ISOLATION AND CHARACTERIZATION OF DICHLORVOS-DEGRADING BACTERIA FROM CONTAMINATED SOIL

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การคัดแยกเชื้อและศึกษาคุณลักษณะของแบคทีเรียที่มีความสามารถในการย่อยสลายสารฆ่าแมลงไดคลอร์วอสจากดินที่ปนเปื้อน

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ไดคลอร์วอส (2,2-dimethyl dichlorovinyl phosphate) เป็นหนึ่งในสารเคมีกำจัดแมลงในกลุ่มออร์แกโนฟอสเฟต ซึ่งถูกใช้อย่างแพร่หลายในการควบคุมแมลงศัตรูของพืช ดังนั้นจึงมีความเป็นไปได้ที่สารไดคลอร์วอสอาจปนเปื้อนลงสู่สิ่งแวดล้อม งานวิจัยนี้มีจุดมุ่งหมายเพื่อศึกษาการย่อยสลายสารไดคลอร์วอสทางชีวภาพ กลุ่มจุลินทรีย์และจุลินทรีย์บริสุทธิ์ที่สามารถย่อยสลายสารไดคลอร์วอสได้คัดแยกมาจากดินปนเปื้อน การศึกษามุ่งเน้นการย่อยสลายสารไดคลอร์วอสภายใต้สภาวะที่มีความเข้มข้นไดคลอร์วอส (50 100 200 400 และ 800 มิลลิกรัมต่อลิตร) และ ค่าความเป็นกรดด่าง (pH 4 5 6 7 และ 8) ต่างกัน นอกจากนี้การศึกษานี้ยังครอบคลุมการคาดการณ์จลนพลศาสตร์การย่อยสลายและการติดตามสารมัธยันต์ กลุ่มจุลินทรีย์และจุลินทรีย์บริสุทธ์สามารถใช้สารไดคลอร์วอสได้ดี จุลินทรีย์บริสุทธ์ที่คัดเลือกได้ 3 ชนิดระบุเป็น *Klebsiella* sp. strain DV1 (DV1) *Enterobacter* sp. strain DV2 (DV2) และ *Klebsiella pneumoniae* strain DV3 (DV3) ในการทดลองภายใต้สภาวะที่มีความเข้มข้นของสารไดคลอร์วอสต่างกันพบว่ากระบวนการทางเคมี (ปฏิกิริยาไฮโดรไลซิส) สามารถกำจัดสารไดคลอร์วอสได้ 0.10 ถึง 0.28 ต่อวัน ในขณะที่การย่อยสลายทางชีวภาพ (ร่วมกับปฏิกิริยาไฮโดรไลซิส) โดยกลุ่มจุลินทรีย์และจุลินทรีย์บริสุทธ์สามารถกำจัดสารไดคลอร์วอสได้ 0.17 ถึง 0.28 และ 0.24 ถึง 0.43 ต่อวัน ตามลำดับ ในสภาวะที่มีสารไดคลอร์วอสความเข้มข้นสูง (มากกว่า 400 มิลลิกรัมต่อลิตร) กระบวนการย่อยสลายด้วยจุลินทรีย์สามารถเพิ่มการกำจัดสารไดคลอร์วอสได้ สำหรับผลของ pH ไฮโดรไลซิสสามารถกำจัดสารไดคลอร์วอสได้ดีที่ pH 7 ถึง 8 (0.67-0.80 ต่อวัน) ในขณะที่การกำจัดสารไดคลอร์วอสที่ pH ในช่วง 4 ถึง 6 ลดลงเหลือเพียง 0.04 ถึง 0.30 ต่อวัน ในสภาวะที่มี pH เป็นกรดการย่อยสลายทางชีวภาพ (ร่วมกับปฏิกิริยาไฮโดรไลซิส) โดยกลุ่มจุลินทรีย์ DV1 DV2 และ DV3 สามารถกำจัดสารไดคลอร์วอสได้ถึง 0.06-0.13 0.05-0.38 0.07-0.40 และ 0.07-0.38 ต่อวัน ตามลำดับ ในระหว่างการทดลองการย่อยสลายสารไดคลอร์วอสตรวจพบสารมัธยันต์ 3 ชนิด ซึ่งได้แก่ 2-chloroethyl dimethyl phosphate triethyl phosphate และ trimethyl phosphate ทั้งในกระบวนการไฮโดรไลซิสและการย่อยสลายทางชีวภาพ จากการศึกษาครั้งนี้สามารถกล่าวได้ว่าการย่อยสลายทางเคมีโดยกระบวนการไฮโดรไลซิสมีอิทธิพลต่อการย่อยสลายสารไดคลอร์วอส การย่อยสลายทางชีวภาพสามารถเพิ่มประสิทธิภาพการกำจัดสารได้ โดยเฉพาะภายใต้สภาวะที่สารปนเปื้อนความเข้มข้นสูงหรือสภาพเป็นกรด กลุ่มจุลินทรีย์และจุลินทรีย์บริสุทธ์มีประสิทธิภาพในการย่อยสลายสารไดคลอร์วอสและมีความเป็นไปได้ในการใช้งานฟื้นฟูพื้นที่ปนเปื้อนสารไดคลอร์วอสได้ต่อไป

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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NUNNAREE THONGSUKMAK: ISOLATION AND CHARACTERIZATION OF DICHLORVOS-DEGRADING BACTERIA FROM CONTAMINATED SOIL. ADVISOR: ASST. PROF. SUMANA RATPUKDI, Ph.D., CO-ADVISOR: ASSOC. PROF. ALISA VANGNAI, Ph.D., 138 pp.

Dichlorvos (2,2-dimethyl dichlorovinyl phosphate) is one of the major organophosphate insecticides. It has been widely used to control various insect pests. There is possibility of dichlorvos contamination to the environment. This study aimed on biodegradation of dichlorvos. Dichlorvos-degrading consortium and pure cultures were enriched from contaminated soil. Dichlorvos degradation under different dichlorvos concentrations (50, 100, 200, 400 and 800 mg/L) and pHs (pH 4, 5, 6, 7 and 8) was focused. In addition, the degradation kinetic estimation and metabolic intermediate monitoring were conducted. A consortium and pure cultures well utilize dichlorvos. Three selected isolates were identified as *Klebsiella* sp. strain DV1 (DV1), *Enterobacter* sp. strain DV2 (DV2) and *Klebsiella pneumoniae* strain DV3 (DV3). Under different dichlorvos concentrations, abiotic process (hydrolysis) could remove dichlorvos for 0.10-0.28 1/d while biodegradation (with hydrolysis) by a consortium and pure cultures well degraded dichlorvos with rate constance of 0.17-0.28 and 0.24-0.43 1/d, respectively. At high dichlorvos concentrations (more than 400 mg/L), microbial degradation could enhance the dichlorvos removal. For effect of pH, hydrolysis well removed dichlorvos at pH of 7 to 8 (0.67-0.80 1/d) while the dichlorvos removal at pHs ranging from 4 to 6 was 0.04 to 0.30 1/d. At acidic pHs, biodegradation (with hydrolysis) by a consortium, DV1, DV2 and DV3 reduced dichlorvos of 0.06-0.13, 0.05-0.38, 0.07-0.40 and 0.07-0.38, respectively. During the dichlorvos degradation experiment, three metabolites including 2-chloroethyl dimethyl phosphate, triethyl phosphate and trimethyl phosphate were detected in both abiotic (hydrolysis) and biodegradation processes. It could say that abiotic process by hydrolysis reaction influenced dichlorvos degradation. Biodegradation could improve the removal performance, espicailly under the conditions with high contaminated concentration or acidic pH. The consortium and isolates are efficient in dichlorvos degradation and have potential for dichlorvos remediation.

Field of Study: Environmental Management Student's Signature .....
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CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION

1.1 Motivation

Organophosphates (OPs) are one of the most commonly used pesticides in agriculture to protect plants and prevent crop damaged from pest weeds, diseases and to increase harvest productivity for more than sixty years (Cycon et al. 2013). It is well known that their effect on non-target organisms including humans since OPs have a high acute toxicity due to the prevention of neural impulse transmission by their inhibition of cholinesterase (Sogorb and Vilanova 2002). Therefore, these compounds considered as environmental pollutants and human health hazard.

Dichlorvos (2,2-dimethyl dichlorovinyl phosphate) which known as DDVP is one of the major organophosphate insecticides, commercially manufactured since 1961 and used in different parts of the world. It has been widely used in order to control a large variety of insect pests on agricultural, commercial, domestic, and industrial sites. It is also used for controlling parasites, insects in houses, aircraft and outdoor areas (as aerosols form or liquid sprays) (Evgenidou et al. 2005). The global production of dichlorvos was approximately 4 million kg/y (World Health Organization [WHO] 1989). In the United States, the production volumes of dichlorvos were quite high (up to 450,000 kg/yr) (United State Department of Health and Human Services [U.S. DHHS] 1997). The demand of dichlorvos in China was over 40,000 tons in 2007 (Liu et al. 2009). In Thailand, the imported quantities of dichlorvos were 623,876 kg with the value of 48,579,810 Baht in 2012. Dichlorvos was the 3rd highest imported insecticide of Thailand (Ministry of Agriculture and cooperatives [MOA] 2013). A large amount of dichlorvos utilization could result high possibility of dichlorvos contamination in the environment via many ways including air, water and soil. It relates from self-reaction of dichlorvos, leaks from storage containers, evaporation, and accidental spill during manufacturing, transportation, landfills and during crop application process (Agency for Toxic Substances and Disease Registry [ATSDR] 1997).

Additionally, dichlorvos also occurs from a metabolic process of other chemicals. Naled (dimethyl 1,2-dibromo-2,2-dichloroethylphosphate) and trichlorfon (dimethyl-(2,2,2-trichloro-1-hydroxyethyl) phosphonate) could metabolize and degrade to dichlorvos in food, water, or the environment (Samuelsen, 1987; U.S. EPA, 2006; Li et al., 2011). Naled is used mainly for mosquito elimination and for insect control in agriculture crops. For trichlorfon, US EPA has banned trichlorfon for farming but it is still continuously used in cattle and fish production. Therefore, using these pesticides resulted to indirect increase of dichlorvos contaminating in

environment (Northest Coalition for Pesticides [NCAP] 2002, Thomaz et al. 2009). The International Agency for Research on Cancer (IARC) has classified dichlorvos as possible carcinogen to human – Group 2B (World Health Organization [WHO] 1999). The poisonous effect of dichlorvos has been shown to inhibit acetylcholinesterase and cholinesterase activities in brain. These enzymes are important for neurological function in nervous system. Therefore, exposure to dichlorvos resulted in neurotoxicity, dermatologic irritation and respiratory disorder effects (United States Department of Health and Human Services [U.S. HHS] 1997, U.S. EPA 2006).

Correspondingly, it is necessary to develop a method that is safe, feasible, convenient, and economical to remediate dichlorvos pesticide residue. Biodegradation has been recognized as an effective bioremediation technique which is a reliable and cost-effective method for the pesticide removal (Yang et al. 2005). A lot of bacteria have been reported to transform various contaminants including organophosphate pesticides (Ramanathan and Lalithakumari 1998, Horne et al. 2002). In the case of dichlorvos, there are studies reported the success on biodegradation. *Flavobacterium* sp. was isolated from the rape phyllosphere which utilized dichlorvos as a sole phosphorus source (Ning et al. 2012). The degradation rate of dichlorvos was 60.89%. Another study by Xiao-Hua et al. (2006) found *Ochrobactrum* sp. isolated from pesticide manufacturing wastewater treatment plant could use dicholrvos as a sole carbon source. The degradation efficiency depends on pH and temperature (Xiao-Hua et al. 2006). Moreover, *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp., and *Acinetobacter* sp. isolated from agricultural soil could utilize dichlorvos as a sole carbon source. Based on the prior works, the biodegradation by isolated bacteria is potential for dichlorvos removal from contaminated environment including water.

However, the rate of degradation depends on many factors including environmental factors such as pH, temperature, moisture content and amount of oxygen. Additionally, concentration of pesticide, type and characteristic of microorganisms are concerned in the biological treatment (Tang and You, 2012). Thus far, it is difficult to obtain previously isolated microbial cultures for pesticide removal. Also, in order to avoid environmental stresses, enriched bacterial consortium and isolates from contaminated sites are effective alternative for the case (Benimeli et al. 2008). Consequently, this study emphasized on isolation of bacteria from contaminated crop field for dichlorvos removal. Also, the effects of dichlorvos concentration and pH on dichlorvos degradation were investigated. The enriched consortium and isolates will be useful for the contaminated site and may be applied for other contaminated fields later on.

1.2 Objectives

Main objective

To isolate and characterize dichlorvos – degrading bacteria from contaminated soil.

Specific objectives

1. To isolate dichlorvos-degrading bacterial consortium and strains from contaminated soil by enrichment technique.
2. To examine degradation of dichlorvos by dichlorvos-degrading bacterial consortium and strains at different pH and dichlorvos concentrations.
3. To monitor intermediate product during dichlorvos biodegradation.

1.3 Scopes of the Study:

1.31 This study is in laboratory level.

1.32 Dichlorvos-degrading bacterial mixed culture and each pure isolated culture in this study enriched from dichlorvos-contaminated soil obtained from agricultural site, Khon Kaen, Thailand.

1.33 Dichlorvos applied in the experiment was a commercial product, Supernox Pratoothong, Thailand while one used for analysis was analytical grade obtained from Fluka, Sigma-Aldrich, Singapore.

1.34 Initial dichlorvos concentrations of 50, 100, 200, 400, and 800 mg/L were varied.

1.35 pH ranging from 4.0 to 8.0 were tested.

1.36 Residual dichlorvos concentration was analyzed by Gas Chromatography coupled with Electron Capture Detector (GC-ECD).

1.37 Cell number was measured by colony plate count technique.

1.38 Initial cell number used in this study was approximate 1.2×10^9 CFU/ml.

1.4 Hypotheses:

1. Dichlorvos-degrading bacterial consortium and strains can be enriched from the contaminated soil.
2. pH and dichlorvos concentrations affect degradation of dichlorvos.
3. Dichlorvos degradation intermediate products could be monitored during the biodegradation.

CHAPTER II
LITERATURE REVIEW

2.1 Organophosphate pesticide

2.1.1 Overview of organophosphate pesticide

Organophosphorus pesticides are the most utilization pesticides these days. They are ester of phosphoric acid, phosphonic acid or thiophosphoric acid. The main structure composes of phosphorthioate (P = S) or phosphate (P=O) (Figure 1). The toxicity of organophosphorus pesticide which composed with P = S (called organothiophosphate) generally less than P = O (called organophosphate). However, the organothiophosphate substances are longer persistant resulting higher accumulation in the environment.

Organophosphorus pesticides are widely used to control pest for agricultural purpose. The most well-known compounds including monocrotophos, mevinphos, chlorpyrifos, dimethoate, dicrotophos, parathion-methyl, and parathion are presented in Table 1. Organophosphorus pesticides normally degrade in the environment with photodegradation or biodegradation by microorganism in soil within a relatively short period of time. However, the integration between these substances with some organic materials or some minerals available in soil may cause the substances remain in the environment for a long time. In addition, it was reported that organophosphorus pesticide contaminating in acidic soil remained longer (Jaga and Dharmani 2003, Centers for Disease Control and Prevention [CDC] 2013).

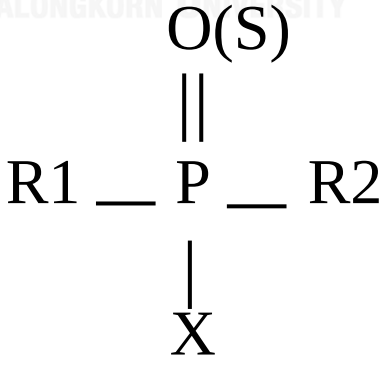


Figure 2.1 General chemical structure of an organophosphate. R1 and R2 are alkyl-, alkoxy-, alkylthio-, or amido-groups. X is the acyl residue (labile fluorine-, cyano-, substituted- or branched-aliphatic, aromatic, or heterocyclic groups)
(Kazemi et al. 2012).

Table 2.1 A list of commonly used organophosphate insecticides

Organophosphate insecticides	Organophosphate insecticides (con.)
Acephate	Isofenphos[a]
Azinphos-methyl[a]	Malathion
Bensulide	Methamidophos[a]
Chlorethoxyphos[a]	Methidathion[a]
Chlorpyrifos[a]	Mevinphos
Coumaphos[a]	Naled[a]
Diazinon[a]	Phosmet
Dichlorvos[a]	Profenofos[a]
Dicrotophos[a]	Propetamphos
Dimethoate	Sulfotepp[a]
Disulfoton[a]	Sulprofos[a]
Ethion[a]	Tebupirimiphos
Ethoprop[a]	Temephos[a]
Ethyl parathion[a]	Terbufos[a]
Fenamiphos[a]	Tetrachlorvinphos
Fenitrothion[a]	Tribufos
Fonofos[a]	Trichlorfon

[a]Use of this organophosphate is restricted by the Environmental Protection Agency of the United States (Sullivan and Blose 1992, U.S. EPA 2013).

2.1.2 Toxicity of organophosphate pesticide

Due to widespread use of organophosphate pesticide, humans can be exposed through various routes including ingestion, inhalation, and dermal absorption (Fortenberry et al. 2014). Organophosphate substances generally cause acute toxicity to human and vertebrates. Acute poisoning can cause symptoms of brain disorders due to abnormality of central nervous system. Common symptoms include dizziness, headache, lethargy, restlessness and severe symptoms may cause seizure, unconsciousness, and dead. Monocrotophos, parathion, methyl-methamidophos, and dicrotophos are high toxic organophosphorus pesticides while chlorpyrifos, dimethoate, malathion, naled, trichlorfon, and dichlorvos are moderately toxic (Tafuri and Roberts 1987, Silva and Samarawickrema 2006).

2.2 Dichlorvos

2.2.1 Physical and chemical properties

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) (also known as DDVP), is a chlorinated volatile organophosphate compound which is a synthetic chemical substance and cannot be found in natural environment (United States Department of Health and Human Services [U.S. HHS] 1997). Dichlorvos was firstly discovered and synthesized in 1961 (World Health Organization [WHO] 1989). It has been subdivided into phosphorylcholines group since dichlorvos comprises chlorine in its structure (Figure 2.2) (Agency for Toxic Substances and Disease Registry [ATSDR] 1997).

Dichlrvos has been commonly used as an insecticides, herbicides, or fungicides in green houses, food storage areas, workplaces, and houses. It occurs as a viscous fluid with colorless to amber liquid. Physical and chemical characteristics of dichlorvos are shown in Table 2.2.

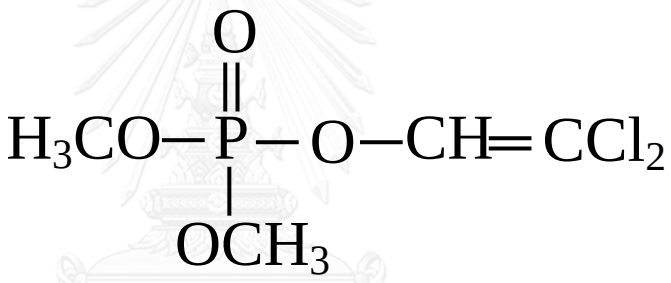


Figure 2.2 Dichlorvos Structure

Table 2.2 Physiochemical properties of dichlorvos

Physiochemical properties	**Information**
Common name	Dichlorvos (DDVP)
Chemical name	2,2-dichlorovinyl dimethyl phosphate (IUPAC) or 2,2-dichlorothenyl dimethyl phosphate (CAS)
Relative density of gas	7.63
Molecular formula	$C_4H_7Cl_2O_4P$
Molecular weight	220.98
Color	colorless or shiny brown
Odor	Mild chemical odor or aromatic odor
Solubility	10,000 mg/L (at 20 °C)

Physiochemical properties	Information
	16,000 mg/L (at 25 °C)
The octanol-water partition coefficient (Log K_{OW})	1.47
Soil adsorption coefficient (Log K_{OC})	1.45
Henry's Law Constant	6.81×10^{-7} atm-m^3/mol (at 25 °C)
Density	1.45 (at 25 °C)
Boiling point	35 °C (at 0.05 mmHg) 120 °C (at 14 mmHg) 140 °C (at 20 mmHg) 221 °C (at 760 mmHg)
Melting point	< 25 °C
Vapor pressure	1.2×10^{-2} mmHg (at 20 °C)
EPA toxicity classification	Class II

Source: (Bowman and Sans 1983, Domine et al. 1992, Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Schram and Hua 2001)

2.2.2 Mode of action

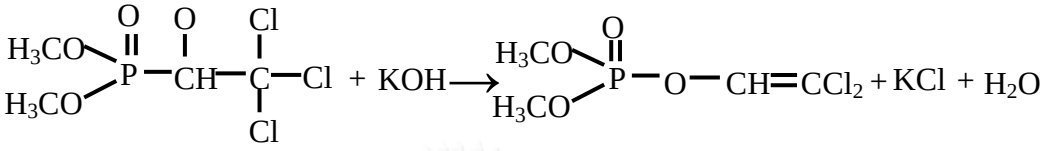
Diclorvos can kill insects via ingested, or absorbed through the spiracles or integument. The major mechanism of dichlorvos inhibits acetyl acetylcholinesterase enzyme (AChE). The enzyme is bonded to molecules of organophosphate pesticides which is called phosphorylated enzyme. The inhibition of AChE associated with an increase in the level of acetylcholine (ACh) in the neuron/neuron junction (synapse) or neuron/muscle junction. This leads to problem in the peripheral and central nervous system of insects resulting in muscle tremors, severe convulsions, paralysis and respiratory failure, and death (Booth et al. 2007, Pancetti et al. 2007, Espeland et al. 2010).

2.2.3 Production and utilization

Dichlorvos is organophosphate insecticide which extensively used around the world especially in developing countries. It was initially synthesized in the late 1940s.

