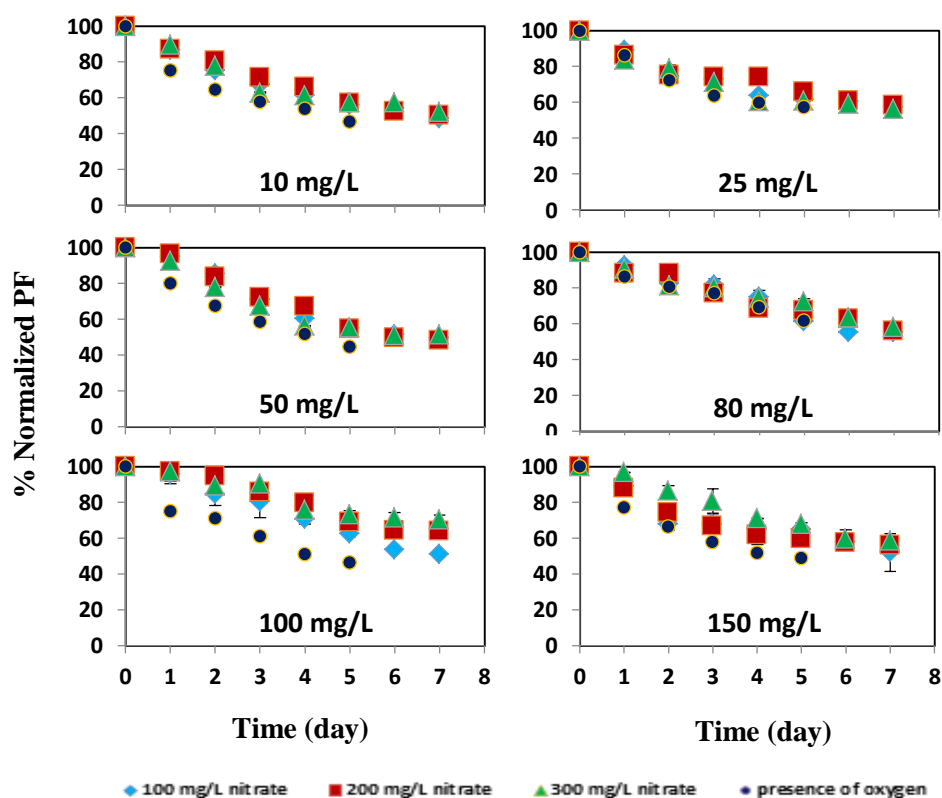


Figure 4.1 Profenofos degradation under presence of oxygen and nitrate by PF1

Table 4.1 Degradation kinetics of PF1 under presence of oxygen

Profenofos conc.(mg/L)	Kinetics		
	Equation	R ²	Rate constant at 3 days (1/d)
10	$y = -0.141x + 2.099$	0.95	0.141
25	$y = -0.114x + 2.973$	0.95	0.114
50	$y = -0.157x + 3.605$	0.99	0.157
80	$y = -0.089x + 4.075$	0.98	0.089
100	$y = -0.150x + 4.240$	0.96	0.150
150	$y = -0.140x + 4.549$	0.95	0.140

x=Time(day), y=ln(profenofos concentration)

Table 4.2 Degradation kinetics of PF1 under presence of nitrate

Profenofos conc.(mg/L)	Nitrate conc.(mg/L)	Kinetics		
		Equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = -0.116x + 2.071$	0.99	0.116
	200	$y = -0.108x + 2.095$	0.99	0.108
	300	$y = -0.118x + 2.102$	0.95	0.118
25	100	$y = -0.098x + 2.922$	0.95	0.098
	200	$y = -0.073x + 2.920$	0.88	0.073
	300	$y = -0.102x + 2.983$	0.95	0.102
50	100	$y = -0.129x + 3.647$	0.98	0.129
	200	$y = -0.121x + 3.649$	0.97	0.121
	300	$y = -0.132x + 3.674$	0.97	0.132
80	100	$y = -0.088x + 4.069$	0.93	0.088
	200	$y = -0.079x + 4.086$	0.97	0.079
	300	$y = -0.063x + 4.044$	0.94	0.063
100	100	$y = -0.095x + 4.387$	0.98	0.095
	200	$y = -0.073x + 4.355$	0.93	0.073
	300	$y = -0.066x + 4.358$	0.91	0.066
150	100	$y = -0.101x + 4.632$	0.88	0.101
	200	$y = -0.107x + 4.657$	0.95	0.107
	300	$y = -0.084x + 4.679$	0.98	0.084

x=Time(day), y=ln(profenofos concentration)

It was found that profenofos degradation under presence of oxygen and nitrate was similar. Profenofos concentration continuously decreased in the first three to five day and gradually for the rest of the experiment. For abiotic control test, profenofos concentration reduced less than 20% (data not shown). It is indicated that profenofos is stable substance and persist for a long time in environment (half-life of 7 days). The physical and chemical reactions were less occurred. Based on experimental data, the profenofos transformation is mainly biodegradation. Based on the kinetic order, the

degradation followed the first kinetic equation which degradation rate depended on initial profenofos concentration. The degradation rates constants under presence of oxygen and nitrate ranged from 0.089 to 0.157 and 0.063 to 0.132 1/day, respectively. It was noticed that the degradation rate constants under presence of oxygen were higher than those of presence of nitrate. This result was related to previous work (Zheng et al., 2013). Under aerobic degradation was likely faster degraded rather than anaerobic degradation.

When compared removal efficiency percentage of profenofos at 5 days under presence of oxygen and nitrate, this result was presented as shown in Table 4.3. PF1 could be degraded profenofos both presence of oxygen and nitrate which percent removal efficiency under presence of oxygen and nitrate range from 38.14 to 55.39 and 27.50 to 45.33%, respectively (Table 4.3) It could imply that profenofos could be served as a carbon source for the enriched cultures (PF1) both under presence of oxygen and nitrate. The average removal efficiency percentages of the tests by PF1 with aerobic condition (49.00%) were likely better than those with presence of nitrate of (37.95%). Moreover, profenofos was less degraded when increasing nitrate concentrations. It is implied that higher nitrate concentration might be inhibited profenofos degradation ability of PF1. The result related to the previous work (Dou et al., 2008). More initial nitrate concentrations were inhibited on degradation under anaerobic condition.

When compared the degradation with different initial profenofos concentrations, the removal efficiencies were quite stable even increasing the profenofos concentration. It is indicated that profenofos concentration did not obviously affect the degradation. In general, each microbial culture has threshold tolerant to hazardous chemicals differently. Low concentration of toxic substance did not inhibit microbial degradation. In this case, there was no inhibition observed in the tests with the concentrations of up to 150 mg/L. It could say that the cultures were efficient and tolerant to profenofos pesticide. They are potential to apply for the contaminated sites in the future. From the result, it could suggest that 150 mg/L of profenofos did not inhibited degradation ability of PF1.

Table 4.3 The percentages of profenofos removal efficiency by PF1 at 5 days

Profenofos conc. (mg/L)	Profenofos removal (%)			
	Presence of oxygen	Presence of nitrate		
		Nitrate conc. (mg/L)		
		100	200	300
10	53.37	44.02	43.03	42.50
25	42.65	38.18	34.02	39.04
50	55.39	44.89	45.33	44.72
80	38.14	38.73	32.56	27.50
100	53.47	37.68	30.94	26.76
150	50.97	40.55	40.38	32.32

The profenofos degradation by strain PF2 under the presence of oxygen and nitrate with various initial profenofos and nitrate concentrations was shown in Figure 4.2. The degradation kinetics under presence of oxygen and nitrate were shown in Table 4.4 and 4.5.

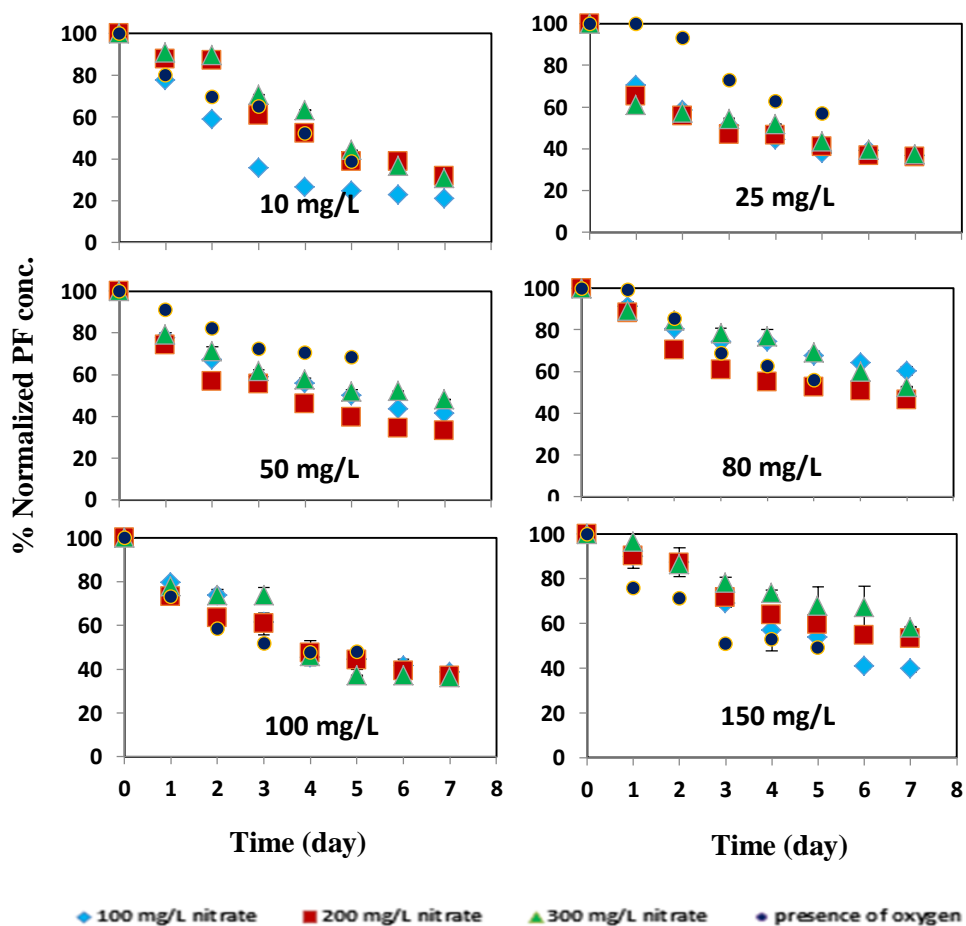


Figure 4.2 Profenofos degradation under presence of oxygen and nitrate by PF2

Table 4.4 Degradation kinetics of PF2 under presence of oxygen

Profenofos conc.(mg/L)	Kinetics		
	Equation	R ²	Rate constant at 3 days (1/d)
10	$y = -0.180x + 2.138$	0.91	0.180
25	$y = -0.142x + 3.008$	0.98	0.142
50	$y = -0.080x + 3.645$	0.95	0.080
80	$y = -0.128x + 4.150$	0.96	0.128
100	$y = -0.146x + 4.173$	0.87	0.146
150	$y = -0.141x + 4.590$	0.89	0.141

x=Time(day), y=ln(profenofos concentration)

Table 4.5 Degradation kinetics of PF2 under presence of nitrate

Profenofos conc.(mg/L)	Nitrate conc.(mg/L)	Kinetics		
		Equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = -0.306x + 2.181$	0.97	0.306
	200	$y = -0.191x + 2.245$	0.94	0.191
	300	$y = -0.154x + 2.128$	0.90	0.154
25	100	$y = -0.181x + 2.866$	0.96	0.181
	200	$y = -0.162x + 2.804$	0.87	0.162
	300	$y = -0.135x + 2.814$	0.80	0.135
50	100	$y = -0.131x + 3.493$	0.95	0.131
	200	$y = -0.174x + 3.490$	0.95	0.174
	300	$y = -0.125x + 3.500$	0.96	0.125
80	100	$y = -0.075x + 4.089$	0.94	0.075
	200	$y = -0.136x + 4.072$	0.96	0.136
	300	$y = -0.067x + 4.083$	0.96	0.067
100	100	$y = -0.170x + 4.235$	0.96	0.170
	200	$y = -0.155x + 4.202$	0.95	0.155
	300	$y = -0.189x + 4.286$	0.93	0.189
150	100	$y = -0.134x + 4.749$	0.95	0.134
	200	$y = -0.110x + 4.686$	0.97	0.110
	300	$y = -0.081x + 4.688$	0.99	0.081

x=Time(day), y=ln(profenofos concentration)

From Figure 4.2, profenofos concentration continuously reduced in the first three to five day. For abiotic degradation test, profenofos concentration decreases less than 20% (data not shown). Based on degradation kinetics, the degradation followed the first kinetic equation which degradation rate constant under presence of oxygen and nitrate ranged from 0.080 to 0.180 and 0.067 to 0.306 1/d. The results showed that degradation rate decreased when increasing the profenofos concentration for both

presences of oxygen and nitrate. It is indicated that PF2 could be inhibited degradation ability at high profenofos concentration. When increasing of initial concentration over the concentration relating to V_{max} , the degradation rate may reduce (Mathur and Majumder, 2010).

When compared between profenofos degradation at 5 days under presence of oxygen and nitrate, the result was shown in Table 4.6 Profenofos was degraded by PF2 under both presence of oxygen and nitrate. The percentages of removal efficiency under presence of oxygen and nitrate range from 31.62 to 61.22 and 32.09 to 75.36 %, respectively. The results showed that higher profenofos concentration likely inhibited the degradation ability of PF2. Higher substance concentration could inhibit the degradation rate (Mathur and Majumder, 2010; Patichot, 2012). Trend of the profenofos degradation under presence of oxygen was lower than that with presence of nitrate at 100 mg/L. It is indicated that the different electron acceptor could affect profenofos degradation. Some prior report showed that the microbial cell degraded substrate under presence of anaerobic (presence of nitrate) better than aerobic condition (Reddy and Sethunathan, 1893). They found that parathion which is an organophosphorus compounds disappeared at a faster rate under anaerobic (presence of nitrate) compared with aerobic condition.

Among the degradations with various initial nitrate concentrations, PF2 less degraded profenofos when increasing nitrate concentration. The results clearly showed the effect of nitrate on profenofos degradation with nitrate concentrations of 200 and 300 mg/L. It is indicated that higher nitrate concentration could affect degradation ability of PF2 resulting in lower profenofos degradation performance. When increasing initial nitrate concentration, the degradation was decreased (Dou et al., 2008).

Table 4.6 The percentages of profenofos removal efficiency by PF2 at 5 days

Profenofos conc. (mg/L)	Profenofos removal (%)			
	Presence of oxygen	Presence of nitrate		
		Nitrate conc. (mg/L)		
		100	200	300
10	61.22	75.36	61.31	55.75
25	48.90	61.99	59.05	56.45
50	31.62	50.01	60.47	48.12
80	43.88	32.26	47.42	30.69
100	52.12	55.67	55.83	62.99
150	50.62	46.13	40.53	32.09

Profenofos degradation by PF3 under the presence of oxygen and nitrate with various initial profenofos and nitrate concentration was shown in Figure 4.3. The degradation kinetics under presence of oxygen and nitrate was shown in Table 4.7 and 4.8.

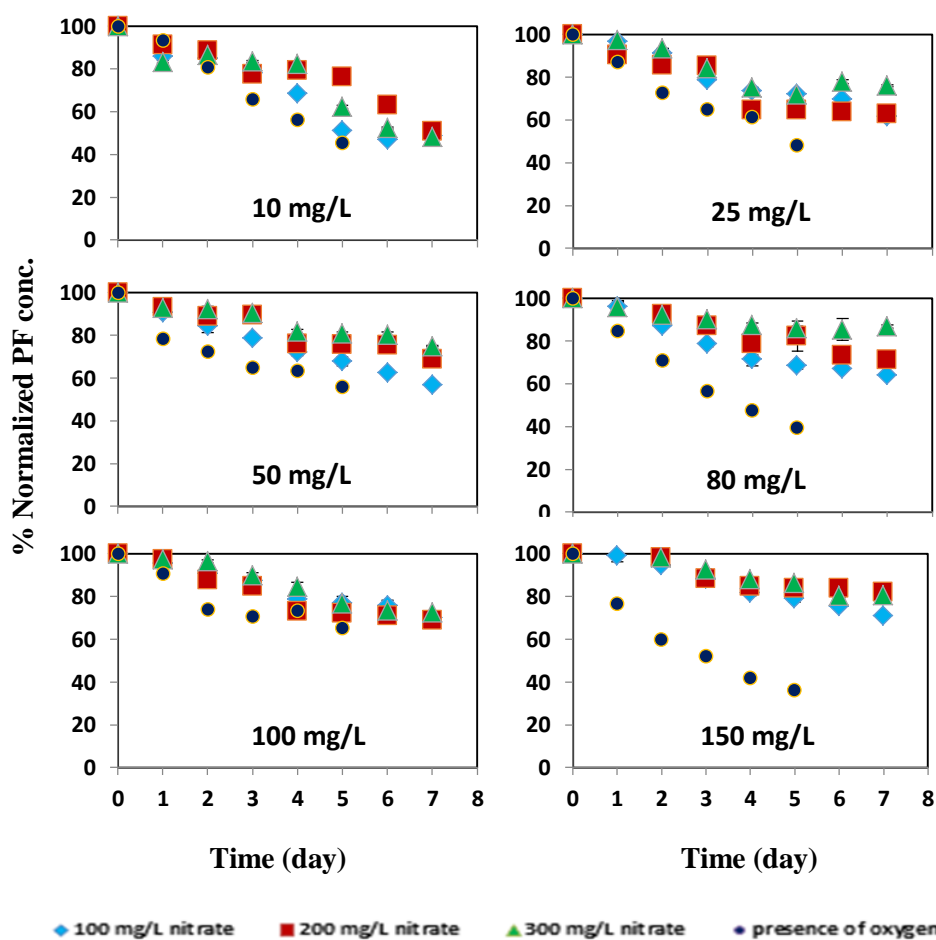


Figure 4.3 Profenofos degradation under presence of oxygen and nitrate by PF3

Table 4.7 Degradation kinetics of PF3 under presence of oxygen

Profenofos conc.(mg/L)	Kinetics		
	Equation	R ²	Rate constant at 3 days (1/d)
10	$y = -0.162x + 2.303$	0.98	0.162
25	$y = -0.137x + 3.178$	0.98	0.137
50	$y = -0.104x + 3.627$	0.93	0.104
80	$y = -0.188x + 4.160$	0.99	0.188
100	$y = -0.081x + 4.269$	0.86	0.081
150	$y = -0.204x + 4.664$	0.99	0.204

x=Time(day), y=ln(profenofos concentration)

Table 4.8 Degradation kinetics of PF3 under presence of nitrate

Profenofos conc.(mg/L)	Nitrate conc.(mg/L)	Kinetics		
		Equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = -0.121x + 2.157$	0.90	0.121
	200	$y = -0.055x + 2.186$	0.89	0.055
	300	$y = -0.070x + 2.208$	0.71	0.070
25	100	$y = -0.074x + 3.091$	0.95	0.074
	200	$y = -0.091x + 3.133$	0.89	0.091
	300	$y = -0.073x + 3.066$	0.96	0.073
50	100	$y = -0.077x + 3.810$	0.99	0.077
	200	$y = -0.057x + 3.804$	0.91	0.057
	300	$y = -0.042x + 3.735$	0.92	0.042
80	100	$y = -0.082x + 4.286$	0.98	0.082
	200	$y = -0.051x + 4.226$	0.87	0.051
	300	$y = -0.031x + 4.206$	0.98	0.031
100	100	$y = -0.055x + 4.501$	0.98	0.055
	200	$y = -0.072x + 4.521$	0.96	0.072
	300	$y = -0.052x + 4.490$	0.92	0.052
150	100	$y = -0.052x + 4.731$	0.96	0.052
	200	$y = -0.045x + 4.695$	0.88	0.045
	300	$y = -0.035x + 4.664$	0.90	0.035

x=Time(day), y=ln(profenofos concentration)

PF3 could degrade profenofos in both presence of oxygen and nitrate. Profenofos concentration continuously decreased in the first three to five day and gradually for the rest of the experiment. For abiotic control test, profenofos concentration reduced less than 20% (data not shown). Based on kinetic order, the degradation followed the first kinetic equation which degradation rate constant under presence of oxygen and nitrate ranged from 0.081 to 0.204 and 0.031 to 0.121 1/d, respectively. The degradation rates under presence of nitrate were declined at high

profenofos concentration. It is indicated that profenofos concentration less affected degradation under presence oxygen; however, it obviously influenced on profenofos degradation under presence of nitrate. Generally, the degradation rate was decreased at high substrate concentrations. This is because bacterial cultures and degradation ability was inhibited at higher substrate concentration (Dou et al., 2008). The profenofos removal efficiency of PF3 at 5 days under presence of oxygen and nitrate was shown in Table 4.9. PF3 could utilize profenofos as carbon source both presence of oxygen and nitrate. The percentages of removal efficiency under presence of oxygen and nitrate range from 34.72 to 63.81 and 13.78 to 48.89%, respectively. The results showed that PF3 less degraded profenofos when increasing profenofos concentration. It is because degradation ability of PF3 was inhibited on profenofos degradation. The result was similar to a previous report (Patichot, 2012). Profenofos degradation ability of mixed cultures including PF1, PF2, and PF3 decreased when concentration reached to 120 mg/L. Profenofos degradation efficiencies under presence of oxygen were higher than those under presence of nitrate. It could conclude that different electron acceptors (oxygen and nitrate) apparently affected profenofos degradation. Moreover, PF3 preferred to use oxygen as an electron acceptor more than nitrate. Generally, most microorganism prefer to use oxygen as electron acceptor rather than nitrate, manganese (IV) oxide, iron (III) hydroxides, sulfate and carbondioxide, respectively. In case of PF3, the removal efficiency percentages under presence of oxygen were higher than nitrate. This is indicated that PF3 favored to use oxygen more than nitrate because oxygen can give more energy for microbial metabolism. This happened in general contaminant removal such as nonylphenol ethoxylates (Lu et al., 2008). For influence of initial nitrate concentration, PF3 degraded profenofos less when increasing nitrate concentration. This should be toxicity of nitrate on degradation (Dou et al., 2008).

Table 4.9 The percentages of profenofos removal efficiency by PF3 at 5 days

Profenofos conc. (mg/L)	Profenofos removal (%)			
	Presence of oxygen	Presence of nitrate		
		Nitrate conc. (mg/L)		
		100	200	300
10	54.64	48.89	23.76	37.77
25	51.71	27.72	35.38	28.17
50	44.04	32.12	24.31	19.01
80	60.44	31.53	17.80	14.19
100	34.72	23.08	27.82	23.26
150	63.81	20.96	16.30	13.78

From all the degradation result, it concluded that PF1, PF2 and PF3 could degrade profenofos under presence of oxygen and nitrate. Profenofos could be served as a carbon source for all isolates. Oxygen and nitrate could be used as an electron acceptor. Based on our knowledge, *Pseudomonas* sp. could degrade substrate both under presence of oxygen and nitrate (Brock et al., 1984). In case of profenofos degradation under presence of nitrate, it was confirmed by nitrate reduction (presented in the next section). Profenofos could be utilized by PF1 and PF3 under presence of oxygen better than nitrate while PF2 could degrade profenofos under presence of nitrate higher than presence of oxygen. It is indicated that the same bacterial genus/species could differently influence the degradation. Also, different microbial strains preferred electron acceptor differently.

4.1.2 Profenofos degradation kinetics

The biodegradation kinetic study is to forecast the remaining substrate concentrations at given time during biodegradation. The Michaelis–Menten model was described in equation 5 which showed the relationship between substrate degradation rate and substrate concentration. Moreover, the Lineweaver-Burk plot (equation 6) was used to calculate kinetic parameter,

$$V = \frac{V_{\max} \times S}{K_s + S} \quad (5)$$

$$\frac{1}{V} = \frac{K_s}{V_{\max}} \times \frac{1}{S} + \frac{1}{V_{\max}} \quad (6)$$

From the results, the Lineweaver-Burk plots of profenofos degradation under presence of oxygen and nitrate by PF1, PF2 and PF3 were shown in Figure 4.4(a), 4.4(b), 4.5(a), 4.5(b), 4.6(a) and 4.6(b), respectively. The kinetic parameter of profenofos degradation under presence of oxygen and nitrate were shown in Table 4.10. From Table 4.10, Michaelis-Menten model of the profenofos degradation under presence of oxygen and nitrate was shown in in Figures 4.7, 4.8 and 4.9.

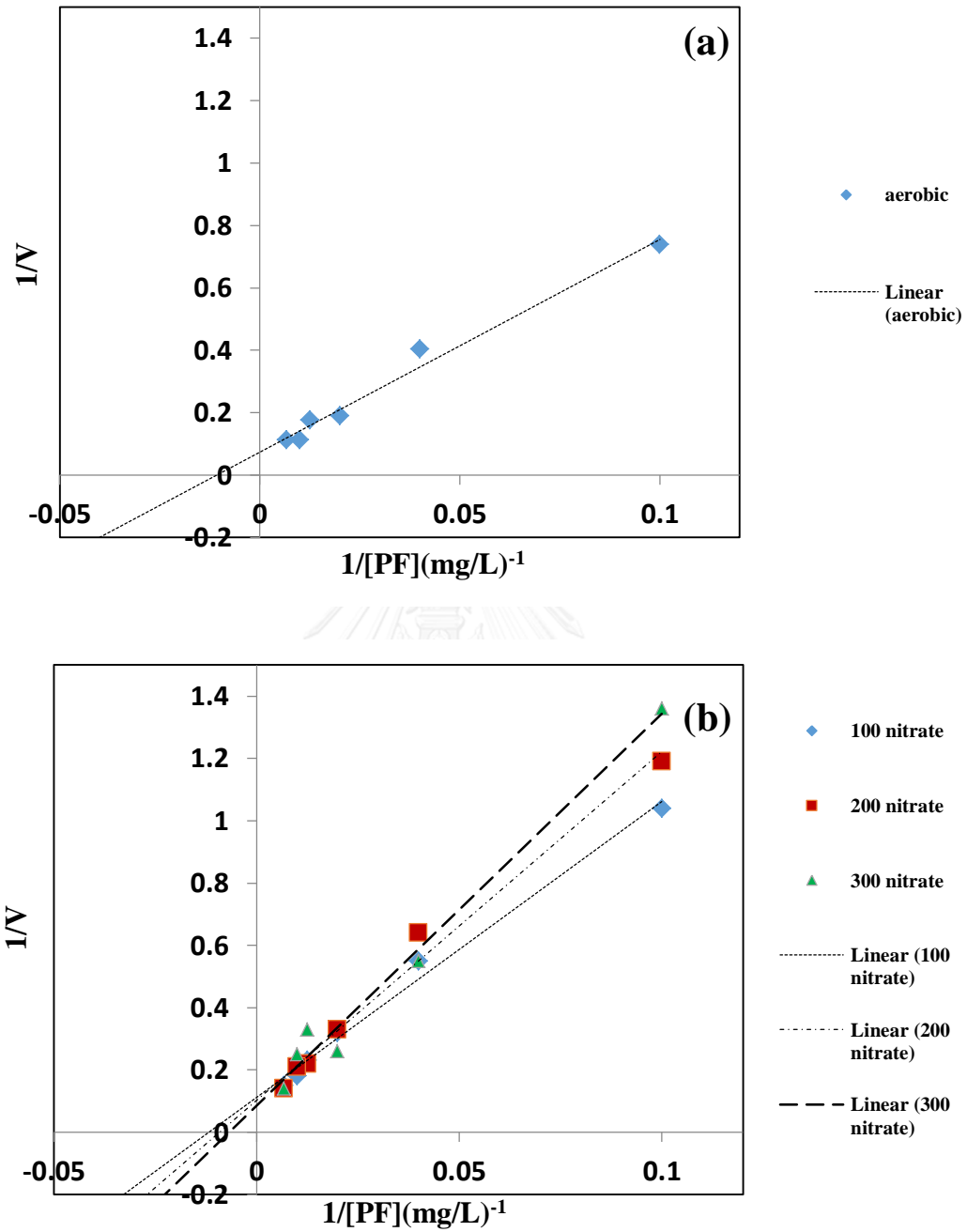


Figure 4.4 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF1 (a) under presence of oxygen (b) under presence of nitrate