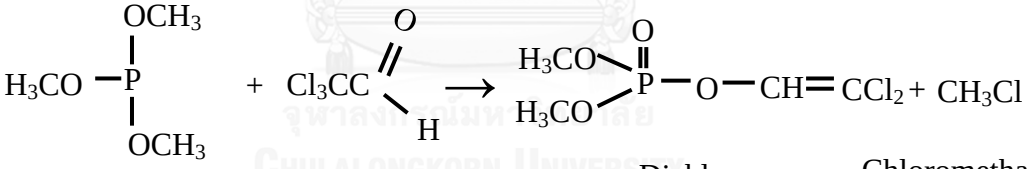
An industrial scale production of dichlorvos began in the late 1950s in the United States. Dichlorvos is produced from two reactions include:

1) Dehydrochlorination of the pesticide trichlorfon (chlorophos) in aqueous alkali (potassium hydroxide) at 40-50 °C as shown in the equation below (World Health Organization [WHO] 1989, Pollution Control Department [PCD] 2008).



Trichlorphon — Potassium hydroxide — Dichlorvos — Potassium chloride — Water

2) Dichlorvos is manufactured by a reaction between trimethylphosphite and chloral as shown in the equation below (Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Pollution Control Department [PCD] 2008). This process is more effective reaction with production yield of 92-93 percent.



Trimethylphosphite — Chloral — Dichlorvos — Chloromethane

In Thailand, there is no production of dichlorvos. It is imported from other countries, such as China, Switzerland and India. Based on the information from Ministry of Agriculture, in overall, the imported dichlorvos quantities are increasing every year (Table 2.3).

Table 2.3 Import dichlorvos to Thailand

Year	Imported volumes (kg)
2002	134,635
2003	264,606
2004	687,441
2005	236,432
2006	340,233
2007	483628
2008	454,110
2009	398,314
2010	417,460
2011	553,746
2012	623,876

Source: (Department of Agriculture [DOA] 2002-2012)

In 2012, Thailand has to import dichlorvos up to 623,876.00 kg with the value of 48,579,810.66 Baht. Dichlorvos is the 3rd highest insecticide imported to Thailand after chlorpyrifos and carbaryl respectively (Department of Agriculture [DOA] 2013). Not only dichlorvos is used as an insecticide for agriculture, but also it is utilized for household and public health purposes. Previous study reported that 60% of total dichlorvos production was implemented in agriculture (Gandhi and Snedeker 1999). It is used to eliminate insect pests and weed pests or to mix with seeds before planting. Dichlorvos of 30% is used for public health. It is used for preventing the harmful or nuisance insects including mosquitoes, ticks, mites, and ants. The rest (10%) is applied to prevent materials and products during storage before processing and after production, respectively.

Most of the dichlorvos utilization is to mix with other insecticides to improve the effectiveness. For example, the mixture of dichlorvos, piperonyl butoxide, and pyrethrins is used for removing the general flying insects. Another example is Piran. It is the mixture product of dichlorvos and dibromochloropropane which apply for removing plant pest (Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Meister 1998). There are numerous trade name of dichlorvos including apavap, benfos, cekusan, cypona, derriban, derribanate, devikol, didivane, duo-kill, duravos,

elastrel, fly-bate, fly-die, flyfighter, herkol, marvex, prentox, vaponite, vapona, verdican, verdipor, and verdisol. Dichlorvos trade names commonly known in the United States are doom, nogos, and nuvan (National Pesticide Information Center [NPIC] 1996, Chaudhary and Bist 2014).

In agriculture, dichlorvos is applied in farms, such as cotton, coffee, cocoa, tobacco, rice, potatoes, corn, sorghum, and soybeans in order to get rid of insects, weeds and rodents such as caterpillars, moths, aphids, mayflies, ladybugs, beetles, oriental fruit fly, rice leaffolders, asiatic corn borer, and mice. It is also applied in orchards, such as grape, apple, peach, orange, etc. Furthermore, it is implemented in vegetable cultivation, such as cucumbers, carrots, cauliflower, cabbage, tomatoes, as well as flowers and many other plants. Dichlorvos is applied in the form of aerosol spraying in cultivation area. Dichlorvos was applied for apple production by 1.5 kg/ha in Switzerland, strawberry by 0.65-1.25 kg/ha in South America, grape in France and Switzerland by 1.0-1.25 kg/ha. To use as an herbicide, dichlorvos was used for grass elimination in a garden plot by 0.9 mg/m^3 (World Health Organization [WHO] 1989, Agency for Toxic Substances and Disease Registry [ATSDR] 1997, U.S. EPA 1999). In Thailand, dichlorvos is used as pesticides for crop protection and parasite control in livestock as shown in Table 2.4.

Table 2.4 Utilization of dichlorvos in Thailand

Crop	Pests	Application rate
Vegetable	Aphids, Diamondback moth, Flea beetle, Bean fly, Leaf-eating ladybird beetle, Corn armyworm, Common cutworm, Gonepteryx rhamni	16-25 mL/ 20 L of water
Cucurbitaceous	Aphids, Rice thrips	
Tomato	Aphids, Corn armyworm	16-25 mL/ 20 L of water
Grain	Aphids, Rice thrips, Leafhopper, Moina, Rice seedling armyworm	
Green stink bug	Rice ear-cutting caterpillar	
Cotton	Aphids, Rice thrips, Leafhopper, Common cutworm	
Flowerer	Aphids, Rice thrips, Moina	
Tobacco	Aphids	

Crop	Pests	Application rate
Grapes	White fly, Rice thrips, Moina	3-5mL/ 20 L of water
Orange	Scale insect, Mealy bug, Leaf eating caterpillar, Citrus Leaf miner	
Banana	Scale insect	
Storage Unit (for grain in Storage houses)	Cockroach, Mayflies, Cigarette beetle, Weevil, Red flour beetle	Emulsions used in concentrations of 50-100 mL / 20 L of water spraying in a storage house.

(Pollution Control Department [PCD] 2008)

Dichlorvos (about 0.02 g/ft^2) is also used to eliminate insects and parasites in livestock farming for cattle, pig, sheep, duck, and chicken farms. Dichlorvos is also applied for freshwater and marine aquaculture for controlling parasites, especially in salmon and carp farm (World Health Organization [WHO] 1988, Grave et al. 1991, U.S. EPA 1995, Binukumar and Gill 2010).

Moreover, dichlorvos is applied in industrial sector. For example, it is used in crop preservation. It is used during production process and storage. In residential area, dichlorvos (about 15 g in 10-15 weeks) can apply to control insects in building, museum, garage, garden, and greenhouse (Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Australian Pesticides & Veterinary Medicines Authority [APVMA] 2008).

2.2.4 Releasing to the environment

Dichlorvos could contaminate to the environment via several process as follows. First of all, from manufacturing process, filling and packaging process may lead to dichlorvos releasing to the atmosphere. Furthermore, storage and transport may cause dichlorvos entering to the environment from accidental leaking. During application process, this is the major route of contamination. Lastly, during disposal process, improper waste disposal may cause the dichlorvos contamination in soil and groundwater.

Dichlovos releasing to the air may be in forms of spray, liquid, and aerosol. It generally degrades through the photodegradation. World Health Organization [WHO] (1989) reported that in 1993, dichlorvos of approximately 593 kg was utilized. It was also found that 84% of dichlorvos was applied as fumigant and could be detected in

the air (Califonia Environmental Protection Agency [Cal. EPA] 1996). Leary et al. (1974) monitored the concentration of dichlorvos contaminating in house in Arizona, USA. The study was reported that dichlorvos of 0.11-0.16 mg/m^3 detected after application for 12-15 days. For longer period, dichlorvos concentration may be lower than detection limit. The removal in the case was from photodegradation.

Dichlorvos contamination in the water mainly occurs in upper layer (approximately 50 cm) of surface water. Howard (1991) reported that dichlorvos could be rapidly degraded in the water depending on pH and temperature of the water. High temperature and alkaline water caused rapid degradation of dichlorvos. Dichlorvos contamination in water could decompose through both abiotic and biolotic degradation. For abiotic process, dichlorvos was mostly degraded by hydrolysis reaction. The dichlorvos metabolites from hydrolysis reaction in the water including dichloroethanol, dichloroacetaldehyde, dichloroacetic acid, dimethyl phosphate, and dimethyl phosphoric acid were reported. Fritz et al. (1984) examined half-life of dichlorvos in the water. Dichlorvos of 10 mg/L at 30 °C and pH of 1, 5, 7, and 9 had half-lives of 74, 50, 18, and 16 hours, respectively. Furthermore, from another study indicate that hydrolysis reaction of dichlorvos cannot occur at pH less than 3.3 (Lamoreaux and Newland 1978). Sakai (2003) explored pesticide contamination in water near agricultural areas in Yokohama, Japan from August 2001 until July 2002. The results of the survey found dichlorvos (0.33-0.5 µg/L) in most water samples.

Dichlorvos contamination in soil may occur during the production process as well as accidental spillage (International Agency for Research [IARC] 1991). Dichlorvos which was sprayed on the soil surface, leached into the ground at depth of 30 cm for about 18-20% within 5 days after spraying (Howard 1991). Dichlorvos contamination in the soil were decomposed by the hydrolysis, biological degradation, and photodegradation processes. Due to the characteristics of the soil are different, dichlorvos half-life in soil varied from 1.5 to 17 days (World Health Organization [WHO] 1989). The soil containing clay and organic matter could adsorb dichlorvos resulting in less contamination in groundwater. Also, it caused difficulty to be degraded leading to longer half- life. Table 2.5 is the summary of dichlorvos contamination in the environment.

Table 2.5 Concentration of dichlorvos detected in the environment of different countries

Place/Country	Year of study	Dichlorvos concentration	Reference
Air			
• Shimizu/Japan	2000 – 2001	1.33-15.0 ng/m^3	Ohura et al. (2006)
Natural Water			
• Jiulong river/China	2000	22.84 ng/L	Zulin et al. (2002)
• Taihu Lake/China	2003-2004	51.6 ng/L	Qu et al. (2011)
Rainwater			
• South Holland/ Netherlands	1998-1999	9.5 ng/L	Hamers et al. (2003)
Vegetables			
• Shaanxi area/China	2003	0.004 mg/kg	Bai et al. (2006)
• Kumasi market/Ghana	2006-2007	0.022-0.151 mg/kg	Darko and Akoto (2008)

2.2.5 Other chemicals relating to Dichlorvos

Dichlorvos can also enter to the environment as degradation product of other chemicals including trichlorfon (O,O-dimethyl(1-hydroxy-2,2,2-trichloroethyl) phosphonate) and naled (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate). In the environment, trichlorfon (trade name as metrifonate) is decomposed to dichlorvos by hydrolysis and photolysis as shown in Figure 2.3. Trichlorfon is used as an insecticide or anthelmintic agents. Dichlorvos is about eight times more toxic than trichlorfon. Oral LD_{50} of dichlorvos in rat is 56-80 mg kg^{-1}, while that of trichlorfon is 630 mg kg^{-1} (Samuelsen, 1987; Li et al., 2011) .

Naled (trade name is dibrom) is used as an insecticide in order to eliminate mosquitoes, mites and flying insects. It could be broken down to dichlorvos in both inside animals and the environment through chemical hydrolysis and biodegradation (U.S. EPA 1995). Naled was rapidly degraded via hydrolysis in water with alkaline condition (half-lives of 15.4 at pH 7 and 1.6 at pH 9). For photolysis, naled was also speedily degraded in water (about 1 d) and normally generated dichlorvos (Hoang and Rand 2015).

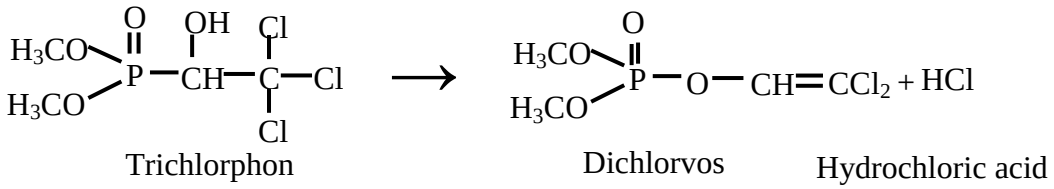


Figure 2.3 Degradation of trichlorphon (Pollution Control Department [PCD] 2008)

2.2.6 Toxicity of dichlorvos

Dichorvos not only causes toxic to insects (target organisms) but mammalian toxicity including human (non-target organisms) are also significant. Acute toxicity of dichlorvos to human is the inhibition of acetylcholinesterase enzyme (AchE) which is an important enzyme in brain and nerves. The acute toxicity symptoms of dichlorvos in the nervous system are shown in Table 2.6 (Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Pollution Control Department [PCD] 2008).

Table 2.6 Acute toxicity of dichlorvos in human

Nervous system	**Organ**	**Symptoms**
Nervous system	Rspiratory system	Productive cough, rhinorrhea, dyapnea from bronchispasm, and pulmonary edema
	Digestive system	Nausea, vomitting, diarrhea, abdominal cramp, and fecal incontinence
	Urinary System	Urinary incontinence
	Eye	Pupil constriction, blurred vision
	Glands	Epiphora, hyperhidrosis, and drooling
Sympathetic and parasympathetic nervous system	Circulatory system	Tachycardia and hypertension
Neuromuscular junction	Skeletal muscles	Ocular and facial muscle spasm, muscle weakness , fatigue, and malaise
Central nervous system	Brain	Lethargy, headache, attention deficit, hypotension, respiratory compression, ataxia, seizure or coma

(Food and Drug Administration [FDA] 2000)

Patients who intake high dose of dichlorvos probably fetal because of respiratory failure, respiratory muscle paralysis and respiratory center dysfunction. For chronic toxicity of dichlorvos, the poisoning symptoms show after repeated exposure of dichlorvos. The symptoms are malaise, vomit, restless, fatigue, weary, pupil constrict, muscle tremors, dizzy, conjunctivitis, and cramp (Agency for Toxic Substances and Disease Registry [ATSDR] 1997, Department of Agriculture [DOA] 2011).

There are many previous studies on toxicity of dichlorvos. Dichlorvos is moderately to highly hazardous to mammals with single ingestion exposure. The acute toxicity values (LD_{50}) of dichlorvos on mammals are shown in Table 2.7.

Table 2.7 Acute toxicity values of dichlorvos to tested animal

Animal test	**Exposure route**	**LD_{50} (mg/kg body weight)**
Rat	Ingestion	30-110
Male mouse	Ingestion	80
Female mouse	Ingestion	56
Male chicken	Ingestion	14.8
Pig	Ingestion	157
Dog	Ingestion	100-316
Cat	Ingestion	28
Rabbit	Ingestion	13-23

(World Health Organization [WHO] 1989)

Environmental Health Criteria [EHC] (1988) reported that dichlorvos was toxic to various animals and insects such as bee (with LD_{50} was 0.29 mg/kg body weight). Moreover, EHC (1988) also found that aquatic animals living in the dichlorvos-contaminated water had dichlorvos of 0.25-1.25 mg/kg body weight in brain and liver. LD_{50} of dichlorvos via ingestion in mouse and chick were 56-108 and 14.8 mg/kg body weight, respectively.

In human, Health and Safety Guide [HSG] (1988) reported that exposed dichlorvos via ingestion was rapidly absorbed into bloodstream and rapidly degraded by metabolism process in liver within 1 hour. Hutson and Hoadley (1972) found decreasing of cholinesterase enzyme levels by 75% from dichlorvos ingestion. Later, Gandhi and Snedeker (1999) investigated risk of farmers using dichlorvos. They

found that farmers had higher abnormality of white blood cells and children living in the area had higher risk of brain cancer.

2.2.7 Degradation of dichlorvos in the environment

1. Chemical degradation

Chemical degradation processes are important to insecticide degradation in nature. Chemical degradation occurs through several reactions, such as hydrolysis, oxidation, reduction, isomerization, ionization, and salt formation. For dichlorvos degradation, important chemical reaction is hydrolysis. The metabolites of this reaction include dimethyl phosphoric acid and dichloroacetaldehyde (via dichlorovinyl alcohol as an unstable intermediate) (Vitthal and Murlidhar 1993). The hydrolysis of dichlorvos depends on pH and temperature. The rate of hydrolysis increases with increasing pH or rapidly degrades under alkaline condition. Conversely, it slowly degrades in acidic condition (Australian Pesticides & Veterinary Medicines Authority [APVMA] 2008). Half-life of dichlorvos by hydrolysis at 20 °C in standard buffers at pH 5, 7, and 9 were approximate 77, 31.4, and 19 hours, respectively. Dichlorvos would be classified as rapid (DT_{50} < 1 days) to ready (DT_{50} in range 1-4 days at pH of 7.8-9.3) hydrolysis (Suter 1981). Lamoreaux and Newland (1978) studied the degradation rate of dichlorvos in buffer solution at a range of pH values. The results showed that hydrolysis occurred very slowly under acidic condition (pH 2-3.3) leading to slight hydrolysis (half-life more than 30 days). At pH of 6.2-6.9, it was moderate hydrolysis with half-life of 10-30 days. Hydrolysis reaction not only depends on pH value, but also depends on the temperature. At higher temperatures, the dichlorvos degradation rate was faster (Dedek et al. 1979).

2. Physical degradation

Photodegradation is not considered as the main process in degradation of dichlorvos. Base on the ability of dichlorvos in UV-adsorption spectrum, it can conclude that direct photolysis of dichlorvos could not occur under normal environmental condition. Wilmes (1983) reported that no degradation occurred after applying dichlorvos solution of 22 mg/L with high-pressure mercury vapor lamps. In addition, Guth and Voss (1970) found that the half-life of dichlorvos (100 mg/L) under filtered UV light conditions was approximately 7 hours in water but it remained stable in methanol after 6 hours exposure. This result implied that dichlorvos degradation apparently due to hydrolysis (in aqueous solution).

The main metabolites of dichlorvos were 2,2-,dichloroacetaldehyde and des-methyl dichlorvos based on the radiocarbon application. For the chemical degradation of dichlorvos on sandy loams, dichlovos mostly degraded at surface layer of soil. At pH of 7, half-lives of the tests with and without (dark) UV-light were 15.5

and 16.5 hours, respectively (U.S. EPA 1998). After 72 hours of irradiation, dichlorvos disappeared from the soil by a combination of degradation and volatilization. The metabolites founded in the irradiated soil were 2,2-dicholoroacetic acid (26.6%) and 2,2-dichloroethanol (4.4%). The degradation product in the absence of light is only 2,2-dichloroacetic acid (U.S. EPA 1998).

3. Mineralization

Mineralization is the process which an organic substance transforms to inorganic substances. Assessment of pesticides mineralization is evaluated by the release of carbon dioxide which is byproduct caused by biochemical processes. Yasuno et al. (1965) reported that dichlorvos was mineralized by a bacteriam, *Bacillus subtilis*, within 16 days. Agency for Toxic Substances and Disease Registry [ATSDR] (1997) measured the accumulation of carbon dioxide (60% within 360 hours) which occurred from the mineralization of dichlorvos. The mineralization process by microorganisms is as shown in Figure 2.4. The equation of the dichlorvos mineralization is as follow,

$$(CH_3O)POOCHCCl_2 + 9/2O_2 \longrightarrow PO_4^{3-} + 4CO_2 + 2Cl^- + 5H^+ + H_2O$$

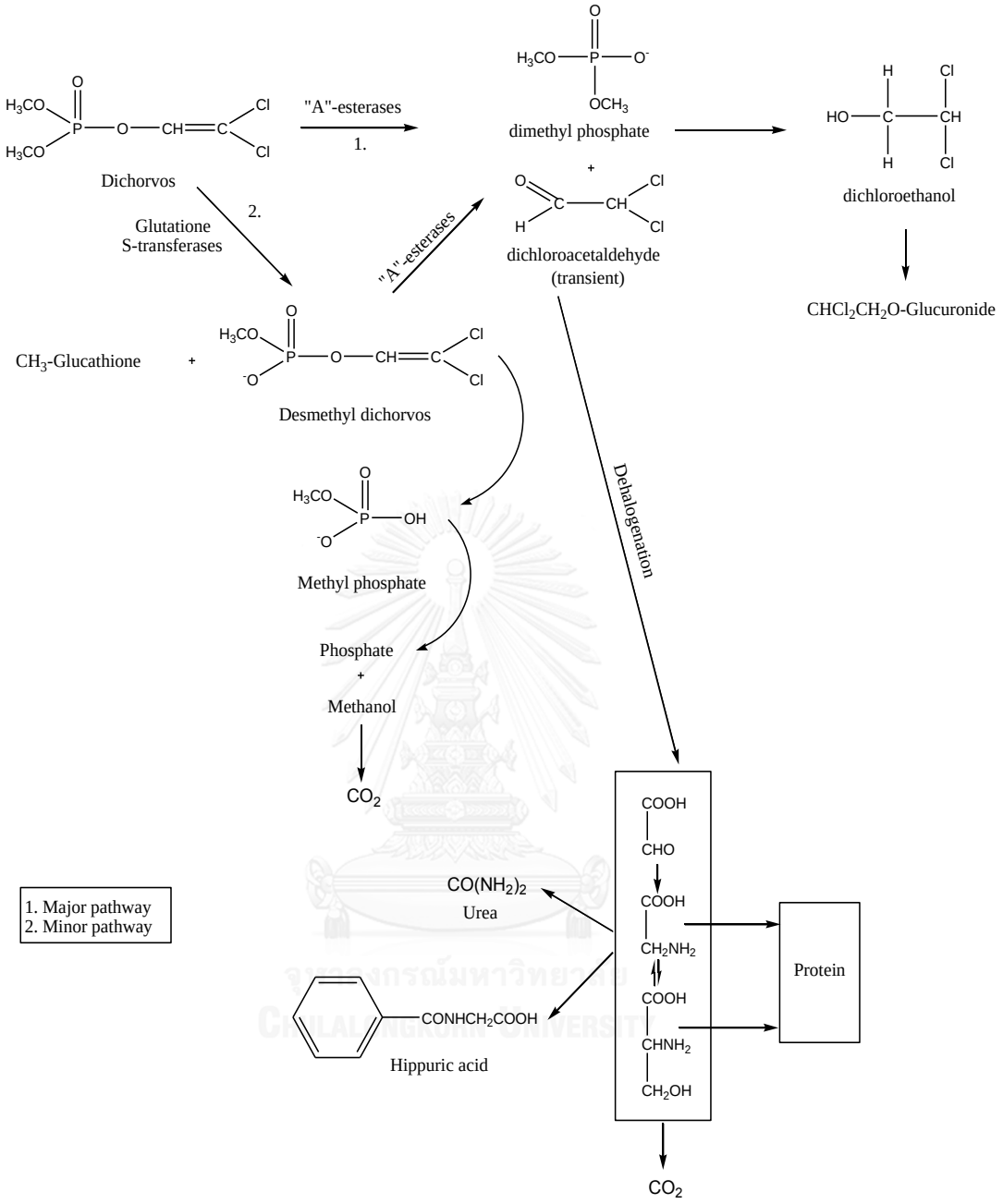


Figure 2.4 Minaeralization of dichlorvos by microorganisms

4. Biodegradation

The degradation of dichlorvos via biological process could occur. Microbes can utilize dichorvos as an energy source. The metabolites from this process could be dichloroethanol, dimethyl phosphoric acid, and dichloroacetaldehyde. Lamoreaux and Newland (1978) reported that *Bacillus cereus* used dichlorvos as carbon source while it could not use this substance as phosphorus source. They found that dichlorvos decreased to 30% after incubation for 10 days. Sattar (1990) investigated the degradation of organophosphate insecticides including dichlorvos,

phosdrin, diazinon, and parathion contaminated in sandy clay. It was found that the half-life of dichlorvos is approximately 10 days. Microbes in sandy clay can use dichlorvos as an energy source. The study also indicated that a bacterial strain under *Pseudomonas melophthora* degraded dichlorvos using esterases enzyme.

Xiao-Hua, Guo-Shun et al. (2006) reported that *Ochrobactrum sp.* strain DDV-1 which isolated from the activated sludge had ability to degrade dichlorvos and utilized it as the sole carbon and energy source. The dichlorvos degradation efficiency of *Ochrobactrum sp.* strain DDV-1 depended on pH and temperature. The maximum degradation efficiency was observed at pH 7.0 and 30 °C. Australian Pesticides & Veterinary Medicines Authority [APVMA] (2008) summarized that dichlorvos degradation in unsterilized water (with indigenous microbes) and soil was faster than those occurred due to hydrolysis alone. Also, the microorganism may be capable for further degradation of the hydrolysis products. The intermediate metabolites of dichlorvos degradation included desmethyldichlorvos, 2,2-dichloroacetaldehyde and dichloroethanol.

Dichlorvos degradation can occur from biological (30%) and chemical (70%) processes (Lamoreaux and Newland 1978). The degradation pathway of dichlorvos in the environment including soil, water, and air is shown in Figure 2.5 (Agency for Toxic Substances and Disease Registry [ATSDR] 1997).

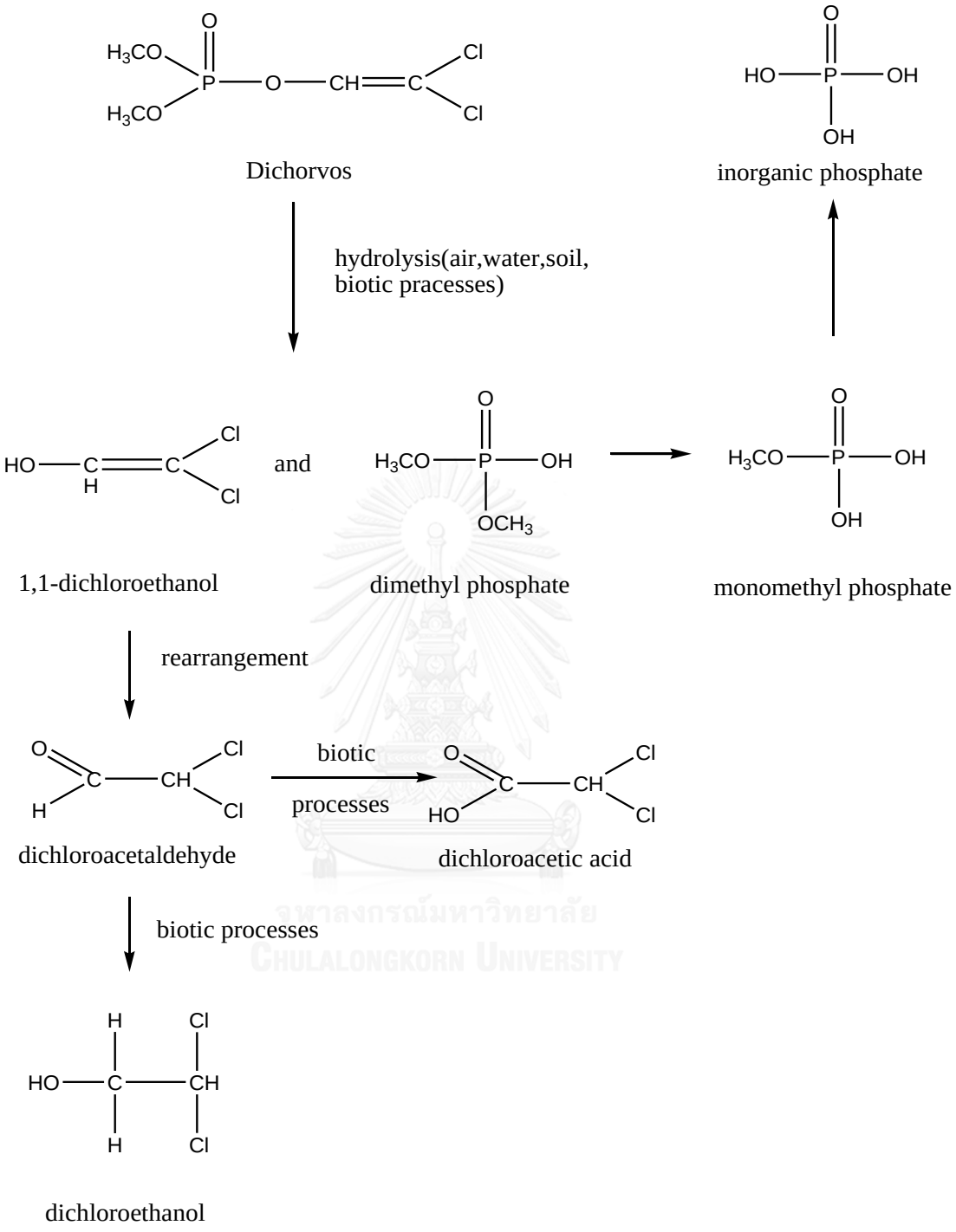


Figure 2.5 Degradation pathway of dichlorvos in the environment

2.3 Biodegradation process in the environment

The mechanisms that affect the transformation of contaminants are the reaction which causes a change in the primary structure and secondary structure of contaminants. For instance, the elimination of side chains and changes in the structure of the contaminants causing them are less complex structure and reduced their

toxicity. Transformation of pollutants consists of chemical transformation, geotransformation, and biodegradation. These reactions may be called biogeochemical reaction which referred to the transformation of natural organic and inorganic substances (Yong and Mulligan 2003). Biodegradation or biotransformation is contaminant structural changes by metabolic pathway of microorganisms which mostly resulted in the alteration or loss in some characteristic properties from the original compound. It may reduce toxic of substances.

2.3.1 Role of microorganisms

Important key on pollutants remediation with biological methods are microorganisms (microflora) and other organisms (microfauna) in the environmental system. These organisms have ability to transform or degrade pollutants that are toxic to less or none toxic. Bacteria of approximately 10^8 cells per gram of soil follow by actinomycetes and fungus could be found in the soil (Raynaud and Nunan 2014). These bacteria and microorganisms play the important role on degradation of organic compounds and releasing inorganic compound in an environmental system. In addition, they may completely decompose organic pollutant until obtain carbon dioxide and water as the final products (called mineralization) (Pepper et al. 2000). Although lot of bacteria can be found in the soil, previous study indicated that bacteria that capable to degrade contaminants (xenobiotic compound) in the environment are minimal (Fetzner 2002).

2.3.2 Factors affecting biodegradation

The ability of microorganisms to decompose contaminants depends on several factors which were divided into abiotic factors including environmental factors, structure and properties of pollutants and biotic factor including characteristics of microorganism.

1. Environmental factors

Physical and chemical characteristics of the environment affect the survival and activity of bacteria. Organic and inorganic sources (such as nitrogen, phosphorus, and vitamins source) are the essential factors influence to heterotroph microorganism. Limitation of these factors leads to decreasing of bacterial growth and number resulting in the reduction of bacterial degradation ability (Das and Chandran 2010).

Other environmental factors including oxygen, temperature, pH, moisture content, heavy metals and various salts may affect bacterial activity depended on individual bacterial behavior (Vidali 2001). In addition, some environmental factors such as moisture content and soil characteristics associate with

the accession of microorganisms to contaminants relating to degradation efficiency (Joutey et al. 2013).

2. Structure, properties and concentration of pollutants

Chemical structure, type and location of side chain group, polarity, and solubility are the properties related to biological degradation (Wiedemeier et al. 1999). These properties are the significant factors resulting in the persistence of substances in the environment and the degradation ability of microbes differently. Moreover, concentration of contaminants is importance to survival of microbes. In extremely diluted environmental pollutants, concentration of pollutants is the limitation factor for microbial growth (Rashid 1974). However, high concentration of contaminants may cause bacterial growth inhibition because of toxic effects.

3. Characteristics of microorganism

The microbial characteristics, their activities, enzymatic system and metabolic pathway are important factors that affect the growth of microorganism and their degradation ability. These factors related to the capacity of microbes to be tolerant to the toxicity of pollutant (Das and Chandran 2010). There are many processes associated with these factor including detoxification, mineralization, co-metabolism, and gratuitous metabolism.

2.3.3 Screening and selecting of microorganism

Microorganism generally screened and isolated from the environment contaminated with target pollutants. The isolation of microbes that have ability to degrade pesticide from agricultural soil or water; for example, isolated bacteria were able to degrade endosulfan insecticide including *Klebsiella, Acinetobacter, Alcaligenes, Flavobacterium,* and *Bacillus* (Kafilzadeh et al. 2014) and profenofos degrading bacteria including *Pseudomonas aeruginosa* (Malghani et al. 2009). Contaminated soil or water samples were collected as a source of isolated microbes before culturing and enriching under controlled conditions in the laboratory. These conditions affect the species and numbers of isolated microorganisms.

2.3.4 Advantages and drawbacks of biological degradation

Advantages (U.S. EPA 2004, Trotsky et al. 2010)

1. Due to this method relies on biodegradation of natural microorganisms and it is implemented as in situ operation, it does not cause to environmental contamination in other nearby areas. Also, it does not destroy the nature of remediated sites (Non-destructive method).
2. It is a low cost treatment compared to other remediation technologies.

3. It can be used alone or applied in combination with other treatment methods.
4. Biodegradation of bacterial isolated is specific to contaminant. Therefore, the remediation is potentially achievable.
5. Biodegradation by bacterial isolated is the process that spends short time compared to other methods. Because this technique can be controlled on type and quantity of augmented microorganisms.
6. This technique is flexible. The microorganisms used in this process could be single isolate or consortium depended on contaminant and remediation condition.

Drawbacks (U.S. EPA 2004)

1. Treatment period is generally longer than other remediation methods
2. Evaluation of remediation performance is difficult because this cannot be predicted the transformation products and remediation efficiency.
3. Geographical change may affect remediation performance.
4. Since this method is applied as an in situ treatment, it is necessary to achieve public acceptance.
5. The bacterial cultures need to be well isolated and studied before real application.
6. Growth and ability of the bacterial cultures may be inconsistent in practice.

CHAPTER II
MATERIALS AND METHODS

3.1 Experimental Framework and scheme

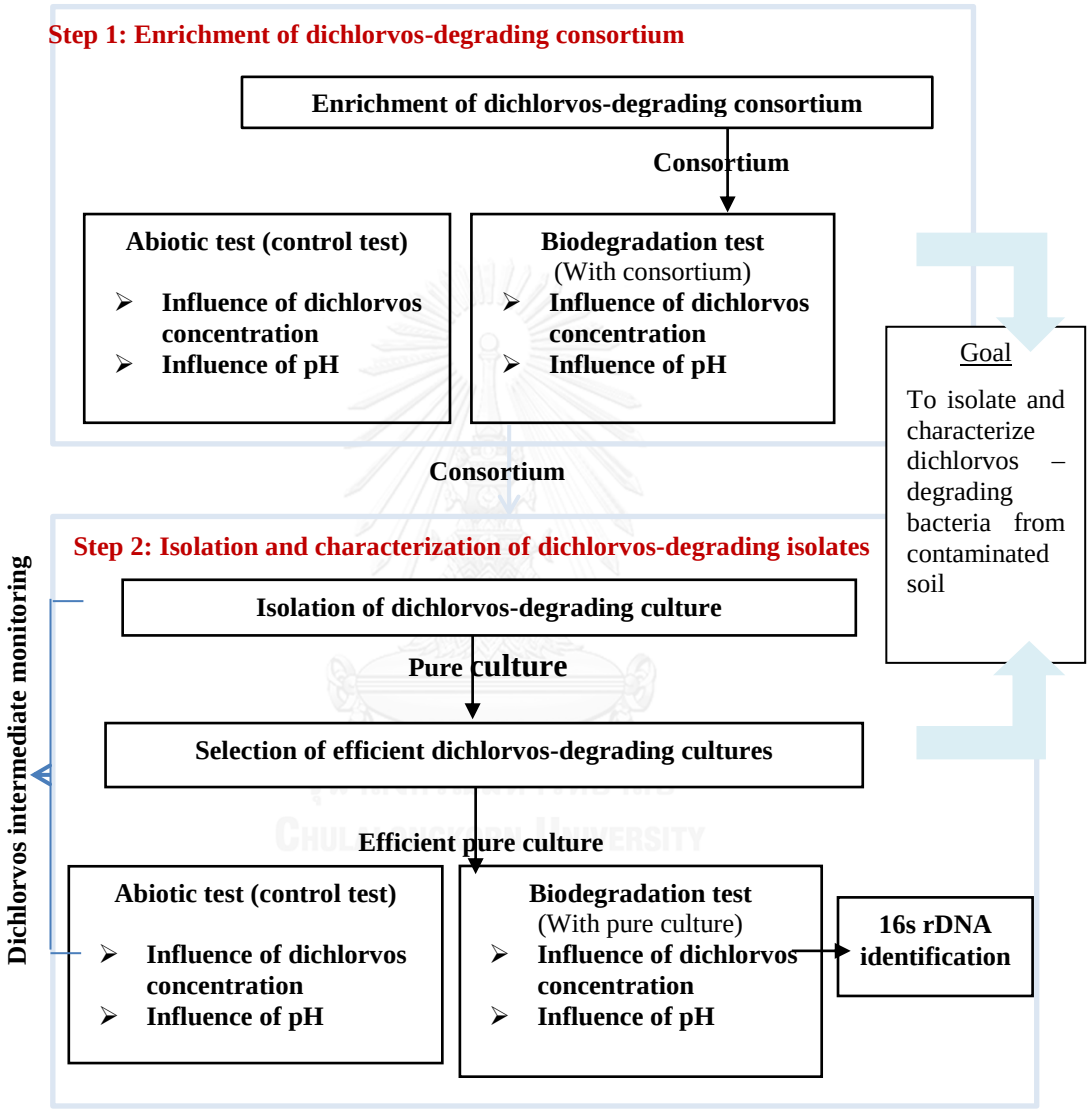


Figure 3.1 Framework of the study

3.2 Chemicals and media

Analytical standards of dichlorvos (99.1% purity) were purchased from Sigma Aldrich, Singapore. Hexane and other chemicals used for preparing medium were analytical grade and obtained from V.S. Chem House, Thailand and Ajex Finechem Pty Ltd, Australia, respectively.

Mineral salt medium (MSM) that contained (in grams per liter of deionized water) 6.814 g $NaH_2PO_4 \cdot 2H_2O$, 3.0 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 2.0 g NH_4Cl, and yeast extract of 0.1% (w/v), was used as liquid medium for biodegradation studies and used for the isolation of bacterial strains. The final pH value was adjusted to 6.8 before autoclaved at 121 °C for 15 min. Sterile filtered dichlorvos insecticide solution (dissolved in water) was supplemented to the medium.

3.3 Enrichment of dichlorvos-degrading mixed culture

Dichlorvos-degrading bacteria were enriched from soil at agricultural site experienced various pesticide exposure, especially dichlorvos for a long period (Khonkaen, Thailand). A sterile MSM supplemented with yeast extract (0.1% (w/v)) of 100 mL was prepared in 250-mL Erlenmeyer flasks. After autoclaving, 2.0 g of air-dried soil and dichlorvos 200 mg/L were mixed in the medium. The flasks were wrapped with aluminum foil to prevent photo degradation and incubated on a rotary shaker at 100 rpm. After that 10% of soil suspension was transferred every 7 days to the fresh MSM and incubated under the same conditions. The re-cultivation was conducted for 10 times consecutively until obtain dichlovos-degrading bacterial mixed-culture.

3.3.1 Degradation of dichlorvos: effect of dichlorvos concentration

The experiment comprised 2 tests including 1) abiotic test (without culture inoculation) and 2) biodegradation test (with mixed culture inoculation). Both tests were run in triplicate. The dichlorvos degradation process was performed in 250 mL Erlenmeyer flasks containing 100 mL of MSM and 0.1% of yeast extract. The medium was sterile by autoclave at 121 °C for 15 min. For biodegradation test, the bacterial culture suspension of 10 mL (1.2×10^9 CFU/mL) was inoculated into MSM with dichlorvos at different concentrations which include 50, 100, 200, 400, and 800 mg/L. Both test flasks were wrapped with aluminum foil and incubated on rotary shaker at 100 rpm for 7 days. The dichlorvos degradation and bacterial growth was regularly checked every day. Growth was measured by colony plate count technique on MSM agar medium while the degradation ability of bacterial mixed culture was using GC-ECD. Dichlorvos intermediate products were monitoring along with dichlorvos detection using GC-ECD. The growth and degradation kinetics were then estimated.

3.3.2 Degradation of dichlorvos: effect of pH

The liquid MSM at different pHs (4, 5, 6, 7, and 8) was prepared. It was adjusted to the pHs by hydrochloric acid (HCl) and sodium hydroxide (NaOH). The growth of bacterial cultures and dichlorvos degradation ability by abiotic and biodegradation process with various pHs were carried out following the protocol described above. The initial concentrations of dichlorvos of 200 and 800 mg/L were selected.

3.4 Isolation and characterization of dichlorvos-degrading isolates

In this process, the rate of dichlorvos degradation by selected bacterial cultures was investigated. For the first task, isolation and selection of the degrading microbes was performed. The present study, dichlorvos degrading bacteria was isolated from dichlorvos degrading mixed culture and identified by the basis of 16s rRNA sequence analysis. Three best isolates based on dichlorvos degradability were selected. Then, effect of dichlorvos concentration and pH on dichlorvos degradation both abiotic and biodegradation test were also determined. The intermediates of dichlorvos were monitored along with the biodegradation test.

3.4.1 Isolation of dichlorvos-degrading culture

Dichlorvos-degrading microorganisms were isolated from the soil contaminated with dichlorvos through the enrichment technique. After a series of ten subculturing, the cultures were transferred through spread plate techniques on the MSM agar supplemented with diclorvos at concentration of 200 mg/L. Then, the colonies were transferred to the MSM agar plate using streak plate technique until single colonies presented. Based on morphological properties, the individual bacterial colonies were selected.

3.4.2 Selection and characterization of dichlorvos-degrading cultures

All purified isolates were pre-selected according to their ability to degrade dichlorvos and their growth. The degradability of isolated strains was estimated in the liquid MSM containing 400 mg/L of dichlorvos and 0.1% of yeast extract. The degradability test was carried out for 7 days under the condition described earlier. A sample was collected to analyze dichlorvos residue on day 7 (compared to the initial concentration at day 0). The bacterial growth was determined by spread plate technique along with the degradability test. Three isolated strains that showed the highest dichlorvos degradation were selected and applied in this experiment.

The isolated bacteria were identified and characterized on the basis of the 16S rRNA sequence analysis. The bacterial colonies of selected isolates were analyzed by Macrogen 16s sequencing service (Korea). The bacterial colonies were

extracted for genomic DNA. The 16S rRNA gene was amplified by PCR using the universal primers. The forward primer, 27F (5'(AGA GTT TGA TCM TGG CTC AG)3'), and reverse primer, 1492R (5'(TAC GGY TAC CTT GTT ACG ACT T)3'), were applied. Twenty nanogram of genomic DNA was used as the template in a 30-µL reaction mixture using a EF-Taq (SolGent, Korea). The PCR conditions were as follows: activation of Taq polymerase at 95 °C for 2 min, then 35 cycles of 95 °C for 1 min, 55°C for 1 min, and 72 °C for 1 min were performed, finishing with a 10-minute step at 72 °C. The amplification products were purified using a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA). The nucleotide sequence was analyzed by BLASTn program (NCBI). A phylogenetic tree was constructed using maximum likelihood analysis (MEGA 6.0).

3.4.3 Degradation of dichlorvos by dichlorvos-degrading isolates

1) Degradation of dichlorvos: effect of dichlorvos concentration

Effect of dichlorvos concentration on the pesticide degradation by dichlorvos-degrading isolates was carried out using the method described in earlier section.

2) Degradation of dichlorvos: effect of pH

To evaluate pH effect on dichlorvos removal by dichlorvos-degrading isolates, the liquid MSM was adjusted to pH ranging from 4.0 to 8.0. The test protocol and sampling was followed method described in previous section.

3.5 Analysis

3.5.1 Evaluation of bacterial growth

Bacterial cell number was measured by spread plate technique. Aseptic technique was used in this procedure to avoid the contamination. 1.5-mL centrifuge tubes filled with 0.9 mL of sterile water were prepared. 0.1 mL of aliquots was serially transferred. 0.01 mL of diluted aliquot was transferred to MSM agar (Benimeli, Fuentes et al. 2008) plate with 200 mg/L of dichlorvos as a sole carbon source. The solution is then spread thoroughly over surface of agar plate. Plates were incubated at 35 °C. After 5-day incubation, the number of bacterial colonies was counted.

3.5.2 Dichlorvos and intermediate product analysis

Dichlorvos residues in a liquid MSM were extracted using a liquid–liquid extraction technique. First, 0.5 mL of the sample was placed into a 1.5-mL centrifuge

tube and then the mixture of 0.5 mL of n-hexane and 0.01% of acetic acid was added. After that, the tube was forcefully mixed in a vortex mixer at 2,500 rpm for 10 min. After solution separated, supernatant (upper layer) was collected and clean up by filtrating through a sterile syringe filter 0.2 μm before transfer to a GC vial. Standard curve was shown in Appendix B.