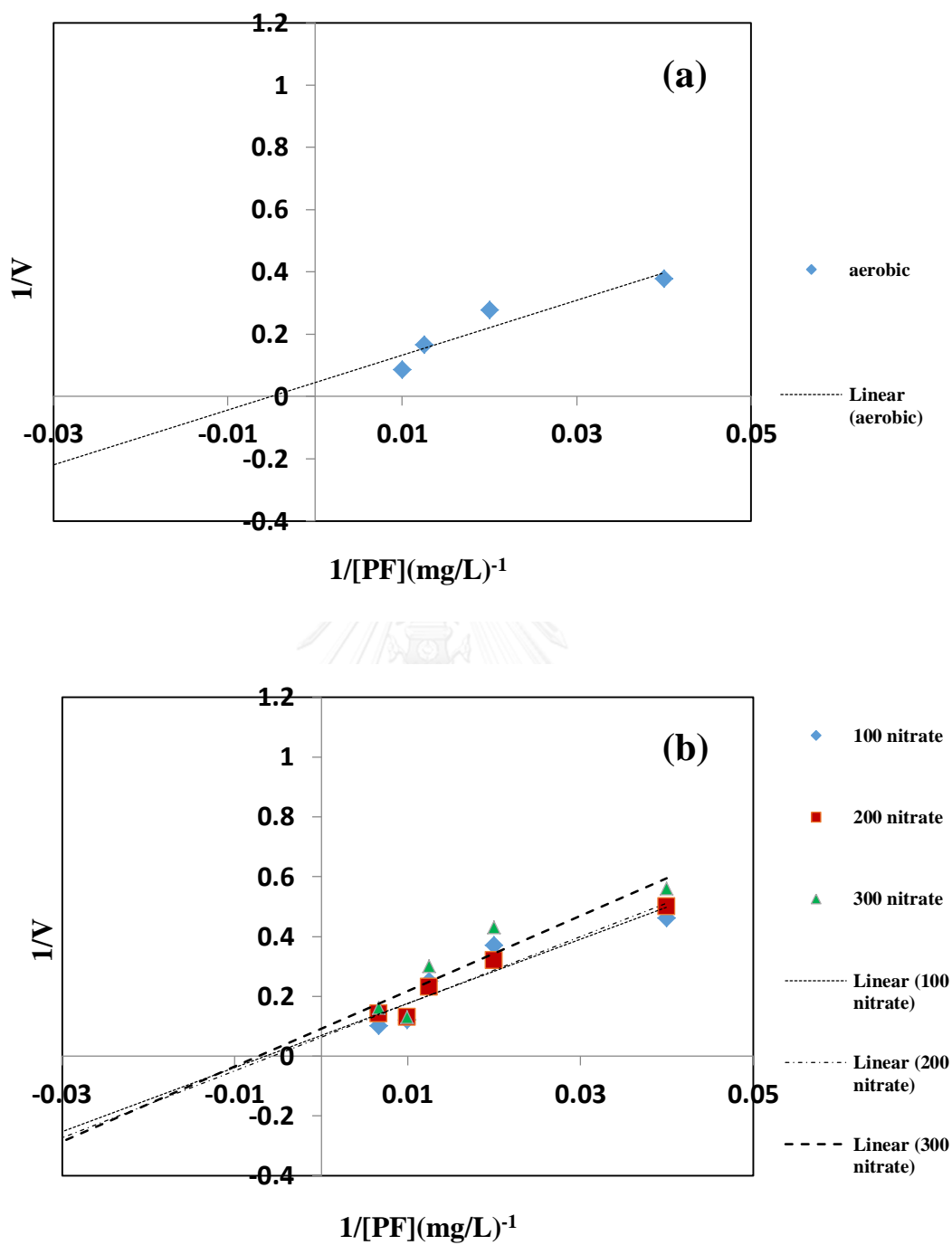


Figure 4.5 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF2 (a) under presence of oxygen (b) under presence of nitrate

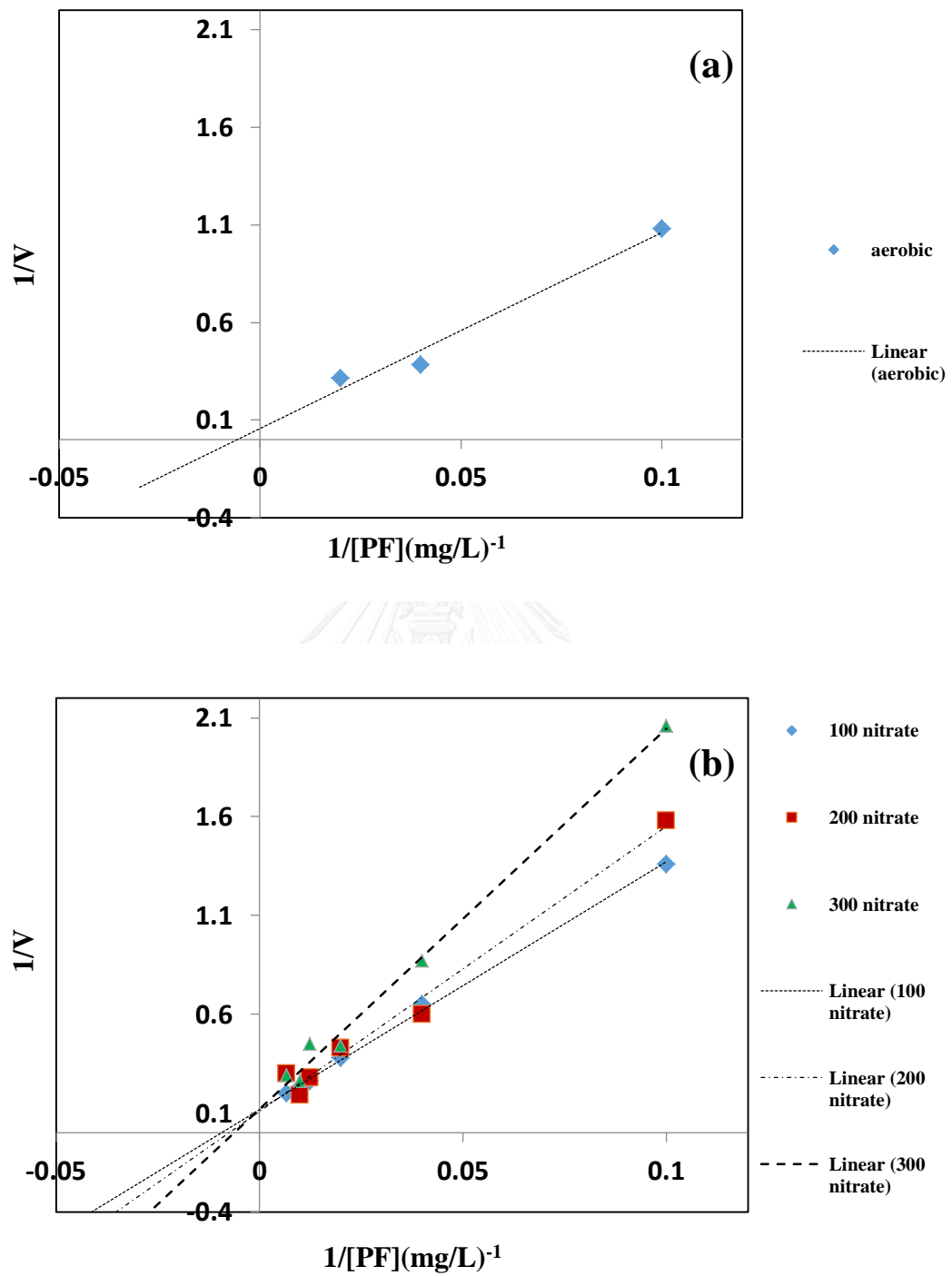


Figure 4.6 The relationship between $1/V$ and $1/[PF]$ of profenofos degradation by PF3 (a) under presence of oxygen (b) under presence of nitrate

Table 4.10 Degradation kinetic parameters

Isolate	Condition				
	Presence of oxygen		Presence of nitrate		
	V_{max} (mg/L/day)	K_s (mg/L)	Nitrate conc. (mg/L)	K_s (mg/L)	V_{max} (mg/L/day)
PF1	13.07	92.07	100	84.76	8.92
			200	109.62	9.79
			300	147.36	11.72
PF2	22.57	199.35	100	155.46	14.51
			200	175.86	15.67
			300	137.72	10.94
PF3	17.79	178.86	100	105.90	8.45
			200	133.38	9.24
			300	158.85	8.25

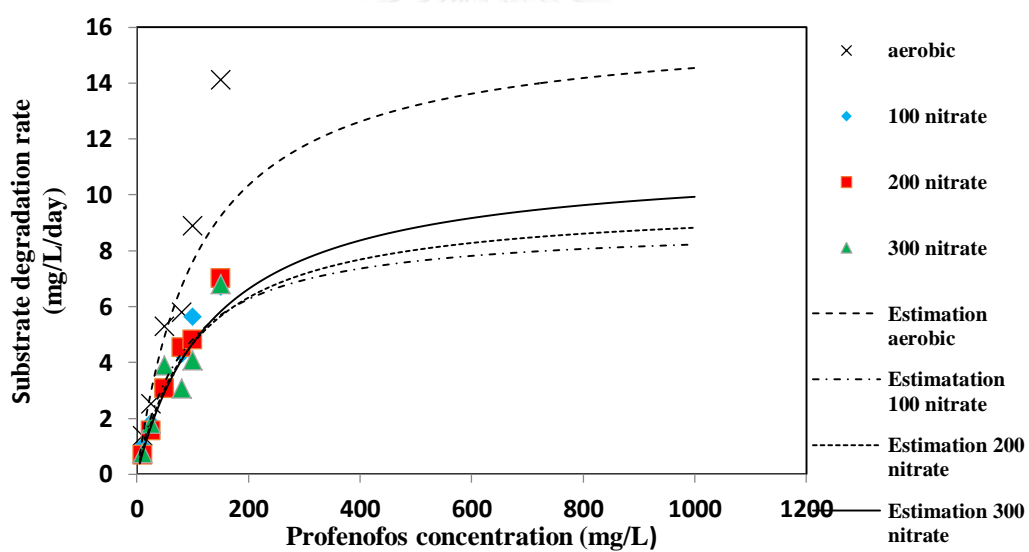


Figure 4.7 The relationship between V and S of profenofos degradation by PF1 under presence of oxygen and nitrate (100, 200 and 300 mg/L)

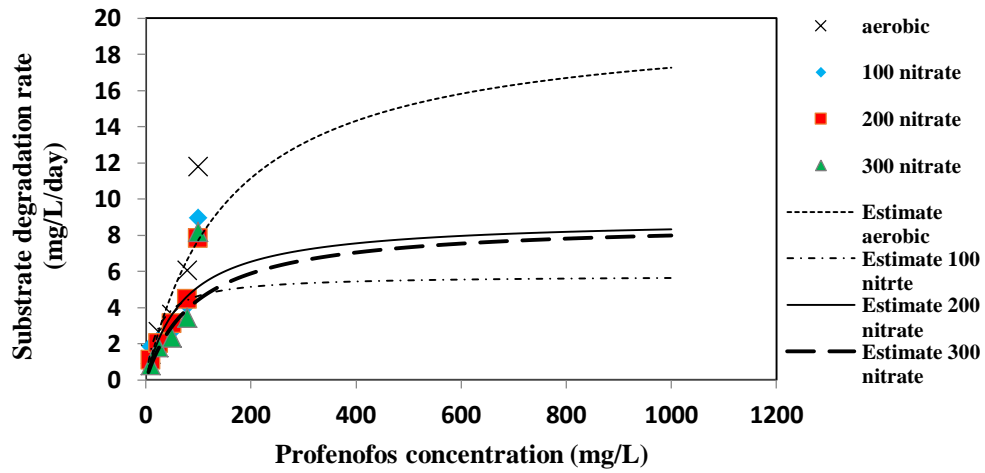


Figure 4.8 The relationship between V and S of profenofos degradation by PF2 under presence of oxygen and nitrate (100, 200 and 300 mg/L)

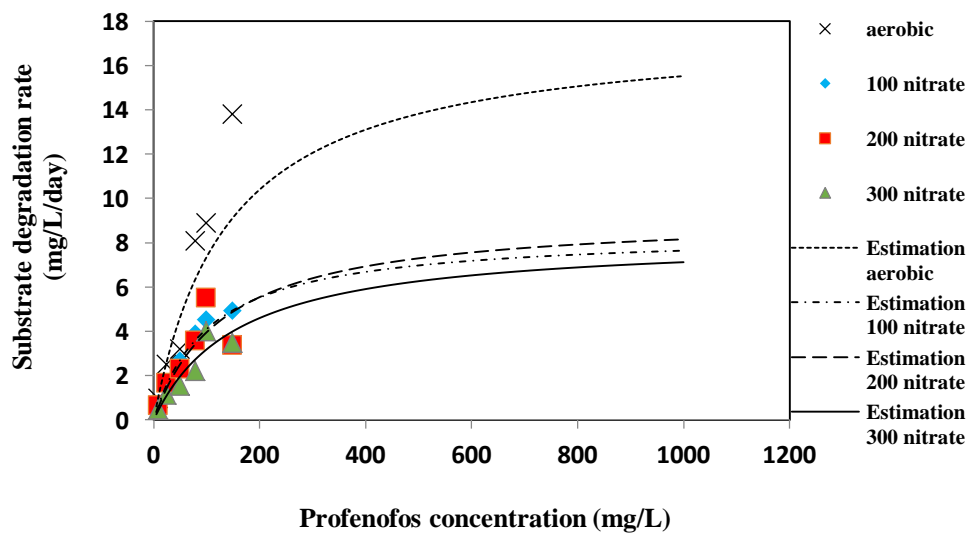


Figure 4.9 The relationship between V and S of profenofos degradation by PF3 under presence of oxygen and nitrate (100, 200 and 300 mg/L)

From degradation kinetic estimation, in overall, the profenofos biodegradation under presence of nitrate was competitive inhibition like (based on Lineweaver-Burk plot in Figures 4.4- 4.6). Competitive inhibition is one of inhibition type which inhibitor was similar shape with substrate and responded to enzymes at active site. It is concluded that nitrate was applied as an electron acceptor for the microbial metabolism, but it could be an inhibitor which react with enzyme at active site as well as profenofos (substrate). It is noted that experiment was performed in the concentration range of 10-150 mg/L did not cover the concentrations in the model. These concentrations were chosen followed the previous study. Patichot (2012) found that at the range of 10-120 mg/L, profenofos degradation of the mixed cultures (which contained the three strains) could be fitted to the model. The previous result showed that the stable range of degradation rate (the graph reached to V_{max}) was found at the concentration lower than 120 mg/L. The different result found in this study might be from the pure cultures more efficient than the mixed cultures. However, it should be further studied at higher range of profenofos concentrations for better fitting the model.

4.2 Microbial growth under presence of oxygen and nitrate

The growth of PF1, PF2 and PF3 was measured during the tests within 6 days under presence of oxygen and 8 days under presence of nitrate at different profenofos (10, 25, 50, 80, 100 and 150 mg/L) and nitrate concentrations (100, 200 and 300 mg/L) using viable cell count technique. This result showed that growth of PF1, PF2 and PF3 under presence of oxygen and nitrate was similar in all experiment. The cell number quickly increased within the first 3 days (exponential phase) and led to stationary phase after day 4. The viable cell number of PF1, PF2 and PF3 under presence of oxygen and nitrate increased from 10^4 to 10^8 , 10^4 to 10^8 and 10^4 to 10^7 CFU/ml, respectively. The microbial growth of PF1, PF2 and PF3 under the presence of oxygen and nitrate with different initial profenofos and nitrate concentration was shown in Figures 4.10, 4.11 and 4.12, respectively. The growth kinetics under presence of oxygen and nitrate (100, 200 and 300) was shown in Tables 4.11, 4.12, 4.13 and 4.14.

Table 4.11 Growth kinetics of PF1, PF2 and PF3 under presence of oxygen

Isolate	Profenofos conc.(mg/L)	Kinetics		
		equation	R ²	Rate constant at 3 days (1/d)
PF1	10	$y = 1.922x + 10.973$	0.68	1.922
	25	$y = 1.540x + 11.281$	0.62	1.450
	50	$y = 2.680x + 9.325$	0.84	2.680
	80	$y = 3.033x + 10.288$	0.97	3.033
	100	$y = 3.597x + 11.065$	0.75	3.597
	150	$y = 2.431x + 11.838$	0.72	2.431
PF2	10	$y = 2.071x + 9.813$	0.87	2.071
	25	$y = 2.832x + 11.294$	0.92	2.832
	50	$y = 2.450x + 11.209$	0.92	2.450
	80	$y = 2.123x + 10.297$	0.99	2.123
	100	$y = 2.556x + 9.790$	0.91	2.556
	150	$y = 2.985x + 8.062$	0.83	2.985
PF3	10	$y = 1.454x + 11.441$	0.66	1.454
	25	$y = 1.958x + 11.386$	0.74	1.958
	50	$y = 2.007x + 11.929$	0.60	2.001
	80	$y = 2.203x + 11.609$	0.65	2.203
	100	$y = 2.068x + 11.993$	0.59	2.068
	150	$y = 2.379x + 11.979$	0.71	2.379

x=Time(day), y=ln(profenofos concentration)

Table 4.12 Growth kinetics of PF1 under presence of nitrate

Profenofos conc. (mg/L)	Nitrate conc. (mg/L)	Kinetics		
		equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = 1.791x + 12.682$	0.91	1.791
	200	$y = 2.158x + 12.558$	0.99	2.158
	300	$y = 1.997x + 13.054$	0.95	1.997
25	100	$y = 2.249x + 12.510$	0.96	2.249
	200	$y = 2.062x + 13.817$	0.60	2.062
	300	$y = 2.373x + 12.427$	0.96	2.373
50	100	$y = 2.577x + 12.111$	0.94	2.577
	200	$y = 2.286x + 12.438$	0.97	2.286
	300	$y = 2.576x + 12.107$	0.98	2.576
80	100	$y = 2.552x + 12.567$	0.89	2.552
	200	$y = 2.133x + 14.153$	0.99	2.133
	300	$y = 1.633x + 14.049$	0.96	1.634
100	100	$y = 2.048x + 12.385$	0.93	2.048
	200	$y = 2.149x + 11.404$	0.91	2.149
	300	$y = 2.392x + 10.964$	0.82	2.392
150	100	$y = 2.087x + 11.367$	0.80	2.087
	200	$y = 2.199x + 11.736$	0.90	2.199
	300	$y = 2.631x + 11.610$	0.89	2.631

x=Time(day), y=ln(profenofos concentration)

Table 4.13 Growth kinetics of PF2 under presence of nitrate

Profenofos conc. (mg/L)	Nitrate conc. (mg/L)	Kinetics		
		equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = 2.52x + 11.06$	0.88	2.52
	200	$y = 2.31x + 11.09$	0.86	2.31
	300	$y = 1.03x + 13.32$	0.79	1.03
25	100	$y = 1.74x + 13.01$	0.84	1.74
	200	$y = 1.89x + 12.51$	0.76	1.89
	300	$y = 1.15x + 10.99$	0.84	1.15
50	100	$y = 2.07x + 11.46$	0.87	2.07
	200	$y = 2.66x + 10.75$	0.95	2.66
	300	$y = 2.05x + 11.12$	0.79	2.05
80	100	$y = 2.36x + 11.22$	0.92	2.36
	200	$y = 2.36x + 11.22$	0.92	2.36
	300	$y = 2.36x + 11.31$	0.87	2.36
100	100	$y = 2.46x + 10.87$	0.85	2.46
	200	$y = 2.11x + 10.99$	0.87	2.11
	300	$y = 2.36x + 11.09$	0.87	2.36
150	100	$y = 2.58x + 10.43$	0.89	2.58
	200	$y = 2.69x + 9.52$	0.93	2.69
	300	$y = 2.74x + 9.47$	0.95	2.74

x=Time(day), y=ln(profenofos concentration)

Table 4..14 Growth kinetics of PF3 under presence of nitrate

Profenofos conc. (mg/L)	Nitrate conc. (mg/L)	Kinetics		
		equation	R ²	Rate constant at 3 days (1/d)
10	100	$y = 1.276x + 13.657$	0.72	1.276
	200	$y = 1.229x + 13.650$	0.67	1.229
	300	$y = 1.189x + 13.532$	0.75	1.189
25	100	$y = 1.149x + 13.196$	0.75	1.149
	200	$y = 1.286x + 13.18$	0.77	1.286
	300	$y = 1.036x + 13.73$	0.51	1.036
50	100	$y = 0.979x + 13.099$	0.54	0.979
	200	$y = 1.079x + 13.738$	0.50	1.079
	300	$y = 1.164x + 12.461$	0.68	1.164
80	100	$y = 1.411x + 11.872$	0.72	1.411
	200	$y = 1.741x + 11.501$	0.68	1.741
	300	$y = 1.496x + 11.993$	0.81	1.496
100	100	$y = 1.462x + 11.948$	0.51	1.462
	200	$y = 2.011x + 10.893$	0.75	2.011
	300	$y = 1.609x + 10.900$	0.56	1.609
150	100	$y = 1.743x + 11.546$	0.55	1.743
	200	$y = 1.784x + 11.391$	0.71	1.784
	300	$y = 1.498x + 12.019$	0.54	1.498

x=Time(day), y=ln(profenofos concentration)

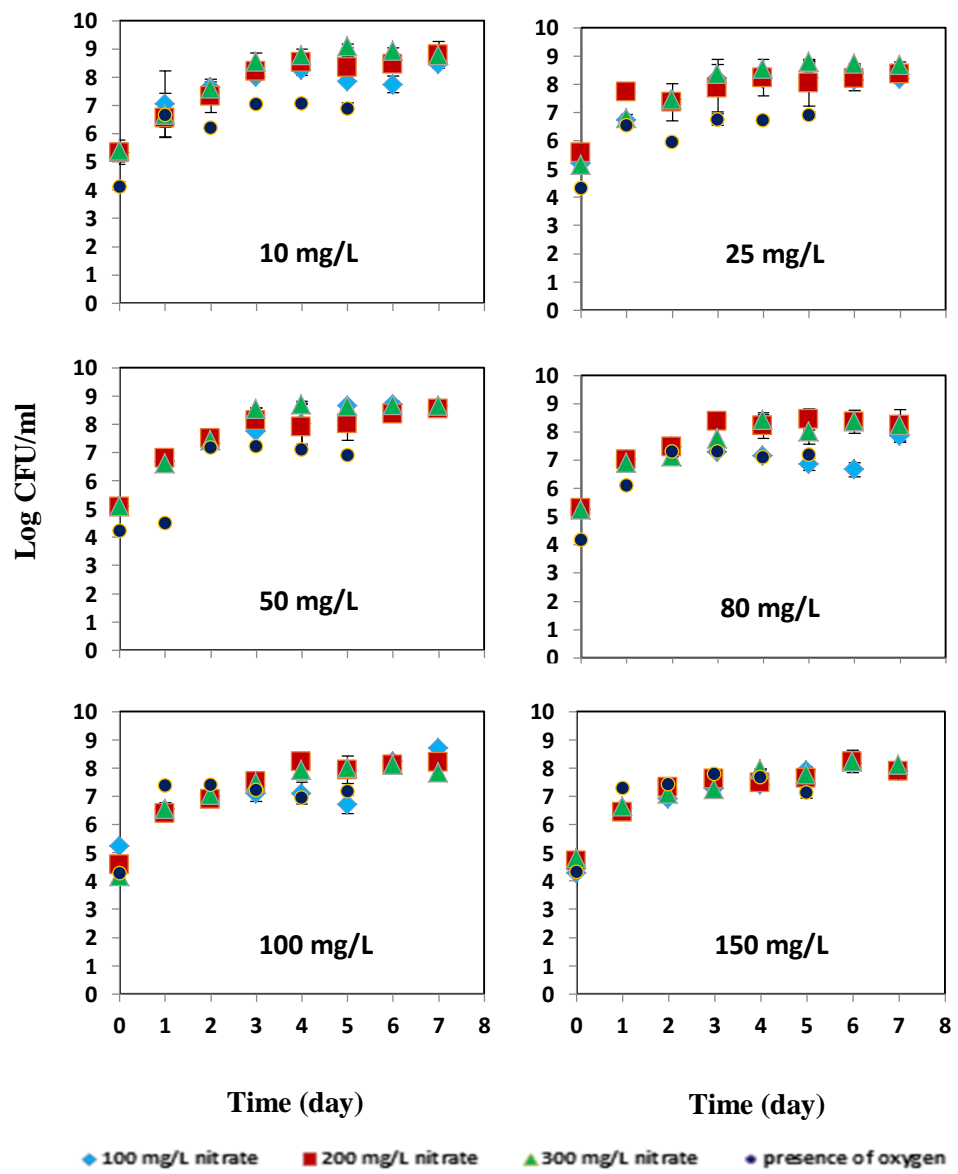


Figure 4.10 Microbial growth of PF1 under presence of oxygen and nitrate

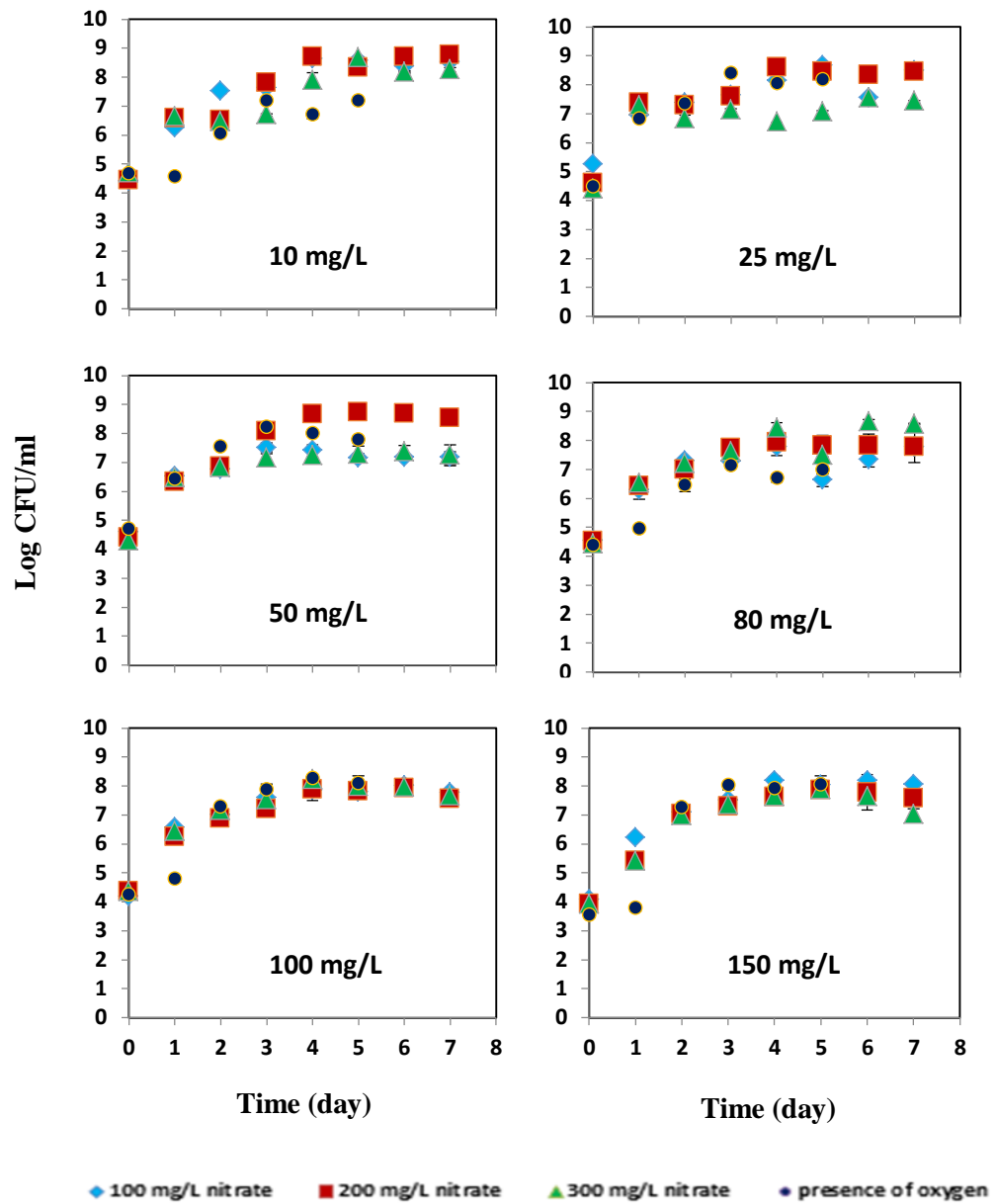


Figure 4.11 Microbial growth of PF2 under presence of oxygen and nitrate

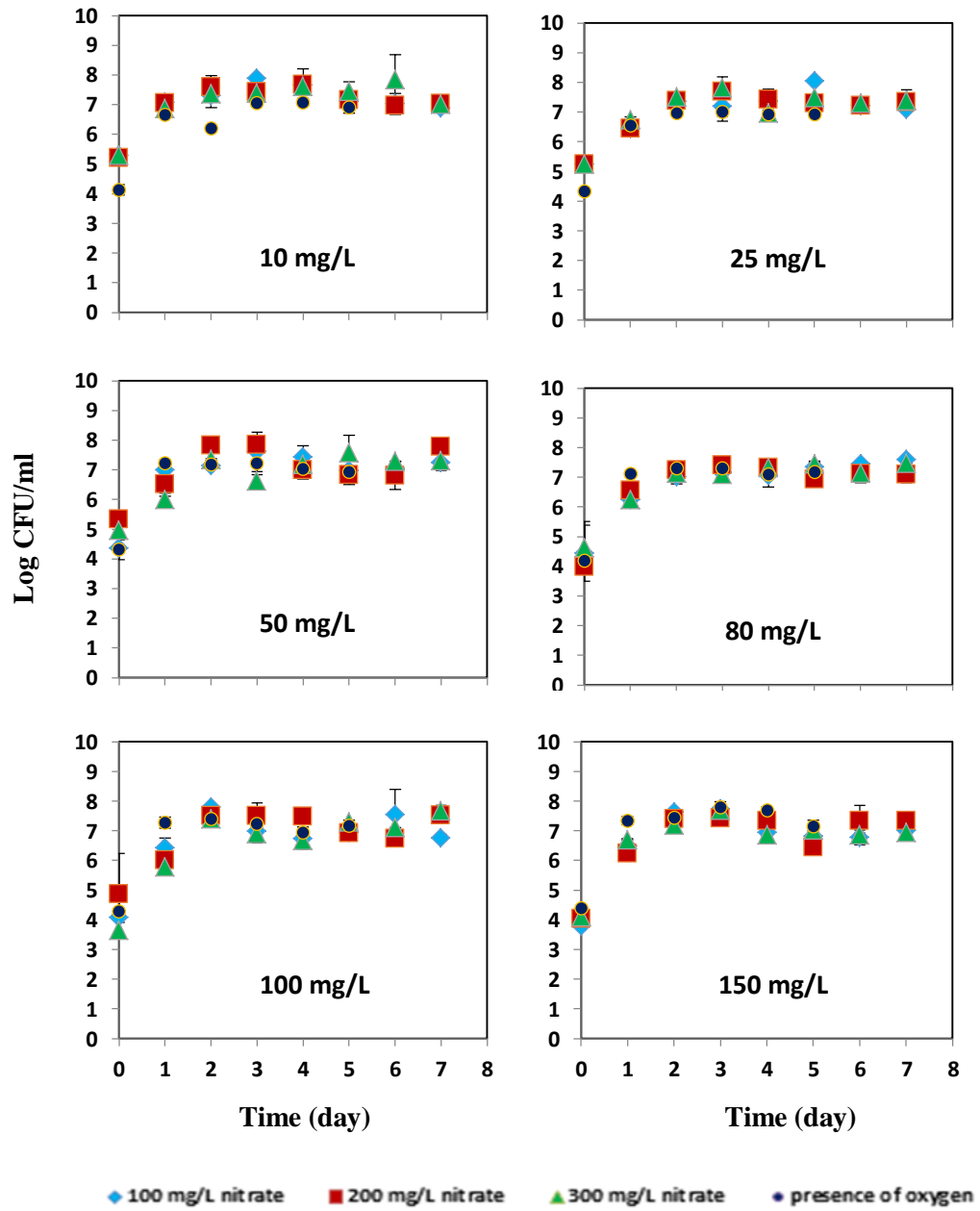


Figure 4.12 Microbial growth of PF3 under presence of oxygen and nitrate

From the results, profenofos could be served as a carbon source under presence of oxygen and nitrate for microbial growth of PF1, PF2 and PF3. All isolates could grow very well in all the experimental conditions. The viable cell growth of PF1 and PF2 (10^8 CFU/ml) was quite higher than PF3 (10^7 CFU/ml). All isolates degraded profenofos biodegradation under both presence of oxygen and nitrate without lag phase resulting in continuously decrease in profenofos degradation and dramatically increase the microbial growth (exponential phase). In addition, they gradually steady in stationary after day 4 (without dead phase). The result pointed that the isolates were able to survive and reproduce in the medium supplemented with profenofos for both presence of oxygen and nitrate. It is indicated that various profenofos and nitrate concentrations were not inhibited to the microbial growth of PF1, PF2 and PF3. This may be because the culture has been acclimated in the medium containing profenofos for a long time (longer than 1 month).

Based on microbial growth kinetics (Tables 4.11 to 4.14), the microbial cell growth under presence of oxygen followed the first kinetic equation which the growth rates of strain PF1, PF2 and PF3 ranged from 1.45 to 3.59, 2.07 to 2.98 and 1.45 to 2.37 1/d, respectively. Under presence of nitrate, the microbial growth of all isolate also followed the first kinetic equation which the growth rates of strain PF1, PF2 and PF3 range from 1.63 to 2.63, 1.03 to 2.74 and 0.97 to 2.01 1/d, respectively. The growth rates of PF1, PF2 and PF3 were quite similar. It is indicated that all isolates could grow well even increasing profenofos concentrations from 10 to 150 mg/L and nitrate concentrations (100-300 mg/L). This result was different with the previous study (Patichot, 2012). The microbial growth of the consortium decreased when increasing profenofos concentration to 120 mg/L. This is because high profenofos concentration (120 mg/L) could be toxic to microbial cell growth of the consortium. Generally, the growth rate increases with an increase in substrate concentration until a maximum value is reached and then the rates decreased. Moreover, high substrate concentration inhibited the bacterial growth.

The effect of nitrate on microbial growth of PF1, PF2 and PF3 showed that various initial nitrate concentrations did not affect growth. When increasing the nitrate concentrations from 100 mg/L to 300 mg/L, the growth rates of all isolate quite stable.

It is indicated that nitrate concentration may not obviously influence on microbial growth. This result was correlated to the previous works (Rhee et al., 1997; Chayabutra and Ju, 2000). The bacterial culture growing in the medium with certain range of nitrate concentrations, the cell growths were similar. However, at too high nitrate concentration, the cultures were inhibited. In this study, based on the tested condition, the system contained enough nitrate concentration for supporting microbial metabolism resulting in well growth of the isolates. This result suggested that nitrate concentration of up to 300 mg/L did not be toxic to microbes.

4.3 Nitrate reduction under presence of nitrate

This study investigated the nitrate reduction by PF1, PF2 and PF3 under presence of nitrate. Influence of six profenofos concentrations (10, 25, 50, 80, 100 and 150 mg/L) and three nitrate concentrations (100, 200 and 300 mg/L) were examined. The normalized nitrate remaining percentages of the tests by PF1, PF2 and PF3 were shown in Figures 4.13, 4.14 and 4.15, respectively.

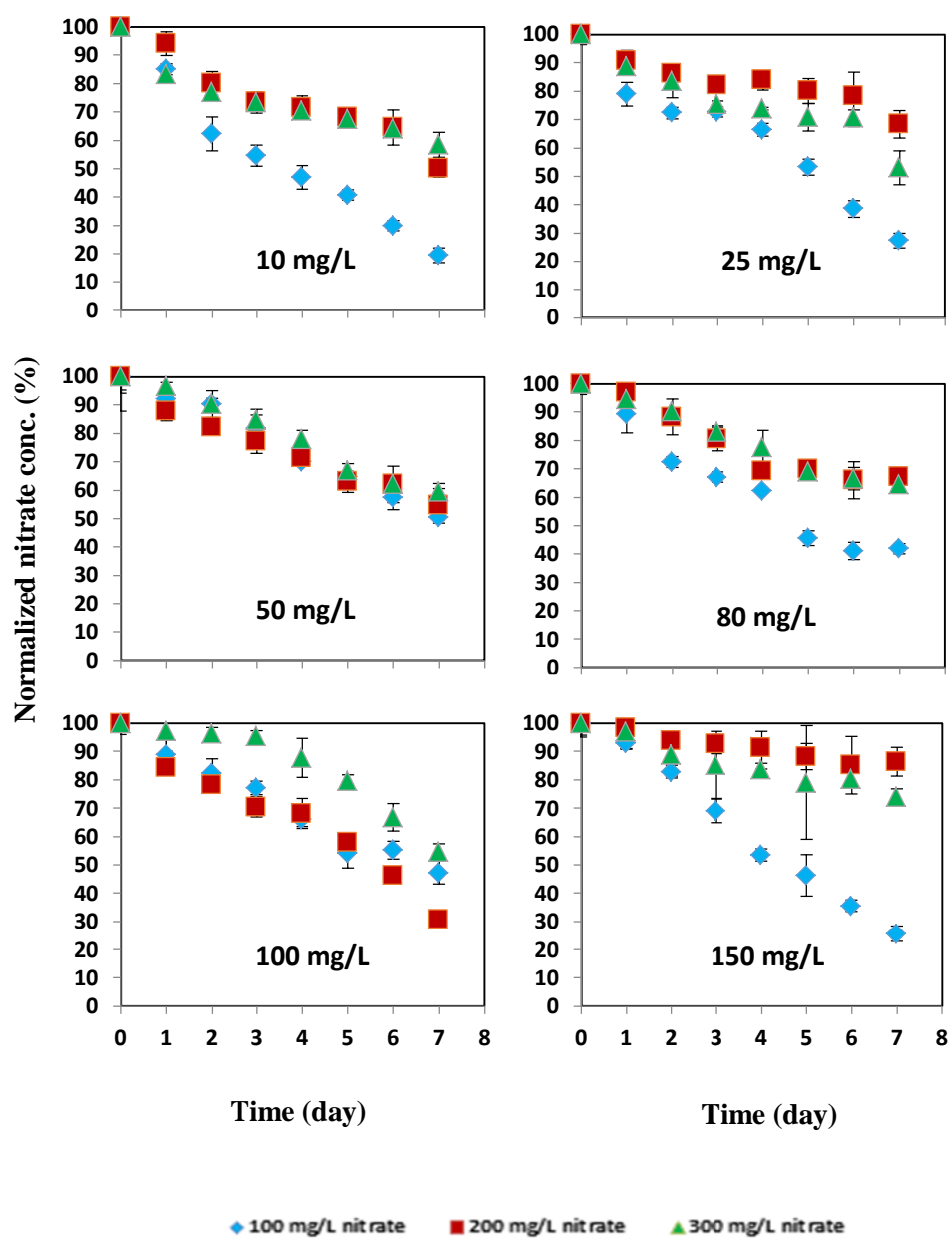


Figure 4.13 The normalized nitrate concentration percentages of PF1 at profenofos 10,25,50,80,100 and 150 mg/L

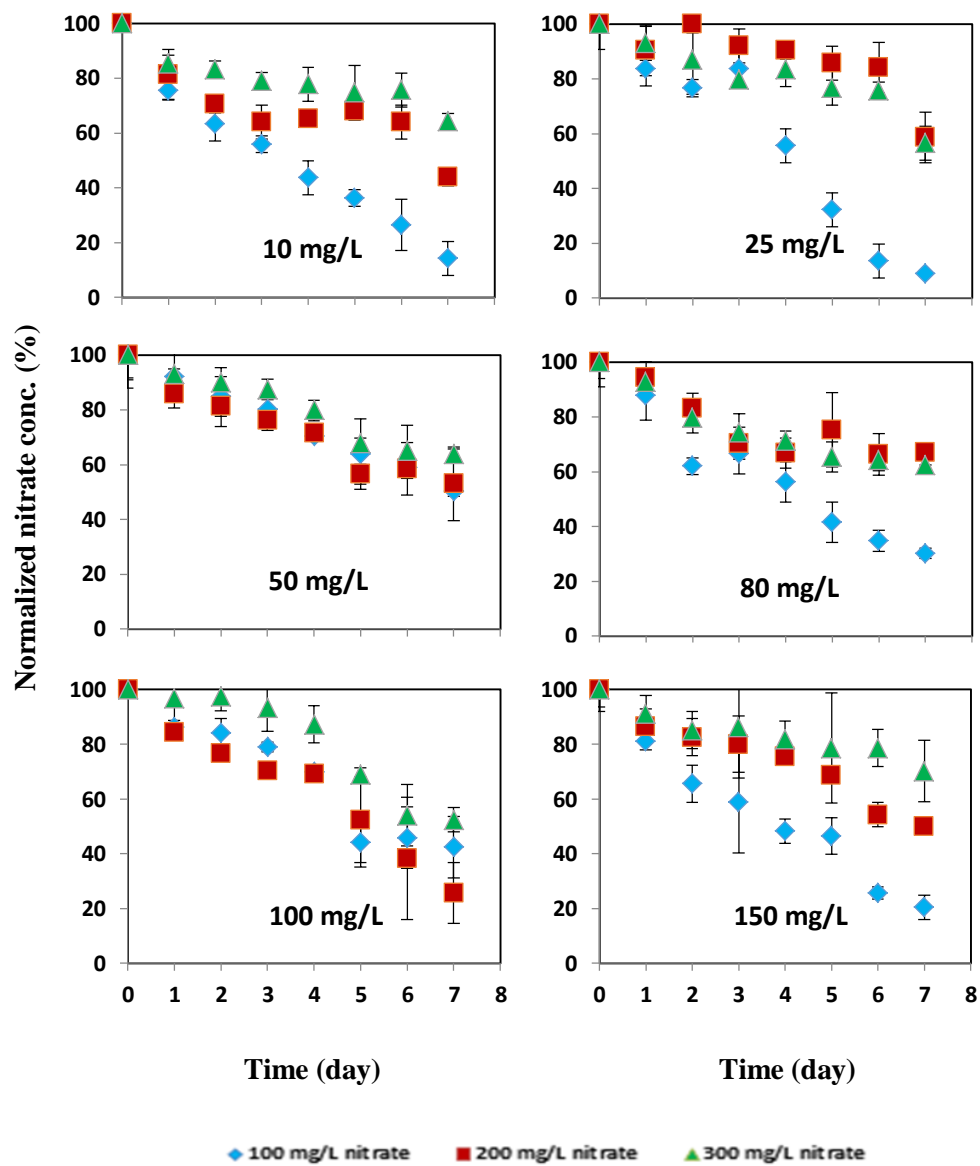


Figure 4.14 The normalized nitrate concentration percentages of PF2 at profenofos 10,25,50,80,100 and 150 mg/L

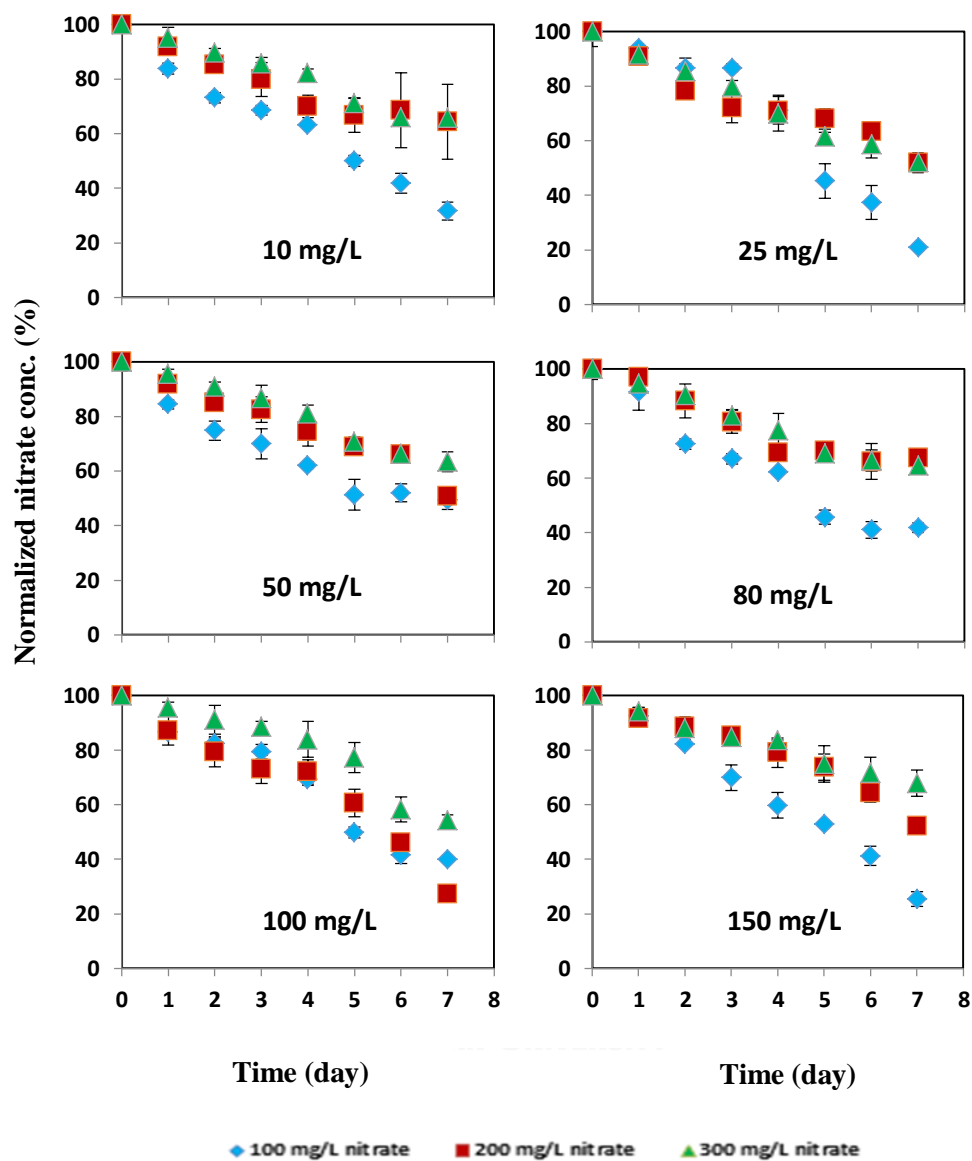


Figure 4.15 The normalized nitrate concentration percentages of PF3 at profenofos 10,25,50,80,100 and 150 mg/L

During profenofos degradation under presence of nitrate, the nitrate concentrations (100, 200 and 300 mg/L) were continuously decreased within 8 days. This result showed the similar patterns of PF1, PF2 and PF3 (Figures 4.13, 4.14 and 4.15). In overall, the nitrate concentrations of the tests at 100 mg/L of nitrate obviously reduced more than those of 200 and 300 mg/L.

It concluded that all isolates could use nitrate as an electron acceptor under profenofos degradation; therefore, profenofos degradation and nitrate reduction occurred due to the biological process. For abiotic control test, nitrate concentration reduced less than 10% (data not shown) in all the experiments. The nitrate reduction percentages of the tests by PF1, PF2 and PF3 ranged from 26.08 to 80.61, 29.86 to 91.12 and 32.23 to 78.96%, respectively as shown in Table 4.15.

Table 4.15 Nitrate reduction (%)

Isolate	Profenofos conc. (mg/L)	Presence of nitrate		
		Nitrate conc. (mg/L)		
		100	200	300
PF1	10	80.61	49.89	41.64
	25	72.71	35.68	46.94
	50	49.74	45.48	40.58
	80	50.11	32.73	35.45
	100	52.78	69.31	45.45
	150	74.38	47.51	26.08
PF2	10	85.79	56.15	35.86
	25	91.12	41.40	43.52
	50	49.69	46.98	36.17
	80	69.81	33.10	37.61
	100	57.57	74.30	47.59
	150	79.52	50.03	29.86
PF3	10	73.94	46.87	51.40
	25	78.96	48.01	47.76
	50	50.77	49.19	36.55
	80	68.35	35.64	34.33
	100	60.12	72.62	45.67
	150	74.48	47.83	32.23

The result was similar to an earlier work (Lu et al., 2008). Nitrate concentration (electron acceptor) were rapidly reduced in the first week of experiment. It was indicated that nitrate could be used as an electron acceptor in the tested conditions (Zhuang et al., 2012).

From this result, nitrate reduction percentage of the test at 100 mg/L of nitrate showed the best reduction (49.69-91.12%). The results showed the similar pattern of all the isolates. The trend of nitrate reduction by PF1, PF2 and PF3 was continuously reduced at various nitrate concentrations. It is indicated that nitrate concentration affected nitrate reduction. This result was similar to previous studies. For example, Rezaee et al (2012) reported that low initial nitrate concentration reduced more than high concentration. When comparing the tests with various initial profenofos concentrations at the same nitrate concentration, it was found that profenofos was not obviously influenced nitrate reduction. This is similar to prior works (Zhuang et al., 2012); contaminant concentration did not play a role on decreasing of nitrate.

It concluded that strains PF1, PF2 and PF3 could use nitrate as an electron acceptor for profenofos biodegradation. In practice, all isolates could be applied in agricultural area containing nitrate from fertilizer. In the case of pesticide and fertilizer-contaminated groundwater (low oxygen condition), the isolated cultures sound promising for bioremediation.

4.4 Profenofos intermediate metabolite under presence of oxygen and nitrate

It was reported that during profenofos degradation, strains PF1, PF2 and PF3 degraded profenofos through hydrolase enzyme and formed 4-bromo-2-chlorophenol (BCP) as a metabolite. In case of high profenofos concentration, BCP was accumulated. Based on a previous study, a consortium containing PF1, PF2 and PF3 degraded both profenofos and BCP (Siripattanakul-Ratpukdi et al., 2015). From this study, BCP was found as an intermediate metabolite under both presence of oxygen and nitrate (Figure 4.16). In addition, it was found that 1,1diethylethylphenol was detected as a metabolite under presence of oxygen and nitrate and triethyl phosphate was accumulated under presence of nitrate. The mass spectrum of BCP, 1,1 diethylethylphenol and triethyl phosphate were shown in Figures 4.16, 4.17 and 4.18, respectively.

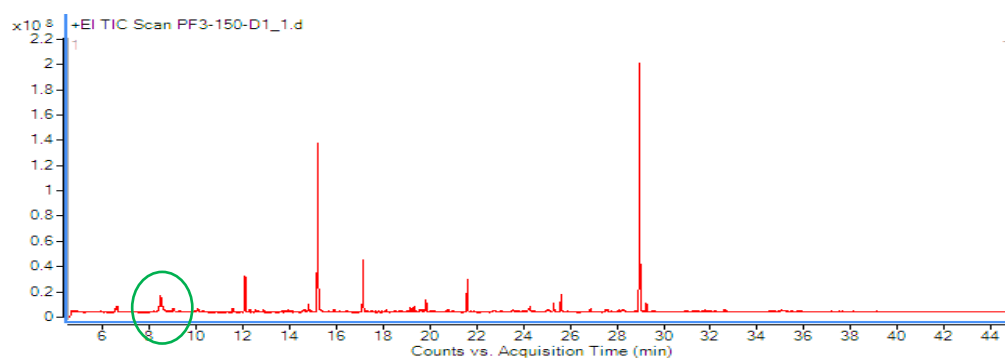
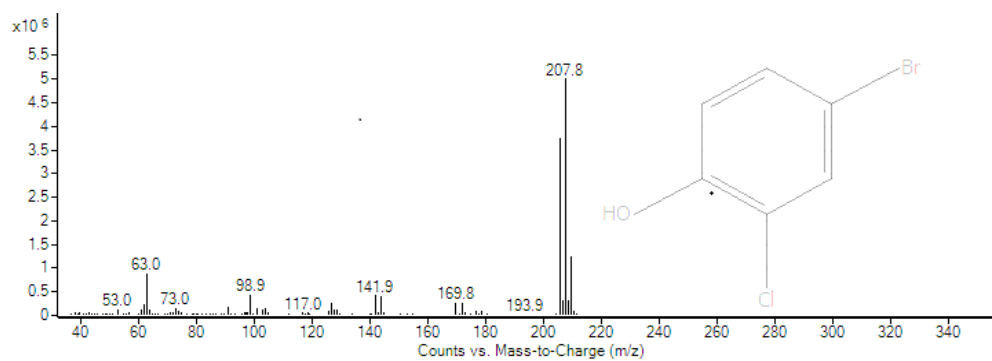


Figure 4.16 Mass spectrum of 4-bromo-chlorophenol (BCP)

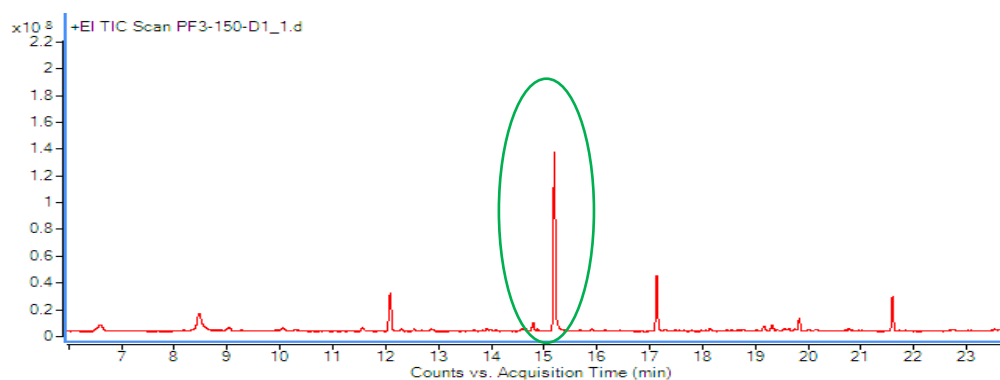
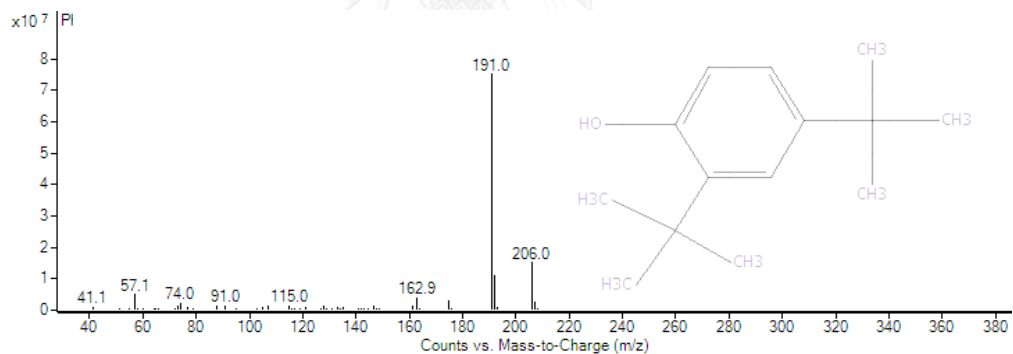


Figure 4.17 Mass spectrum of 1,1 diethylethylphenol

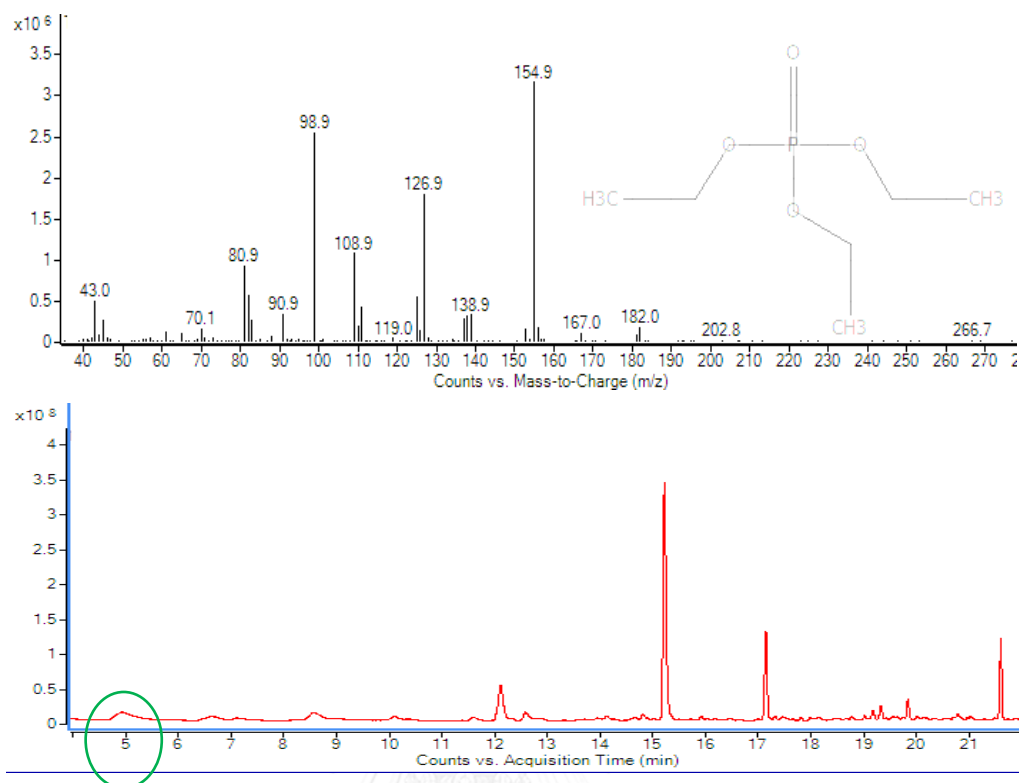


Figure 4.18 Mass spectrum of triethyl phosphate

During the test with profenofos concentration of 150 mg/L in abiotic control test (no cell), profenofos concentration decreased in day 1, BCP was accumulated under presence of oxygen. It was indicated that abiotic hydrolytic process occurred. However, it was found that BCP did not accumulate under presence of nitrate. This was unclear whether BCP was not accumulated or profenofos degraded to be other metabolites. This may be like the proposed pathway previously reported (Silva et al., 2013). Profenofos could be degraded to BCP or other metabolites such as 4-bromo-2-chlorophenyl ethyl hydrogen phosphate, 4-bromo-2-chlorophenyl dihydrogen phosphate and O-4-bromo-2-chlorophenyl-S-propyl O-hydrogen phosphorothioate.

During profenofos biodegradation by PF1, PF2 and PF3, BCP was accumulated under presence of oxygen which was correlated to the previous work. Profenofos was transformed via organophosphorus hydrolase enzyme (Siripattanakul-Ratpukdi et al., 2015). Formed BCP was taken place by breaking ester bond of profenofos (Malghani et al., 2009). When comparing between area peaks of BCP on profenofos biodegradation and abiotic degradation, it was found that the peak area of

BCP on profenofos biodegradation was higher than abiotic hydrolysis (data not shown). It was indicated that biodegradation could enhance profenofos degradation. Based on the peak area, BCP-profenofos ratio by PF1, PF2 and PF3 under presence of oxygen and nitrate was demonstrated in Table 4.16.