After the extraction process, the analysis was performed using Agilent gas chromatograph (GC) (Model 4890D, Agilent Technologies, USA) equipped with a SPB-608 fused silica capillary column (length 30.0 m, diameter 0.25 mm, film thickness 0.25 μm) and electron capture detector (ECD). The operating conditions were: initial temperature 100 °C, then heated at 40 °C/min to a final temperature of 220 °C and retained for 5 min. The total run time was 10.0 min. The splitless mode was used for injection and one-microliter of the extract was injected to the GC. The injector temperature was set at 240 °C and the detector temperature was at 250 °C. Nitrogen and helium gas was used as the carrier gas with a helium gas flow and total flow at 7.8 and 46.6 mL/min respectively. Dichlorvos peak was detected at 3.8 min. Degradation intermediate product of dichlorvos was also monitored along with dichlorvos detection by gas chromatography-mass spectrum (GC-MS) after extracted by ethyl acetate. The recovery percentage of the extraction technique was 70-95% as shown in Appendix A.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Dichlorvos degradation by mixed cultures

4.1.1 Influence of bacterial medium on dichlorvos degradation

This experiment is a preliminary study conducted to compare bacterial growth and dichlorvos degradation of mixed cultures enriched from agricultural soil under two conditions: enrichment with and without additional carbon source. Yeast extract of 0.1% was selected as an additional carbon source. The growths of the consortium under the conditions were shown in Figure 4.1 (raw data presented in Appendix C). Bacterial growth of the test without the additional carbon source (dichlorvos added as a sole carbon source) was relatively low compared to one with the additional carbon source. For the test with yeast extract, viable cell number of dichlorvos-degrading mixed cultures sharply increased from 7.7 to 8.8 logCFU/mL in the first two days. After that, the mixed cultures reached the stationary phase and declined since day 5. For the test without yeast extract, the consortium grew from 5.9 to 7.2 logCFU/mL at the first day and being stable afterward. This finding pointed out that the enriched consortium was able to grow in the both dichlorvos-containing medium with and without additional carbon source.

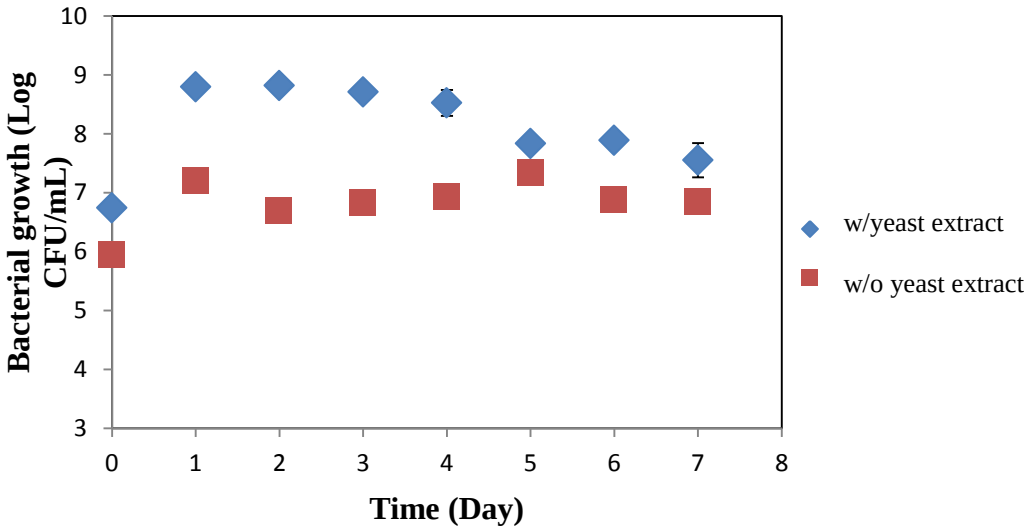


Figure 4.1 Bacterial growth of mixed cultures in different media (with and without yeast extract as additional carbon source) at pH 5.5

The degradation of dichlorvos by a consortium cultured in the medium with and without yeast extract was shown in Figure 4.2 (raw data presented in Appendix C). The dicholrvos concentration continuously reduced in both tests. It is clearly that a consortium was potential for dichlorvos degradation. Under presence of yeast extract, a consortium grew up faster resulting in more effective on dichlorvos removal (dichlorvos removal percentage of the test with and without yeast extract = 87.50 and 59.09%, respectively).

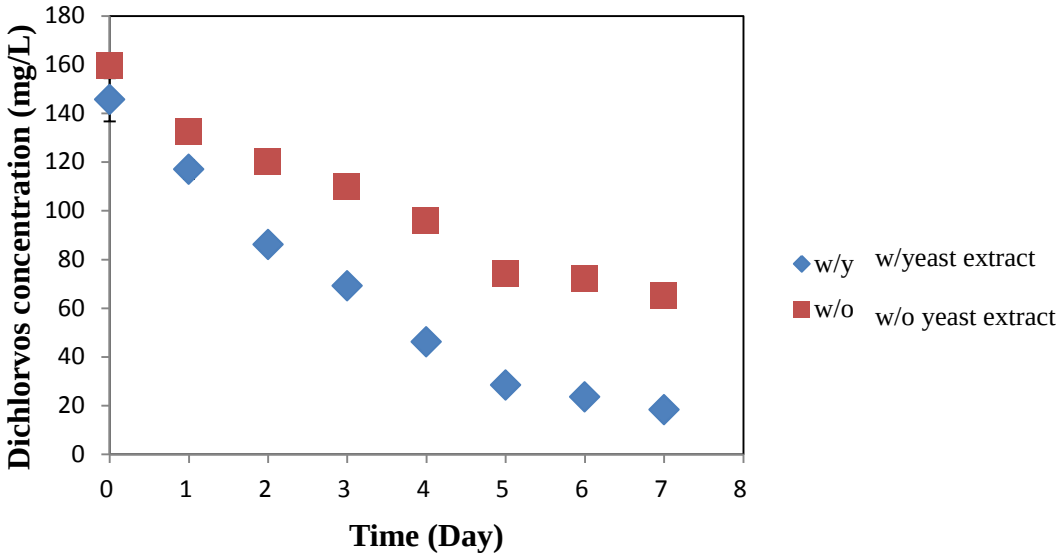


Figure 4.2 Dichlorvos degradation by mixed cultures in different media (with and without yeast extract as additional carbon source) at pH 5.5

It is obvious that the consortium cultured in medium with yeast extract provided the better results in growth and dichlorvos degradation rates. Therefore, this condition was selected as culturing medium for the rest of the study. These experiments were consistent with the research by Ning et al. (2012). It was reported that the growth of dichlorvos-degrading isolate strain YD4 was inhibited when applying dichlorvos as a sole carbon source. The additional carbon supplement could lessen the problem. These results also correlated with Lamoreaux and Newland (1978). They found that *Bacillus cereus* isolated from soil could utilize dichlorvos either as a sole carbon source or with additional carbon source, but the degradation of the experiment with dichlorvos supplement as a sole carbon source was slightly less than that with the additional carbon source.

4.1.2 Influence of dichlorvos concentration on dichlorvos degradation by dichlorvos-degrading mixed cultures

1. Enrichment of dichlorvos degrading cultures

The dichlorvos-degrading consortiums were screened by enrichment technique in aerobic condition at pH of 6.8 and temperature of 30 ± 2 °C. It was found that the consortium could utilize dichlorvos as a sole carbon source. But in the case with additional carbon source (yeast extract of 0.1%), the dichlorvos degradation was obviously better than that without the carbon source supplement (data shown in previous section). The consortium plated onto the medium agar consisted of numerous types of bacterial colonies as shown in Figure 4.3. This indicates that the agricultural soil used in this study was rich in dichlorvos-degrading cultures. Previously dichlorvos-degrading cultures have been enriched and identified. For example, *Flavobacterium* sp. strain YD4, *Ochrobactrum* sp. strain DDV-1, *Bacillus cereus*, *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp. and *Acinetobacter* sp. were enriched from contaminated sites experienced with dichlorvos and were proved their degradability (Lamoreaux and Newland 1978, Xiao-Hua, Guo-Shun et al. 2006, Ning, Gang et al. 2012, Agarry et al. 2013)

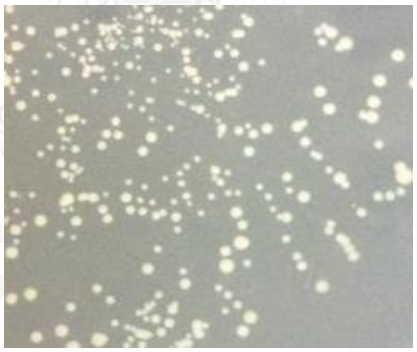


Figure 4.3 Dichlorvos-degrading bacterial colonies

2. Bacterial growth of mixed cultures under different dichlorvos concentrations

The growth of dichlorvos-degrading mixed culture used in biodegradation process was measured by viable cell counting which is shown in Figure 4.4 (raw data presented in Appendix C). The consortiums which were cultured under different dichlorvos concentrations (50, 100, 200, 400 and 800 mg/L) provided similar results. The viable cell number of mixed culture grew from 7.7 to 10.2, 8.3 to 10.2, 7.7 to 10.1, 7.7 to 10.1 and 7.5 to 9.5 for the tests with dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L respectively. All cultures reached the stationary phase after 2 days. This indicated that consortium was able to survive and endure in the medium containing dichlorvos at different concentrations. The bacterial growth rates of

dichlorvos-degrading consortium (1.22-2.83 1/d) followed the first-order kinetics (Table 4.1) (raw data presented in Appendix C). Bacterial growth rate of the test with dichlorvos concentration at 800 mg/L was relatively low compared to other tests. According to previous finding by Ning et al. (2012), it was reported that dichlorvos concentrations of higher than 800 mg/L were toxic to bacteria and might led to bacterial growth inhibition.

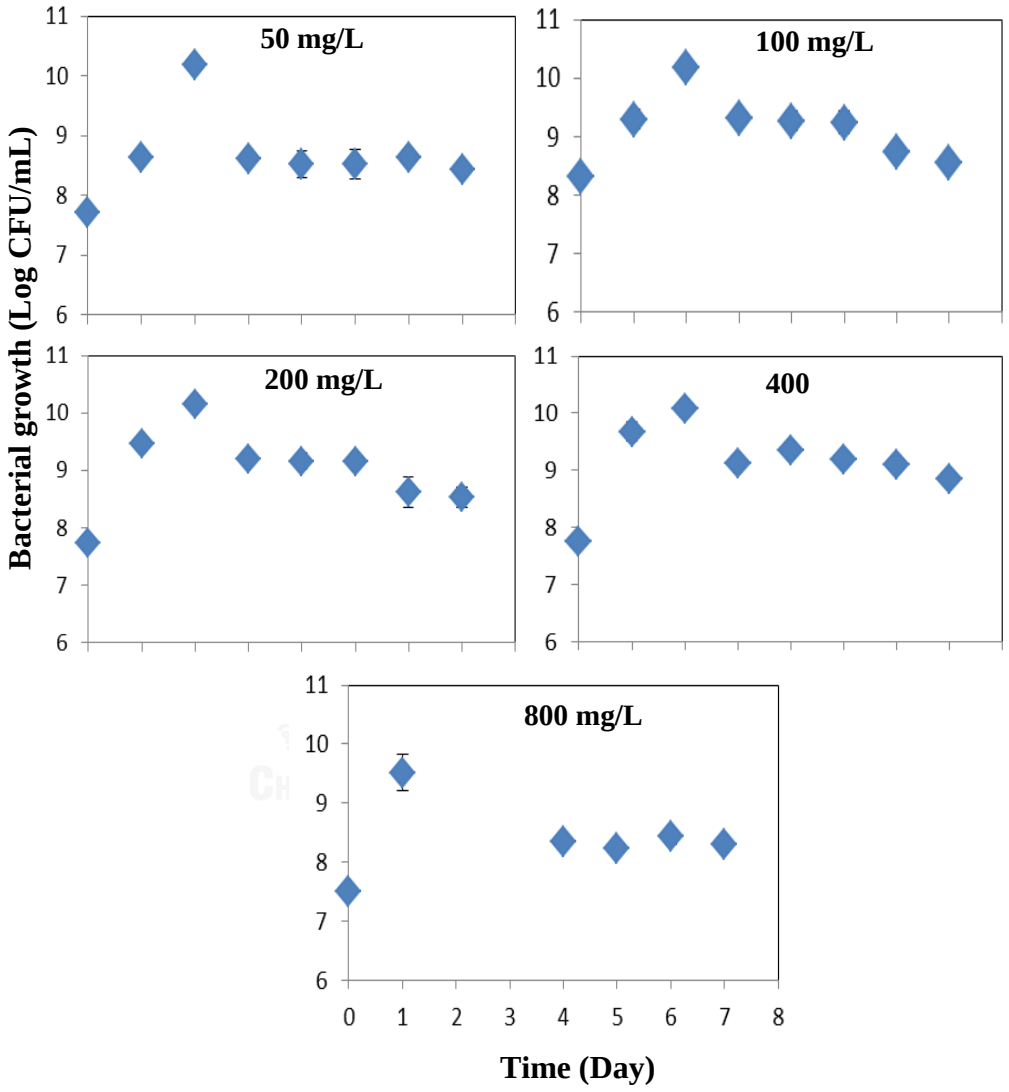


Figure 4.4 Bacterial growths under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

Table 4.1 Bacterial growth rates of dichlorvos-degrading consortium under different dichlorvos concentrations

Dichlorvos Concentration (mg/L)	**Growth kinetic equation***	**k (1/d)**	**R^2**
50	y = 2.83x + 17.53	2.83	0.98
100	y = 2.10x + 19.20	2.10	0.99
200	y = 2.78x + 18.21	2.78	0.94
400	y = 2.67x + 18.45	2.67	0.86
800	y = 1.22x + 1.00	1.22	1.00

***** y = ln (bacterial number)
x= time (day)

3. Dichlorvos degradation of mixed cultures under different dichlorvos concentrations

The reduction of dichlorvos concentration by abiotic (control) and biodegradation processes under different dichlorvos concentrations was shown in Figure 4.5 (raw data presented in Appendix C). It is observed in Figure 4.5 that dichlorvos concentration dramatically decreased every day in all treatment. These results could imply that abiotic and biodegradation processes obviously removed dichlorvos even at the high concentrations.

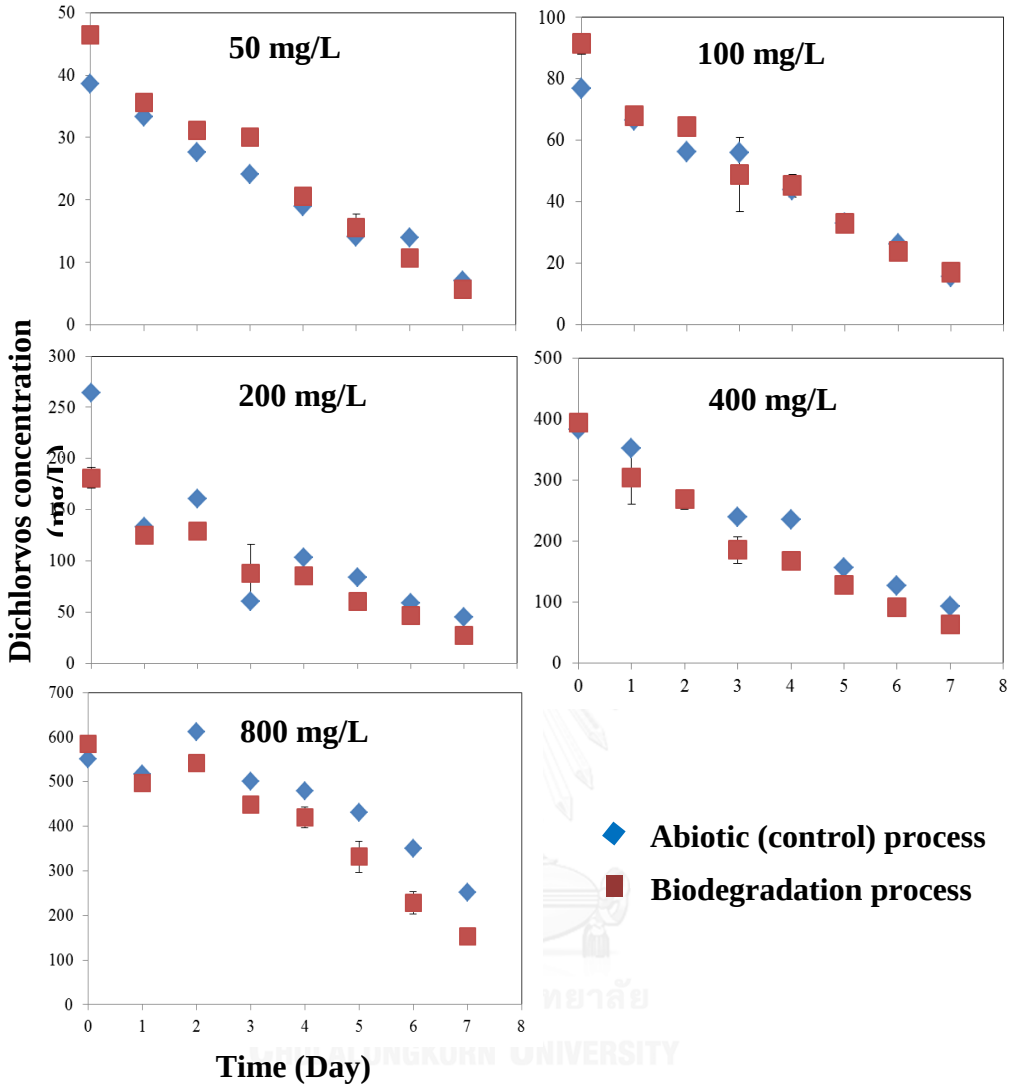


Figure 4.5 Dichlorvos degradation by abiotic and biodegradation processes under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

Table 4.2 (raw data presented in Appendix C) shows rate of removal and removal percentage of dichlorvos by abiotic and biodegradation processes under different concentrations of dichlorvos. Based on the removal percentage, dichlorvos concentration greatly influenced the degradation of dichlorvos by abiotic (control) process (no inoculation). The removal percentage of dichlorvos markedly decreased along with the increasing of dichlorvos concentration. After the 7-d experiment, maximum degradation efficiency by abiotic process of 82.99% was observed at dichlorvos concentration of 200 mg/L while lowest one of 54.48% occurred in the test at 800 mg/L. For the biodegradation test, inoculated bacterial consortium and abiotic process could degrade dichlorvos. Dichlorvos removal percentages of 73.73% -

87.75%% were detected. The result turned out that the concentration at low to moderate concentrations (50-400 mg/L) was not an important role on biodegradation process. However, during the test with high concentration (800 mg/L), it was obvious that the removal efficiency reduced. Abiotic and biodegradation processes performed the dichlorvos treatment (dichlorvos removal rate) at the rate of 4.50 to 42.87 and 5.82 to 61.63 mg/L/d, respectively (Table 4.2). The removal rates of dichlorvos by abiotic and biodegradation process were similar at low pesticide concentrations (50-400 mg/L) while different results were observed at high concentrations (800 mg/L).

Table 4.2 Dichlorvos removal rates and dichlorvos removal percentages of abiotic and biodegradation processes

Dichlorvos concentration (mg/L)	Abiotic (control) process		Biodegradation process		Biodegradation alone
	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal (%)
50	4.50	81.71	5.82	87.75	6.04
100	8.74	79.59	10.63	81.42	1.83
200	31.31	82.99	22.00	85.00	2.01
400	41.42	75.72	47.20	84.00	8.28
800	42.87	54.48	61.63	73.73	19.25

Table 4.3 (raw data presented in Appendix C) shows dichlorvos degradation kinetic equation by all cultures which followed the first-order kinetics. Biodegradation process performed better than abiotic process. For abiotic process, it was reported that dichlorvos could be degraded rapidly by hydrolysis reaction (Suter 1981). However, when the concentrations increased, biodegradation by microorganism could accelerate the degradation (Australian Pesticides & Veterinary Medicines Authority [APVMA] 2008).

Because of technical limitation, the experiment cannot eliminate hydrolysis reaction (abiotic degradation) from biodegradation process. Therefore, the dichlorvos degradation occurred from the activity of bacteria (biodegradation alone) was calculated from the efficiency of the biodegradation process (biotic plus abiotic reactions) subtracted by the abiotic process. The result in Table 4.2 may imply that after subtracting abiotic degradation, biodegradation process alone could decrease dichlorvos of up to 6.04, 1.83, 2.01, 8.28 and 19.25% mg/L/d at dichlorvos concentration of 50, 100, 200, 400 and 800 mg/L, respectively. Since dichlorvos has high water solubility of 18,000 mg/L. This results in high possibility of dichlorvos

contamination in aquatic environment and groundwater systems. In some cases such as an accidental spill case with high pesticide concentration, hydrolysis alone might not be enough for remediating dichlorvos contamination. Therefore, biodegradation is an alternative technique to improve the dichlorvos degradation.

The result from this section can indicate that at high concentration of dichlorvos (800 mg/L), biodegradation process by bacterial mixed cultures play a role on dichlorvos degradation. Moreover, they can enhance the degradation of dichlorvos. Therefore, the dichlorvos concentration of 800 mg/L will be selected to apply in the later experiment of study the influence of pH on dichlorvos degradation.