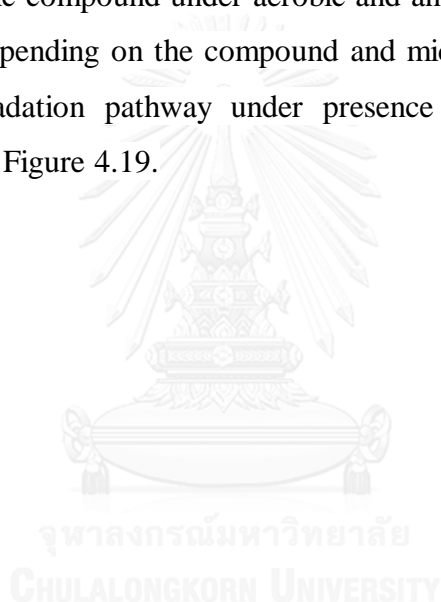
Table 4.16 BCP-profenofos ratio on profenofos degradation

Isolates	Profenofos concentration (mg/L) in day 1		BCP-profenofos ratio	
	Under presence of oxygen	Under presence of nitrate	Under presence of oxygen	Under presence of nitrate
PF1	79.036	101.836	0.321	0.343
PF2	81.413	103.082	0.187	0.206
PF3	85.104	104.719	0.191	0.390

Between presence of oxygen and nitrate, it was found that profenofos remaining under presence of oxygen was lower than under presence of nitrate. The potential BCP degradation under presence of oxygen was higher than one under presence of nitrate. Therefore, BCP accumulation (BCP-profenofos ratio) under presence of oxygen was lower than presence of nitrate. Based on profenofos biodegradation by PF1, PF2 and PF3, profenofos degradation under each condition was quite similar whereas BCP accumulation under presence of oxygen and nitrate was different. Accumulated BCP by PF1 was higher than PF3 and PF2. It was indicated that the profenofos-degrading enzyme of PF2 and PF3 may be more efficient than that of PF1 under presence of oxygen. On the other hand, under presence of nitrate, strain PF2 accumulated BCP less than PF1 and PF3. It was likely that the profenofos-degrading enzyme of PF2 performed better than PF1 and PF3. In overall, all the cultures worked well for both aerobic and anaerobic conditions. However, it sounds that PF2 was more efficient and adaptable for profenofos and intermediate degradation compared to PF1 and PF3. To the best of our knowledge, there was no report on profenofos biodegradation pathway under anaerobic condition (presence of nitrate). Therefore, this study investigated intermediate metabolites under presence of nitrate. The result presented that BCP, 1,1diethylethylphenol and triethyl

phosphate were found as the metabolites on profenofos degradation under presence of nitrate.

It has been reported on organic compound degradation pathways under aerobic and anaerobic conditions. Most of biodegradation pathway under aerobic and anaerobic conditions showed the different pathway such as parathion (Munnecke and Hsieh, 1976), Endosulfan (Tiwari and Guha, 2013) and 17α -estradiol-3-sulfate (Zheng et al., 2013). Moreover, some compounds presented the same pathway even different conditions (aerobic and anaerobic conditions) such as chlorpyrifos (Yadav et al., 2015). Based on the literatures, it could be pointed out that the degradation pathway of the organic compound under aerobic and anaerobic conditions may be the same or difference depending on the compound and microbial cultures. In this study, the profenofos degradation pathway under presence of oxygen and nitrate was proposed as shown in Figure 4.19.



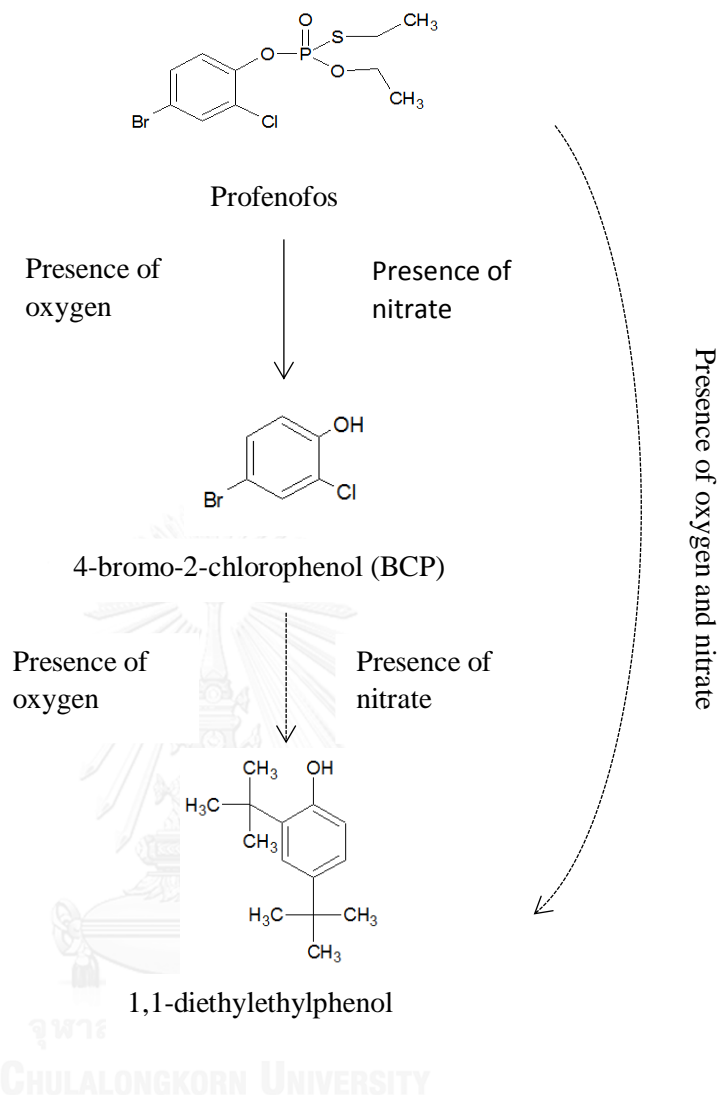


Figure 4.19 Proposed commercial profenofos degradation pathway under presence of oxygen and nitrate

Strains PF1, PF2 and PF3 showed the same degradation pathway as presented in Figure 4.19. In presence of oxygen and nitrate, some intermediate metabolites (BCP and 1,1 diethylethylphenol) were discovered. A metabolite (Triethyl phosphate) was found only presence of nitrate. It was indicated that hydrolase enzyme worked on profenofos degradation by all isolates even different conditions. From this study, profenofos was transformed to BCP via hydrolysis reaction. The conjugation reaction was occurred and form 1,1 diethylethylphenol as the intermediate metabolite. It is noted that the conjugation took place since the commercial profenofos may contain some other unknown hydrocarbons to react with profenofos intermediate product.

From the previous study, Siripattanakul-Ratpukdi et al. (2015) reported that profenofos was converted to BCP on profenofos biodegradation under presence of oxygen through hydrolysis. The 3-methoxyphenol was found as an intermediate metabolite. This study was reported some more intermediate metabolites. However, it is inconclusive for the degradation pathway order. Further study on profenofos degradation pathway by the cultures was recommended.



CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Conclusion

5.1.1 Profenofos degradation and degradation kinetics under presence of oxygen and nitrate

Profenofos could be served as a carbon sources under presence of oxygen and nitrate by PF1, PF2 and PF3. The degradation rate followed the first kinetic order for both presence of oxygen and nitrate. The removal efficiency percentages under presence of oxygen by PF1, PF2 and PF3 ranged from 38.14 to 55.39, 31.62 to 61.22 and 34.72-63.81%, respectively. Under presence of nitrate, PF1, PF2 and PF3 were removed profenofos for 27.50 to 45.33, 39.09 to 75.36 and 13.78 to 48.89%, respectively. Based on percent removal, profenofos was higher degraded by PF1 and PF3 under presence of oxygen than presence of nitrate whereas PF2 utilized profenofos under presence of nitrate better than presence of oxygen.

From Michalis-Menten kinetic model, maximum profenofos degradation rate (V_{max}) of PF1, PF2 and PF3 under presence of oxygen was 13.07, 22.57 and 17.79 mg/L/day, respectively. The V_{max} of PF1, PF2 and PF3 under presence of nitrate was 10.14, 13.71 and 8.65 mg/L/day, respectively. Under presence of oxygen by PF1, PF2 and PF3 was 92.07, 199.35, 178.86 mg/L, respectively. The apparent K_s under presence of nitrate by PF1, PF2 and PF3 ranged from 84.76 to 147.36, 137.86 to 175.86 and 105.90 to 158.85 mg/L, respectively. Moreover, profenofos degradation under presence of nitrate showed the competitive inhibition like.

5.1.2 Microbial growth under presence of oxygen and nitrate

The viable cell number of PF1, PF2 and PF3 under presence of oxygen and nitrate increased from 10^4 to 10^8 , 10^4 to 10^8 and 10^4 to 10^7 CFU/ml, respectively. The growth kinetics followed the first kinetic order for both presence of oxygen and nitrate. The Growth rate of PF1, PF2 and PF3 under presence of oxygen ranged from 1.45 to 3.59, 2.07 to 2.98 and 1.45 to 2.37 1/d, respectively whereas growth rates of

strains PF1, PF2 and PF3 ranged from 1.63 to 2.63, 1.03 to 2.74 and 0.97- 2.01 1/d, respectively.

5.1.3 Nitrate reduction (under presence of nitrate)

Strains PF1, PF2 and PF3 could use nitrate as an electron acceptor under presence of nitrate. The nitrate reduction percentages of PF1, PF2 and PF3 ranged from 26.08 to 80.61, 39.86 to 91.12 and 32.23 to 78.96%, respectively.

5.1.4 Profenofos intermediate metabolite monitoring under presence of oxygen and nitrate

During profenofos degradation under presence oxygen and nitrate, BCP and 1,1,diethylethylphenol were detected as intermediate metabolites and triethyl phosphate was found as a metabolite on profenofos degradation under presence of nitrate. Profenofos degradation under presence of oxygen and nitrate occurred by hydrolysis reaction.

5.2 Recommendations for future work

5.2.1 Various types of electron acceptors such as sulfate and iron (III) should be studied on profenofos degradation under anaerobic condition.

5.2.2 Based on degradation under presence of nitrate, oxygen and nitrite concentrations during the experiment should be investigated.

5.2.3 The stoichiometry between nitrate consumption and profenofos degradation should be studied to ensure the reaction of nitrate on profenofos degradation.

5.2.4 Surface water and groundwater which contained nitrate from fertilizer should be used in the pilot experiment before real application.

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APPENDIX

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APPENDIX A

Medium preparation and aseptic technique

1. Medium preparation

1.1 Minimal salt medium (MSM) for bacteria cultivation

Chemical

Phosphate buffer 10 mM

- 1) $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 20.9565 g
- 2) $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ 31.00g

Minimal salt medium (MSM)

- | | | |
|--|--------|------|
| 1) $\text{NaHPO}_4 \cdot 12\text{H}_2\text{O}$ | 14.678 | g |
| 2) KH_2PO_4 | 3 | g |
| 3) NaCl | 0.5 | g |
| 4) NH_4Cl | 2 | g |
| 5) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ | 0.513 | g |
| 6) Profenofos | 20 | mg/L |

Procedure

Dissolved all chemicals in 10 mM Phosphate buffer Solution and dilute to 1 liter. The medium was autoclaved at 121°C for 15 min and then supplemented with 20 mg/L of filtered sterile profenofos solution.

Note: 1.5% of Agar and 0.1% of yeast extract were added for solid medium

1.2. Nutrient Agar (NA) for bacteria plate count

Chemical

Nutrient agar 28 g

Procedure

Dissolved nutrient agar in distilled water and dilute to 1 liter. Then the medium was autoclaved at 121°C for 15 min and supplemented with 20 mg/L of filtered sterile profenofos solution.

1.3 Spread plate technique

Spread plate technique is a common method which distributes the bacteria cell number over the surface of an agar plate. The processing of spread plate method consists of 10 serial dilution and spread plate. The 10 serial dilutions and Spread plate were shown in Figure A.1 and A.2

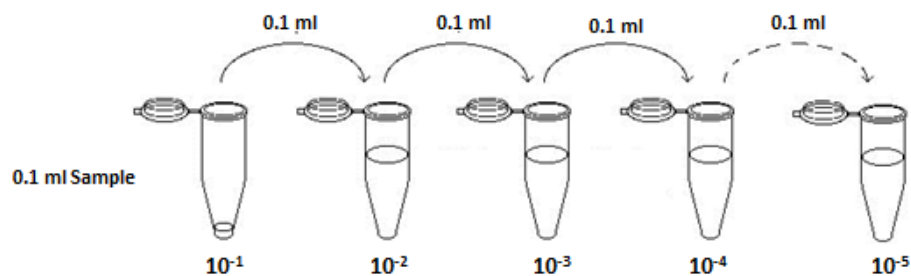


Figure A.1 10 Serial dilutions

The procedure of 10 serial dilutions

1. Pipette 0.1 mL of cell suspension with aseptic technique in 0.9 ml of sterilizes distilled water, then mix it. The dilution of cell suspension obtains at 10^{-1} dilution.
2. Pipette 0.1 mL of cell suspension from (1) in 0.9 ml of sterilizes distilled water, then mix it. The dilution of cell suspension obtains at 10^{-2} dilution.
3. Pipette 0.1 mL of cell suspension from (2) in 0.9 ml of sterilizes distilled water, then mix it. The dilution of cell suspension obtains at 10^{-3} dilution. Keep doing until expected dilution.
4. The serial dilution was used to cultivate microbial cell

The methods of Spread Plate

1. Preparation agar plate
2. Pipette 0.1 ml of sample on agar plate (Figure A.2)
3. Put glass spreader into 95% alcohol and burned. After cooling at room temperature, spread plate, reverse plate upside down and incubate at 35 °C in the incubator
4. Obverse the morphology of bacterial colonies and count the cell number

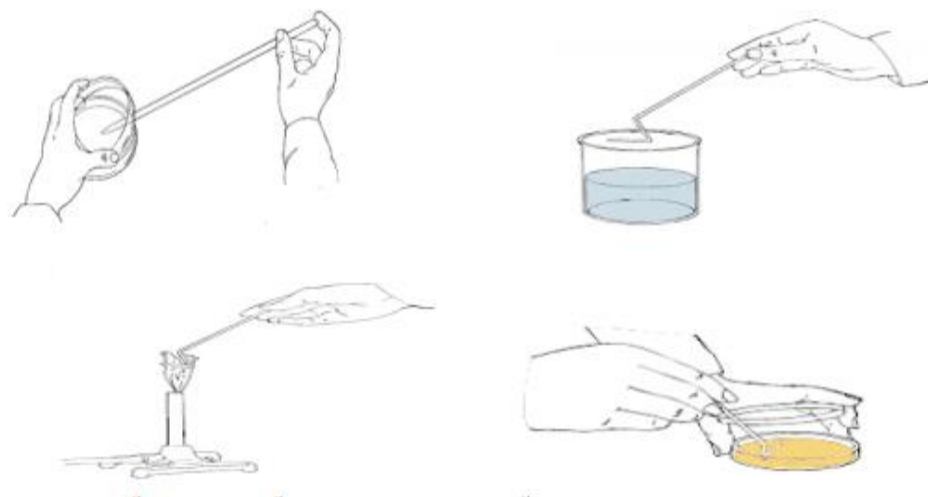


Figure A.2 Spread plate technique (King Mongkut's University of Technology North Bangkok, 2010)

2. Medium preparation of presence of nitrate

The procedure of medium preparation under presence of nitrate

1. The medium was carried out using 100 mL of serum bottles containing 70-mL medium (without profenofos and nitrate). The serum bottles were sealed with silicone rubber and then autoclaved at 121°C for 15 min (Figures A.3 and A.4).



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Figure A.3 serum bottle



Figure A.4 serum bottle after autoclaved

2. After sterilization, the bottles were purged with filter sterilized nitrogen gas for 15 min to ensure the absence of oxygen and capped with aluminium crimps (Figure A.5 and A.6). Finally, the serum bottle under presence of nitrate was done (Figure A.7). Then, profenofos, nitrate and the isolated bacteria were injected into the bottles, respectively.



Figure A.5 Purging nitrogen gas



Figure A.6 Capped with aluminium crimps



Figure A.7 Medium for degradation under presence of nitrate

APPENDIX B

Chemical Analysis

1. Profenofos analysis

1.1 Profenofos preparation

1.1.1 Standard Profenofos

Dissolve 0.25 g of standard profenofos (Dr. Ehrenstorfer GmbH, Germany) and dilute with hexane 2.5 ml to 100 mg/L for analysis

1.1.2 Commercial grade profenofos

Dilute 50% v/v (500,000 mg/L) of commercial grade profenofos with hexane to 200,000 mg/L. Then, Dilute profenofos with distilled water to 20,000 mg/L. Finally, the stock solution for experiment is 2,000 mg/L profenofos. The percentages of profenofos recovery were shown in Table B.1.

Table B.1 Profenofos recovery percentage (%)

PF conc. (mg/L)	Profenofos recovery percentage (%)			
	Presence of oxygen	Presence of nitrate		
		Nitrate concentration (mg/L)		
		100	200	300
10	87.31	87.50	88.27	85.72
25	84.50	79.64	80.21	79.25
50	76.59	74.59	73.34	74.49
80	73.12	76.10	74.24	74.81
100	72.75	72.37	70.53	71.74
150	72.37	71.46	73.42	73.41

1.2 Storage and Preservation

Collected sample without light and preserve sample at 4 °C

1.3 Sample preparation (Extraction)

The sample was extracted using liquid-liquid extraction technique. 0.5 ml of sample was added in 1.5 ml of microcentrifuge tube with 0.5 ml of

hexane and 0.1% acetic acid. Then, the sample was mixed on vortex at 2500 rpm for 10 min. Upper solution (hexane) was transferred to 2-mL GC vial. The extracted sample was analyzed using gas chromatography.

1.4 Analysis

The sample was analyzed by GC (Agilent 4890D, USA) equipped with Ni⁶³ electron capture detector. The column was a SPBTM-608 fused silica capillary column (15 m × 0.53 mm × 0.25 μm film thickness). The temperatures of injection plot and detector were set at 240 °C and 250 °C, respectively. The temperature condition was started at 120 °C, hold at 120 °C for 2 min, ramped up 40 °C/min to final temperature 240°C and then hold at 240 °C for 5 mins. Total retention time was 10 min. Helium and nitrogen gas were used as the carrier gas with flow rate 8 mL/min and 47 mL/min, respectively. Profenofos peak was shown at 3.1 min in chromatogram and profenofos standard curve was shown in Figure B.1 and B.2, respectively.

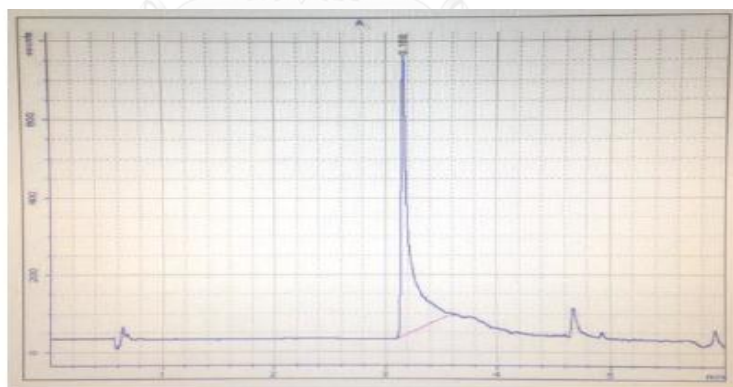
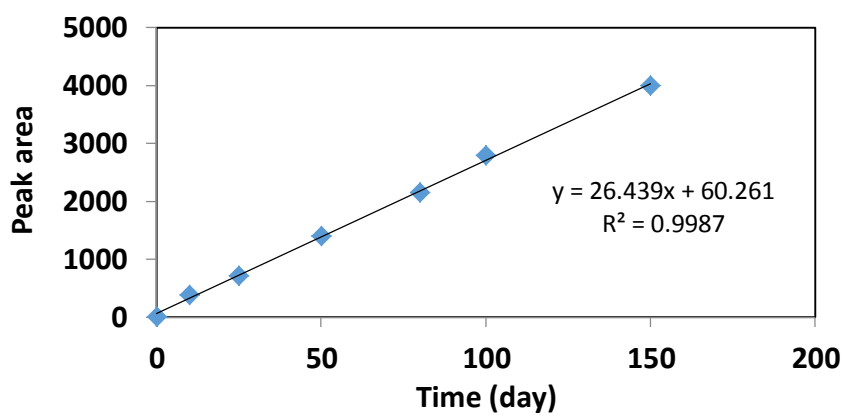


Figure B.1 Profenofos Chromatogram



FigureB.2 Profenofos standard curve



2. Nitrate analysis

Nitrate (NaNO_3 , RCI Labscan, Thailand) was analyzed by Colorimetric Method (Brucine) according to standard method for the examination of water and wastewater 14th Edition (APHA, 1975). The nitrate standard curve and %recovery was shown in Figure B.3 and Table B.2.

2.1 Scope and Application

2.1.1 This method is applied to analyze drinking, surface and saline water, domestic and industrial wastes.

2.1.2 The applicable range of concentrations is 0.1 to 2 mg/L NO_3^- -N/liter

2.2 Sample Handling and Preservation

2.2.1 Analysis should be done as soon as possible. If analysis can be made within 24 hours, the sample should be preserved at 4 °C. If sample can be stored more than 24 hours, it should be preserved with sulfuric acid

2.3 Interference

2.3.1 Dissolved organic matter will cause an off color in 13 N H_2SO_4 and must be compensated for by additions of all reagents except the brucine-sulfanilic acid reagent.

2.3.2 Salinity is eliminated by addition of sodium chloride to the blanks, standard and sample.

2.3.3. All strong oxidizing or reducing agents interfere. They may be determined with a total residual chlorine test kit.

2.3.4 Residual chlorine interference is eliminated by the addition of sodium arsenite.

2.4 Reagents

2.4.1 Potassium nitrate stock solution: 1.0 mL = 0.1 mg NO₃-N.

Dissolve 0.7218 g anhydrous potassium nitrate (KNO₃) in distilled water and dilute to 1 liter. This solution is stable for at least 6 months.

2.4.2 Potassium nitrate standard solution: 1.0 mL = 0.001 mg NO₃-N.

Dilute 10.0 mL of the stock solution (2.4.1) to 1 liter. This standard solution should be prepared fresh weekly.

2.4.3 Sodium chloride solution (30%)

Dissolve 300 g NaCl in distilled water and dilute to 1 liter.

2.4.4 4+1 Sulfuric acid solution

Carefully add 500 mL conc. H₂SO₄ to 125 ml distilled water. Cool and keep tightly stoppered to prevent absorption of atmospheric moisture.

2.4.5 Brucine-sulfanilic acid reagent: Dissolve 1 g brucine sulfate [(C₂₃H₂₆N₂O₄)₂ H₂SO₄·7H₂O] and 0.1 g sulfanilic acid (NH₂C₆H₄SO₃H·H₂O) in 70 mL hot distilled water. Add 3 mL conc. HCl, cool, mix and dilute to 100 mL with distilled water. This solution is stable for several months. Note: the pink color that develops slowly does not affect to analyze..

2.5 Procedure

2.5.1 Pipette 10.0 mL of standards and samples into the test tube (Pyrex, Mexico).

2.5.2 Added 2 ml of 30% sodium chloride solution (Analytical grade, Ajax Finechem Pty Ltd, Thailand), to the reagent blank, standards and samples.

2.5.3 Pipette 10.0 mL of 4+1 sulfuric acid solution (AR grade, RCI Labscan, Thailand)

2.5.4 Added 0.5 mL brucine-sulfanilic acid reagent (Himedia, India and Loba chemie, India, respectively) in each test tube and carefully mix by

swirling. Then, the tubes were soaked into the water bath at 95 °C for 20 min. After cooling the sample to room temperature, the sample was measured the absorbance using spectrophotometer at wavelength of 410 nanometer.

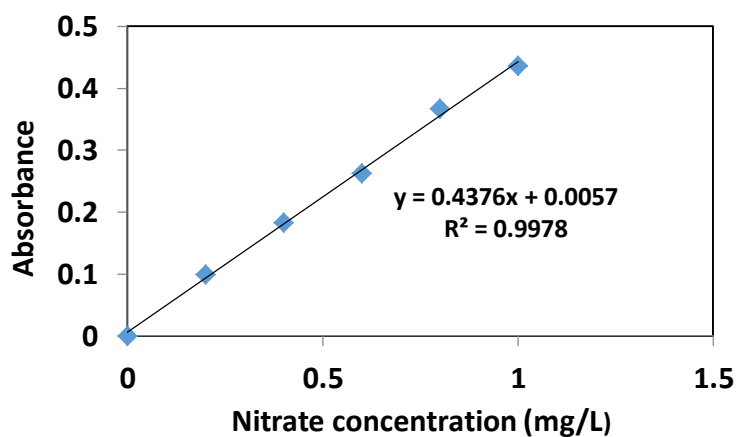


Figure B.3 Nitrate standard curve

Table B.2 Nitrate recovery percentage (%)

Sample	Initial nitrate concentration (mg/L)	Average nitrate concentration (mg/L)	% Recovery
Nitrate (100 mg/L)	84	86.00	86.00
	88		
Nitrate (200 mg/L)	195	189.50	94.75
	184		
Nitrate (300 mg/L)	290	282.50	94.17
	275		

APPENDIX C

Raw data

Results of profenofos degradation under presence of oxygen and nitrate by PF1, PF2 and PF3

Table C.1 Profenofos degradation by PF1 at 10mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	8.690	8.545	8.618	100.00	8.880	8.959	8.959	100.00
1	8.484	8.532	8.508	98.724	6.554	6.554	6.716	75.291
2	8.624	8.543	8.548	99.605	5.911	5.626	5.769	64.675
3	8.422	8.542	8.482	98.422	5.181	5.131	5.156	57.803
4	8.119	8.342	8.231	95.509	4.963	4.669	7.938	53.991
5	7.833	8.043	7.938	92.110	4.271	4.046	4.159	46.626

Table C.2 Profenofos degradation by PF1 at 25mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.079	19.782	19.931	100.00	20.236	20.678	20.457	100.00
1	19.218	18.923	19.071	95.685	17.593	17.751	17.672	86.386
2	19.018	18.923	18.971	95.183	15.169	14.504	14.837	72.528
3	18.670	18.643	18.657	93.608	13.458	12.688	13.073	63.905
4	17.726	18.002	17.864	89.629	12.342	12.130	12.236	59.813
5	16.960	17.892	17.426	87.432	11.709	11.756	11.756	57.354

Table C.3. Profenofos degradation by PF1 at 50mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	37.163	37.002	37.083	100.00	36.684	40.286	38.485	100.00
1	36.403	37.720	37.062	99.943	30.515	31.054	30.785	79.992
2	35.103	36.843	35.973	97.007	24.743	27.136	25.940	67.403
3	34.915	35.832	35.374	95.391	21.383	23.637	22.510	58.490
4	34.604	35.832	35.218	94.971	19.474	20.268	19.871	51.633
5	33.263	32.829	33.046	89.114	16.741	17.598	17.170	44.615

Table C.4 Profenofos degradation by PF1 at 80mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	59.661	60.232	59.947	100.00	60.256	60.194	60.225	100.00
1	58.575	59.782	59.179	98.719	52.953	50.993	51.973	86.298
2	58.691	59.873	59.282	98.891	49.854	47.454	48.654	80.787
3	55.085	56.321	55.703	92.920	46.477	46.348	46.412	77.064
4	51.280	52.384	51.832	86.463	40.244	43.052	41.648	69.154
5	49.788	50.324	50.056	83.500	37.519	36.989	37.254	61.858

Table C.5 Profenofos degradation by PF1 at 100 mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	71.919	72.834	72.377	100.00	72.295	74.833	73.564	71.919
1	64.013	68.432	66.223	91.497	53.054	57.360	55.207	64.013
2	60.184	60.002	60.093	83.028	50.558	53.731	52.144	60.184
3	57.797	58.320	58.059	80.217	44.592	45.401	44.997	57.797
4	54.767	55.893	55.330	76.447	39.186	35.979	35.979	54.767
5	51.651	50.432	51.042	70.522	34.608	33.855	34.232	51.651

Table C.6. Profenofos degradation by PF1 at 150mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	102.494	102.432	102.463	100.00	102.322	102.322	102.653	100.00
1	92.799	95.893	94.346	92.078	80.040	78.012	79.036	76.993
2	87.722	90.342	89.032	86.892	68.364	67.698	68.031	66.273
3	82.034	80.453	81.244	79.291	59.192	59.414	59.303	57.770
4	79.734	80.412	80.073	78.148	53.472	52.862	53.167	51.793
5	76.896	78.903	77.900	76.027	51.351	49.313	50.332	49.031

Table C.7. Profenofos degradation by PF2 at 10mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	8.652	8.598	8.625	100.00	8.160	7.930	8.045	100.00
1	8.319	8.932	8.626	100.012	5.478	7.430	6.454	80.223
2	7.642	8.084	7.863	91.165	4.220	6.995	6.995	69.695
3	7.242	7.584	7.413	85.948	5.301	5.189	5.245	65.196
4	6.995	7.940	7.468	86.586	4.171	4.259	4.215	52.393
5	6.818	7.843	7.331	84.997	3.175	3.066	3.120	38.782

Table C.8 Profenofos degradation by PF2 at 25mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.930	20.782	20.782	100.00	20.230	19.950	20.090	100.00
1	20.306	19.562	19.934	95.579	18.559	18.828	18.693	93.046
2	19.632	18.732	18.732	91.974	15.727	13.522	14.624	72.792
3	17.421	18.932	18.170	87.121	12.447	12.817	12.632	62.877
4	16.826	17.782	17.304	82.969	11.629	11.268	11.448	56.984
5	16.308	17.782	17.045	81.727	10.828	9.704	10.266	51.100

Table C.9. Profenofos degradation by PF2 at 50mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	39.920	38.543	39.232	100.00	39.390	39.140	39.265	100.00
1	38.627	37.784	38.206	97.385	35.570	35.891	35.730	90.997
2	38.026	37.674	37.850	96.477	30.078	34.338	32.208	82.027
3	35.691	36.894	36.293	92.509	28.753	28.033	28.393	72.311
4	35.415	35.637	35.526	90.554	27.045	28.337	27.691	70.523
5	34.853	35.001	34.927	89.027	25.619	28.084	26.851	68.384

Table C.10 Profenofos degradation by PF2 at 80mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	63.490	60.423	61.957	100.00	59.380	60.500	59.940	100.00
1	62.538	60.432	61.485	99.238	60.652	58.445	59.548	99.346
2	62.122	60.432	61.277	98.902	54.712	47.832	51.272	85.539
3	60.463	59.342	59.903	96.685	42.215	40.348	41.281	68.871
4	59.967	59.432	59.700	96.357	38.045	37.399	37.722	62.933
5	59.439	58.432	58.936	95.124	32.992	34.284	33.638	56.119

Table C.11 Profenofos degradation by PF2 at 100 mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	74.922	73.782	74.352	100.00	73.260	75.090	74.175	100.00
1	73.276	72.483	72.880	98.020	58.419	50.306	54.367	73.296
2	71.856	72.335	72.096	96.966	46.147	40.579	43.363	58.460
3	69.172	70.324	69.748	93.808	40.542	36.439	38.490	51.891
4	67.848	69.543	68.696	92.393	32.657	38.036	35.346	47.652
5	67.354	68.432	74.352	91.313	37.118	33.908	35.513	47.877

Table C.12. Profenofos degradation by PF2 at 150mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	108.502	109.543	109.023	100.00	107.015	107.188	107.101	100.00
1	106.567	105.242	105.905	97.140	82.146	80.680	81.413	76.015
2	104.964	105.321	105.143	96.441	77.467	75.483	76.477	71.406
3	100.356	100.432	100.394	92.085	58.612	50.885	54.748	51.118
4	99.124	100.443	99.784	91.526	56.422	57.346	56.884	53.112
5	98.576	97.892	98.234	90.104	54.203	51.578	52.891	49.383

Table C.13. Profenofos degradation by PF3 at 10mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.554	9.465	9.510	100.00	9.632	9.195	9.413	100.00
1	9.391	9.254	9.323	98.034	8.743	8.853	8.798	93.466
2	8.902	9.043	8.973	94.353	7.518	7.684	7.601	80.750
3	8.573	8.854	8.714	91.630	6.049	6.321	6.185	65.707
4	8.179	8.483	8.331	87.603	5.237	5.375	5.306	56.369
5	7.702	8.043	7.873	82.787	4.454	4.086	4.270	45.363

Table C.14. Profenofos degradation by PF3 at 25mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	23.989	22.843	23.416	100.00	23.804	24.500	24.152	100.00
1	21.537	20.523	21.030	89.810	21.010	21.098	21.054	87.173
2	20.228	20.003	20.116	85.907	17.773	17.424	17.598	72.864
3	19.118	19.832	19.475	83.169	16.301	15.057	15.679	64.918
4	20.137	19.432	19.785	84.494	14.474	15.171	14.822	61.370
5	19.622	18.732	19.177	81.897	11.352	11.974	11.663	48.290

Table C.15. Profenofos degradation by PF3 at 50mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	41.848	40.523	41.186	100.00	40.909	40.448	40.678	100.00
1	36.049	38.674	37.362	90.715	31.605	32.256	31.931	78.497
2	36.620	37.843	37.232	90.400	27.747	31.123	29.435	72.361
3	34.798	35.732	35.265	85.624	24.578	28.208	26.393	64.883
4	35.333	35.998	35.666	86.597	26.125	25.531	25.828	63.494
5	34.895	34.266	34.581	83.963	23.668	21.861	22.764	55.961

Table C.16 Profenofos degradation by PF3 at 80mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	66.881	67.893	67.387	100.00	60.622	65.827	63.224	100.00
1	65.540	65.893	65.717	97.522	52.886	54.149	53.517	84.647
2	60.439	60.554	60.497	89.775	44.789	45.081	44.935	71.073
3	55.119	58.902	57.011	84.602	35.591	36.021	35.806	56.634
4	48.558	50.783	49.671	73.710	31.049	29.046	30.047	47.525
5	45.773	48.783	67.387	70.159	25.163	24.866	25.014	39.564

Table C.17 Profenofos degradation by PF3 at 100 mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	74.479	74.783	74.631	100.00	72.068	77.579	74.823	100.00
1	72.098	70.343	71.221	95.431	69.369	66.312	67.840	90.667
2	68.426	70.532	69.479	93.097	54.226	52.625	55.426	74.076
3	65.447	68.793	67.120	89.936	53.097	52.504	52.801	70.568
4	61.458	60.874	61.166	81.958	54.340	55.220	54.780	73.212
5	61.114	60.664	74.631	100.00	47.105	50.591	48.848	65.285

Table C.18. Profenofos degradation by PF3 at 150mg/L under presence of oxygen

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	106.284	110.532	108.408	100.00	113.824	108.423	111.123	100.00
1	106.308	104.853	105.581	97.392	86.041	84.161	85.104	76.585
2	88.780	92.893	90.837	83.792	66.461	66.333	66.397	59.751
3	86.736	89.048	87.892	81.075	57.486	58.372	57.929	52.130
4	81.806	85.932	83.869	77.364	43.251	50.006	46.628	41.961
5	81.150	80.893	81.022	74.738	39.539	38.936	39.237	36.194

Table C.19 Profenofos degradation by PF1 at 10mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.183	8.636	8.910	100.000	8.438	7.833	8.135	100.000
1	8.900	8.516	8.708	97.732	7.877	6.209	7.043	86.580
2	9.035	8.457	8.746	98.159	6.142	6.132	6.137	75.440
3	8.990	8.426	8.708	97.732	5.557	5.423	5.490	67.490
4	7.993	8.317	8.155	91.526	5.073	4.898	4.985	61.280
5	7.559	7.931	7.745	86.924	4.522	4.586	4.554	55.980
6	7.234	7.559	7.397	83.019	4.188	4.610	4.399	54.070
7	7.241	7.616	7.429	83.378	4.002	3.914	3.958	48.650

Table C.20 Profenofos degradation by PF1 at 10mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	8.344	8.132	8.238	100.00	7.967	8.429	8.198	100.00
1	8.275	8.126	8.201	99.551	6.905	7.380	7.143	87.130
2	7.752	7.998	7.87	95.594	6.183	7.017	6.600	80.510
3	7.694	7.862	7.778	94.416	5.884	5.828	5.857	71.440
4	7.479	7.160	7.320	88.857	5.732	5.077	5.404	65.920
5	7.143	7.511	7.327	88.941	4.951	4.391	4.670	56.970
6	6.903	6.756	6.830	82.908	4.341	4.251	4.296	52.40
7	6.471	6.748	6.610	80.238	4.467	3.793	4.130	50.38

Table C.21 Profenofos degradation by PF1 at 10mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	8.393	8.122	8.258	100.00	8.259	8.374	8.317	100.00
1	8.074	7.653	7.864	95.229	6.908	8.009	7.459	89.68
2	7.524	7.683	7.604	92.080	6.312	6.646	6.480	77.91
3	7.344	7.558	7.451	90.228	5.018	5.444	5.231	62.900
4	7.526	7.796	7.661	92.771	5.102	5.086	5.093	61.240
5	7.286	7.402	7.344	88.932	4.833	4.733	4.782	57.500
6	7.008	7.247	7.128	86.316	4.705	4.844	4.774	57.400
7	6.865	7.082	6.974	84.451	4.507	4.167	4.337	52.150

Table C.22 Profenofos degradation by PF1 at 25mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	19.295	19.424	19.360	100.00	19.186	19.134	19.161	100.00
1	18.989	19.428	19.209	99.220	16.147	18.060	17.104	89.260
2	16.203	16.682	16.443	84.933	14.210	14.372	14.291	74.580
3	15.598	15.901	15.750	81.353	13.875	14.147	14.011	73.120
4	15.804	15.755	15.780	81.508	11.437	13.053	12.245	63.910
5	15.801	15.681	15.741	81.307	11.716	11.976	11.846	61.820
6	16.522	16.147	16.335	84.375	11.719	11.514	11.617	60.630
7	16.371	15.504	15.938	82.324	10.732	11.250	10.991	57.360

Table C.23 Profenofos degradation by PF1 at 25mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	18.614	19.025	18.820	100.00	19.052	20.226	19.639	100.00
1	17.670	17.914	17.792	94.538	16.864	17.126	16.995	86.540
2	17.862	17.704	17.783	94.490	14.907	14.714	14.811	75.420
3	17.143	17.047	17.095	90.834	14.571	14.637	14.604	74.360
4	16.692	17.163	16.928	89.947	14.527	13.596	14.601	74.350
5	16.532	16.228	16.380	87.035	13.279	12.636	12.958	65.980
6	15.994	16.124	16.059	85.329	12.149	11.827	11.988	61.040
7	15.803	16.214	16.009	85.064	11.437	11.481	11.459	58.350

Table C.24 Profenofos degradation by PF1 at 25mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.330	19.817	20.074	100.00	20.842	20.127	20.484	100.00
1	20.226	20.011	20.119	100.224	16.030	18.381	17.206	83.997
2	18.753	18.603	18.678	93.046	17.369	15.007	16.188	79.028
3	18.589	18.614	18.602	92.667	14.371	14.944	14.658	71.558
4	16.681	17.301	16.991	84.642	12.056	12.768	12.41	60.594
5	16.558	16.420	16.489	82.214	12.764	12.211	12.487	60.960
6	16.003	15.924	15.960	79.526	11.812	12.503	12.158	59.354
7	15.954	15.651	15.803	78.724	11.504	11.582	11.543	56.351

Table C.25 Profenofos degradation by PF1 at 50mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	36.019	35.774	35.897	100.00	36.406	36.805	36.605	100.00
1	36.866	37.097	36.982	103.023	34.479	34.964	34.722	94.860
2	32.719	33.351	33.035	92.027	31.274	31.159	31.217	85.280
3	33.291	33.502	33.397	93.035	24.862	27.126	25.993	71.010
4	32.339	32.425	32.382	90.208	22.504	21.564	22.034	60.190
5	29.780	30.299	30.040	83.684	19.259	21.089	20.173	55.110
6	29.002	29.353	29.178	81.283	17.492	20.066	18.779	51.300
7	28.825	28.985	28.905	80.522	17.638	18.751	18.194	49.700

Table C.26 Profenofos degradation by PF1 at 50mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	35.862	36.323	36.093	100.00	37.175	36.328	36.752	100.00
1	35.514	33.795	34.655	96.016	35.691	35.102	35.397	96.310
2	33.621	33.887	33.754	93.520	29.399	31.903	30.652	83.400
3	32.595	32.998	32.797	90.868	26.964	26.153	26.559	72.270
4	32.823	33.354	33.089	91.677	24.240	24.240	24.678	67.180
5	31.837	32.033	31.935	88.480	19.320	20.867	20.093	54.670
6	30.068	30.492	30.280	83.894	18.000	18.508	18.254	49.670
7	29.805	29.961	29.883	82.794	16.978	18.373	17.676	48.100

Table C.27 Profenofos degradation by PF1 at 50mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	37.001	37.105	37.053	100.00	39.338	38.383	38.860	100.00
1	36.467	36.796	36.632	98.864	36.173	35.772	35.972	92.570
2	31.191	32.135	31.663	85.453	30.539	30.044	30.292	77.950
3	28.144	29.366	28.755	77.605	26.235	26.235	26.187	67.390
4	28.658	28.418	28.538	77.019	21.992	21.701	21.847	56.220
5	28.019	28.422	28.221	76.164	21.346	21.616	21.481	55.280
6	26.732	27.262	26.997	72.860	19.282	20.413	19.848	51.080
7	26.538	26.911	26.725	72.126	20.109	19.784	19.946	51.330

Table C.28 Profenofos degradation by PF1 at 80mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	58.759	58.541	58.650	100.00	56.852	58.890	57.871	100.00
1	56.633	57.004	56.819	96.878	54.029	52.830	53.430	92.330
2	54.607	54.813	54.710	93.282	49.288	46.136	47.713	82.450
3	54.191	53.906	54.049	92.155	49.737	44.719	47.228	81.610
4	52.654	52.175	52.415	89.369	45.928	40.986	43.457	75.090
5	52.467	52.350	52.409	89.359	35.701	35.211	35.456	61.270
6	51.824	52.289	52.057	88.759	31.056	32.924	31.990	55.280
7	49.029	48.708	48.869	83.323	24.921	27.073	25.997	44.920

Table C.29 Profenofos degradation by PF1 at 80mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	62.628	62.821	62.725	100.00	61.951	60.627	61.289	100.00
1	62.409	62.281	62.345	99.394	54.411	53.576	53.993	88.100
2	57.661	57.432	57.547	91.745	51.013	48.499	49.756	81.180
3	54.122	54.581	54.352	86.651	46.405	47.906	47.155	76.940
4	51.539	51.336	51.438	82.006	43.669	40.456	42.063	68.630
5	50.771	52.720	51.746	82.497	44.041	38.628	41.335	67.440
6	50.552	50.639	50.596	80.663	38.492	38.432	38.462	62.760
7	48.218	48.311	48.265	76.947	33.764	35.119	34.442	56.200

Table C.30 Profenofos degradation by PF1 at 80 mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	61.607	61.486	61.547	100.00	58.205	59.620	58.913	100.00
1	61.125	61.040	61.083	99.246	51.920	54.926	53.423	90.680
2	58.147	57.971	58.059	94.332	45.557	50.187	47.872	81.26
3	57.532	57.242	57.387	93.240	47.667	46.842	47.255	80.210
4	52.944	52.774	52.859	85.884	43.593	44.356	43.974	74.640
5	52.562	52.441	52.502	85.304	41.717	43.704	42.710	72.500
6	51.116	50.435	50.776	82.499	36.041	38.622	37.331	63.370
7	48.839	48.625	48.732	79.179	34.112	34.536	34.324	58.270

Table C.31 Profenofos degradation by PF1 at 100mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	73.441	73.214	73.328	100.00	80.580	75.614	78.097	100.00
1	73.798	73.023	73.411	99.887	72.151	77.356	74.753	95.720
2	70.944	70.529	70.737	96.467	61.755	70.247	66.001	84.510
3	69.215	68.828	69.022	94.128	56.609	68.644	62.62	80.190
4	69.544	69.506	69.525	94.814	57.290	53.376	55.333	70.850
5	69.176	68.994	69.085	94.214	47.724	49.612	48.667	62.320
6	68.064	67.855	67.960	92.679	41.770	42.088	41.929	53.690
7	67.493	67.855	67.674	92.289	39.057	40.424	39.740	50.890

Table C.32 Profenofos degradation by PF1 at 100mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	83.731	84.409	84.070	100.00	73.663	75.588	74.625	100.00
1	82.384	81.918	82.151	97.717	67.927	77.161	72.544	97.210
2	78.900	78.620	78.760	93.683	70.094	70.990	70.542	94.530
3	76.160	75.687	75.924	90.310	66.721	61.239	63.980	85.740
4	74.138	74.783	74.461	88.570	58.805	60.022	59.413	79.620
5	71.179	72.752	71.966	85.602	50.403	52.670	51.536	69.060
6	65.243	70.644	67.944	80.818	50.380	45.372	47.876	64.160
7	63.621	66.719	65.170	77.519	49.596	45.803	47.700	63.920

Table C.33 Profenofos degradation by PF1 at 100mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	80.362	81.082	80.722	100.00	76.868	75.548	76.208	100.00
1	78.869	79.658	79.264	97.717	74.870	73.198	74.034	97.150
2	71.978	74.347	73.163	93.683	64.638	71.274	67.956	89.170
3	70.006	70.248	70.127	90.310	68.812	69.069	68.940	90.460
4	69.165	69.733	69.449	88.570	57.991	57.615	57.803	75.850
5	68.721	68.868	68.795	85.602	57.298	54.145	55.721	73.120
6	67.935	69.018	68.477	80.818	56.490	52.085	54.287	71.240
7	67.098	67.788	67.443	77.519	55.565	51.444	53.504	70.210

Table C.34 Profenofos degradation by PF1 at 150mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	110.140	109.853	109.997	100.00	110.140	106.760	108.450	100.00
1	106.142	106.317	106.230	96.575	94.094	98.753	96.423	88.910
2	101.707	102.063	101.885	92.625	76.087	71.277	73.682	67.940
3	99.622	99.918	99.770	90.702	74.610	72.684	73.647	67.910
4	98.314	99.077	98.696	89.726	73.445	67.458	70.452	64.960
5	97.130	97.789	97.460	88.602	65.573	63.367	64.470	59.450
6	93.999	94.646	94.323	85.750	64.905	60.772	62.838	57.940
7	89.392	89.103	89.248	81.137	64.079	48.956	56.517	52.110

Table C.35 Profenofos degradation by PF1 at 150mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	109.098	108.693	108.896	100.00	109.676	109.092	109.384	100.00
1	104.902	105.260	105.081	96.497	97.232	94.610	95.921	87.690
2	102.197	101.668	101.933	93.606	78.629	83.674	81.151	74.180
3	100.261	100.448	100.355	92.157	78.166	67.487	72.826	66.570
4	94.613	96.061	95.337	87.549	63.871	71.031	67.451	61.660
5	93.788	94.401	94.095	86.408	64.502	65.947	65.224	59.620
6	90.080	88.952	89.516	82.203	63.104	62.239	62.671	57.290
7	82.492	83.735	83.114	76.324	63.513	59.052	61.282	56.020

Table C.36 Profenofos degradation by PF1 at 150mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	106.814	105.981	106.398	100.00	104.558	105.867	105.212	100.00
1	103.878	103.434	103.656	97.423	102.000	101.673	101.836	96.790
2	100.218	100.472	100.345	94.311	93.073	88.981	91.027	86.510
3	96.207	97.736	96.972	91.141	79.912	89.895	84.903	80.690
4	94.881	96.406	95.644	89.893	74.813	74.620	74.716	71.010
5	93.005	94.529	93.767	88.129	70.523	71.901	71.212	67.680
6	92.775	92.120	92.448	86.889	66.412	59.212	62.812	59.700
7	89.518	89.813	89.666	84.274	63.653	59.504	61.578	58.520

Table C.37 Profenofos degradation by PF2 at 10mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.130	9.510	9.320	100.000	8.886	8.660	8.773	100.000
1	8.463	8.416	8.440	90.560	6.667	6.945	6.806	77.578
2	7.728	7.601	7.665	82.242	4.853	5.489	5.171	58.942
3	6.397	6.481	6.439	69.088	3.032	3.239	3.135	35.734
4	5.490	5.938	5.714	61.309	2.433	2.224	2.337	26.638
5	4.988	5.659	5.324	57.124	2.169	2.156	2.162	24.643
6	5.096	5.504	5.300	56.867	2.071	1.921	2.00	22.797
7	4.739	5.096	4.918	49.175	1.600	2.083	1.841	20.984

Table C.38 Profenofos degradation by PF2 at 10mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.091	9.253	9.172	100.000	8.919	8.424	8.672	100.000
1	8.735	8.813	8.774	95.661	7.383	7.938	7.661	87.704
2	8.892	8.728	8.810	96.053	7.381	7.735	7.558	87.154
3	8.651	8.606	8.629	94.080	5.079	5.469	5.274	60.816
4	8.034	8.211	8.126	88.596	4.364	4.722	4.543	52.387
5	7.062	7.267	7.165	78.119	3.797	2.912	3.355	38.687
6	6.935	7.102	7.019	76.526	3.413	3.310	3.362	38.768
7	6.605	6.773	6.689	72.928	2.588	2.931	2.760	31.826

Table C.39 Profenofos degradation by PF2 at 10mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.130	9.510	9.320	100.000	8.886	8.660	8.773	100.000
1	8.463	8.416	8.440	90.560	6.667	6.945	6.806	77.578
2	7.728	7.601	7.665	82.242	4.853	5.489	5.171	58.942
3	6.397	6.481	6.439	69.088	3.032	3.239	3.135	35.734
4	5.490	5.938	5.714	61.309	2.433	2.224	2.337	26.638
5	4.988	5.659	5.324	57.124	2.169	2.156	2.162	24.643
6	5.096	5.504	5.300	56.867	2.071	1.921	2.00	22.797
7	4.739	5.096	4.918	49.175	1.600	2.083	1.841	20.984

Table C.40 Profenofos degradation by PF2 at 25 mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.276	20.204	20.240	100.000	19.367	19.529	19.448	100.000
1	19.465	19.757	19.611	96.892	13.452	13.975	13.714	70.449
2	18.539	18.867	18.703	92.406	11.736	11.016	11.376	58.494
3	16.912	17.834	17.373	85.835	9.988	9.890	9.939	51.105
4	15.281	16.057	15.669	77.416	8.435	8.893	8.664	44.549
5	14.344	14.229	14.287	70.588	7.030	7.755	7.393	38.014
6	15.641	15.656	15.649	77.317	7.620	7.589	7.605	39.104
7	14.796	15.140	14.968	73.953	7.012	7.244	7.128	36.651

Table C.41 Profenofos degradation by PF2 at 25 mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	19.268	19.384	19.326	100.00	19.514	19.544	19.529	100.00
1	16.654	17.910	17.282	89.423	12.118	13.359	12.739	65.231
2	18.120	17.177	17.649	91.322	10.331	11.414	10.872	55.671
3	17.331	16.657	16.994	87.933	8.133	10.134	9.134	46.771
4	16.265	16.024	16.144	83.535	9.439	8.724	9.082	46.505
5	15.855	15.485	15.670	81.082	7.958	8.035	7.997	40.949
6	15.520	15.284	15.402	79.696	7.553	6.820	7.187	36.801
7	15.301	15.158	15.230	78.806	7.367	6.718	7.043	36.064

Table C.42 Profenofos degradation by PF2 at 25mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	22.562	21.018	21.790	100.00	19.914	20.339	20.127	100.00
1	17.453	17.854	17.654	81.019	11.557	13.021	12.289	61.057
2	15.285	15.843	15.564	71.427	12.250	10.873	11.561	57.440
3	14.552	15.030	14.791	67.879	10.637	11.182	10.910	54.205
4	14.379	14.416	14.398	66.076	10.198	10.600	10.399	51.666
5	12.949	13.059	13.004	59.679	8.810	8.722	8.766	43.553
6	12.586	12.662	12.624	57.934	8.058	7.825	7.942	39.459
7	12.331	12.158	12.245	56.196	7.658	7.368	7.513	37.327

Table C.43 Profenofos degradation by PF2 at 50mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	37.158	37.124	37.141	100.00	36.121	35.234	35.677	100.00
1	33.632	37.099	35.366	95.221	27.397	28.029	27.713	77.677
2	31.914	31.488	31.701	85.353	24.507	23.356	23.931	67.076
3	31.350	31.005	31.178	83.945	21.529	20.521	21.025	58.931
4	29.550	29.460	29.505	79.441	20.022	19.721	19.872	55.699
5	29.241	28.980	29.111	78.379	17.804	17.868	17.836	49.992
6	29.257	29.131	29.194	78.603	15.968	14.977	15.473	43.369
7	28.998	29.030	29.014	78.119	14.172	15.410	14.791	41.458

Table C.44 Profenofos degradation by PF2 at 50mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	36.013	35.940	35.977	100.00	35.750	36.084	35.917	100.00
1	37.472	37.269	37.371	103.875	26.203	26.989	26.596	74.048
2	35.538	35.310	35.424	98.463	19.251	21.499	20.375	56.728
3	35.064	34.655	34.860	96.895	18.738	21.197	19.968	55.594
4	34.364	34.139	34.252	95.205	16.133	16.946	16.539	46.047
5	33.681	33.400	34.541	96.010	14.115	14.278	14.197	39.527
6	31.526	31.759	31.643	87.95	12.644	12.106	12.375	34.454
7	31.244	31.167	31.206	86.739	11.540	12.190	11.865	33.034

Table C.45 Profenofos degradation by PF2 at 50mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	39.307	37.315	38.311	100.00	35.117	35.615	35.366	100.00
1	38.501	36.878	37.690	98.379	27.208	28.664	27.936	78.991
2	36.140	35.564	35.852	93.581	23.526	26.729	25.128	71.051
3	35.312	35.083	35.198	91.874	21.327	22.284	21.806	61.658
4	34.319	33.946	34.132	89.092	19.901	20.932	20.417	57.730
5	32.595	32.444	32.520	84.884	17.652	19.042	18.347	51.877
6	32.927	32.810	32.869	85.795	18.222	18.572	18.397	52.018
7	32.672	32.585	32.629	85.169	16.840	17.194	17.017	48.116

Table C.46 Profenofos degradation by PF2 at 80 mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	59.644	59.406	59.525	100.00	62.407	59.983	61.195	100.00
1	54.137	56.575	55.356	92.996	57.276	54.631	55.954	91.435
2	51.391	50.836	51.114	85.870	49.807	48.907	49.357	80.655
3	50.668	50.472	50.570	84.956	44.251	47.015	45.633	74.569
4	46.180	46.066	46.12	77.485	45.437	45.802	45.620	74.548
5	45.692	45.455	45.574	76.563	40.866	42.040	41.453	67.739
6	44.690	44.466	44.578	74.890	39.077	39.555	39.316	64.247
7	44.141	44.068	44.105	74.095	38.376	35.508	36.942	60.367

Table C.47 Profenofos degradation by PF2 at 80 mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	58.846	58.723	58.785	100.00	60.003	60.583	60.293	100.00
1	55.719	56.189	55.954	95.184	53.085	53.209	53.147	88.147
2	49.320	49.112	49.216	83.722	40.358	44.676	42.517	70.517
3	50.236	49.930	50.083	85.197	36.348	37.080	36.714	60.892
4	46.271	47.648	46.960	79.884	33.353	33.157	33.255	55.155
5	45.673	45.808	45.741	77.811	31.848	31.568	31.708	52.583
6	44.191	44.078	44.135	75.079	30.312	30.888	30.600	50.752
7	43.239	43.623	43.431	73.881	28.310	27.850	28.080	46.582

Table C.48 Profenofos degradation by PF1 at 80 mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	60.901	60.785	60.843	100.00	59.179	62.328	60.754	100.00
1	57.746	58.111	57.92	95.211	53.938	54.619	54.279	89.342
2	50.478	51.717	51.098	83.983	52.078	50.866	51.472	84.721
3	48.725	49.039	48.882	80.341	45.855	49.307	47.581	78.317
4	46.945	47.366	47.156	77.504	49.109	44.488	46.799	77.030
5	46.541	46.423	46.482	76.397	41.851	42.362	42.107	69.307
6	44.302	44.350	44.326	72.853	38.565	34.536	36.551	60.162
7	41.980	42.808	42.394	69.678	31.738	32.131	31.935	52.564

Table C.49 Profenofos degradation by PF2 at 100mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	71.937	72.565	72.251	100.00	69.955	70.034	69.995	100.00
1	68.528	69.113	68.821	95.253	54.863	56.547	55.705	79.584
2	60.958	63.212	62.085	85.930	50.243	53.019	51.631	73.763
3	58.412	59.140	58.776	81.350	40.243	45.821	43.032	61.478
4	57.446	57.646	57.546	79.647	34.188	29.100	31.644	45.208
5	53.338	54.937	54.138	74.930	33.023	29.031	31.027	44.327
6	52.693	52.912	52.803	73.083	26.964	31.175	29.070	41.531
7	48.023	50.001	49.012	67.836	26.414	27.829	27.122	38.748

Table C.50 Profenofos degradation by PF2 at 100mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	69.820	70.431	70.126	100.00	72.406	72.981	72.694	100.00
1	66.546	67.544	67.045	95.606	53.357	52.866	53.112	73.062
2	62.586	64.110	63.348	90.335	47.967	44.508	46.238	63.606
3	57.961	59.123	58.542	83.481	47.703	40.680	44.192	60.791
4	62.921	59.857	61.389	87.541	38.485	30.580	34.533	47.504
5	57.900	58.562	58.231	83.038	35.179	29.181	32.180	44.168
6	50.655	53.876	52.266	74.532	28.352	28.693	28.523	39.237
7	45.601	46.687	46.144	65.802	28.284	25.295	26.790	36.853