Table 4.3 Dichlorvos degradation rates of abiotic and biodegradation processes

Dichlorvos concentration (mg/L)	**Abiotic (control) process**			**Biodegradation process**		
	Dichlorvos degradation kinetic equation*	**k (1/d)**	**R^2**	**Dichlorvos degradation kinetic equation***	**k (1/d)**	**R^2**
50	y = -0.22x + 3.75	0.22	0.94	y = -0.28x + 3.98	0.28	0.93
100	y = -0.21x + 4.47	0.21	0.93	y = -0.23x + 4.56	0.23	0.97
200	y = -0.21x + 5.32	0.21	0.78	y = -0.24x + 5.24	0.24	0.95
400	y = -0.20x + 6.05	0.20	0.96	y = -0.25x + 6.02	0.25	0.98
800	y = -0.10x + 6.46	0.10	0.76	y = -0.17x + 6.53	0.17	0.86

***** y = ln (dichlorvos concentration)
x= time (day)

4.1.3 Influence of pH on dichlorvos degradation by dichlorvos-degrading mixed cultures

1. Bacterial growth of mixed cultures under different pHs

In order to study the influence of pH on the growth of Dichlorvos-degrading mixed culture which was used in biodegradation process, pH of MSM liquid medium was adjusted to pH 4, pH 5, pH 6, pH 7 and pH 8 by hydrochloric acid and sodium hydroxide. The initial dichlorvos concentration was fixed at 800 mg/L. These cultures were incubated for 7 days. The growth of dichlorvos-degrading consortium showed in Figure 4.6 (raw data presented in Appendix C). The viable cell number increased from 7.3 to 8.7, 7.6 to 8.6, 7.7 to 8.5, 7.8 to 8.4 and 7.7 to 8.6 in the medium at pH 4, 5, 6, 7 and 8, respectively. All cultures reached the stationary phase after 1 day. Table 4.4 (raw data presented in Appendix C) shows bacterial growth rates of dichlorvos-

degrading consortium (0.81-1.42 1/d) which followed the first-order kinetics. It can be indicated that pHs have no significant influence of the bacterial growth.

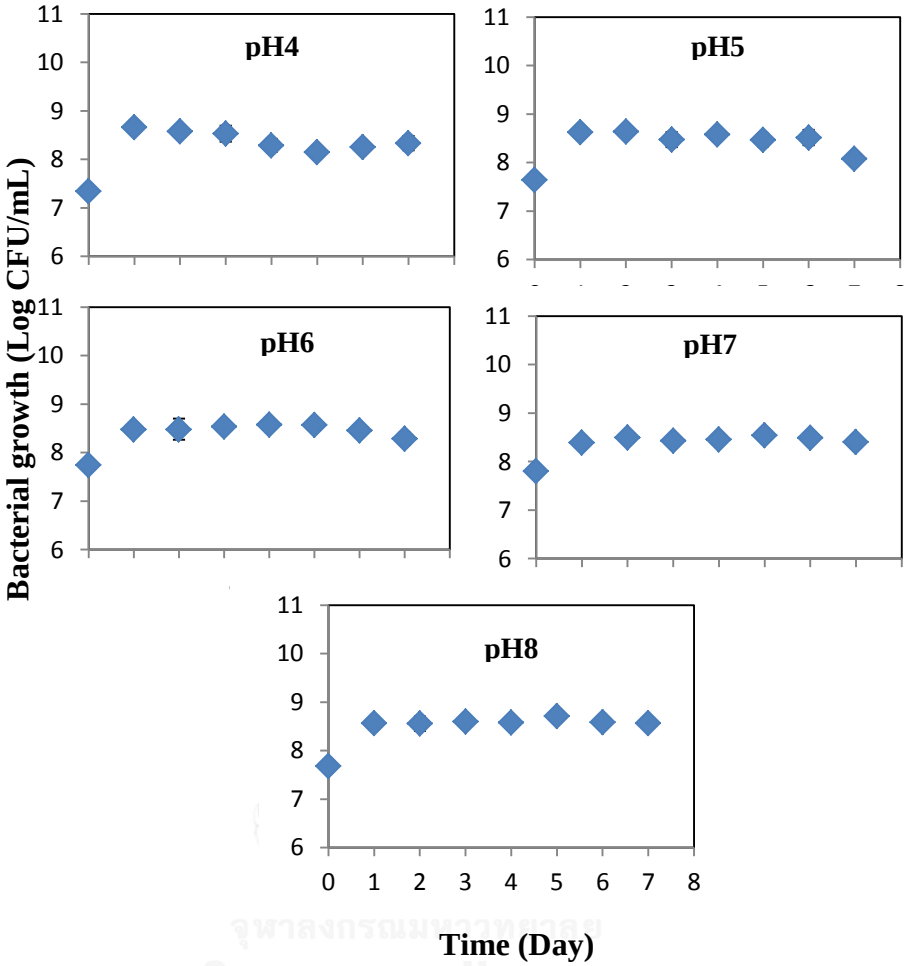


Figure 4.6 Bacterial growths under pH of 4, 5, 6, 7 and 8

Table 4.4 Bacterial growth rates of dichlorvos-degrading consortium under different pH

pH	Growth kinetic equation*	k (1/d)	R^2
pH4	y = 1.42x + 16.03	1.42	0.70
pH5	y = 1.15x + 16.82	1.15	0.76
pH6	y = 0.89x + 17.23	0.89	0.79
pH7	y = 0.81x + 17.36	0.81	0.87
pH8	y = 1.03x + 16.98	1.03	0.76

* y = ln (bacterial number)
x= time (day)

2. Dichlorvos degradation of mixed cultures under different pHs

The influence of pHs (pH 4-8) on dichlorvos degradation between abiotic (control) process and biodegradation process during 7 days of experimental period was shown in Figure 4.7 (raw data presented in Appendix C). From Figure 4.7, although dichlorvos concentration tend to decrease every day in all treatments, but at acidic conditions (pH 4-6), the degradations were relatively low compared to those at the higher pHs (pH7 and pH8). These results indicated that pHs had important effect to the dichlorvos degradation. Dichlorvos removal rates and dichlorvos removal percentage by abiotic (control) and biodegradation processes under different pH were showed in Table 4.5. The removal percentages of dichlorvos of 20 - 60% were found in acidic pH (pH 4- 6) while the treatments at pH 7 and pH 8 could remove dichlorvos by over 90%.

Dichlorvos degradation kinetic equations were showed in Table 4.6 by all cultures which followed the first-order kinetics. Based on the results in table 4.6, dichlorvos degradation kinetic rate of abiotic and biodegradation process increased depending on the increasing of pHs which the lowest one found at pH 4 while highest one found at pH 8. Dichlorvos kinetic rates of 0.03 to 0.77 and 0.06 to 0.74 were observed in abiotic and biodegradation, respectively.

The difference of dichlorvos degradation by abiotic and biodegradation processes can be clearly seen at acidic pH (pH 4-6) which biodegradation had higher efficiency in dichlorvos degradation. At neutral and slightly alkaline pH (pH 7 and pH 8), abiotic process by hydrolysis reaction played a significant role on dichlorvos degradation. However, after subtracting the degradation by abiotic process, biodegradation alone was able to remove dichlorvos of up to 14.94, 11.98 and 7.19% at pH 4, 5 and 6, respectively. These results indicated that biodegradation by bacterial consortium can improve the dichlorvos degradation at acidic condition. The results from the present study were beneficial for remediating dichlorvos pesticide contaminating in acidic environment. Acidic environment including groundwater has been reported in various parts of the world. For example, low pH of groundwater in Australia spread over 200,000 km^2, especially in coastal areas (Indraratna et al. 2014). Groundwater at the U.S. Department of Energy's Savannah River Site (South Carolina, USA) had acidic pH of 3.20 to 5.20 (Otosaka et al. 2011).

The results from present study were against from the study by Ning et al. (2012). It was found that there was insignificant in dichlorvos degradation by abiotic process (approximately 19%). They reported that pH was not important for dichlorvos degradation by abiotic process. Ning et al. (2012) also tested the dichlorvos biodegradation at pH of 4 to 8. The lowest biodegradation rate (< 30%) was observed at pH 4. The biodegradation process by dichlorvos-degrading bacteria could rapidly degrade dichlorvos at pH ranging from 5.5 to 7.0 (the highest degradation was found at pH 6.0). This conflict may be from difference in enriched culture and tested

conditions. Ning et al. (2012) performed the experiment in contaminated soil, so dichlorvos degradation by hydrolysis occurred less than that in water.

However, dichlorvos had been classified as rapidly hydrolysis substance in alkaline pH (Yasuno, Hirahoso et al. 1965). Later literatures also confirmed that hydrolysis was really significant on dichlorvos degradation (Lamoreaux and Newland 1978, Suter 1981, Australian Pesticides & Veterinary Medicines Authority [APVMA] 2008). Therefore, based on the results from the present study and literature review, it could be suggested that biodegradation by dichlorvos-degrading mixed culture should be applied in acidic environment. From the information in previous section, the biodegradation by the enriched cultures will be useful in the site with high contaminated concentration.

Table 4.5 Dichlorvos removal rates and dichlorvos removal percentages of abiotic and biodegradation processes

pH	Abiotic (control) process		Biodegradation process		Biodegradation alone
	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal (%)
pH4	20.82	20.37	35.06	35.31	14.94
pH5	27.96	27.50	39.71	39.48	11.98
pH6	52.64	54.81	59.71	62.00	7.19
pH7	93.24	97.16	92.96	96.05	N.A.
pH8	94.26	99.36	95.87	99.16	N.A.

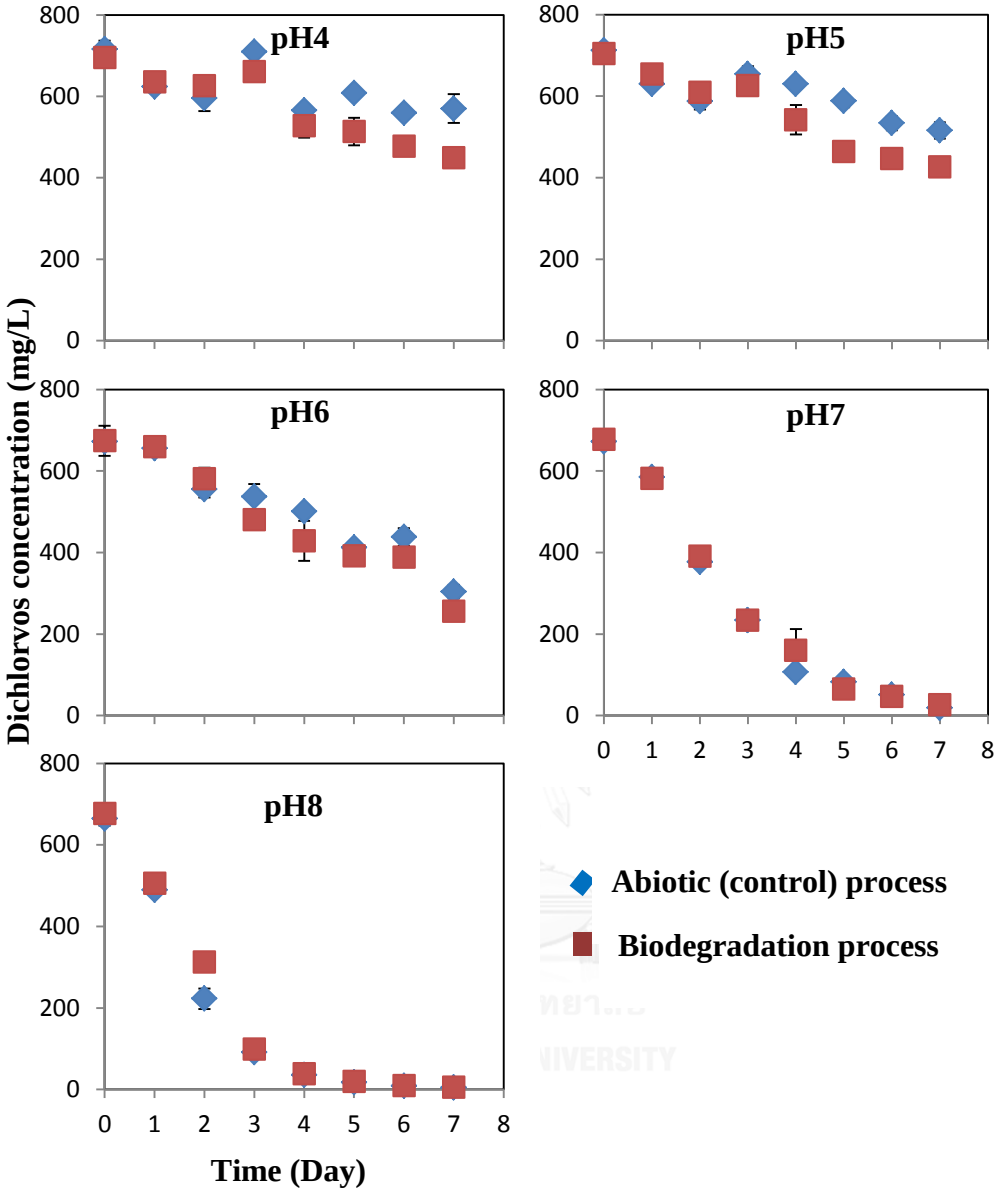


Figure 4.7 Dichlorvos degradation by abiotic and biodegradation processes under different pH

Table 4.6 Dichlorvos degradation rates of abiotic and biodegradation processes

pH	Abiotic (control) process			Biodegradation process		
	Dichlorvos degradation kinetic equation*	**k (1/d)**	**R^2**	**Dichlorvos degradation kinetic equation***	**k (1/d)**	**R^2**
pH4	y = -0.03x + 6.49	0.03	0.68	y = -0.06x + 6.56	0.06	0.90
pH5	y = -0.04x + 6.53	0.04	0.74	y = -0.08x + 6.58	0.08	0.95
pH6	y = -0.10x + 6.56	0.10	0.91	y = -0.13x + 6.58	0.13	0.93
pH7	y = -0.50x + 6.80	0.50	0.97	y = -0.49x + 6.79	0.49	0.98
pH8	y = -0.77x + 6.75	0.77	0.99	y = -0.74x + 6.82	0.74	0.98

***** y = ln (dichlorvos concentration)
x= time (day)

4.2 Dichlorvos degradation by pure cultures

4.2.1 Isolation of dichlorvos-degrading pure cultures

Bacterial strains were isolated by streak plate technique from dichlorvos-degrading mixed cultures mentioned previously. Nine different bacterial colonies (designed as A1 – A9) were purified by re-steaking for several times until the single colony was presented.

In order to select the best three of bacterial strains based on growth and dichlorvos degradability, nine purified isolates were tested in MSM liquid medium supplemented with dichlorvos (400 mg/L) followed method described in Chapter 3. The result presents in Figure 4.8 and Figure 4.9 (raw data presented in Appendix C). Dichlorvos removal percentages, growth rates, and specific degradation rates of each strain were demonstrated in Table 4.7 (raw data presented in Appendix C). Specific degradation rate of each strain was calculated followed the equation shown below:

$$\text{Specific rate } \left(\frac{\text{mg} \cdot \text{mL}}{\text{L} \cdot \text{CFU}}\right) = \frac{\text{Initial concentration (mg/L)} - \text{Final concentration(mg/L)}}{\text{Initial cell number of bacteria (CFU/mL)}}$$

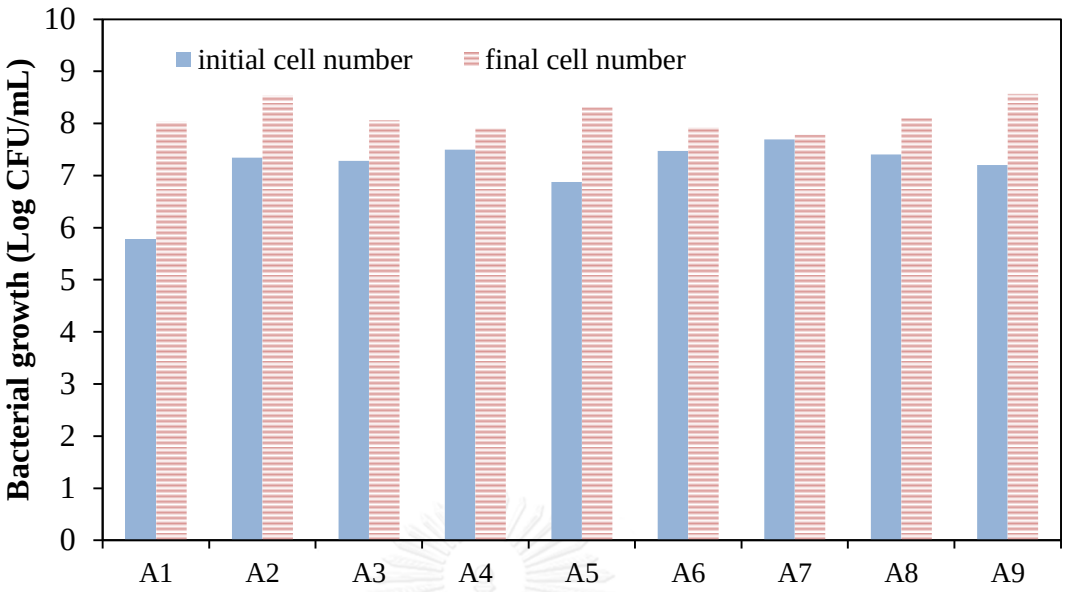


Figure 4.8 Initial cell number and final cell number of purified isolates

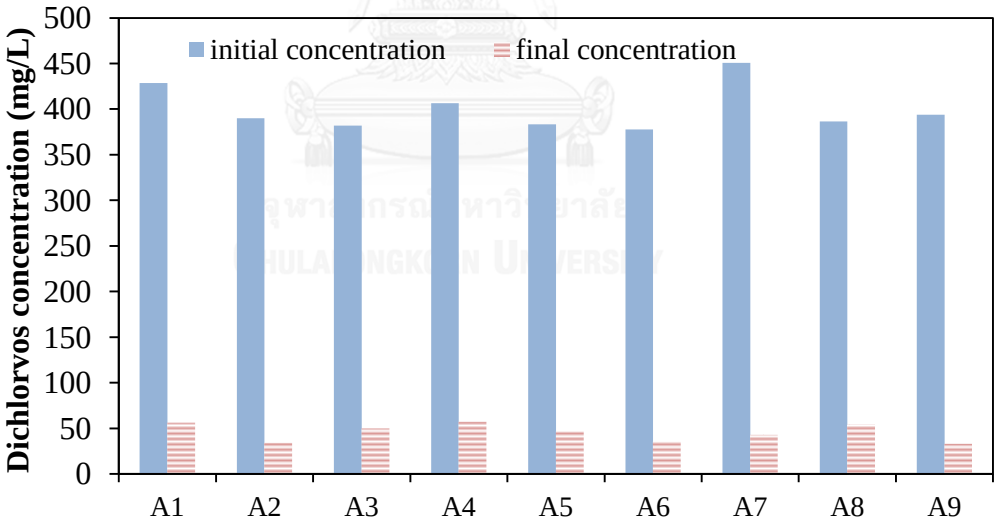


Figure 4.9 Initial dichlorvos concentration and final dichlorvos concentration of purified isolates

Table 4.7 dichlorvos removal percentage, growth rate, and specific rate of the isolates

Strains	Dichlorvos removal (%)	Growth rate (1/d)	Specific rate ($\frac{mg \cdot mL}{L \cdot CFU}$)
A1	86.82	0.32	5.73×10^{-4}
A2	91.20	0.17	1.42×10^{-5}
A3	86.90	0.11	1.7×10^{-5}
A4	85.86	0.06	1.0910^{-5}
A5	87.68	0.20	4.4810^{-5}
A6	90.49	0.06	1.1410^{-5}
A7	90.45	0.01	8.1510^{-5}
A8	85.92	0.10	1.2810^{-5}
A9	91.50	0.20	2.1810^{-5}

Based on the growth rates and specific rates of each strain as reported in Table 4.7, the isolates A1, A5 and A9 performed the best. These results implied that these three isolates were potential as dichlorvos-degrading cultures. Therefore, these three single cultures were selected for further testing.

Three isolates were re-designed as DV1, DV2 and DV3 for A5, A9 and A1, respectively. They were observed colony morphology after 2 days of incubation. All isolates were different in colony morphology (Figure 4.10). Morphological observation of three isolated strains was summarized in Table 4.8.

a) **b)** **c)**

Figure 4.10 Bacterial colonies of pure isolates a) DV1, b) DV2, and c) DV3

Table 4.8 Morphological characteristics of dichlorvos-degrading bacterial isolated strains DV1, DV2 and DV3

Characteristics	**Isolated strain**		
	DV1	**DV2**	**DV3**
Colony color	Cream at center and white periphery	Cream	White
Colony form	Circular and convex	Spindle and crateriform	Circular and convex
Colony elevation	Raised	Flat	Raised
Colony margin	Entire	Entire	Entire
Colony diameter (at 48 h)	2.0 mm	4.0 – 5.0 mm	1.0 mm

After that, the isolates (DV1, DV2 and DV3) were identified by 16S rRNA analysis. Phylogenetic trees of DV1, DV2 and DV3 strains were constructed (Figures 4.11-4.13). Based on Figure 4.11, DV1 showed high similarity to *Klebsiella pneumoniae* (GenBank accession No. CP007731.1) and *Klebsiella* sp. (such as strains ok119S with GenBank accession No. JF274779.1 and JF274760.1) (Table 4.9). For DV2, it was similar to *Enterobacter* sp. (GenBank accession No. GQ478272.1) with similarity of 99%, (Figure 4.12 and Table 4.9). DV3 was similar to *Klebsiella pneumoniae* (GenBank accession No. JQ837267.1) with similarity of 99% (Figure 4.13 and Table 4.9). The results from the basis of 16S rRNA gene sequence analysis, three isolated strains were summarized in Table 4.10. DV1, DV2 and DV3 strains were identified as *Klebsiella* sp., *Enterobacter* sp. and *Klebsiella pneumoniae*, respectively.

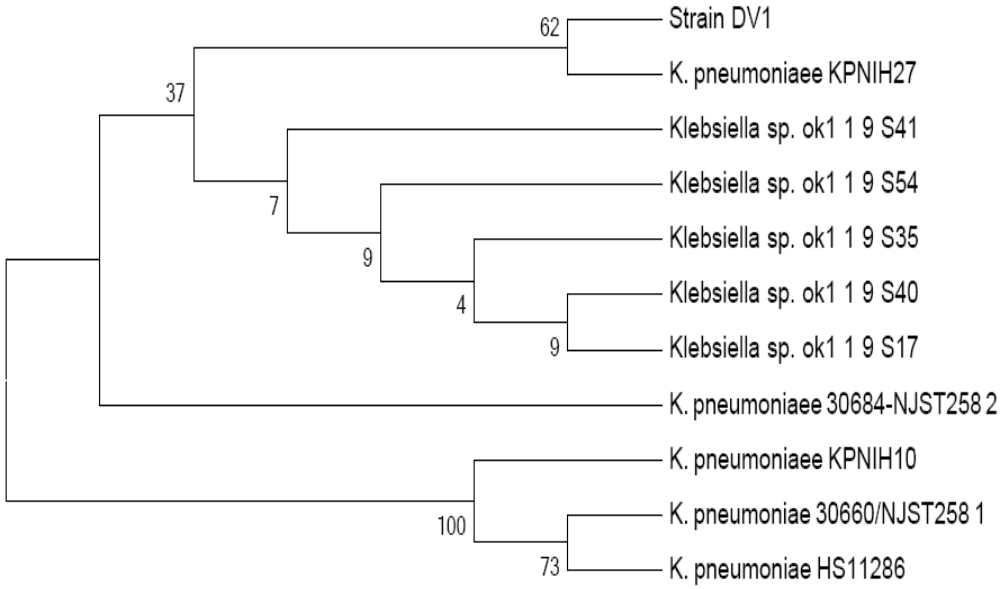


Figure 4.11 Phylogenetic tree of DV1 and related species constructed on the basis of 16S rRNA gene sequence using the maximum likelihood analysis.