Table C.51 Profenofos degradation by PF2 at 100mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	72.932	73.357	73.145	100.00	72.348	69.847	71.098	100.00
1	71.521	71.852	71.687	98.007	54.486	55.993	55.240	77.695
2	64.230	65.325	64.778	88.561	50.497	54.297	52.397	73.696
3	62.777	63.005	62.891	85.981	46.149	51.567	48.858	68.719
4	57.945	61.697	59.821	81.784	31.799	33.579	32.689	45.977
5	56.958	57.408	57.183	78.178	26.077	26.554	26.316	37.013
6	55.048	54.930	54.989	75.178	25.343	27.422	26.383	37.107
7	52.934	52.815	52.875	72.288	26.637	25.086	25.862	36.375

Table C.52 Profenofos degradation by PF2 at 150mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	112.192	112.897	112.545	100.00	110.405	112.265	111.335	100.00
1	101.723	102.645	102.184	90.794	103.730	96.230	99.980	89.801
2	95.651	94.848	95.250	84.633	92.839	101.918	97.379	87.464
3	83.382	86.713	85.048	75.568	75.620	78.416	77.018	69.176
4	80.778	82.868	81.823	72.702	56.932	70.287	63.610	57.133
5	76.863	78.511	77.687	69.028	55.959	63.993	59.976	53.869
6	75.358	75.030	75.194	66.812	44.593	46.488	45.541	40.904
7	71.476	72.757	72.117	64.078	43.138	45.318	44.228	39.725

Table C.53 Profenofos degradation by PF21 at 150mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	107.019	106.166	106.593	100.00	104.266	109.073	106.670	100.00
1	103.550	103.901	103.726	97.310	96.257	96.309	96.283	90.262
2	101.147	99.952	100.550	94.331	91.648	93.851	92.750	86.950
3	92.750	93.739	93.245	87.478	75.965	76.623	76.294	71.523
4	84.921	86.928	85.925	80.610	67.748	68.519	68.134	63.873
5	85.018	84.869	84.944	79.690	66.021	60.863	63.442	59.475
6	83.860	83.753	83.807	78.623	57.442	59.117	58.280	54.635
7	82.487	83.366	82.927	77.798	57.973	55.841	56.907	53.348

Table C.54 Profenofos degradation by PF2 at 150mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	110.443	107.284	108.863	100.00	104.299	109.502	106.901	100.00
1	107.536	106.081	106.809	98.113	100.531	105.632	103.082	96.427
2	98.815	97.920	98.368	90.359	94.572	90.237	92.405	86.439
3	98.815	96.876	97.846	89.880	81.887	85.437	83.662	78.261
4	97.408	96.562	96.985	89.089	79.559	77.980	78.770	73.684
5	93.959	94.861	94.410	86.724	66.683	78.503	72.593	67.906
6	85.711	87.083	86.397	79.363	65.478	78.480	71.979	67.332
7	84.327	83.595	83.961	77.125	62.164	62.421	62.293	58.271

Table C.55 Profenofos degradation by PF3 at 10mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	10.156	9.460	9.808	100.00	9.759	8.934	9.347	100.00
1	9.264	8.847	9.056	92.333	8.059	7.967	8.013	85.780
2	8.986	8.813	8.900	90.742	8.183	7.707	7.945	85.000
3	8.798	8.608	8.703	88.734	7.334	7.247	7.291	78.004
4	7.953	8.196	8.075	82.331	5.824	7.009	6.412	68.600
5	8.188	8.235	8.212	83.728	4.411	5.143	4.777	51.107
6	8.397	8.189	8.293	84.553	4.617	4.171	4.394	47.010
7	8.155	7.994	8.075	82.331	8.155	7.994	4.549	48.668

Table C.56 Profenofos degradation by PF3 at 10mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.372	8.983	9.178	100.00	9.302	8.948	9.125	100.00
1	9.131	8.886	9.009	98.159	8.570	8.070	8.320	91.178
2	8.840	9.029	8.935	97.352	8.125	8.070	8.098	88.745
3	8.805	8.716	8.761	95.457	6.958	7.181	7.070	77.479
4	8.563	8.385	8.474	92.329	6.738	7.724	7.231	79.244
5	8.465	8.318	8.392	91.436	6.308	7.617	6.963	76.307
6	8.733	7.997	8.365	91.142	5.972	5.556	5.764	63.167
7	8.222	7.804	8.013	87.307	4.341	4.955	4.648	50.937

Table C.57 Profenofos degradation by PF3 at 10mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	9.322	9.057	9.190	100.00	9.598	8.990	9.294	100.00
1	9.298	9.116	9.207	100.184	8.256	7.237	7.747	83.355
2	9.122	8.681	8.902	96.866	7.770	8.321	8.046	86.572
3	9.020	8.673	8.847	96.268	7.532	7.991	7.762	83.516
4	8.783	8.953	8.868	96.496	7.515	7.800	7.658	82.397
5	8.644	8.647	8.646	94.081	6.247	5.329	5.788	62.228
6	8.330	8.636	8.483	92.307	5.182	4.566	4.874	52.442
7	5.182	4.566	8.340	90.751	4.552	4.376	4.464	48.031

Table C.58 Profenofos degradation by PF3 at 25 mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.687	20.565	20.626	100.00	22.666	20.511	21.589	100.00
1	20.685	20.490	20.58	99.816	20.665	21.071	20.868	96.660
2	20.426	20.315	20.371	98.764	19.565	19.859	19.712	91.306
3	21.596	21.398	21.497	104.222	17.469	16.548	17.009	78.785
4	20.387	19.820	20.104	97.469	14.814	16.942	15.878	73.547
5	20.156	19.955	20.056	97.236	14.882	16.328	15.605	72.282
6	20.592	20.275	20.434	99.069	15.073	15.067	15.070	69.804
7	20.400	20.183	20.292	98.381	12.966	13.655	13.311	61.656

Table C.59 Profenofos degradation by PF3 at 25 mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.704	20.831	20.768	100.00	22.798	22.511	22.655	100.00
1	20.480	20.662	20.571	99.051	21.642	19.277	20.460	90.311
2	21.175	20.900	21.038	101.300	18.464	20.284	19.374	85.518
3	21.108	20.533	20.821	100.255	18.076	18.005	19.305	85.213
4	20.003	19.420	19.712	94.915	14.319	15.019	14.669	64.750
5	20.035	18.795	19.415	93.485	14.319	14.956	14.638	64.613
6	19.367	19.324	19.346	93.153	14.578	14.323	14.451	63.787
7	20.066	19.527	19.797	95.325	15.108	13.312	14.210	62.723

Table C.60 Profenofos degradation by PF3 at 25mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	20.981	20.944	20.963	100.00	20.914	20.590	20.752	100.00
1	20.667	20.530	20.599	98.264	19.970	20.418	20.194	97.311
2	20.274	19.659	19.967	95.249	19.199	19.567	19.383	93.403
3	20.343	20.042	20.193	96.327	17.572	17.376	17.474	84.204
4	19.096	19.537	19.317	92.148	15.744	15.402	15.573	75.043
5	19.659	19.184	19.422	92.649	15.329	14.483	14.906	71.829
6	19.820	19.531	19.676	93.861	15.447	16.916	16.182	77.978
7	19.625	19.277	19.451	92.787	15.507	16.037	15.772	76.002

Table C.61 Profenofos degradation by PF3 at 50mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	46.092	46.290	46.191	100.00	45.110	46.128	45.619	100.00
1	46.402	45.611	46.007	99.602	41.779	40.881	41.330	90.598
2	46.033	46.883	46.458	100.578	40.933	36.209	38.571	84.550
3	42.557	43.741	43.149	93.414	36.360	35.545	35.953	78.811
4	41.278	41.505	41.392	89.610	31.781	33.910	32.846	72.000
5	37.915	41.808	39.862	86.298	29.519	32.410	30.965	67.877
6	38.404	38.521	38.463	83.269	27.830	29.374	28.602	62.698
7	38.294	38.139	38.217	82.737	26.335	25.566	25.951	56.886

Table C.62 Profenofos degradation by PF3 at 50mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	40.566	40.704	40.635	100.00	44.261	45.438	44.850	100.00
1	40.566	40.704	38.003	93.522	44.261	45.438	41.771	93.135
2	40.981	40.641	40.811	100.433	41.835	37.895	39.865	88.885
3	38.484	40.386	39.435	97.047	40.899	39.294	40.097	89.402
4	38.571	38.064	38.318	94.298	33.801	34.489	34.145	76.132
5	37.798	37.711	37.755	92.913	34.333	33.561	33.947	75.690
6	38.463	40.432	39.448	97.079	32.795	34.738	33.767	75.289
7	38.270	38.317	38.294	94.239	30.714	31.083	30.899	68.894

Table C.63 Profenofos degradation by PF3 at 50mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	44.373	43.833	44.103	100.00	41.652	42.814	42.233	100.00
1	44.135	44.514	44.325	100.503	39.263	39.024	39.144	92.686
2	44.412	43.930	44.171	100.154	39.867	37.961	38.914	92.141
3	44.721	44.278	44.500	100.900	38.330	38.006	38.168	90.375
4	40.937	42.114	41.526	94.157	35.218	33.776	34.497	81.683
5	38.415	38.653	38.534	87.372	33.760	34.601	34.181	80.934
6	38.321	39.575	38.948	88.311	33.022	34.819	33.921	80.319
7	37.837	38.306	38.072	86.325	31.877	31.404	31.641	74.920

Table C.64 Profenofos degradation by PF3 at 80mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	71.003	71.451	71.227	100.00	73.299	69.452	71.376	100.00
1	69.503	69.315	69.409	97.448	66.699	70.670	68.685	96.230
2	65.397	66.277	65.837	92.433	62.312	61.846	62.079	86.975
3	63.617	63.957	63.787	89.554	57.252	55.225	56.239	78.793
4	61.768	62.449	62.109	87.199	48.802	53.228	51.015	71.474
5	61.812	62.252	62.032	87.091	49.969	47.766	48.868	68.466
6	61.914	62.858	62.386	87.588	48.253	47.532	47.893	67.100
7	61.092	60.561	60.827	85.399	45.419	46.159	45.789	64.152

Table C.65 Profenofos degradation by PF3 at 80mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	70.397	70.461	70.429	100.00	66.971	67.215	67.093	100.00
1	68.802	69.182	68.992	97.860	66.061	69.151	67.606	100.765
2	65.004	66.195	65.600	93.143	62.531	61.708	62.120	92.588
3	63.292	63.532	63.412	90.037	58.162	58.869	58.516	87.216
4	61.336	61.890	61.613	87.482	49.035	56.442	52.739	78.606
5	60.139	60.138	60.139	85.390	50.167	46.668	55.153	82.204
6	56.154	57.107	56.631	80.409	48.646	49.700	49.173	73.291
7	53.787	54.822	54.305	77.106	46.474	49.098	47.786	71.223

Table C.66 Profenofos degradation by PF3 at 80mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	66.898	67.294	67.096	100.00	68.677	66.751	67.714	100.00
1	66.735	64.437	65.586	97.749	63.341	66.467	64.904	95.850
2	64.341	64.420	64.381	95.953	63.632	61.454	62.543	92.363
3	64.182	63.815	63.999	95.384	61.332	60.864	61.098	90.229
4	64.480	71.778	68.129	101.540	60.016	58.239	59.128	87.320
5	63.003	62.945	62.974	93.857	58.920	57.281	58.101	85.803
6	62.563	63.328	62.945	93.813	61.413	54.210	57.812	85.377
7	62.760	62.566	62.663	93.393	59.422	58.319	58.871	86.940

Table C.67 Profenofos degradation by PF3 at 100mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	90.616	87.811	89.214	100.00	89.218	89.881	89.550	100.00
1	90.875	90.375	90.625	101.582	88.045	82.209	85.127	95.419
2	89.761	87.142	88.452	99.146	81.517	80.782	81.150	90.620
3	87.653	86.904	87.279	97.831	79.539	76.782	78.161	87.282
4	88.173	87.381	87.777	98.389	68.049	72.826	70.438	78.658
5	86.657	86.186	86.422	96.870	66.604	71.162	68.883	76.921
6	85.644	85.441	85.543	95.885	66.115	69.570	67.842	75.759
7	82.738	82.241	82.490	92.463	63.523	62.176	62.850	70.184

Table C.68 Profenofos degradation by PF3 at 100mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	92.442	92.760	92.601	100.00	90.769	89.575	90.172	92.442
1	93.503	92.587	93.045	100.479	86.560	88.836	87.698	93.503
2	88.747	88.897	88.822	95.919	77.620	80.651	79.136	88.747
3	86.913	86.499	86.706	93.634	75.907	76.987	76.477	86.913
4	84.974	84.469	84.722	91.491	64.973	67.016	65.995	84.974
5	84.733	84.310	84.522	91.275	66.777	63.402	65.090	84.733
6	83.662	83.328	83.495	90.166	65.349	62.491	63.920	83.662
7	81.389	82.009	81.699	88.227	61.883	62.271	62.077	81.389

Table C.69 Profenofos degradation by PF3 at 100mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	90.180	86.791	88.486	100.00	87.586	85.495	86.541	90.180
1	88.125	87.263	87.694	99.105	85.495	82.773	84.134	88.125
2	88.506	88.384	88.445	99.954	83.022	83.988	83.505	88.506
3	87.764	87.842	87.803	99.228	76.701	78.704	77.703	87.764
4	86.396	85.945	86.171	97.384	71.222	74.679	72.951	86.396
5	84.429	84.158	84.294	95.263	65.736	67.093	66.415	84.429
6	83.760	82.623	83.192	94.017	64.155	62.871	63.513	83.760
7	83.091	82.936	83.014	93.816	62.108	63.319	62.714	83.091

Table C.70 Profenofos degradation by PF3 at 150mg/L under presence of nitrate (100 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	113.486	112.781	113.134	100.00	110.412	110.689	110.551	100.00
1	113.040	112.270	112.655	99.577	107.558	111.449	109.504	99.053
2	112.858	112.426	109.504	96.791	104.577	104.396	104.487	94.515
3	107.154	106.763	106.959	94.542	96.532	98.622	97.577	88.264
4	104.872	104.713	104.793	92.627	91.141	89.441	90.291	81.674
5	100.033	98.865	99.44	87.904	86.195	88.575	87.385	79.045
6	98.809	98.275	98.542	87.102	83.459	83.296	83.378	75.420
7	97.514	97.130	97.322	86.024	78.181	78.371	78.276	70.805

Table C.71 Profenofos degradation by PF3 at 150mg/L under presence of nitrate (200 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	110.481	109.902	110.192	100.00	105.371	106.044	105.708	100.00
1	111.426	110.459	110.943	100.682	107.399	109.001	108.200	102.357
2	111.355	110.884	111.120	100.842	103.503	103.817	103.660	98.063
3	108.604	108.169	108.387	98.362	91.779	95.211	93.495	88.446
4	108.315	109.112	108.714	98.659	89.911	89.020	89.466	84.635
5	108.224	107.943	108.084	98.087	89.172	87.790	88.481	83.703
6	102.077	101.562	101.820	92.402	88.222	87.586	87.904	83.157
7	98.243	98.826	98.535	89.666	86.773	86.837	86.805	82.118

Table C.72 Profenofos degradation by PF3 at 150mg/L under presence of nitrate (300 mg/L)

Day	Control				Test			
	1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	104.402	105.007	104.705	100.00	104.948	101.064	103.006	100.00
1	105.623	105.363	105.493	100.752	102.511	106.927	104.719	101.663
2	109.137	105.078	107.108	102.295	101.253	101.028	101.141	98.189
3	107.351	105.907	106.629	101.838	95.109	95.723	95.416	92.631
4	103.516	103.662	103.589	98.934	90.623	90.456	90.540	87.898
5	103.343	102.897	103.120	98.486	87.886	89.740	88.813	86.221
6	99.146	98.788	98.967	94.520	82.020	83.005	82.513	80.105
7	97.426	97.699	97.563	93.179	82.980	82.899	82.940	80.520

Results of microbial growth under presence of oxygen and nitrate

Table C.73 Cell number of PF1 at 10mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.80E+04	1.00E+04	1.40E+04
1	4.50E+06	4.80E+06	4.65E+06
2	1.50E+06	1.80E+06	1.65E+06
3	1.20E+07	1.10E+07	1.15E+07
4	1.00E+07	1.50E+07	1.25E+07
5	6.00E+06	1.10E+07	8.50E+06

Table C.74 Cell number of PF1 at 25mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.40E+04	1.90E+04	2.15E+04
1	3.90E+06	3.30E+06	3.60E+06
2	8.00E+05	1.10E+06	9.50E+05
3	8.00E+06	4.00E+06	6.00E+06
4	7.00E+06	1.00E+07	8.50E+06
5	1.00E+07	7.00E+06	8.50E+06

Table C.75 Cell number of PF1 at 50mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.00E+04	1.50E+04	1.75E+04
1	3.50E+04	3.20E+04	3.35E+04
2	1.40E+07	1.70E+07	1.55E+07
3	1.80E+07	1.60E+07	1.70E+07
4	1.10E+07	1.60E+07	1.35E+07
5	7.00E+06	1.00E+07	8.50E+06

Table C.76 Cell number of PF1 at 80mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.80E+04	1.30E+04	1.55E+04
1	1.20E+06	1.40E+06	1.30E+06
2	1.80E+07	2.20E+07	2.00E+07
3	2.00E+07	2.10E+07	2.05E+07
4	1.40E+07	1.10E+07	1.25E+07
5	1.60E+07	1.50E+07	1.55E+07

Table C.77 Cell number of PF1 at 100 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.90E+04	2.00E+04	1.95E+04
1	2.60E+07	2.40E+07	2.50E+07
2	2.80E+07	2.40E+07	2.60E+07
3	1.50E+07	2.00E+07	1.50E+07
4	7.00E+06	1.20E+07	1.90E+07
5	1.60E+07	1.50E+07	3.10E+07

Table C.78 Cell number of PF1 at 150mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.10E+04	2.30E+04	2.20E+04
1	1.80E+07	2.20E+07	2.00E+07
2	2.60E+07	3.00E+07	2.80E+07
3	7.00E+07	6.00E+07	6.50E+07
4	6.00E+07	4.00E+07	5.00E+07
5	2.00E+07	1.00E+07	1.50E+07

Table C.79 Cell number of PF2 at 10mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	4.80E+04	4.60E+04	4.70E+04
1	3.50E+04	3.70E+04	3.60E+04
2	1.20E+06	1.00E+06	1.10E+06
3	1.60E+07	1.40E+07	1.50E+07
4	6.00E+06	4.00E+06	5.00E+06
5	1.50E+07	1.60E+07	1.55E+07

Table C.80 Cell number of PF2 at 25 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.80E+04	3.10E+04	2.95E+04
1	6.20E+06	6.40E+06	6.30E+06
2	2.30E+07	2.10E+07	2.20E+07
3	2.30E+08	2.60E+08	2.45E+08
4	1.20E+08	1.00E+08	1.10E+08
5	1.60E+08	1.30E+08	1.45E+08

Table C.81 Cell number of PF2 at 50mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	4.90E+04	5.10E+04	5.00E+04
1	2.50E+06	2.70E+06	2.60E+06
2	3.40E+07	3.50E+07	3.45E+07
3	1.60E+08	1.60E+08	1.60E+08
4	9.00E+07	1.10E+08	1.00E+08
5	7.00E+07	5.00E+07	6.00E+07

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Table C.82 Cell number of PF2 at 80mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.60E+04	2.30E+04	2.45E+04
1	1.00E+05	8.00E+04	9.00E+04
2	4.00E+06	2.00E+06	3.00E+06
3	1.20E+07	1.50E+07	1.35E+07
4	6.00E+06	4.00E+06	5.00E+06
5	1.00E+07	9.00E+06	9.50E+06

Table C.83 Cell number of PF2 at 100 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.80E+04	1.60E+04	1.70E+04
1	5.00E+04	7.00E+04	6.00E+04
2	1.90E+07	1.80E+07	1.85E+07
3	1.00E+08	5.00E+07	7.50E+07
4	1.90E+08	1.60E+08	1.75E+08
5	1.10E+08	1.30E+08	1.20E+08

Table C.84 Cell number of PF2 at 150mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	4.00E+03	3.00E+03	3.50E+03
1	5.00E+03	7.00E+03	6.00E+03
2	1.70E+07	1.80E+07	1.75E+07
3	9.00E+07	1.20E+08	1.05E+08
4	9.00E+07	7.00E+07	8.00E+07
5	1.40E+08	8.00E+07	1.10E+08

Table C.85 Cell number of PF3 at 10mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.80E+04	1.00E+04	1.40E+04
1	4.50E+06	4.80E+06	4.65E+06
2	1.50E+06	1.80E+06	1.65E+06
3	1.20E+07	1.10E+07	1.15E+07
4	1.00E+07	1.50E+07	1.25E+07
5	6.00E+06	1.10E+07	8.50E+06

Table C.86 Cell number of PF3 at 25 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.40E+04	1.90E+04	2.15E+04
1	3.90E+06	3.30E+06	3.60E+06
2	8.00E+06	1.10E+07	9.50E+06
3	8.00E+06	1.40E+07	1.10E+07
4	7.00E+06	1.00E+07	8.50E+06
5	1.00E+07	7.00E+06	8.50E+06

Table C.87 Cell number of PF3 at 50mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	3.50E+04	1.20E+04	2.35E+04
1	2.00E+07	1.40E+07	1.70E+07
2	1.40E+07	1.70E+07	1.55E+07
3	1.80E+07	1.60E+07	1.70E+07
4	1.10E+07	1.10E+07	1.10E+07
5	7.00E+06	1.00E+07	8.50E+06

Table C.88 Cell number of PF3 at 80 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.80E+04	1.3 E+4	1.80E+04
1	1.20E+07	1.40E+07	1.30E+07
2	1.80E+07	2.20E+07	2.00E+07
3	2.00E+07	2.10E+07	2.05E+07
4	1.40E+07	1.10E+07	1.25E+07
5	1.60E+07	1.50E+07	1.55E+07

Table C.89 Cell number of PF3 at 100 mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	1.90E+04	2.00E+04	1.95E+04
1	2.60E+07	1.40E+07	2.00E+07
2	2.80E+07	2.40E+07	2.60E+07
3	1.50E+07	2.00E+07	1.75E+07
4	7.00E+06	1.20E+07	9.50E+06
5	1.60E+07	1.50E+07	1.55E+07

Table C.90 Cell number of PF3 at 150mg/L profenofos under presence of oxygen

Day	Test		
	1	2	Average
0	2.10E+04	3.00E+04	2.55E+04
1	1.80E+07	2.80E+07	2.30E+07
2	2.60E+07	3.00E+07	2.80E+07
3	7.00E+07	6.00E+07	6.50E+07
4	6.00E+07	4.00E+07	5.00E+07
5	2.00E+07	1.00E+07	1.50E+07

Table C.91 Cell number of PF1 at 10mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	2.10E+05	2.30E+05	2.20E+05
1		1.60E+05	4.08E+06	4.08E+06
2		4.30E+07	4.80E+07	4.55E+07
3		4.00E+07	2.60E+08	1.50E+08
4		4.00E+08	8.00E+07	2.40E+08
5		1.30E+07	3.70E+08	1.92E+08
6		7.00E+06	4.40E+08	2.24E+08
7		2.90E+08	2.90E+08	2.90E+08

Table C.92 Cell number of PF1 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.40E+05	2.00E+05	2.20E+05
1		3.70E+06	3.50E+06	3.60E+06
2		2.80E+07	1.70E+07	2.25E+07
3		1.50E+08	1.70E+08	1.60E+08
4		1.50E+08	7.70E+08	4.60E+08
5		6.00E+07	8.00E+08	4.30E+08
6		1.00E+08	8.20E+08	4.60E+08
7		6.00E+08	6.40E+08	6.20E+08

Table C.93 Cell number of PF1 at 10mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	3.40E+05	1.90E+05	2.65E+05
1		6.60E+06	3.20E+06	4.90E+06
2		5.20E+07	3.00E+07	4.10E+07
3		3.80E+08	3.50E+08	3.65E+08
4		7.80E+08	4.90E+08	6.35E+08
5		1.60E+09	1.00E+09	1.30E+09
6		1.20E+09	6.50E+08	9.25E+08
7		8.00E+08	4.40E+08	6.20E+08

Table C.94 Cell number of PF1 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.70E+05	1.40E+05	1.55E+05
1		5.90E+06	5.10E+06	5.50E+06
2		2.60E+07	3.20E+07	2.90E+07
3		1.50E+08	1.70E+08	1.60E+08
4		1.50E+08	3.00E+08	2.25E+08
5		3.90E+08	8.00E+07	2.35E+08
6		1.00E+08	1.90E+08	1.45E+08
7		1.30E+08	1.80E+08	1.55E+08

Table C.95 Cell number of PF1 at 10mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	3.40E+05	1.90E+05	2.65E+05
1		6.60E+06	3.20E+06	4.90E+06
2		5.20E+07	3.00E+07	4.10E+07
3		3.80E+08	3.50E+08	3.65E+08
4		7.80E+08	4.90E+08	6.35E+08
5		1.60E+09	1.00E+09	1.30E+09
6		1.20E+09	6.50E+08	9.25E+08
7		8.00E+08	4.40E+08	6.20E+08

Table C.96 Cell number of PF1 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.70E+05	1.40E+05	1.55E+05
1		5.90E+06	5.10E+06	5.50E+06
2		2.60E+07	3.20E+07	2.90E+07
3		1.50E+08	1.70E+08	1.60E+08
4		1.50E+08	3.00E+08	2.25E+08
5		3.90E+08	8.00E+07	2.35E+08
6		1.00E+08	1.90E+08	1.45E+08
7		1.30E+08	1.80E+08	1.55E+08

Table C.97 Cell number of PF1 at 25mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	6.20E+05	2.30E+05	4.25E+05
1		6.40E+07	4.80E+07	5.60E+07
2		8.00E+06	6.80E+07	3.80E+07
3		2.90E+08	1.90E+07	1.55E+08
4		6.00E+07	5.00E+08	2.80E+08
5		3.00E+07	4.30E+08	2.30E+08
6		8.00E+07	3.30E+08	2.05E+08
7		3.50E+08	1.60E+08	2.55E+08

Table C.98 Cell number of PF1 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.40E+05	1.40E+05	1.40E+05
1		7.90E+06	5.10E+06	6.50E+06
2		3.80E+07	2.20E+07	3.00E+07
3		5.30E+08	1.00E+08	3.15E+08
4		3.50E+08	3.70E+08	3.60E+08
5		6.70E+08	6.20E+08	6.45E+08
6		5.30E+08	5.30E+08	5.30E+08
7		4.20E+08	5.90E+08	5.05E+08

Table C.99 Cell number of PF1 at 50mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.60E+05	1.00E+05	1.30E+05
1		5.70E+06	4.30E+06	5.00E+06
2		2.00E+07	2.40E+07	2.20E+07
3		1.60E+08	2.00E+07	9.00E+07
4		3.90E+08	3.00E+07	2.10E+08
5		4.80E+08	4.50E+08	4.65E+08
6		5.20E+08	5.00E+08	5.10E+08
7		4.30E+08	4.50E+08	4.40E+08

Table C.100 Cell number of PF1 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	8.00E+04	1.80E+05	1.30E+05
1		5.70E+06	7.40E+06	6.55E+06
2		2.50E+07	4.40E+07	3.45E+07
3		7.00E+07	2.90E+08	1.80E+08
4		3.00E+07	2.40E+08	1.35E+08
5		4.00E+07	3.00E+08	1.70E+08
6		2.00E+08	2.70E+08	2.35E+08
7		3.50E+08	3.80E+08	3.65E+08

Table C.101 Cell number of PF1 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.80E+05	9.00E+04	1.35E+05
1		3.80E+06	4.80E+06	4.30E+06
2		2.80E+07	2.80E+07	2.80E+07
3		3.60E+08	3.70E+08	3.65E+08
4		5.00E+08	5.40E+08	5.20E+08
5		4.30E+08	4.40E+08	4.35E+08
6		6.30E+08	4.00E+08	5.15E+08
7		4.00E+08	5.60E+08	4.80E+08

Table C.102 Cell number of PF1 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.50E+05	2.00E+05	1.75E+05
1		1.70E+07	6.00E+06	1.15E+07
2		2.90E+07	2.80E+07	2.85E+07
3		2.00E+07	1.90E+07	1.95E+07
4		1.60E+07	1.30E+07	1.45E+07
5		5.00E+06	1.00E+07	7.50E+06
6		3.00E+06	7.00E+06	5.00E+06
7		1.00E+08	5.00E+07	7.50E+07

Table C.103 Cell number of PF1 at 80mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.20E+05	1.70E+05	1.95E+05
1		1.00E+07	1.10E+07	1.05E+07
2		1.70E+07	4.80E+07	3.25E+07
3		1.80E+08	3.00E+08	2.40E+08
4		8.00E+07	3.50E+08	2.15E+08
5		1.50E+08	5.00E+08	3.25E+08
6		1.20E+08	4.40E+08	2.80E+08
7		7.00E+07	4.20E+08	2.45E+08