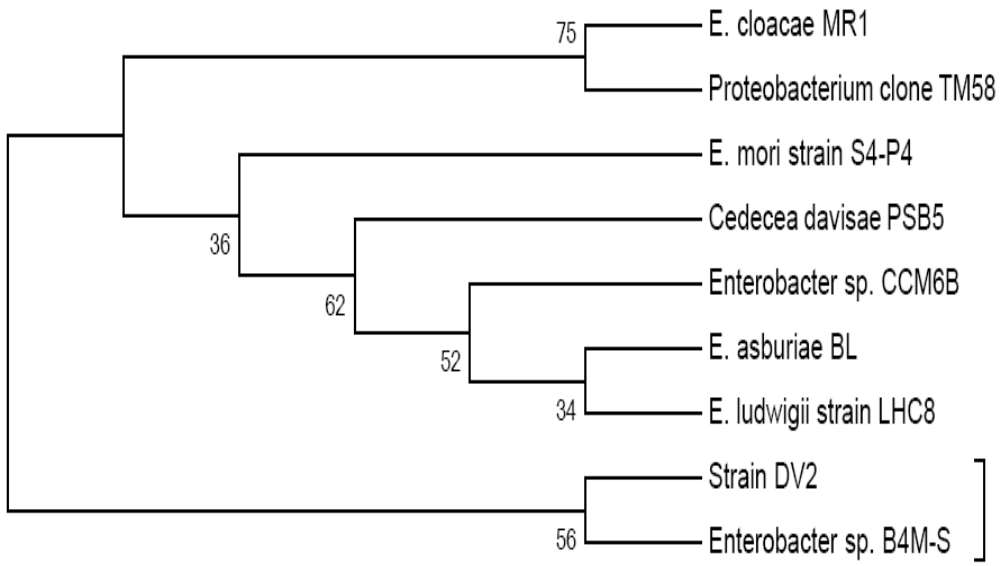


Figure 4.12 Phylogenetic tree of DV2 and related species constructed on the basis of 16S rRNA gene sequence using the maximum likelihood analysis.

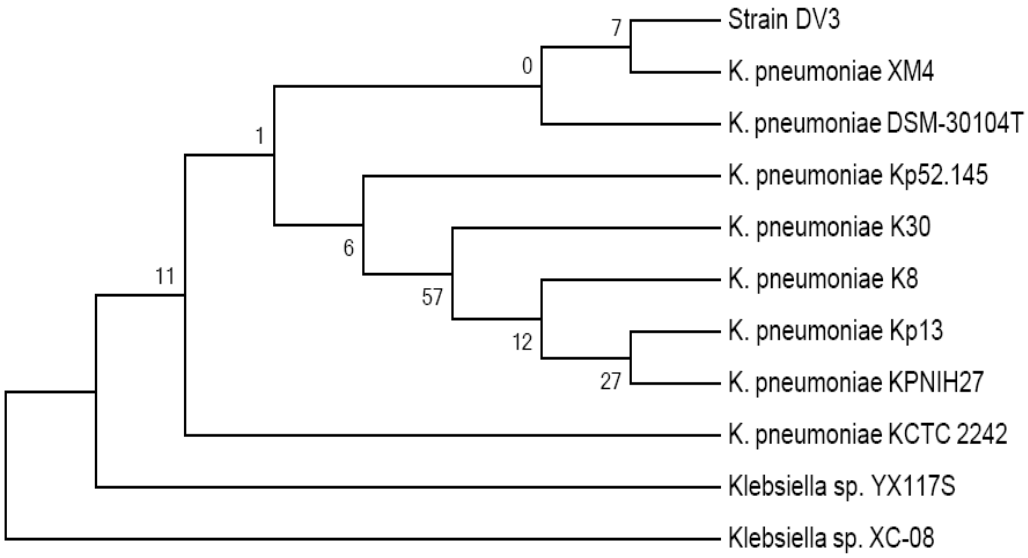


Figure 4.13 Phylogenetic tree of DV3 and related species constructed on the basis of 16S rRNA gene sequence using the maximum likelihood analysis.

Table 4.9 BlastN report for isolated strains DV1, DV2 and DV3

Strains	Description	Accession number	Identities		
			Match	Total	%
DV1	*Klebsiella pneumoniae* 30660/NJST258_1	CP006923.1	1474	1475	99
	Klebsiella pneumoniae subsp. *pneumoniae* HS11286	CP003200.1	1474	1475	99
	Klebsiella sp. ok1_1_9_S54 16S	JF274779.1	1472	1473	99
	Klebsiella sp. ok1_1_9_S35 16S	JF274760.1	1472	1473	99
	Klebsiella sp. ok1_1_9_S40 16S	JF274765.1	1472	1473	99
	Klebsiella sp. ok1_1_9_S41 16S	JF274766.1	1472	1473	99
	Klebsiella sp. ok1_1_9_S17 16S	JF274742.1	1472	1473	99
	Klebsiella pneumoniae subsp. *pneumoniae* KPNIH27	CP007731.1	1473	1475	99
	Klebsiella pneumoniae subsp. *pneumoniae* KPNIH10	CP007727.1	1473	1475	99
	Klebsiella pneumoniae 30684/NJST258_2	CP006918.1	1473	1475	99

Table 4.9 BlastN report for isolated strains DV1, DV2 and DV3 (cont.)

Strains	Description	Accession number	Identities		
			Match	Total	%
DV2	*Proteobacterium symbiont* of *Nilaparvata lugens* clone TM58	FJ774962.1	1472	1478	99
	Enterobacter sp. CCM6B	FN433019.1	1471	1478	99
	Enterobacter ludwigii strain LHC8	KC951920.1	1470	1477	99
	Cedecea davisae isolate PSB5	HQ242718.1	1470	1478	99
	Enterobacter sp. B4M-S	GQ478272.1	1466	1472	99
	Enterobacter mori strain S4-P4	KC851827.1	1461	1467	99
	Enterobacter asburiae strain BL	KF747681.1	1469	1478	99
	Enterobacter cloacae strain MR1	KC999878.1	1469	1478	99
DV3	*Klebsiella pneumoniae* str. Kp52.145	FO834906.1	1473	1473	100
	Klebsiella sp. YX117S	HQ204294.2	1473	1473	100
	Klebsiella pneumoniae KCTC 2242	CP002910.1	1473	1473	100
	Klebsiella pneumoniae strain XM4	JQ837267.1	1472	1473	99
	Klebsiella pneumoniae subsp. pneumoniae	HF543828.1	1472	1473	99
	Klebsiella pneumoniae strain K8	EU661374.1	1472	1473	99
	Klebsiella pneumoniae strain K30	EU661377.1	1472	1473	99
	Klebsiella pneumoniae subsp. pneumoniae KPNIH27	CP007731.1	1471	1473	99
	Klebsiella pneumoniae subsp. pneumoniae Kp13	CP003999.1	1471	1473	99
	Klebsiella sp. XC-08	KC787534.1	1471	1473	99

Table 4.10 Identification of isolated strains DV1, DV2 and DV3 by the basis of 16S rRNA gene sequence analysis

Strains	Genus	Species
DV1	*Klebsiella*	*Klebsiella* sp.
DV2	*Enterobacter*	*Enterobacter* sp.
DV3	*Klebsiella*	*Klebsiella pneumonia*

Previous studies reported the dichlorvos-degrading bacteria from the contaminated sites experienced with dichlorvos Lieberman and Alexander (1981)

showed that *Pseudomonas aeruginosa* could utilize and convert dichlorvos to its metabolites. Later, *Ochrobactrum* sp. strain DDV-1 isolated by Xiao-Hua et al. (2006) was able to degraded dichlorvos in contaminated soil. Ning et al. (2012) found the dichlorvos degradation ability of *Flavobacterium* sp. strain YD4 isolated from the rape phyllosphere. Recently, Agarry et al. (2013) isolated *Proteus vulgaris*, *Vibrio* sp., *Serratia* sp., and *Acinetobacter* sp. from agricultural soil. These four bacteria isolates were able to remove dichlorvos under presence of additional carbon source. To the best of our knowledge, this is the first discovery on dichlorvos degradation by *Klebsiella pneumoniae* (strain DV1), *Enterobacter* sp. (strain DV2) and *Klebsiella pneumonia* (strain DV3).

Some previous works reported that various contaminants could be utilized by species in *Klebsiella* genus. For example, *Klebsiella pneumoniae* was able to degrade penicillin (Wang et al., 2015) and tributyl phosphate (Kulkarni et al. 2014). *Klebsiella* sp. was capable for degrading of Chlorpyrifos (Wang et al., 2013) , endosulfan (Singh and Singh 2014), and carbazole (nitrogen heteroaromatic in many petroleum fractions) (Li et al. 2008). Also, *Enterobacter* sp. could remove numerous contaminants including methyl tert-butyl ether (MTBE) (Chen et al. 2011), 2-methyl-4-chlorophenoxyacetic acid (MCPA) (Tan et al. 2013), pyrene (Sheng et al. 2008) and polychlorinated biphenyl (PCB) (Jia et al. 2008). Based on the literature and the result from this study, it could say that these bacterial isolates were able to degrade various hazardous chemicals including dichlorvos pesticide.

4.2.2 Influence of dichlorvos concentration on dichlorvos degradation by dichlorvos-degrading pure cultures

1. Bacterial growth of pure cultures under different dichlorvos concentrations

After obtaining the dichlorvos-degrading bacteria (DV1, DV2 and DV3), influence of dichlorvos concentration on microbial growth and dichlorvos degradation were investigated. Three dichlorvos-degrading isolates provided different results as shown in Figure 4.14 (raw data presented in Appendix C).

During 7 days of experimental period, under dichlorvos concentrations of 50, 100 and 200 mg/L, the growths of DV1 composed two phases including log and stationary phases. For the log phase, DV1 grew from 7.9 to 8.7, 7.8 to 8.8 and 7.6 to 8.6 logCFU/mL within 1 day under dichlorvos concentrations of 50, 100 and 200 mg/L, respectively. Conversely, the growth of DV1 cultured under dichlorvos concentrations of 400 and 800 mg/L composed three phases including log, stationary and declined phases (Figure 4.14). This finding indicated that strain DV1 was able to survive and reproduce in the medium containing dichlorvos at different concentration (50-800

mg/L) but it is likely that DV1 was more endurable in low concentrations (50-200 mg/L) than high concentrations (400-800 mg/L).

For strain DV2, under different dichlorvos concentrations (50, 100, 200, 400 and 800 mg/L), an increase in bacterial population was observed at the first day. The initial cell numbers of 7.3, 7.2, 7.5, 7.1 and 7.0 logCFU/ml were detected and the number reached stable at 8.8, 8.6, 8.6, 8.6 and 8.2 logCFU/ml for the treatment under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L, respectively. During the experiment, strain DV2 in all treatments reached the stationary phase for 1 or 2 days (day 1 to day 3) before starting the declining phase.

For strain DV3, during the tests with low dichlorvos concentrations of 50 and 100 mg/L, it seemed to no obvious effect of the microbial growth. The log phase occurred at the first day of experiment. DV3 grew from 7.3 to 8.9 and 7.6 to 8.9 logCFU/mL under dichlorvos concentrations of 50 and 100 mg/L, respectively. After that, the stationary phase took place. For the tests under dichlorvos concentrations of 200 and 400 mg/L, the bacterial number of strain DV3 also sharply increased at the first day. DV3 grew from 7.7 to 8.9 and 7.6 to 9.1 logCFU/mL under dichlorvos concentrations of 200 and 400 mg/L, respectively. DV3 reached the stationary phase after 1 day. After day 4, DV3 dramatically decreased in cell number. Similar to the test at 800 mg/L of dichlorvos concentration, DV3 reached stationary phase at day 1 (grew from 7.3 to 8.7 logCFU/ml) but the stationary phase took place only 1 day (day 1 to day 2) before markedly reducing in bacterial cell growth.

The growth rates of strains DV1, DV2 and DV3 followed the first order kinetics. The growth rates of three isolated strains were showed in Table 4.11 (raw data presented in Appendix C). The isolates, DV1, DV2 and DV3, were able to grow in 50, 100, 200, 400 and 800 mg/L concentrations of MSM liquid medium supplemented with dichlorvos and an additional carbon source (yeast extract). The bacterial population of three isolates increased in the first day implied that dichlorvos and yeast extract could stimulate the growth of different isolates (strains DV1, DV2 and DV3). Based on the bacterial growth of three isolates under different dichlorvos concentrations, it was found that dichlorvos concentrations affect to these three strains differently. Dichlorvos concentrations of 50 and 100 mg/L did not affect bacterial population of DV1 and DV3 but at higher concentrations, the declining phase obviously occurred. In case of strain DV2, at all of dichlorvos concentrations (50-800 mg/L), the dead phase clearly observed. The declining phase may be from the shortage of yeast extract after testing for some period. Moreover, it might be toxicity effect of dichlorvos in the test with higher concentrations. The results could summarize that DV2 was more sensitivity to dichlorvos concentrations than strains DV1 and DV3.

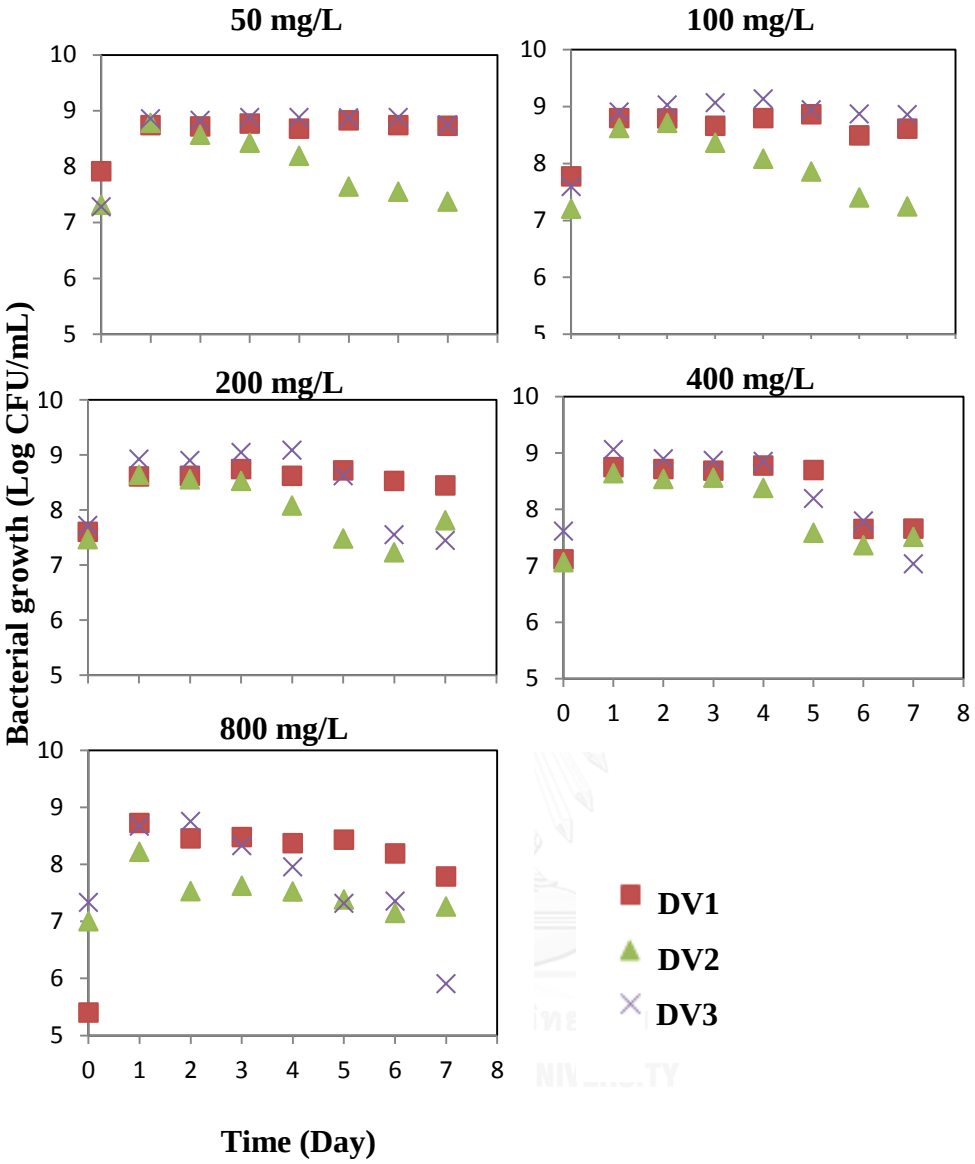


Figure 4.14 Growth of DV1 under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

Effect of contaminant concentrations on biodegradation (including pesticide degradation) has been reported (Kale et al. 1989, Kryuchkova et al. 2014, Peter et al. 2014). Too high concentration may cause toxic to microorganism while too low concentration might not be enough for cell growth. For dichlorvos degradation, the result in this study corresponded to previous finding by Ning et al. (2012). It was reported that dichlorvos concentrations of higher than 800 mg/L were toxic to *Flavobacterium* sp. resulting in cell number decreasing. In addition, Lieberman and Alexander (1981) observed that low concentrations of dichlorvos (0.1-100 mg/L) had only slight or no toxicity effect to microorganism enriched from sewage sludge.

Table 4.11 Growth rates of DV1, DV2 and DV3 under different dichlorvos concentrations

Bacterial isolated strain	Dichlorvos Concentration (mg/L)	Growth kinetic equation*	k (1/d)	R^2
DV1	50	y = 0.92x + 18.55	0.92	0.73
	100	y = 1.17x + 18.29	1.17	0.74
	200	y = 1.17x + 17.89	1.17	0.76
	400	y = 1.84x + 17.02	1.84	0.73
	800	y = 3.52x + 13.81	3.52	0.68
DV2	50	y = 1.45x + 17.48	1.45	0.63
	100	y = 1.73x + 17.10	1.73	0.79
	200	y = 1.25x + 17.68	1.25	0.69
	400	y = 1.70x + 16.91	1.70	0.70
	800	y = 0.72x + 16.28	0.72	0.86
DV3	50	y = 1.78x + 17.38	1.78	0.73
	100	y = 1.65x + 17.93	1.65	0.82
	200	y = 1.36x + 18.24	1.36	0.74
	400	y = 1.44x + 18.03	1.44	0.73
	800	y = 1.63x + 17.36	1.63	0.79

***** y = ln (bacterial number)
x= time (day)

2. Dichlorvos degradation of pure cultures under different dichlorvos concentrations

The dichlorvos degradation was presented in Figures 4.15, 4.16 and 4.17 (raw data presented in Appendix C) for strains DV1, DV2 and DV3, respectively. As shown in the figures, in all tested conditions (all initial concentrations of dichlorvos), dichlorvos was well degraded. For biodegradation by all pure isolates and abiotic control test (without inoculation), dichlorvos concentration dramatically decreased everyday throughout experimental period. However, biodegradation process by all pure cultures performed more efficiently on dichlovos degradation than those with abiotic process, especially at high concentrations of dichlorvos (400 and 800 mg/L).

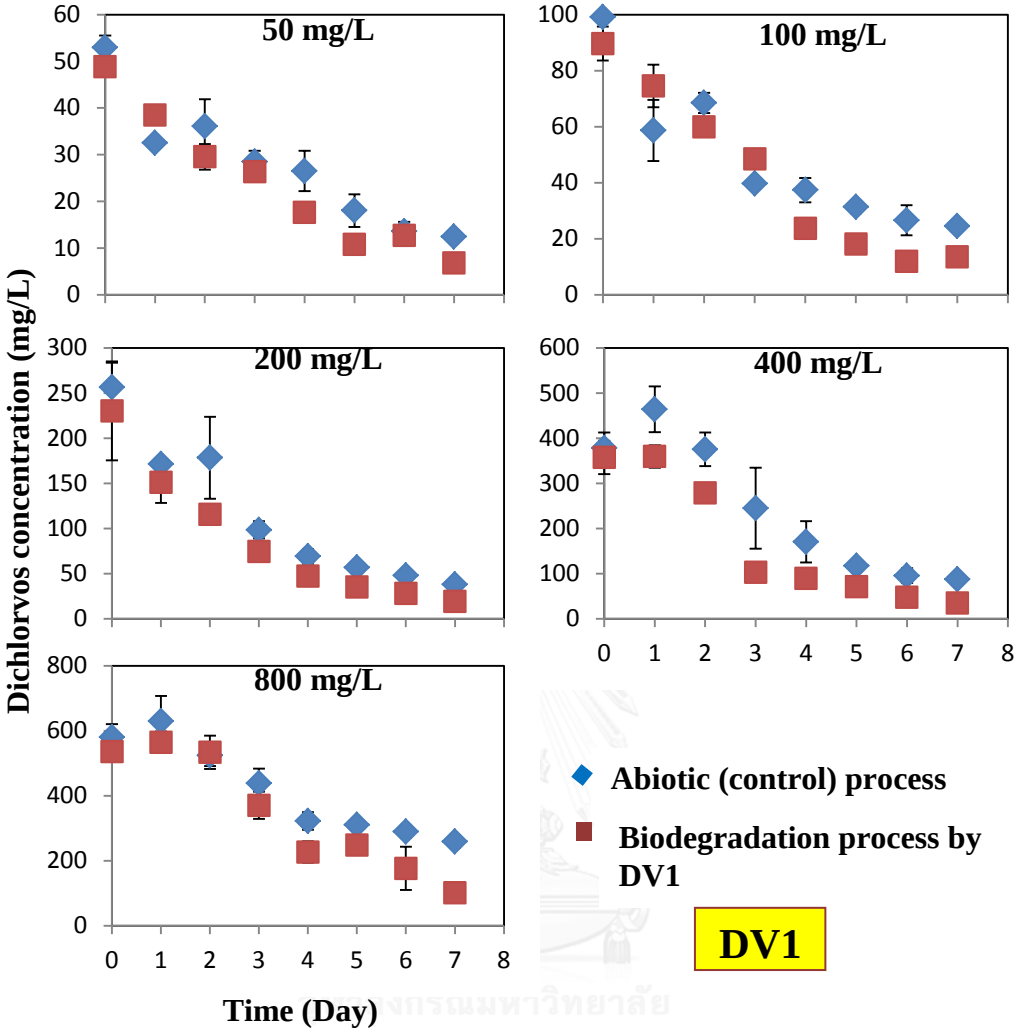


Figure 4.15 Dichlorvos degradation by abiotic and biodegradation processes (strain DV1) under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

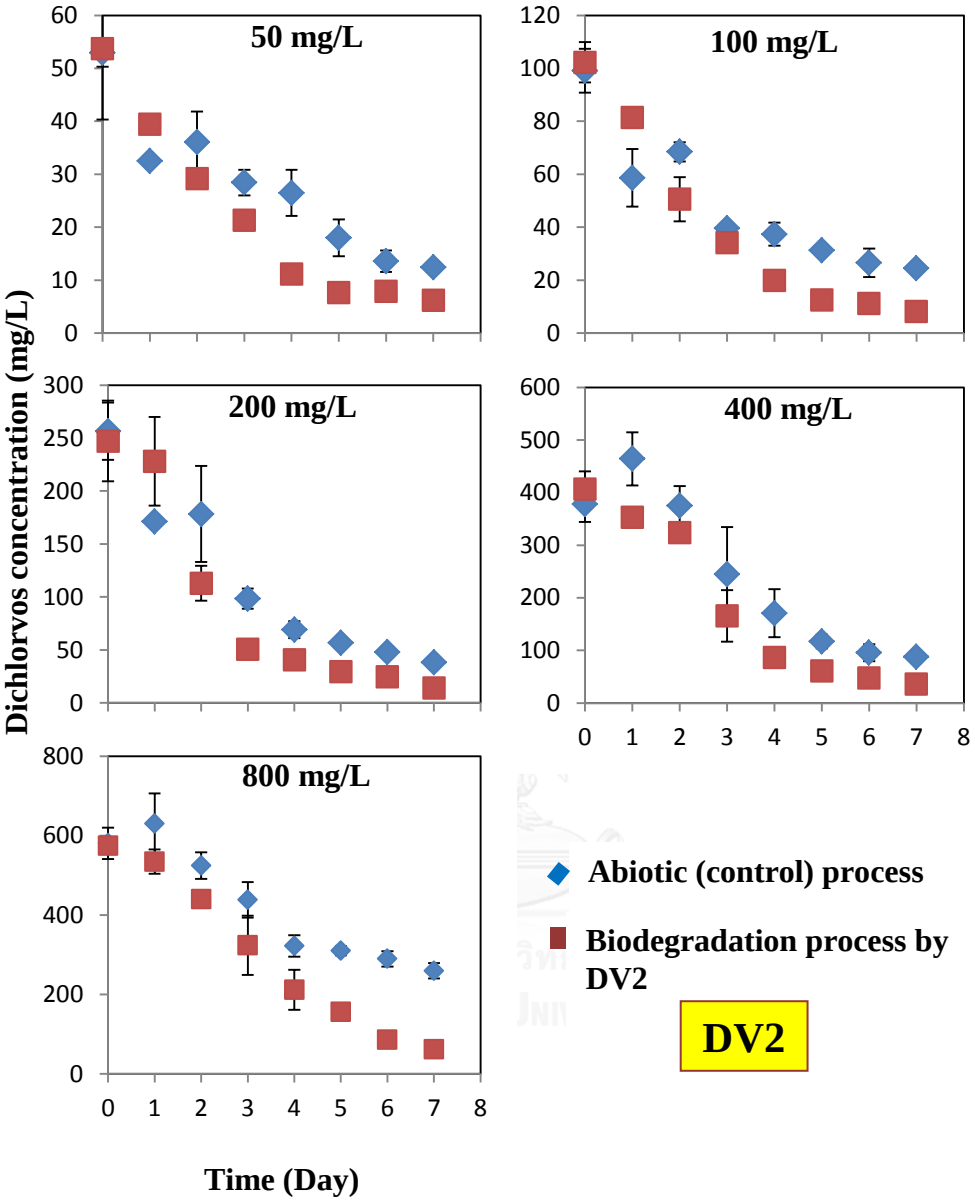


Figure 4.16 Dichlorvos degradation by abiotic and biodegradation processes (strain DV2) under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

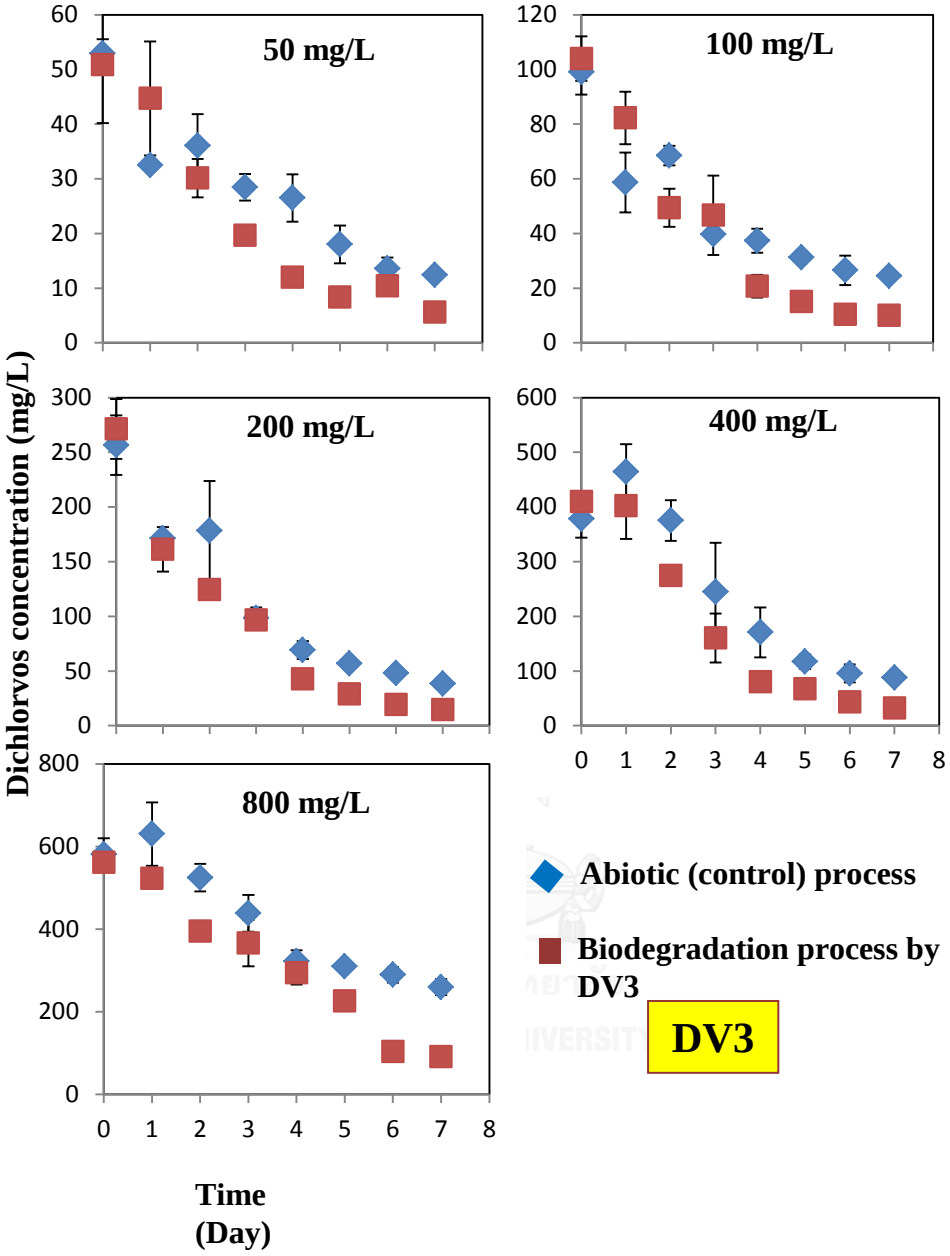


Figure 4.17 Dichlorvos degradation by abiotic and biodegradation processes (strain DV3) under dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L at pH 5.5

Dichlorvos removal rates and dichlorvos removal percentages of abiotic (control) test and biodegradation test by three isolates under dichlorvos concentrations of ranging from 50-800 mg/L were presented in Table 4.12 (raw data presented in Appendix C). Dichlorvos removal rates increased along with the increasing of dichlorvos concentrations. The difference between abiotic and biodegradation processes can be clearly seen at high dichlorvos concentration (800 mg/L). However, among three isolated strains, the dichlorvos removal rates were not considerably

differenct. Dichlorvos removal rates by abiotic process of 5, 10, 31, 41 and 45 mg/L/d were evaluated while the removal rates by biodegradation treatment of 6, 13, 33, 51 and 85 mg/L/d were found in the tests with dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L, respectively (Table 4.12). It is noted that the abiotic degradation percentage in this subsection was higher than that of previous section. This is because of the influence of temperatures. The experiment was performed in room temperature which ranged from approximately 15-40°C. This correlated to Suter (1981) and Australian Pesticides & Veterinary Medicines Authority [APVMA] (2008) which reported higher temperature leading to higher hydrolysis.

Refer to the dichlorvos removal percentage (Table 4.12), initial dichlorvos concentrations affected dichlorvos removal by both abiotic and biodegradation processes. After 7-d experiment, the removal percentages of abiotic (76.54%, 75.25%, 85.20%, 76.87% and 55.31%), DV1 (86.13%, 85.00%, 91.69%, 90.12% and 81.11%), DV2 (88.38%, 92.02%, 94.23%, 91.11% and 89.26%) and DV3 (88.96% 90.27%, 94.65%, 92.08% and 83.80%) under dichlorvos concentration of 50, 100, 200, 400 and 800 mg/L, respectively were found. The highest removal percentage was found at dichlorvos concentration of 200 mg/L in all cultures. The tests with the concentrations of higher or lower than 200 mg/L seemed to be less removal performance. The lowest one was detected in the test with dichlorvos concentration of 800 mg/L for abiotic test and biodegradation tests with strains DV1 and DV3 while it was detected in the test with 50 mg/L of dichlorvos concentration for DV2.

Biodegradation process (biotic and abiotic reactions) exhibited higher of dichlorvos removal percentage than abiotic process. The activity of bacteria (biodegradation alone) after subtracted by abiotic process was shown on Table 4.12. Based on these values, it can be confirmed that the degradation by bacterial isolates, strains DV1, DV2 and DV3, played an important role in the degradation especially at high concentration of dichlorvos. DV1, DV2 and DV3 could degrade dichlorvos of up to 25%, 33% and 28% respectively. These findings can be interpreted that dichlorvos degrading bacteria isoalated in this study were potential for remediation particularly in the contaminated environment with high concentration of dichlorvos.

Table 4.12 Dichlorvos removal rates and dichlorvos removal percentages of abiotic and biodegradation processes (strains DV1, DV2 and DV3)

Bacterial isolated strain	Dichlorvos concentration (mg/L)	Abiotic (control) process		Biodegradation process		Biodegradation alone
		Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal (%)
DV1	50	5.79	76.54	6.01	86.13	**9.59**
	100	10.65	75.25	10.88	85.00	**9.75**
	200	31.18	85.09	30.14	91.69	**6.60**
	400	41.53	76.87	46.00	90.12	**13.25**
	800	45.87	55.31	62.08	81.11	**25.80**
DV2	50	5.79	76.54	6.78	88.38	**11.84**
	100	10.65	75.25	13.44	92.02	**16.77**
	200	31.18	85.09	33.28	94.23	**9.14**
	400	41.53	76.87	52.91	91.11	**14.24**
	800	45.87	55.31	73.25	89.26	**33.95**
DV3	50	5.79	76.54	6.47	88.96	**12.42**
	100	10.65	75.25	13.41	90.27	**15.02**
	200	31.18	85.09	36.70	94.65	**9.56**
	400	41.53	76.87	53.92	92.08	**15.21**
	800	45.87	55.31	67.19	83.80	**28.49**

It was estimated dichlorvos degradation kinetics followed the reaction kinetic order as shown in Table 4.13 (raw data presented in Appendix C). The result indicated that the degradation was the function of the initial concentration. These finding went together with other earlier studies. Dichlorvos of 71% could remove

from soil after 10 days of incubation in the treatment with bacteria while loss of dichlorvos only 50% from soil after the same period occurred in treatment without bacterial inoculation (Lamoreaux and Newland, 1978). They also mentioned that greater loss of dichlorvos was related to the increasing of bacterial growth. Fritz (1987) compared the dichlorvos mineralization to $^{14}CO_2$ between sterile and non-sterile soils. In sterile soil, the mineralization hardly occurred even after 60 days of incubation and dichlorvos degradation (the half-life of 8.7 days) was much slower than that in non-sterile soil (the half-life of less than 2 days). The study suggested that dichlorvos degradation in sterile soil occurred by abiotic hydrolysis. The degradation result also associated with the review report published by Australian Pesticide & Veterinary Medicine Authority (2008). It informed that dichlorvos degradation in aquatic situation mainly occurred by abiotic hydrolysis but the degradation of this pesticide in unsterilized water with microorganism available in an environmental system could accelerate the degradation.

Initial dichlorvos concentration had effect on dichlorvos degradation either by abiotic hydrolysis or by biodegradation. The removal percentage of dichlorvos by abiotic process in the present study was governed by the concentration similar to Drevenkar et al. (1976). In biodegradation process by all pure cultures, the removal percentage gradually increased with the increasing of dichlorvos concentrations ranging from 50 to 200 mg/L. But it decreased in the test with higher dichlorvos concentrations. These finding agreed with the studies by Ning et al. (2012). Too high (800 mg/L) or too low (50 mg/L) concentrations could reduce bacterial growth and activity resulting in less degradation.

However the biodegradation test in present study, after subtracting by the abiotic degradation as presented in Table 4.12, the biodegradation alone was estimated. The biodegradation played an important role on dichlorvos degradation when the concentration increased. At high concentration (800 mg/L), the biodegradation (alone) obviously increased. The isolates worked well even in the high concentration. This result could imply that biodegradation was suitable for contaminated site with high dichlorvos concentration because abiotic hydrolysis alone required longer time. The biodegradation by dichlorvos-degrading isolates could accelerate the dichlorvos degradation.

Table 4.13 Dichlorvos degradation rates of abiotic and biodegradation processes (strains DV1, DV2 and DV3)

Bacterial isolated strain	Dichlorvos concentration (mg/L)	Abiotic (control) process			Biodegradation process		
		Dichlorvos degradation kinetic equation*	k (1/d)	R^2	Dichlorvos degradation kinetic equation*	k (1/d)	R^2
DV1	50	y = -0.20x + 3.90	0.20	0.94	y = -0.27x + 3.94	0.27	0.96
	100	y = -0.19x + 4.44	0.19	0.92	y = -0.32x + 4.60	0.32	0.95
	200	y = -0.28x + 5.51	0.28	0.97	y = -0.35x + 5.40	0.35	0.99
	400	y = -0.26x + 6.22	0.26	0.93	y = -0.36x + 6.05	0.36	0.95
	800	y = -0.13x + 6.46	0.13	0.93	y = -0.24x + 6.54	0.24	0.91
DV2	50	y = -0.20x + 3.90	0.20	0.94	y = -0.33x + 3.96	0.33	0.96
	100	y = -0.19x + 4.44	0.19	0.92	y = -0.38x + 4.66	0.38	0.98
	200	y = -0.28x + 5.51	0.28	0.97	y = -0.42x + 5.54	0.42	0.96
	400	y = -0.26x + 6.22	0.26	0.93	y = -0.39x + 6.21	0.39	0.96
	800	y = -0.13x + 6.46	0.13	0.93	y = -0.34x + 6.61	0.34	0.96
DV3	50	y = -0.20x + 3.90	0.20	0.94	y = -0.32x + 3.98	0.32	0.95
	100	y = -0.19x + 4.44	0.19	0.92	y = -0.37x + 4.69	0.37	0.97
	200	y = -0.28x + 5.51	0.28	0.97	y = -0.43x + 5.62	0.43	0.98
	400	y = -0.26x + 6.22	0.26	0.93	y = -0.40x + 6.23	0.40	0.97
	800	y = -0.13x + 6.46	0.13	0.93	y = -0.27x + 6.54	0.27	0.92

***** y = ln (dichlorvos concentration)
x= time (day)

3. Comparison of dichlorvos degradation by mixed cultures and pure isolates under different dichlorvos concentrations

The different in bacterial growth and dichlorvos degradation ability under various dichlorvos concentrations of mixed cultures, isolated strains DV1, DV2 and DV3 were presented in Figure 4.18 and Figure 4.19, respectively (raw data presented in Appendix C). As seen in Figure 4.18, in overall, bacterial population of mixed cultures seemed to grow better than isolated strains. The results on bacterial growth have opposing relationship with dichlorvos degradation ability. In degradation ability as shown in Figure 4.19, mixed cultures seemed to have lowest efficiency on dichlorvos degradation. It could indicate that mixed cultures preferred to use yeast extract supplemented as additional carbon source rather than dichlorvos solely to support their growth. Also, the growth showing in mixed cultures could be some other dichlorvos-tolerant cultures which were not able to utilize dichlorvos leading to less dichlorvos degradation.

In practice, the mixed cultures generally performed more efficient for biodegradation than pure cultures (Alvey and Crowley 1996, Nestler et al. 2001, Smith et al. 2005). Due to the mixed cultures might compose different bacterial strains resisting in stress condition including the toxic pollutant metabolites. Therefore, biodiversity of mixed cultures can enhance the survival of bacteria. Furthermore, some species in the mixed cultures were possible to further degrade the metabolites (Alvey and Crowley 1996, Nestler, Hansen et al. 2001, Smith, Alvey et al. 2005). For example, Jabeen et al. (2015) reported that the degradation capability of bacterial mixed cultures was much higher compared to those of the pure isolates. Mixed cultures could metabolize profenofos of 37% within 1 day and completely degrade after 3 days whereas, individual strains, *Achromobacter xylosoxidans* PF1, *Pseudomonas aeruginosa* PF2, *Bacillus* sp. PF3 and *Citrobacter koseri* PF4 degraded profenofos only 32%, 44%, 36% and 27%, respectively after 4 days of incubation. However, in present study, the isolated strains DV1, DV2 and DV3 were acclimated in the synthetic medium with high concentration of dichlorvos leading to better performance in dichlorvos degradation. The pure isolates were likely to show higher efficiency in the dichlorvos degradation. This may be due to the competition between the cultures in the consortium. It was similar to previous study by Cheela et al. (2014). They indicated that the phenol removal efficiency of pure culture was found to be higher than that of mixed cultures even in phenol concentrations of 100 mg/L or 200 mg/L.