Table C.104 Cell number of PF1 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.90E+05	1.60E+05	1.75E+05
1		7.00E+06	9.00E+06	8.00E+06
2		1.30E+07	1.40E+07	1.35E+07
3		2.90E+07	1.10E+08	6.95E+07
4		3.60E+08	1.90E+08	2.75E+08
5		5.00E+07	2.00E+08	1.25E+08
6		2.70E+08	2.00E+08	2.35E+08
7		1.80E+08	1.70E+08	1.75E+08

Table C.105 Cell number of PF1 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.90E+05	1.60E+05	1.75E+05
1		3.70E+06	3.30E+06	3.50E+06
2		1.10E+07	1.00E+07	1.05E+07
3		2.00E+07	8.00E+06	1.40E+07
4		2.40E+07	7.00E+06	1.55E+07
5		3.00E+06	9.00E+06	6.00E+06
6		2.00E+08	1.50E+08	1.75E+08
7		5.00E+08	5.40E+08	5.20E+08

Table C.106 Cell number of PF1 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	3.80E+04	4.00E+04	3.90E+04
1		2.30E+06	2.60E+06	2.45E+06
2		9.00E+06	7.00E+06	8.00E+06
3		3.20E+07	3.60E+07	3.40E+07
4		2.00E+08	1.50E+08	1.75E+08
5		1.90E+08	4.00E+07	1.15E+08
6		1.80E+08	1.00E+08	1.40E+08
7		1.60E+08	1.70E+08	1.65E+08

Table C.117 Cell number of PF1 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.90E+04	1.20E+04	1.55E+04
1		2.60E+06	5.20E+06	3.90E+06
2		1.30E+07	1.00E+07	1.15E+07
3		3.00E+07	3.00E+07	3.00E+07
4		5.00E+07	1.60E+08	1.05E+08
5		1.00E+08	1.00E+08	1.00E+08
6		1.30E+08	1.30E+08	1.30E+08
7		5.00E+07	1.00E+08	7.50E+07

Table C.118 Cell number of PF1 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	3.00E+04	2.00E+04	2.50E+04
1		4.10E+06	3.50E+06	3.80E+06
2		1.00E+07	7.00E+06	8.50E+06
3		1.70E+07	2.30E+07	2.00E+07
4		1.90E+07	3.80E+07	2.85E+07
5		7.00E+07	1.00E+08	8.50E+07
6		1.50E+08	1.50E+08	1.50E+08
7		8.00E+07	8.00E+07	8.00E+07

Table C.119 Cell number of PF1 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	7.00E+04	4.00E+04	5.50E+04
1		3.00E+06	2.70E+06	2.85E+06
2		3.40E+07	1.30E+07	2.35E+07
3		3.90E+07	4.40E+07	4.15E+07
4		3.00E+07	3.20E+07	3.10E+07
5		5.00E+07	4.00E+07	4.50E+07
6		2.00E+08	1.50E+08	1.75E+08
7		7.00E+07	9.00E+07	8.00E+07

Table C.110 Cell number of PF1 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	6.00E+04	7.00E+04	6.50E+04
1		4.90E+06	4.00E+06	4.45E+06
2		1.30E+07	1.20E+07	1.25E+07
3		1.20E+07	2.70E+07	1.95E+07
4		2.60E+07	2.80E+07	2.70E+07
5		4.00E+07	9.00E+07	6.50E+07
6		3.30E+08	9.00E+07	2.10E+08
7		1.20E+08	1.40E+08	1.30E+08

Table C.111 Cell number of PF2 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	2.70E+04	2.30E+04	2.50E+04
1		1.90E+06	1.60E+06	1.75E+06
2		3.70E+07	2.90E+07	3.30E+07
3		3.50E+07	4.80E+07	4.15E+07
4		3.90E+09	4.40E+08	2.17E+09
5		3.00E+08	4.50E+08	3.75E+08
6		1.80E+08	3.00E+08	2.40E+08
7		3.00E+08	3.50E+08	3.25E+08

Table C.112 Cell number of PF2 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	3.00E+04	2.30E+04	2.65E+04
1		3.50E+06	4.00E+06	3.75E+06
2		2.90E+06	3.50E+06	3.20E+06
3		5.90E+07	6.30E+07	6.10E+07
4		3.60E+08	6.90E+08	5.25E+08
5		1.70E+08	2.50E+08	2.10E+08
6		8.00E+08	3.00E+08	5.50E+08
7		4.60E+08	6.80E+08	5.70E+08

Table C.113 Cell number of PF2 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	4.00E+04	6.00E+04	5.00E+04
1		4.20E+06	4.50E+06	4.35E+06
2		3.30E+06	2.60E+06	2.95E+06
3		4.60E+06	5.20E+06	4.90E+06
4		1.20E+08	5.00E+07	8.50E+07
5		4.80E+08	4.50E+08	4.65E+08
6		1.40E+08	1.50E+08	1.45E+08
7		2.00E+08	1.60E+08	1.80E+08

Table C.114 Cell number of PF2 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.90E+05	1.60E+05	1.75E+05
1		1.00E+07	7.00E+06	8.50E+06
2		2.40E+07	2.10E+07	2.25E+07
3		3.80E+07	4.50E+07	4.15E+07
4		1.40E+08	1.20E+08	1.30E+08
5		4.30E+08	4.50E+08	4.40E+08
6		3.00E+07	4.00E+07	3.50E+07
7		2.80E+08	3.00E+08	2.90E+08

Table C.115 Cell number of PF2 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	5.00E+04	3.00E+04	4.00E+04
1		2.70E+07	1.90E+07	2.30E+07
2		1.80E+07	1.90E+07	1.85E+07
3		3.80E+07	3.60E+07	3.70E+07
4		3.60E+08	4.00E+08	3.80E+08
5		3.10E+08	2.20E+08	2.65E+08
6		2.80E+08	1.60E+08	2.20E+08
7		2.50E+08	2.60E+08	2.55E+08

Table C.116 Cell number of PF2 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	3.00E+04	2.00E+04	2.50E+04
1		2.00E+07	1.80E+07	1.90E+07
2		5.00E+06	8.00E+06	6.50E+06
3		1.20E+07	1.40E+07	1.30E+07
4		5.00E+06	5.00E+06	5.00E+06
5		1.00E+07	1.20E+07	1.10E+07
6		3.50E+07	3.30E+07	2.40E+07
7		2.40E+07	2.80E+07	2.60E+07

Table C.117 Cell number of PF2 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	3.60E+04	3.90E+05	2.13E+05
1		3.50E+06	2.80E+06	3.15E+06
2		5.70E+06	5.65E+06	5.65E+06
3		4.10E+07	2.30E+07	3.20E+07
4		3.50E+07	1.80E+07	2.65E+07
5		1.80E+07	1.10E+07	1.45E+07
6		1.20E+07	1.70E+07	1.45E+07
7		2.00E+07	1.00E+07	1.50E+07

Table C.118 Cell number of PF2 at 50 mg/L profenofos under presence of nitrate

Day	Ntrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.10E+04	2.90E+04	2.50E+04
1		2.30E+06	1.80E+06	2.05E+06
2		5.60E+06	8.00E+06	6.80E+06
3		1.17E+08	1.15E+08	1.16E+08
4		5.40E+08	3.50E+08	4.45E+08
5		6.80E+08	3.90E+08	5.35E+08
6		5.30E+08	3.90E+08	4.60E+08
7		3.10E+08	3.50E+08	3.30E+08

Table C.119 Cell number of PF2 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.90E+04	1.80E+04	1.85E+04
1		3.40E+06	2.50E+06	2.50E+06
2		4.50E+06	9.00E+06	6.75E+06
3		9.00E+06	2.00E+07	1.45E+07
4		1.10E+07	2.50E+07	1.80E+07
5		1.20E+07	3.00E+07	2.10E+07
6		1.50E+07	3.30E+07	2.40E+07
7		1.00E+07	3.20E+07	3.20E+07

Table C.120 Cell number of PF2 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	3.20E+04	3.70E+04	3.45E+04
1		2.50E+06	1.50E+06	1.50E+06
2		1.50E+06	1.20E+07	2.15E+07
3		3.10E+07	1.10E+07	2.10E+07
4		7.00E+07	5.10E+07	6.05E+07
5		6.00E+06	3.00E+06	4.50E+06
6		4.00E+07	1.20E+07	2.60E+07
7		4.00E+07	9.00E+07	6.50E+07

Table C.121 Cell number of PF2 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	3.00E+04	3.40E+04	3.40E+04
1		2.60E+06	2.70E+06	2.65E+06
2		9.00E+06	1.00E+07	9.50E+06
3		3.40E+07	8.40E+07	5.90E+07
4		5.70E+07	1.25E+08	1.25E+08
5		6.00E+07	7.00E+07	6.50E+07
6		5.00E+07	9.00E+07	7.00E+07
7		9.00E+07	4.00E+07	6.50E+07

Table C.122 Cell number of PF2 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	2.80E+04	2.60E+04	2.70E+04
1		3.30E+06	3.40E+06	3.40E+06
2		3.40E+06	2.00E+07	1.65E+07
3		2.60E+07	6.90E+07	4.75E+07
4		3.40E+08	2.00E+08	2.70E+08
5		2.90E+07	3.30E+07	3.30E+07
6		4.90E+08	3.80E+08	3.80E+08
7		5.00E+08	2.70E+08	3.85E+08

Table C.123 Cell number of PF2 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.40E+04	1.60E+04	1.50E+04
1		4.10E+06	2.90E+06	3.50E+06
2		6.00E+06	1.50E+07	1.05E+07
3		4.90E+07	3.00E+07	3.95E+07
4		4.00E+07	1.20E+08	8.00E+07
5		5.30E+07	6.80E+07	6.05E+07
6		9.00E+07	1.10E+08	1.10E+08
7		5.80E+07	5.80E+07	5.55E+07

Table C.124 Cell number of PF2 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.60E+04	1.80E+04	2.20E+04
1		1.90E+06	1.50E+06	1.70E+06
2		7.00E+06	7.00E+06	7.00E+06
3		1.50E+07	1.60E+07	1.55E+07
4		6.00E+07	9.00E+07	7.50E+07
5		5.30E+07	6.90E+07	6.10E+07
6		6.00E+07	1.10E+08	1.10E+08
7		3.00E+07	3.80E+07	3.40E+07

Table C.125 Cell number of PF2 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.90E+04	2.50E+04	2.20E+04
1		2.50E+06	2.70E+06	2.60E+06
2		1.50E+07	1.30E+07	1.40E+07
3		3.60E+07	2.90E+07	3.25E+07
4		1.20E+08	2.10E+08	2.10E+08
5		1.70E+08	5.00E+07	1.10E+08
6		1.10E+08	7.00E+07	9.00E+07
7		6.60E+07	3.00E+07	4.80E+07

Table C.126 Cell number of PF2 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.30E+04	1.00E+04	1.15E+04
1		1.70E+06	1.50E+06	1.60E+06
2		1.30E+07	1.10E+07	1.20E+07
3		1.70E+07	5.80E+07	3.75E+07
4		1.10E+08	1.90E+08	1.50E+08
5		6.00E+07	1.80E+08	1.80E+08
6		1.00E+08	2.10E+08	1.55E+08
7		1.00E+08	1.20E+08	1.10E+08

Table C.127 Cell number of PF2 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	9.00E+03	7.00E+03	8.00E+03
1		2.00E+05	3.00E+05	2.50E+05
2		9.00E+06	1.10E+07	1.00E+07
3		1.40E+07	2.40E+07	1.90E+07
4		3.00E+07	5.00E+07	4.00E+07
5		9.00E+07	5.00E+07	7.00E+07
6		6.00E+07	5.00E+07	5.50E+07
7		3.80E+07	3.40E+07	3.60E+07

Table C.128 Cell number of PF2 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	9.00E+03	7.00E+03	8.00E+03
1		2.00E+05	3.00E+05	2.50E+05
2		8.00E+06	1.00E+07	9.60E+06
3		2.30E+07	2.10E+07	2.02E+07
4		6.00E+07	3.00E+07	4.20E+07
5		6.00E+07	9.00E+07	7.20E+07
6		9.00E+07	2.00E+07	5.50E+07
7		1.40E+07	7.00E+06	2.58E+07

Table C.129 Cell number of PF3 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	2.20E+05	1.70E+05	1.95E+05
1		1.20E+07	1.20E+07	1.20E+07
2		1.40E+07	2.80E+07	2.10E+07
3		8.20E+07	7.10E+07	7.65E+07
4		5.10E+07	4.00E+07	4.55E+07
5		1.20E+07	1.50E+07	1.35E+07
6		1.90E+07	6.00E+06	1.25E+07
7		9.00E+06	7.00E+06	8.00E+06

Table C.130 Cell number of PF3 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.30E+05	1.10E+05	1.10E+05
1		1.40E+07	9.10E+06	1.16E+07
2		7.40E+07	2.20E+07	4.80E+07
3		4.00E+07	1.90E+07	2.95E+07
4		4.40E+07	5.10E+07	4.75E+07
5		1.30E+07	1.60E+07	1.45E+07
6		1.00E+07	9.00E+07	5.00E+07
7		9.00E+06	1.30E+07	1.10E+07

Table C.131 Cell number of PF3 at 10 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	2.30E+05	1.70E+05	2.00E+05
1		8.20E+06	7.00E+06	7.60E+06
2		4.80E+07	1.10E+07	2.95E+07
3		4.30E+07	1.40E+07	2.85E+07
4		1.10E+08	1.60E+07	6.30E+07
5		1.70E+07	4.80E+07	3.25E+07
6		1.80E+07	2.80E+08	1.49E+08
7		1.10E+07	1.00E+07	1.05E+07

Table C.132 Cell number of PF3 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.40E+05	2.30E+05	1.85E+05
1		2.60E+06	3.00E+06	2.80E+06
2		3.20E+07	1.70E+07	2.45E+07
3		3.80E+07	7.00E+06	2.25E+07
4		2.00E+07	2.70E+07	2.35E+07
5		1.10E+08	1.20E+08	1.15E+08
6		1.80E+07	1.50E+07	1.65E+07
7		1.10E+07	1.40E+07	1.25E+07

Table C.133 Cell number of PF3 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	1.50E+05	2.10E+05	1.80E+05
1		2.40E+06	3.40E+06	2.90E+06
2		2.70E+07	2.20E+07	2.45E+07
3		5.00E+07	4.80E+07	4.90E+07
4		4.70E+07	1.50E+07	3.10E+07
5		2.50E+07	1.60E+07	2.05E+07
6		1.90E+07	1.50E+07	1.70E+07
7		3.20E+07	1.50E+07	2.35E+07

Table C.134 Cell number of PF3 at 25 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.70E+05	1.90E+05	1.80E+05
1		6.40E+06	5.00E+06	5.00E+06
2		3.20E+07	3.40E+07	3.30E+07
3		3.60E+07	1.20E+08	7.80E+07
4		1.10E+07	8.00E+06	9.50E+06
5		3.70E+07	2.60E+07	3.15E+07
6		1.90E+07	2.10E+07	2.00E+07
7		1.40E+07	4.40E+07	2.90E+07

Table C.135 Cell number of PF3 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	2.00E+04	2.60E+04	2.30E+04
1		9.50E+06	1.00E+07	9.75E+06
2		1.30E+07	1.50E+07	1.40E+07
3		1.20E+08	1.40E+07	6.70E+07
4		5.00E+07	1.50E+07	3.25E+07
5		8.00E+06	1.10E+07	9.50E+06
6		1.00E+07	1.20E+07	1.10E+07
7		1.90E+07	1.60E+07	1.75E+07

Table C.136 Cell number of PF3 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	2.30E+05	2.00E+05	2.15E+05
1		2.00E+06	5.30E+06	3.65E+06
2		6.70E+07	6.50E+07	6.60E+07
3		1.00E+08	5.00E+07	7.50E+07
4		1.70E+07	6.00E+06	1.15E+07
5		4.00E+06	1.10E+07	7.50E+06
6		3.00E+06	1.40E+07	8.50E+06
7		4.80E+07	7.50E+07	6.15E+07

Table C.137 Cell number of PF3 at 50 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	8.00E+04	1.10E+05	9.50E+04
1		1.20E+06	8.00E+05	1.00E+06
2		2.30E+07	2.10E+07	2.20E+07
3		6.00E+06	3.00E+06	4.50E+06
4		1.10E+07	2.10E+07	1.60E+07
5		1.40E+07	1.00E+08	5.70E+07
6		2.00E+07	1.90E+07	1.95E+07
7		3.50E+07	1.20E+07	2.35E+07

Table C.138 Cell number of PF3 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	6.00E+03	1.30E+05	6.80E+04
1		1.90E+06	1.60E+06	1.75E+06
2		7.00E+06	1.50E+07	1.10E+07
3		1.40E+07	1.50E+07	1.45E+07
4		6.00E+06	2.10E+07	1.35E+07
5		1.60E+07	3.10E+07	2.35E+07
6		3.10E+07	2.50E+07	3.20E+07
7		4.00E+07	3.80E+07	3.90E+07

Table C.139 Cell number of PF3 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	9.00E+03	1.00E+04	9.50E+03
1		4.00E+06	3.40E+06	3.70E+06
2		1.70E+07	1.80E+07	1.75E+07
3		2.20E+07	3.10E+07	2.65E+07
4		1.40E+07	3.30E+07	2.35E+07
5		8.00E+06	9.00E+06	8.50E+06
6		1.00E+07	1.70E+07	1.35E+07

Table C.140 Cell number of PF3 at 80 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.00E+04	1.80E+05	9.50E+04
1		1.30E+06	2.30E+06	1.80E+06
2		1.40E+07	1.30E+07	1.35E+07
3		1.60E+07	1.00E+07	1.30E+07
4		1.60E+07	2.70E+07	2.15E+07
5		3.10E+07	2.50E+07	2.80E+07
6		2.30E+07	8.00E+06	1.55E+07
7		2.90E+07	3.00E+07	2.95E+07

Table C.141 Cell number of PF3 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	1.10E+04	1.40E+04	1.25E+04
1		4.50E+06	1.60E+06	3.05E+06
2		6.50E+07	6.00E+07	6.25E+07
3		1.90E+07	5.00E+06	1.20E+07
4		5.00E+06	6.00E+06	5.50E+06
5		1.00E+07	8.00E+06	9.00E+06
6		9.00E+06	1.40E+08	7.45E+07
7		5.00E+06	7.00E+06	6.00E+06

Table C.142 Cell number of PF3 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	8.00E+03	7.00E+03	7.50E+03
1		7.00E+05	1.50E+06	1.10E+06
2		3.00E+07	3.40E+07	3.20E+07
3		1.60E+07	6.50E+07	4.05E+07
4		4.40E+07	2.20E+07	3.30E+07
5		1.00E+07	7.00E+06	8.50E+06
6		6.00E+06	5.00E+06	5.50E+06
7		4.00E+07	3.00E+07	3.50E+07

Table C.143 Cell number of PF3 at 100 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	3.00E+03	7.00E+03	5.00E+03
1		1.30E+06	7.00E+05	1.00E+06
2		1.70E+07	4.00E+07	2.85E+07
3		6.00E+06	1.10E+07	8.50E+06
4		3.00E+06	8.00E+06	5.50E+06
5		1.80E+07	2.20E+07	2.00E+07
6		1.30E+07	1.30E+07	1.30E+07
7		3.70E+07	6.40E+07	5.05E+07

Table C.144 Cell number of PF3 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	100	8.00E+03	5.00E+03	6.50E+03
1		2.90E+06	3.40E+06	3.15E+06
2		4.00E+07	4.70E+07	4.35E+07
3		5.90E+07	5.20E+07	5.55E+07
4		7.00E+06	1.20E+07	9.50E+06
5		5.00E+06	9.00E+06	7.00E+06
6		4.00E+06	9.00E+06	6.50E+06
7		1.60E+07	7.00E+06	1.15E+07

Table C.145 Cell number of PF3 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	200	1.30E+04	1.00E+04	1.15E+04
1		1.70E+06	1.80E+06	1.75E+06
2		3.30E+07	2.10E+07	2.70E+07
3		2.60E+07	2.70E+07	2.65E+07
4		2.40E+07	2.00E+07	2.20E+07
5		2.00E+06	4.00E+06	3.00E+06
6		1.00E+07	5.10E+07	3.05E+07
7		2.50E+07	1.90E+07	2.20E+07

Table C.146 Cell number of PF3 at 150 mg/L profenofos under presence of nitrate

Day	Nitrate conc. (mg/L)	Test		
		1	2	Average
0	300	1.70E+04	1.00E+04	1.35E+04
1		5.00E+06	5.30E+06	5.15E+06
2		1.50E+07	1.70E+07	1.60E+07
3		7.80E+07	3.20E+07	5.50E+07
4		7.00E+06	8.00E+06	7.50E+06
5		1.30E+07	9.00E+06	1.10E+07
6		7.00E+06	8.00E+06	7.50E+06
7		1.20E+07	7.00E+06	9.50E+06

Results of nitration reduction (%) by PF1, PF2 and PF3 under presence of nitrate

Table C.147 Nitrate reduction (%) of PF1 at 10 mg/L of profenofos

Day	Control						Test					
	Nitrate Conc. (mg/L)	1	2	Average	%Normalized PF conc.		1	2	Average	%Normalized PF conc.		
0	100	97.598	99.089	98.344	100.00		90.896	90.896	90.896	100.00		
1		90.896	90.896	90.896	92.427		75.890	78.574	77.232	84.967		
2		90.432	90.432	90.432	91.954		60.784	52.345	56.565	62.230		
3		85.897	82.765	84.331	85.751		47.078	52.345	49.712	54.691		
4		85.897	82.765	84.331	85.751		39.708	45.687	42.698	46.975		
5		82.822	86.757	84.790	86.218		35.660	38.238	36.949	40.650		
6		82.822	86.757	84.790	86.218		25.899	28.445	27.172	29.894		
7	82.822	86.757	84.790	86.218		15.798	19.456	17.627	19.392			
0	200	160.897	164.897	162.897	100.00		161.675	165.072	163.374	100.00		
1		157.789	143.806	150.798	92.573		150.708	156.689	153.699	94.078		
2		135.408	142.798	139.103	85.393		127.790	133.687	130.739	80.024		
3		144.171	132.786	138.479	85.010		120.789	119.446	120.118	73.523		
4		138.780	132.786	135.783	83.355		119.897	114.078	116.988	71.607		
5		135.045	132.798	133.922	82.213		109.897	113.008	111.453	68.220		
6		132.045	125.896	128.971	79.173		109.878	100.989	105.433	64.535		
7	135.045	125.896	130.471	80.094		84.089	79.645	81.867	50.110			
0	300	230.050	235.078	232.564	100.00		235.078	239.789	237.434	100.00		
1		225.078	223.576	224.327	96.458		195.583	199.875	197.729	83.277		
2		213.798	211.086	212.442	91.348		180.574	184.894	182.734	76.962		
3		213.890	212.678	213.284	91.710		171.586	176.875	174.231	73.381		
4		209.890	209.890	209.890	90.250		167.786	166.780	167.283	70.455		
5		209.890	203.789	206.840	88.939		161.890	158.899	160.395	67.554		
6		204.453	204.453	204.453	87.913		152.678	151.221	151.950	63.997		
7	204.453	200.243	202.348	87.007		141.709	135.445	138.577	58.364			

Table C.148 Nitrate reduction (%) of PF1 at 25 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	95.987	90.044	93.016	100.00	97.896	99.234	98.565	100.00
1		88.743	85.897	87.320	93.876	74.878	80.789	77.834	78.967
2		88.743	83.087	85.915	92.366	69.897	72.655	71.276	72.314
3		78.678	82.008	80.343	86.375	72.890	70.332	71.611	72.653
4		82.008	80.785	81.397	87.509	66.897	63.779	65.338	66.389
5		78.678	80.785	79.732	85.719	54.564	50.567	52.566	53.331
6		78.678	80.785	79.732	85.719	40.090	35.890	37.990	38.543
7	75.775	75.775	75.775	81.464	28.678	25.112	26.895	27.287	
0	200	150.895	150.895	150.895	100.00	150.895	145.784	148.340	100.00
1		149.875	144.890	147.383	97.672	131.894	137.231	134.563	90.713
2		134.895	135.689	135.292	89.660	125.784	129.894	127.839	86.180
3		135.689	130.678	133.184	88.263	121.897	122.006	121.952	82.211
4		135.689	138.594	137.142	90.886	122.006	126.786	124.396	83.859
5		129.089	124.098	126.594	83.895	122.006	115.705	118.856	80.124
6		122.890	125.342	124.116	82.253	122.006	109.890	115.948	78.164
7	125.342	125.342	125.342	83.066	97.897	104.786	101.342	68.317	
0	300	230.778	235.009	235.894	100.00	230.778	233.223	232.001	100.00
1		219.760	215.678	217.719	92.295	205.776	205.776	205.776	88.696
2		205.899	209.009	207.454	87.944	189.463	197.590	193.527	83.416
3		209.009	205.789	207.399	87.920	175.776	174.009	174.893	75.385
4		209.009	200.700	204.855	86.842	171.678	170.990	171.334	73.850
5		205.665	205.665	205.665	87.185	167.890	161.009	164.450	70.883
6		95.987	90.044	93.016	100.00	97.896	99.234	98.565	100.00
7	88.743	85.897	87.320	93.876	74.878	80.789	77.834	78.967	

Table C.149 Nitrate reduction (%) of PF1 at 50 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	87.890	91.342	89.616	100.00	71.490	88.613	80.052	100.00
1		87.890	88.890	88.390	98.632	71.490	75.771	73.631	91.979
2		80.934	84.332	82.633	92.208	70.897	73.675	72.286	90.299
3		73.152	78.432	75.792	84.574	68.798	62.089	65.444	81.752
4		67.963	70.658	69.311	77.342	55.889	56.009	55.949	69.891
5		67.964	70.658	69.311	77.342	52.890	50.342	51.616	64.478
6		65.945	70.658	68.302	76.216	48.897	42.897	45.897	57.334
7	63.243	64.785	64.014	71.431	39.678	40.789	40.234	50.260	
0	200	205.556	205.556	205.556	100.00	205.556	198.897	202.227	100.00
1		199.087	199.087	199.087	96.853	179.564	175.031	177.298	87.673
2		198.876	195.089	196.983	95.829	166.087	166.087	166.087	82.129
3		192.887	195.089	193.988	94.372	158.854	153.123	155.989	77.136
4		188.897	190.078	189.488	92.183	144.908	143.088	143.998	71.207
5		184.695	184.695	184.695	89.851	129.897	124.708	127.303	62.951
6		184.695	184.695	184.695	89.851	120.756	129.897	125.327	61.973
7	180.876	180.876	180.876	87.994	114.595	105.897	110.246	54.516	
0	300	298.854	295.089	296.972	100.00	286.876	295.089	290.983	100.00
1		285.531	285.531	285.531	96.147	279.876	282.009	280.943	96.550
2		274.087	280.113	277.100	93.308	266.087	259.443	262.765	90.303
3		270.002	270.002	270.002	90.918	249.076	243.432	246.254	84.628
4		270.002	268.765	269.384	90.710	228.576	223.876	226.226	77.745
5		265.110	265.110	265.110	89.271	195.954	192.112	194.033	66.682
6		260.541	265.110	262.826	88.502	178.708	182.786	180.747	62.116
7	260.541	265.110	262.826	88.502	170.865	174.954	172.910	59.423	

Table C.150 Nitrate reduction (%) of PF1 at 80 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	92.894	85.093	88.994	100.00	85.093	90.574	87.834	100.00
1		80.893	78.084	79.489	89.320	75.685	84.944	80.315	91.440
2		77.894	71.049	74.472	83.682	64.909	62.312	63.611	72.422
3		67.885	67.885	67.885	76.280	60.181	57.588	58.885	67.041
4		67.832	67.832	67.832	76.221	54.665	54.665	54.665	62.237
5		65.890	67.832	66.861	75.130	41.895	38.221	40.058	45.606
6		65.890	67.832	66.861	75.129	33.864	38.221	36.043	41.035
7	67.832	67.832	67.832	76.221	35.543	38.043	36.793	41.889	
0	200	195.983	195.983	195.983	100.00	188.534	190.983	189.759	100.00
1		195.983	190.853	193.418	98.691	184.784	182.932	183.858	96.890
2		189.334	185.895	187.615	95.730	171.883	163.003	167.443	88.240
3		184.333	185.895	185.114	94.454	155.889	150.003	152.946	80.600
4		184.333	184.333	184.333	94.056	132.892	130.132	131.512	69.305
5		182.101	182.101	182.101	92.917	132.887	132.887	132.887	70.029
6		174.757	174.757	174.757	89.169	129.897	120.678	125.288	66.025
7	171.889	176.089	173.989	88.778	125.405	129.897	127.651	67.270	
0	300	284.576	290.965	287.771	100.00	285.894	284.576	285.235	100.00
1		275.565	270.435	273.000	94.867	268.564	270.903	269.734	94.566
2		265.115	270.903	268.009	93.132	256.687	258.013	257.350	90.224
3		265.115	265.115	265.115	92.127	235.032	238.132	236.582	82.943
4		259.904	255.567	257.736	89.563	225.432	216.743	221.088	77.511
5		252.353	259.904	256.129	89.004	197.542	195.932	196.737	68.974
6		252.353	250.943	251.648	87.447	192.424	186.903	189.664	66.493
7	252.353	252.353	252.353	87.692	184.112	184.112	184.112	64.547	

Table C.151 Nitrate reduction (%) of PF1 at 100 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	100.335	100.335	100.335	100.00	95.007	95.007	95.007	100.00
1		99.314	99.645	99.480	99.148	80.564	88.345	84.455	88.893
2		100.564	102.546	101.555	101.216	74.653	81.764	78.209	82.319
3		95.556	95.556	95.556	95.237	71.563	74.989	73.276	77.127
4		91.534	94.786	93.160	92.849	64.543	61.002	62.773	66.072
5		84.765	88.097	86.431	86.142	47.743	55.132	51.438	54.141
6		81.780	84.765	83.273	82.995	50.205	54.654	52.430	55.185
7	81.780	81.780	81.780	81.507	47.765	41.954	44.860	47.218	
0	200	190.083	190.083	190.083	100.00	188.071	190.083	189.077	100.00
1		169.790	165.487	167.639	88.193	160.649	158.364	159.507	84.361
2		167.980	165.567	166.774	87.737	148.908	146.890	147.899	78.222
3		163.078	165.567	164.323	86.448	130.678	135.890	133.284	70.492
4		157.789	160.008	158.899	83.595	132.678	125.098	128.888	68.167
5		158.720	158.720	158.720	83.500	110.199	108.908	109.554	57.941
6		157.562	160.008	158.785	83.535	88.889	86.008	87.449	46.250
7	151.247	157.562	154.405	81.230	56.986	59.078	58.032	30.692	
0	300	295.789	295.789	295.789	100.00	290.004	295.789	292.897	100.00
1		292.330	295.789	294.060	99.415	290.004	279.873	284.939	97.283
2		297.285	292.330	294.808	99.668	280.797	283.809	282.303	96.383
3		292.330	292.330	292.330	98.830	280.797	278.006	279.402	95.393
4		295.968	292.330	294.149	99.531	261.789	252.075	256.932	87.721
5		299.653	290.879	295.266	99.823	231.743	234.867	233.305	79.654
6		290.180	299.653	294.917	99.705	198.790	191.897	195.344	66.694
7	287.055	290.180	288.618	97.576	161.790	157.768	159.779	54.551	

Table C.152 Nitrate reduction (%) of PF1 at 150 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	95.132	93.802	94.467	100.00	90.893	93.802	92.348	100.00
1		95.132	95.132	95.132	100.704	87.083	84.382	85.733	92.837
2		88.490	88.490	88.490	93.672	74.943	78.154	76.549	82.892
3		90.112	90.112	90.112	95.390	66.843	60.783	63.813	69.101
4		84.334	80.356	82.345	87.168	47.892	50.893	49.393	53.485
5		81.781	81.781	81.781	86.571	37.575	47.892	42.734	46.275
6		84.334	80.584	82.459	87.289	31.354	34.265	32.810	35.529
7	82.454	75.466	78.960	83.585	25.544	21.776	23.660	25.620	
0	200	184.889	184.889	184.889	100.00	181.554	181.554	181.554	100.00
1		182.543	180.894	181.719	98.285	165.465	176.143	170.804	94.079
2		175.043	172.246	173.645	93.919	158.211	158.211	158.211	87.143
3		170.233	172.246	171.240	92.618	155.784	151.049	153.417	84.502
4		167.780	170.233	169.007	91.410	148.896	141.009	144.953	79.840
5		160.720	165.732	163.226	88.283	135.907	129.354	132.631	73.053
6		154.405	160.720	157.563	85.220	117.768	103.365	110.567	60.900
7	158.782	160.584	159.683	86.367	91.709	98.897	95.303	52.493	
0	300	293.894	293.894	293.894	100.00	300.893	293.894	297.394	100.00
1		300.893	290.896	295.895	100.681	288.732	287.732	288.232	96.919
2		300.893	300.893	300.893	102.381	269.578	259.007	264.293	88.870
3		297.008	297.008	297.008	101.060	244.778	261.756	253.267	85.162
4		280.785	297.008	288.897	98.300	249.132	249.132	249.132	83.772
5		274.392	280.785	277.589	94.452	249.132	220.714	234.923	78.994
6		274.392	270.567	272.480	92.713	240.778	236.947	238.863	80.319
7	260.778	260.778	260.778	88.732	217.789	221.870	219.830	73.919	

Table C.153 Nitrate reduction (%) of PF2 at 10 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	95.968	94.357	95.163	100.00	87.204	91.586	89.395	100.00
1		87.204	90.089	88.647	93.153	65.293	69.676	67.485	75.490
2		87.204	88.967	88.086	92.563	60.911	52.147	56.529	63.235
3		82.822	85.897	84.360	88.648	47.765	52.147	49.956	55.882
4		78.440	80.675	79.558	83.601	34.619	43.383	39.001	43.627
5		82.822	85.806	84.314	88.600	34.619	30.237	32.428	36.275
6		82.822	83.586	83.204	87.433	17.090	30.237	23.664	26.471
7	78.440	80.806	79.623	83.670	8.326	17.090	12.708	14.215	
0	200	152.936	160.374	156.655	100.00	161.700	166.082	163.891	100.00
1		131.025	143.806	137.416	87.719	139.790	126.643	133.217	81.283
2		135.408	140.783	138.096	88.719	117.879	113.497	115.688	70.588
3		144.171	142.784	143.478	91.589	100.350	109.115	104.733	63.904
4		139.790	142.465	141.128	90.088	109.115	104.732	106.924	65.241
5		131.025	137.854	134.440	85.819	109.115	113.497	111.306	67.915
6		131.025	143.795	137.410	87.715	109.115	100.350	104.733	63.904
7	135.408	142.743	139.076	88.779	74.058	69.676	71.867	43.850	
0	300	223.049	225.443	224.246	100.00	205.521	209.904	207.713	100.00
1		205.521	213.586	209.554	93.448	174.846	179.229	177.038	85.232
2		214.286	210.465	212.376	94.707	170.464	174.846	172.656	83.121
3		214.286	212.803	213.545	95.228	161.700	166.082	163.891	78.902
4		209.904	209.904	209.904	93.604	157.318	166.082	161.700	77.847
5		209.904	205.793	207.849	92.688	161.700	148.553	155.127	74.683
6		205.521	205.521	205.521	91.650	152.936	161.700	157.318	75.738
7	205.521	200.684	203.103	90.571	131.035	135.407	133.221	64.137	

Table C.154 Nitrate reduction (%) of PF2 at 25 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	91.586	90.643	91.115	100.00	87.204	100.351	93.778	100.00
1		78.440	85.809	82.125	90.133	74.057	82.822	78.440	83.644
2		78.440	83.794	81.117	89.028	69.675	74.057	71.866	76.634
3		78.440	82.794	80.617	88.478	82.822	74.057	78.440	83.644
4		82.822	80.785	81.804	89.780	56.529	47.765	52.147	55.606
5		78.440	80.785	79.613	87.376	34.619	25.855	30.237	32.243
6		78.440	80.785	79.613	87.376	17.090	8.326	12.708	13.550
7	78.440	78.440	78.440	86.089	8.326	8.326	8.326	8.878	
0	200	148.554	148.554	148.554	100.00	139.789	135.408	137.599	100.00
1		139.789	144.798	142.294	95.892	131.025	117.879	124.452	90.445
2		139.790	139.790	139.790	94.100	135.408	139.790	137.599	100.00
3		135.408	135.697	135.553	91.248	131.025	122.261	126.643	92.037
4		135.408	138.906	137.157	92.328	122.261	126.643	124.452	90.445
5		139.789	138.607	139.198	93.702	122.261	113.497	117.879	85.668
6		131.025	135.864	133.445	89.829	122.261	109.115	115.688	84.076
7	135.408	136.795	136.102	91.618	87.204	74.058	80.631	58.598	
0	300	209.904	209.904	209.904	100.00	214.286	218.668	216.477	100.00
1		209.904	205.521	207.713	98.956	196.757	205.521	201.139	92.914
2		205.521	209.904	207.713	98.956	179.229	196.757	187.993	86.842
3		209.904	205.521	207.713	98.956	170.464	174.847	172.656	79.757
4		209.904	200.434	205.169	97.744	179.229	170.464	174.847	83.298
5		205.521	205.521	205.521	97.744	170.464	161.700	166.082	76.720
6		201.139	201.139	205.521	97.744	166.082	161.700	163.891	75.708
7	201.139	201.139	205.521	97.744	117.879	126.643	122.261	56.477	

Table C.155 Nitrate reduction (%) of PF2 at 50 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	88.613	90.754	89.684	100.00	71.490	88.613	80.052	100.00
1		84.332	88.613	86.473	96.420	71.490	75.771	73.631	91.978
2		80.934	84.332	82.633	92.138	62.776	73.152	67.964	84.899
3		73.152	78.432	75.792	84.510	67.963	60.181	64.072	80.037
4		67.963	70.658	69.311	77.283	54.994	57.588	56.291	70.318
5		67.964	70.658	69.311	77.283	42.023	60.182	51.103	63.837
6		65.945	70.658	68.302	76.159	49.730	44.324	47.027	58.745
7	63.243	64.785	64.014	71.377	38.919	41.622	40.271	50.306	
0	200	199.914	198.573	199.244	100.00	204.195	192.353	198.274	100.00
1		199.914	198.683	199.299	100.03	169.949	169.949	169.949	85.714
2		197.665	196.683	197.174	98.961	156.161	166.537	161.349	81.376
3		192.477	190.392	191.435	96.080	148.378	153.567	150.973	76.143
4		187.289	190.563	188.926	94.821	140.597	143.191	141.894	71.564
5		184.695	184.682	184.689	92.695	109.468	114.656	112.062	56.518
6		184.865	184.865	184.865	92.783	122.703	109.189	115.946	58.477
7	179.459	179.459	179.459	90.069	114.595	95.676	105.136	53.025	
0	300	298.373	285.531	291.952	100.00	276.969	264.127	270.548	100.00
1		285.531	285.531	285.531	97.801	259.846	242.723	251.285	92.880
2		275.486	280.789	278.138	95.268	246.952	239.170	243.061	89.840
3		270.298	270.298	270.298	92.583	239.170	233.982	236.576	87.443
4		270.298	268.798	269.548	92.326	218.418	213.229	215.824	79.773
5		265.110	265.110	265.110	90.806	182.101	184.695	183.398	67.788
6		260.541	265.110	262.826	90.024	168.649	182.162	175.406	64.833
7	260.541	265.110	262.826	90.024	171.351	174.054	172.703	63.834	

Tab3 C:156 Nitrate reduction (%) of PF2 at 80 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0		71.490	71.490	71.490	100.00	84.332	92.894	88.613	100.00
1		71.490	71.490	71.490	100.00	71.490	84.332	77.911	87.922
2		67.964	71.490	69.727	97.534	54.994	62.776	58.885	62.060
3		67.964	67.964	67.964	95.068	60.181	57.588	58.885	66.451
4	100	67.964	67.964	67.964	95.068	44.617	54.994	49.806	56.206
5		65.370	67.964	66.667	93.254	31.647	42.023	36.835	41.568
6		65.946	67.964	66.955	93.656	33.514	28.108	30.811	34.770
7		68.649	67.964	68.307	95.548	25.405	28.108	26.757	30.195
0		199.910	199.910	199.910	100.00	187.072	191.353	189.213	100.00
1		195.634	199.910	197.772	98.931	174.229	182.791	178.510	94.343
2		179.507	185.658	182.583	91.333	161.349	153.567	157.458	83.217
3		174.319	183.075	178.697	89.389	125.032	140.597	132.815	70.193
4	200	184.695	183.085	183.890	91.986	122.438	130.220	126.329	66.765
5		182.101	182.101	182.101	91.091	132.815	152.062	142.439	75.279
6		176.757	176.757	176.757	88.418	149.730	160.540	125.405	66.277
7		171.351	176.757	174.054	87.066	125.405	128.108	126.757	66.991
0		264.126	270.156	267.141	100.00	285.531	298.373	291.952	100.00
1		255.565	264.126	259.846	97.269	268.408	272.688	270.548	92.669
2		265.110	278.874	271.992	101.815	236.576	228.794	232.685	79.700
3	300	265.110	265.110	265.110	99.240	215.824	218.418	217.121	74.368
4		259.922	255.689	257.806	96.506	205.447	210.636	208.042	71.259
5		262.516	259.922	261.219	97.783	187.289	195.071	191.180	65.483
6		263.243	260.687	261.965	98.062	190.270	184.864	187.567	64.246
7		257.837	257.837	257.837	96.517	182.162	182.162	182.162	62.394

Table C.157 Nitrate reduction (%) of PF2 at 100 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	101.443	100.254	100.849	100.00	89.993	94.573	92.283	100.00
1		103.519	101.443	102.481	101.618	80.667	78.382	79.525	86.175
2		102.909	101.443	102.176	101.316	74.056	81.270	77.663	84.157
3		95.696	101.443	98.570	97.740	71.652	74.056	72.854	78.946
4		91.253	95.696	93.475	92.688	65.993	62.835	64.414	69.801
5		84.938	91.253	88.096	87.354	47.048	34.417	40.733	44.139
6		81.781	84.938	83.360	82.658	50.205	34.417	42.311	45.849
7	72.308	72.308	72.308	71.699	47.048	31.260	39.154	42.428	
0	200	177.009	170.764	173.887	100.00	188.071	190.357	189.214	100.00
1		169.790	165.487	167.639	96.407	160.649	158.364	159.506	84.299
2		167.829	160.543	164.186	94.421	143.785	146.189	144.987	76.626
3		163.020	160.754	161.887	93.099	131.762	134.167	132.965	70.272
4		157.562	160.754	159.158	91.530	132.302	129.144	130.723	69.087
5		160.720	160.720	160.720	92.428	110.199	88.096	99.147	52.399
6		157.562	160.720	159.141	91.520	88.096	56.520	72.308	38.214
7	151.247	157.562	154.405	88.796	40.732	56.520	48.626	25.699	
0	300	302.954	302.954	302.954	100.00	293.794	295.475	294.635	100.00
1		302.331	302.954	302.643	99.897	290.905	279.479	285.192	96.795
2		307.285	302.331	304.808	100.612	290.454	283.241	286.848	97.357
3		312.094	312.094	312.094	103.017	280.837	268.815	274.826	93.277
4		305.968	302.331	304.150	100.395	261.762	252.289	257.026	87.235
5		299.653	300.332	299.993	99.022	201.768	204.926	203.347	69.017
6		290.180	299.653	294.917	97.347	167.035	151.247	159.141	54.013
7	287.022	290.180	288.601	95.262	151.247	157.562	154.405	52.406	

Table C.158 Nitrate reduction (%) of PF2 at 150 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control			Test				
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	92.093	95.895	93.994	100.00	89.808	92.093	90.951	100.00
1		94.378	95.895	95.137	101.216	71.527	76.097	73.812	81.156
2		88.483	88.483	88.483	94.137	64.439	54.821	59.630	65.563
3		90.887	90.887	90.887	96.694	66.843	40.394	53.619	58.954
4		84.938	80.584	82.761	88.049	47.048	40.733	43.891	48.258
5		81.781	80.584	81.183	86.370	37.575	47.048	42.312	46.522
6		75.466	80.584	78.025	83.011	21.787	24.945	23.366	25.691
7	72.308	75.466	73.887	78.608	15.472	21.787	18.630	20.483	
0	200	174.360	174.360	174.360	100.00	181.216	190.357	185.787	100.00
1		172.075	170.687	171.381	98.291	165.219	156.079	160.649	86.469
2		175.042	172.075	173.559	99.541	158.211	148.593	153.402	82.569
3		170.233	172.075	171.154	98.161	155.807	141.380	148.594	79.981
4		167.035	170.233	168.634	96.716	138.617	141.775	140.196	75.461
5		160.720	165.732	163.226	93.614	125.987	129.144	127.566	68.662
6		154.405	160.720	157.563	90.366	97.569	103.884	100.727	54.216
7	148.090	160.720	154.405	88.555	91.254	94.411	92.833	49.967	
0	300	290.905	290.905	290.905	100.00	304.616	293.190	298.903	100.00
1		300.046	290.905	295.476	101.571	268.053	277.194	272.624	91.208
2		307.285	300.046	303.666	104.387	249.579	259.197	254.388	85.107
3		297.668	300.046	298.857	102.733	244.770	271.219	257.995	86.314
4		280.707	297.668	289.188	99.410	249.132	239.659	244.396	81.764
5		274.392	280.707	277.550	95.409	249.132	220.714	234.923	78.595
6		271.235	270.567	270.901	93.124	230.186	239.659	234.923	78.595
7	261.762	261.762	261.762	89.982	217.556	201.768	209.662	70.144	

Table C.159 Nitrate reduction (%) of PF3 at 10 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	92.783	90.252	91.518	100.00	89.213	92.783	90.998	100.00
1		87.893	90.252	89.073	97.328	75.783	78.943	77.363	85.016
2		88.893	88.893	88.893	97.132	60.738	64.992	62.865	69.084
3		82.783	85.662	84.223	92.029	57.893	52.223	55.058	60.505
4		82.990	80.423	81.707	89.279	44.545	41.804	43.175	47.446
5		82.990	80.423	81.707	89.279	35.832	31.904	33.868	37.218
6		82.023	82.023	82.023	89.625	27.783	30.893	29.338	32.240
7	77.983	80.028	79.006	86.328	28.389	19.032	23.711	26.057	
0	200	182.445	180.782	181.614	100.00	182.445	184.213	183.329	100.00
1		171.673	173.790	172.732	95.346	169.893	166.132	168.013	91.646
2		165.543	160.893	163.218	89.871	157.453	153.821	155.637	84.895
3		165.543	160.893	163.218	89.871	140.893	149.783	145.338	79.277
4		159.721	152.093	155.907	85.845	139.783	144.488	142.136	77.531
5		159.721	155.832	157.777	86.875	129.893	133.973	131.933	71.965
6		151.009	153.243	152.126	83.763	119.783	110.982	115.383	62.938
7	155.783	153.243	154.513	85.078	94.982	99.836	97.409	53.133	
0	300	273.903	275.883	274.893	100.00	275.883	279.241	277.562	100.00
1		265.932	263.674	264.803	96.329	254.784	259.028	256.906	92.558
2		254.286	250.465	252.376	91.809	226.849	234.352	230.601	83.081
3		254.286	252.896	253.591	92.251	201.783	206.054	203.919	73.468
4		249.903	249.903	249.903	90.909	177.182	177.182	177.182	63.835
5		249.903	245.743	247.823	90.152	161.893	168.084	164.989	59.442
6		245.354	245.354	245.354	89.254	152.893	160.764	156.829	56.502
7	245.354	240.885	243.120	88.442	133.894	135.903	134.899	48.601	

Table C.160 Nitrate reduction (%) of PF3 at 25 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	91.082	89.892	90.487	100.00	87.443	91.082	89.263	100.00
1		88.893	85.115	87.004	96.151	84.662	82.902	83.782	93.860
2		79.723	83.917	81.820	90.422	79.904	74.845	77.375	86.682
3		78.832	80.003	79.418	87.767	72.829	74.845	73.837	82.718
4		82.903	80.003	81.453	90.016	59.892	67.133	63.513	71.153
5		78.832	80.003	79.418	87.767	44.832	35.937	40.385	45.243
6		79.903	80.786	80.345	88.792	37.782	28.920	33.351	37.363
7	79.903	79.903	79.903	88.303	18.782	18.782	18.782	21.041	
0	200	178.993	178.993	178.993	100.00	175.883	175.883	175.883	100.00
1		169.009	164.321	166.665	93.113	161.778	157.923	159.851	90.885
2		159.844	159.844	159.844	89.302	135.893	139.099	137.496	78.175
3		145.735	147.930	146.833	82.033	130.554	122.893	126.724	72.050
4		138.778	138.778	138.778	77.533	122.893	126.554	124.724	70.913
5		140.908	138.778	139.843	78.128	122.261	116.893	119.577	67.987
6		141.908	135.783	138.846	77.570	112.893	109.902	111.398	63.337
7	135.783	135.783	135.783	75.859	88.893	94.002	91.448	51.994	
0	300	279.689	275.006	277.348	100.00	275.006	282.894	278.950	100.00
1		269.443	265.672	267.558	96.470	256.893	254.094	255.494	91.591
2		255.895	259.903	257.899	92.988	239.884	236.032	237.958	85.305
3		259.334	255.895	257.615	92.885	220.832	224.143	222.488	79.759
4		259.889	250.032	254.961	91.928	199.902	190.672	195.287	70.008
5		255.892	255.892	255.892	92.264	170.894	172.903	171.899	61.624
6		251.311	251.311	251.311	90.612	166.893	160.029	163.461	58.598
7	251.311	251.311	251.311	90.612	147.903	143.532	145.718	52.238	

Table C.161 Nitrate reduction (%) of PF3 at 50 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	89.902	92.891	91.397	100.00	92.891	89.902	91.397	100.00
1		85.732	89.902	87.817	96.083	75.893	78.323	77.108	84.366
2		80.893	84.432	82.663	90.444	78.004	78.004	68.398	74.836
3		65.893	70.902	78.004	85.346	77.893	70.892	63.948	69.967
4		67.892	60.003	74.393	81.395	77.893	70.892	56.729	62.068
5		55.902	57.556	74.393	81.395	42.893	50.902	46.898	51.312
6		75.903	70.654	73.279	80.177	49.783	45.132	47.458	51.925
7	73.782	70.654	72.218	79.016	48.893	41.092	44.993	49.228	
0	200	178.665	178.665	178.665	100.00	184.906	182.843	183.875	100.00
1		172.905	177.544	175.225	98.075	169.885	167.223	168.554	91.668
2		167.546	166.804	167.175	93.569	156.331	156.331	156.331	85.020
3		162.903	160.673	161.788	90.554	148.223	154.881	151.552	82.421
4		157.894	160.002	158.948	88.964	140.745	133.144	136.945	74.477
5		164.783	154.984	159.884	89.488	128.563	124.785	126.674	68.891
6		164.778	164.778	164.778	92.227	122.893	119.432	121.163	65.894
7	159.905	157.674	158.790	88.875	94.784	92.054	93.419	50.806	
0	300	278.903	275.811	277.357	100.00	270.923	275.811	273.367	100.00
1		275.811	275.811	275.811	99.443	259.432	262.142	260.787	95.398
2		270.782	268.902	269.842	97.290	246.892	249.322	248.107	90.760
3		270.782	270.782	270.782	97.629	239.891	233.035	236.463	86.500
4		270.723	268.823	269.773	97.266	218.781	223.332	221.057	80.864
5		268.823	268.823	268.823	96.923	192.112	194.415	193.264	70.698
6		265.001	265.001	265.001	95.545	178.782	182.932	180.857	66.159
7	260.773	265.001	262.887	94.783	170.892	175.990	173.441	63.446	

Table C.162 Nitrate reduction (%) of PF3 at 80 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	91.854	91.854	91.854	100.00	85.443	88.214	86.829	100.00
1		81.895	78.054	79.975	87.068	71.435	74.243	72.839	83.888
2		77.905	72.554	75.230	81.902	64.894	62.094	63.494	73.125
3		72.554	72.554	72.554	78.988	60.675	58.231	59.453	68.471
4		71.783	68.894	70.339	76.577	54.889	54.889	54.889	63.215
5		69.334	67.121	68.228	74.279	44.773	42.043	43.408	49.993
6		65.783	67.121	66.452	72.345	33.782	38.892	36.337	41.849
7	68.649	67.964	68.307	74.365	29.782	25.188	27.485	31.654	
0	200	189.994	185.443	187.719	100.00	187.893	191.920	189.907	100.00
1		185.443	189.994	187.719	100.00	175.893	172.541	174.217	91.738
2		179.432	185.893	182.663	97.307	160.443	163.221	161.832	85.216
3		174.319	185.893	180.106	95.944	155.773	147.004	151.389	79.717
4		174.442	180.576	177.509	94.561	135.891	130.052	132.972	70.020
5		180.576	180.576	180.576	96.195	130.892	122.083	126.488	66.605
6		176.757	174.873	175.815	93.659	139.903	120.443	130.173	68.546
7	171.894	176.757	174.326	92.865	125.332	119.114	122.223	64.359	
0	300	274.892	270.453	272.673	100.00	274.892	278.431	276.662	100.00
1		268.783	268.783	268.783	98.573	260.154	265.688	262.921	95.033
2		265.110	270.432	267.771	98.202	246.564	248.902	247.733	89.544
3		265.110	265.110	265.110	97.226	235.843	238.714	237.279	85.765
4		259.330	259.330	259.330	95.107	225.882	228.073	226.978	82.042
5		252.516	259.330	255.923	93.857	197.892	195.093	196.493	71.023
6		253.839	250.902	252.371	92.554	180.232	184.892	182.562	65.987
7	247.002	247.002	247.002	90.585	180.903	182.443	181.673	65.666	

Table C.163 Nitrate reduction (%) of PF3 at 100 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	91.672	91.672	91.672	100.00	89.998	93.094	91.546	100.00
1		88.890	91.672	91.546	99.863	80.554	78.009	79.282	86.603
2		82.785	91.886	87.336	95.270	74.008	76.897	75.453	82.421
3		85.697	82.785	84.241	91.893	70.775	74.541	72.658	79.368
4		81.253	85.559	83.406	90.983	65.034	61.892	63.463	69.324
5		85.559	81.253	83.406	90.983	47.048	44.116	45.582	49.791
6		81.903	83.894	82.899	90.430	40.200	35.893	38.047	41.561
7	78.553	78.553	78.553	85.689	37.124	35.893	36.509	39.880	
0	200	187.778	190.083	188.931	100.00	187.778	190.083	188.931	100.00
1		179.432	175.772	177.602	94.004	160.902	168.492	164.697	87.173
2		167.893	165.113	166.503	88.129	153.824	146.002	149.913	79.348
3		163.892	163.892	163.892	86.747	141.443	134.057	137.750	72.910
4		158.443	160.115	159.279	84.305	132.829	139.049	135.939	71.952
5		160.115	160.115	160.115	84.748	110.892	118.003	114.448	60.577
6		158.007	160.902	159.455	84.398	88.032	86.164	87.098	46.100
7	151.841	156.742	154.292	81.665	50.732	52.723	51.728	27.379	
0	300	282.778	282.778	282.778	100.00	284.354	280.121	282.238	100.00
1		282.778	280.453	281.616	99.589	270.885	268.005	269.445	95.467
2		277.889	272.787	275.338	97.369	260.894	253.443	257.169	91.118
3		262.094	272.787	267.441	94.576	250.893	248.002	249.448	88.382
4		265.672	262.892	264.282	93.459	241.332	232.113	236.723	83.873
5		269.814	260.332	265.073	93.739	221.782	214.083	217.933	77.216
6		260.892	258.990	259.941	91.924	167.443	161.029	164.236	58.191
7	257.990	250.332	254.161	89.880	151.990	154.667	153.329	54.326	

Table C.164 Nitrate reduction (%) of PF3 at 150 mg/L of profenofos

Day	Nitrate Conc. (mg/L)	Control				Test			
		1	2	Average	%Normalized PF conc.	1	2	Average	%Normalized PF conc.
0	100	90.834	95.003	92.919	100.00	89.432	92.112	90.772	100.00
1		90.834	90.834	90.834	97.756	81.902	86.132	84.017	92.558
2		88.834	85.902	87.368	94.026	74.554	74.554	74.554	82.133
3		80.003	78.124	79.064	85.089	66.783	60.163	63.473	69.926
4		80.778	80.778	80.778	86.934	57.542	50.893	54.218	59.730
5		82.781	80.778	81.780	88.012	47.783	47.982	47.883	52.750
6		77.243	80.092	78.668	84.662	39.930	34.893	37.412	41.215
7	75.554	75.554	75.554	81.312	25.078	21.253	23.166	25.521	
0	200	184.903	180.554	182.729	100.00	180.554	178.834	179.694	100.00
1		172.893	178.904	175.899	96.262	165.543	168.615	167.079	91.435
2		175.489	172.903	174.196	95.330	158.889	158.889	158.889	88.421
3		170.224	172.903	171.564	93.890	155.465	151.092	153.279	85.300
4		167.453	170.893	169.173	92.581	138.048	145.783	141.916	78.976
5		160.720	167.453	164.087	89.798	135.892	129.083	132.488	73.730
6		154.990	160.893	157.942	86.435	117.893	113.094	115.494	64.273
7	152.883	160.893	156.888	85.858	95.342	92.153	93.748	52.171	
0	300	285.892	289.132	287.512	100.00	284.616	289.132	286.874	100.00
1		280.883	280.883	280.883	97.694	269.053	270.894	269.974	94.109
2		277.223	270.943	274.083	95.329	249.323	255.141	252.232	87.924
3		277.223	270.943	274.083	95.329	244.042	241.889	268.961	84.694
4		270.893	267.028	242.966	93.548	239.887	239.887	272.643	83.621
5		274.392	270.893	239.887	94.828	219.788	210.321	215.055	74.964
6		272.893	270.884	271.889	94.566	200.892	209.213	205.053	71.478
7	265.352	268.153	266.753	92.780	197.782	191.032	194.407	67.767	

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