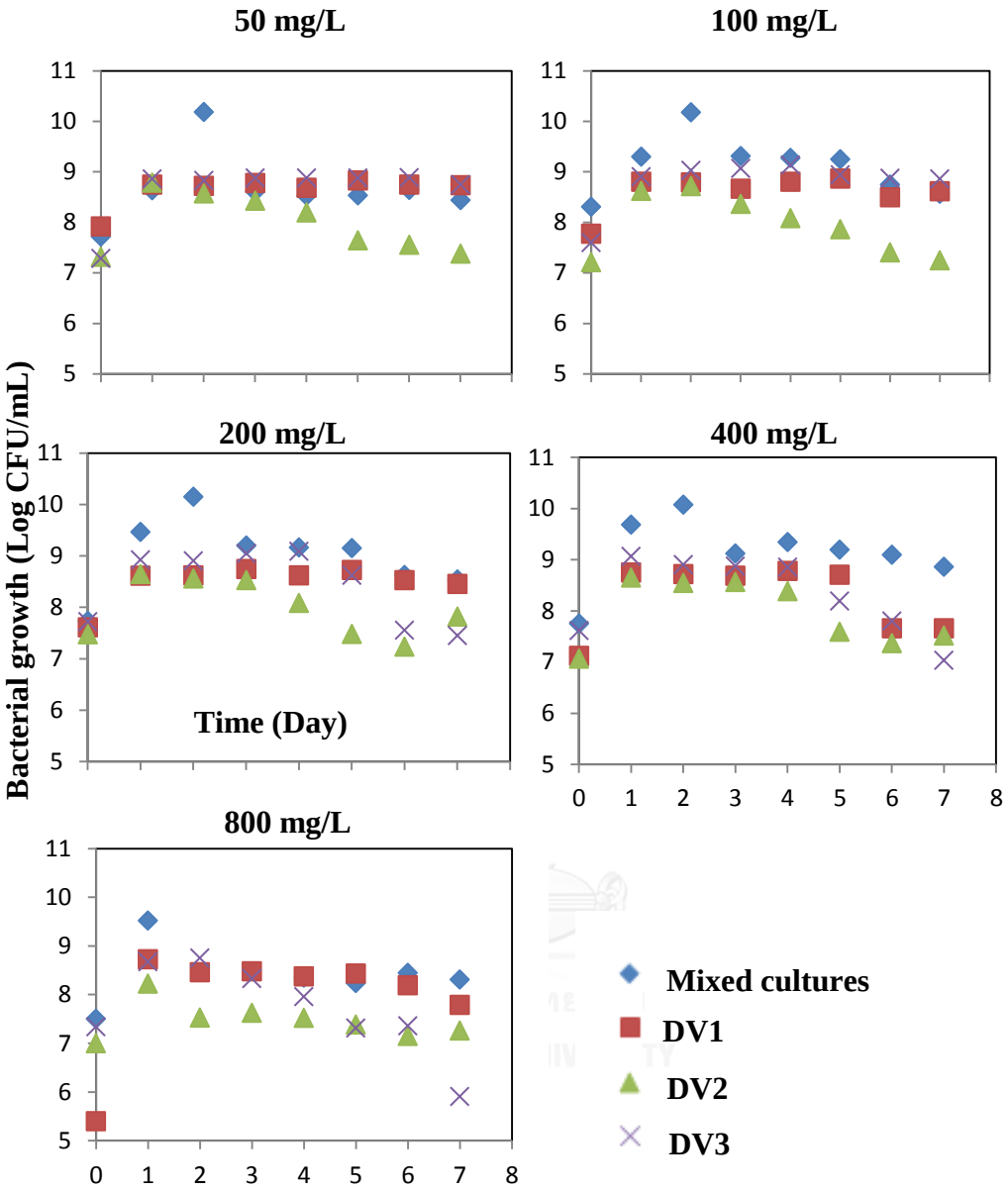


Figure 4.18 Bacterial growth comparison between mixed cultures and isolated strains DV1, DV2 and DV3

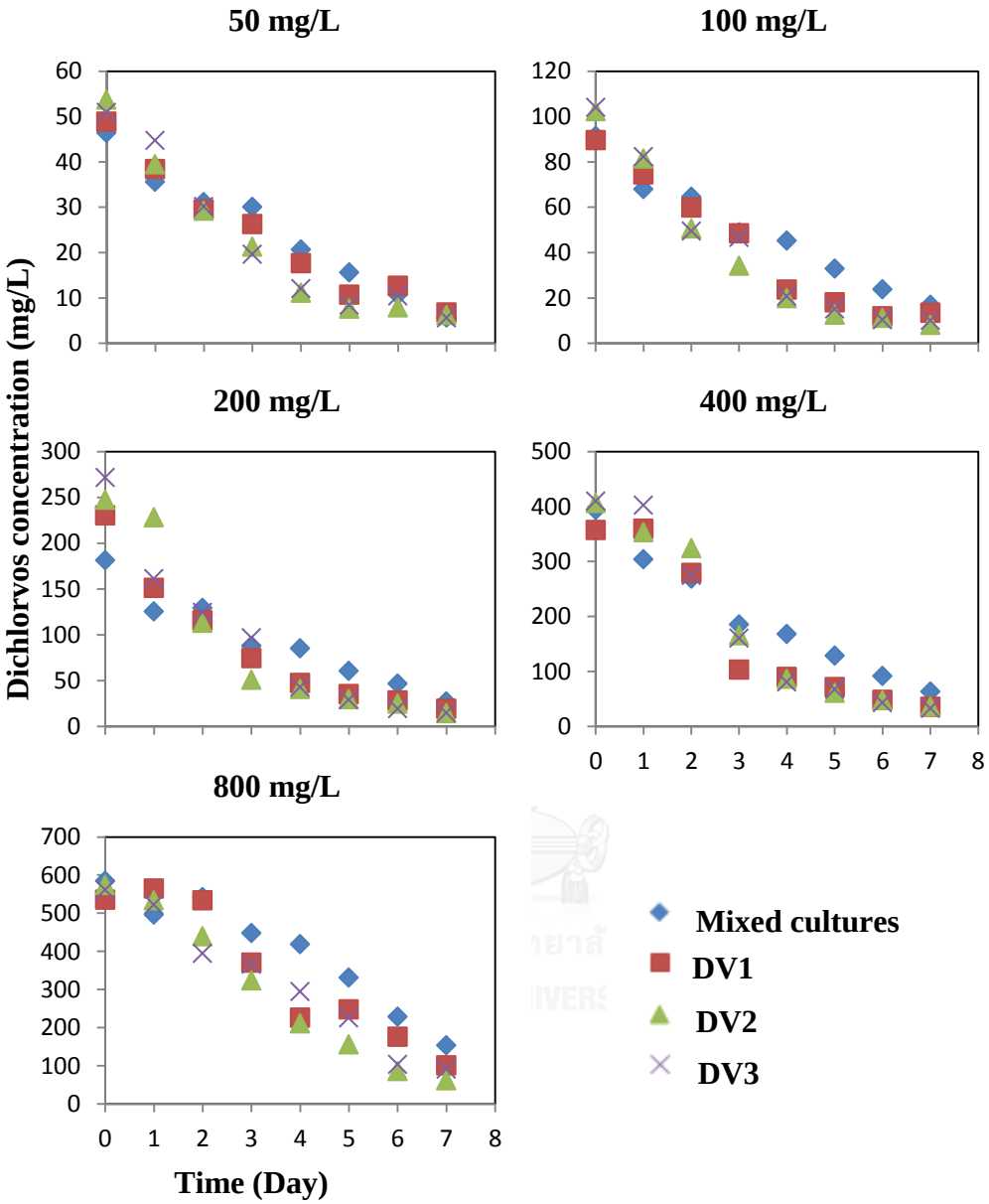


Figure 4.19 Dichlorvos degradation comparisons between bacterial mixed cultures and isolated strains DV1, DV2 and DV3

4.2.3 Influence of pH on dichlorvos degradation by dichlorvos-degrading pure cultures

1. Bacterial growth of pure cultures under different pHs

Influence of various pHs of culture medium on bacterial growth of strain DV1, DV2 and DV3 was also investigated in this study. All bacterial strains were cultured in MSM liquid medium with pHs of 4, 5, 6, 7 and 8 and dichlorvos concentration of 800 mg/L. Figure 4.20 (raw data presented in Appendix C) presents the population growth of isolated strains DV1, DV2 and DV3. At pH ranging from 4-8, the bacterial cell number of DV1, DV2 and DV3 approximately increased from 6 to 8 logCFU/mL. They reached the stationary phase at the first or second days of experimental period. Under acidic condition (pH 4-6), declining phase took place in later period (days 4 to 8). Growth kinetic rates of all tests which followed the first order kinetics were exhibited in Table 4.14 (raw data presented in Appendix C). The kinetic rates of 2.07 to 2.91, 1.20 to 2.21 and 1.88 to 4.78 1/d were detected from the tests with DV1, DV2 and DV3, respectively.

pH is a factor that particularly affects to all living microbial growth and their activities. Researchers have examined effect of pH on the microbial growth kinetics. For example, Salunkhe et al. (2013) found that in the biodegradation process of organophosphorus pesticides, four *Bacillus subtilis* strains were able to proliferate in the alkaline condition. Tang et al. (1989) examined the influence of pH on the growth kinetics of *Clostridium formicoaceticum*. They found that the specific growth rate increased along with the increasing of pH. Another example is a study by Yuan et al. (2014). They reported that the highest growth of pyrene-degrading bacterium strain USTB-X presented at pH 7. The reduction of bacterial growth was found in the tests with pH of lower and higher than 7. Based on the review, it could state that each microorganism prefers the environmental (including pH) condition differently. In this study, the isolates were likely to grow in pH of 7-8 resulting in long stationary phase.

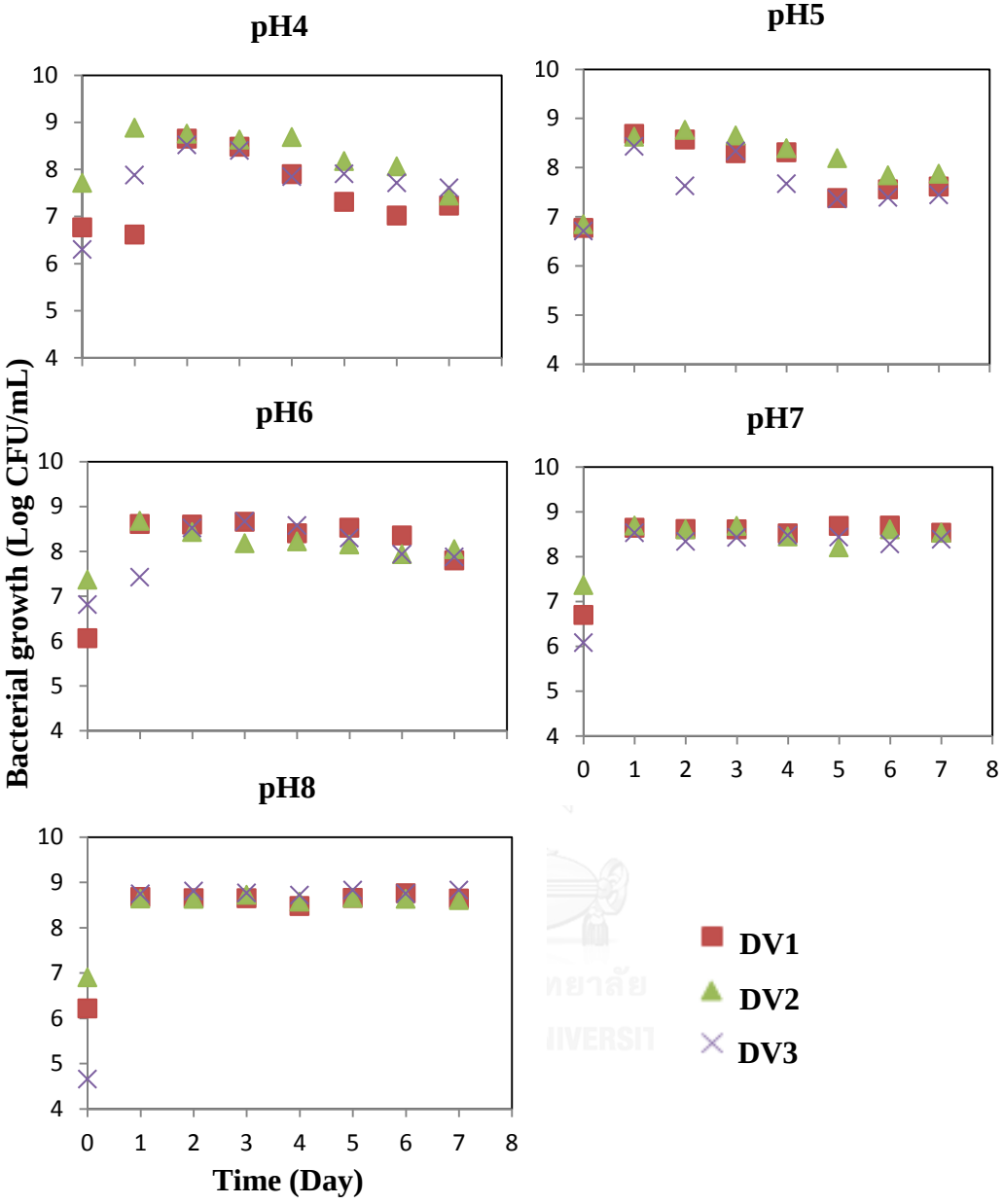


Figure 4.20 Bacterial growth of bacterial isolated strains DV1, DV2 and DV3 under different pHs

Table 4.14 Bacterial growth rates of dichlorvos-degrading isolated strains DV1, DV2 and DV3 under different pHs

Bacterial isolated strain	pH	Growth kinetic equation*	k (1/d)	R^2
DV1	pH4	y = 2.17x + 14.75	2.17	0.69
	pH5	y = 2.07x + 16.37	2.07	0.70
	pH6	y = 2.91x + 14.94	2.91	0.75
	pH7	y = 2.20x + 16.18	2.20	0.74
	pH8	y = 2.79x + 15.273	2.79	0.74
DV2	pH4	y = 1.20x + 18.26	1.20	0.66
	pH5	y = 2.21x + 16.39	2.21	0.80
	pH6	y = 1.22x + 17.55	1.22	0.58
	pH7	y = 1.42x + 17.49	1.42	0.70
	pH8	y = 2.00x + 16.56	2.00	0.75
DV3	pH4	y = 2.55x + 14.87	2.55	0.94
	pH5	y = 1.88x + 15.53	1.88	0.99
	pH6	y = 1.96x + 15.49	1.96	0.97
	pH7	y = 2.59x + 15.01	2.59	0.68
	pH8	y = 4.78x + 12.26	4.78	0.76

***** y = ln (bacterial number)
x= time (day)

2. Dichlorvos degradation of pure cultures under different pHs

Figures 4.21–4.23 (raw data presented in Appendix C) demonstrate the influence of pH ranging from 4 - 8 on the reduction of dichlorvos concentration (initial concentration of 800 mg/L) by abiotic process (hydrolysis reaction) and biodegradation process (the degradation activity of isolated strains with abiotic reaction). As shown in these figures, it is clearly seen that pHs of cultural medium significantly influenced the degradation of dichlorvos. In all testes, the concentration of dichlorvos markedly decreased for entire of the study but the higher pH showed the more dichlorvos reduction.

The dichlorvos removal by abiotic and biodegradation processes under different pH was shown in Table 4.15 (raw data presented in Appendix C). At the end of experiment, dichlorvos was removed from liquid culture medium by hydrolysis (abiotic process) of 24.31%, 38.86%, 87.72%, 99.30% and 99.87% under culture medium at pH4, pH5, pH6, pH7 and pH8, respectively. For biodegradation by DV1, DV2 and DV3, dichlorvos decreased for about 35%, 54%, 92%, 99% and 99% at pH4, pH5, pH6, pH7 and pH8, respectively. The finding agreed with the studies by Suter (1981) and Lamoreaux and Newland (1978). They stated that dichorvos hydrolysis increased with the increasing of pH. This revealed that the hydrolysis of dichlorvos preferred in the presence of hydroxide ion. It was similar to some other pesticides such as profenofos and carbofuran (Bailey et al. 1996, Zamy et al. 2004).

Difference of pHs also affected the biodegradation of dichlorvos by all isolates differently. However as mentioned previously, dichlorvos degradation by biodegradation process included biotic and abiotic reactions. Therefore, in order to obtain the efficiency of biodegradation (alone) by all isolates, the removal percentage of dichlorvos biodegradation was subtracted by the dichlorvos removal percentage occurred from abiotic process (Table 4.15). From Table 4.15, it was indicated that biodegradation alone played an important role on the degradation of dichlorvos in the treatments at pHs ranging from 4-6 while at pH 7 and pH 8, dichlorvos degradation mostly occurred *via* chemical hydrolysis.

Strain DV1 improved the degradation of 4.34, 20.11 and 4.13% at pH 4, 5 and 6, respectively compared to abiotic process. In case of biodegradation (alone) by strain DV2, it enhanced the dichlorvos degradation of 17.06, 14.42 and 6.74% while strain DV3 reduced dichlorvos for 12.61%, 11.25% and 4.64% under the treatments at pH 4, 5 and 6, respectively (Table 4.15). It was noticed that the maximum removal percentage of DV1 presented at pH 5 while those of DV2 and DV3 were at pH 4. In overall, DV2 had higher efficiency in dichlorvos degradation than other two strains.

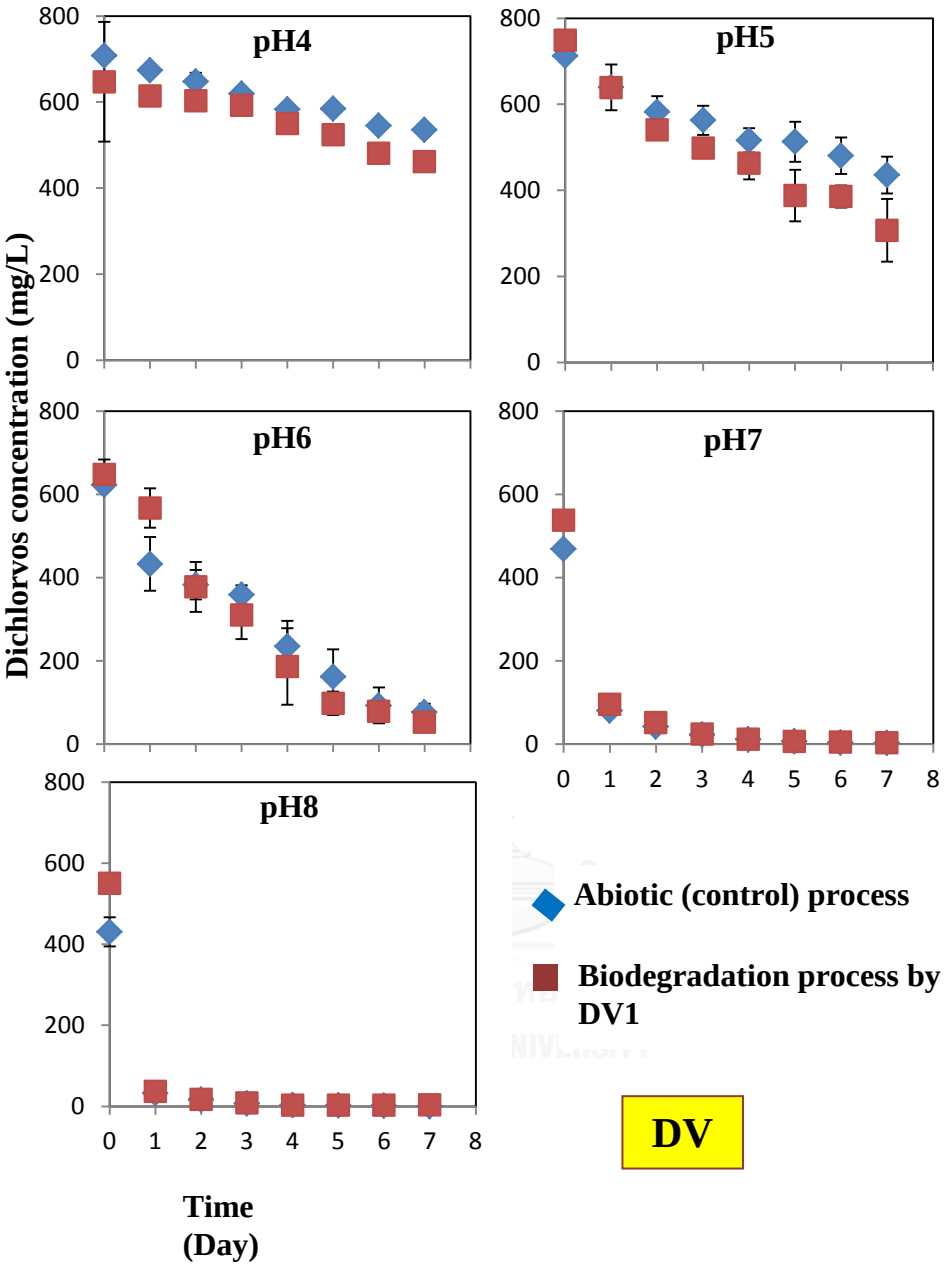


Figure 4.21 Dichlorvos degradation by abiotic and biodegradation processes (strain DV1) under different pHs

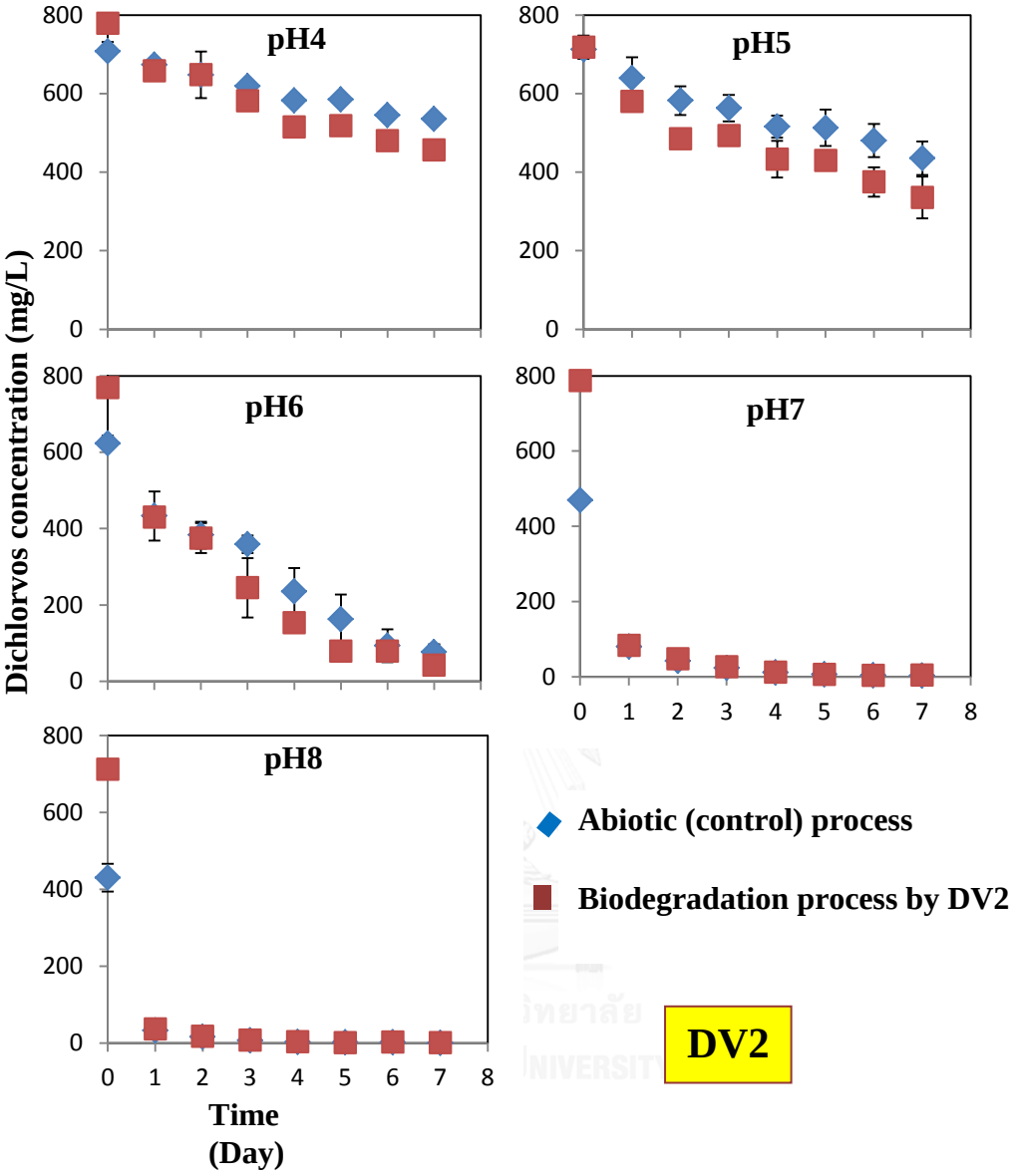


Figure 4.22 Dichlorvos degradation by abiotic and biodegradation processes (strain DV2) under different pHs

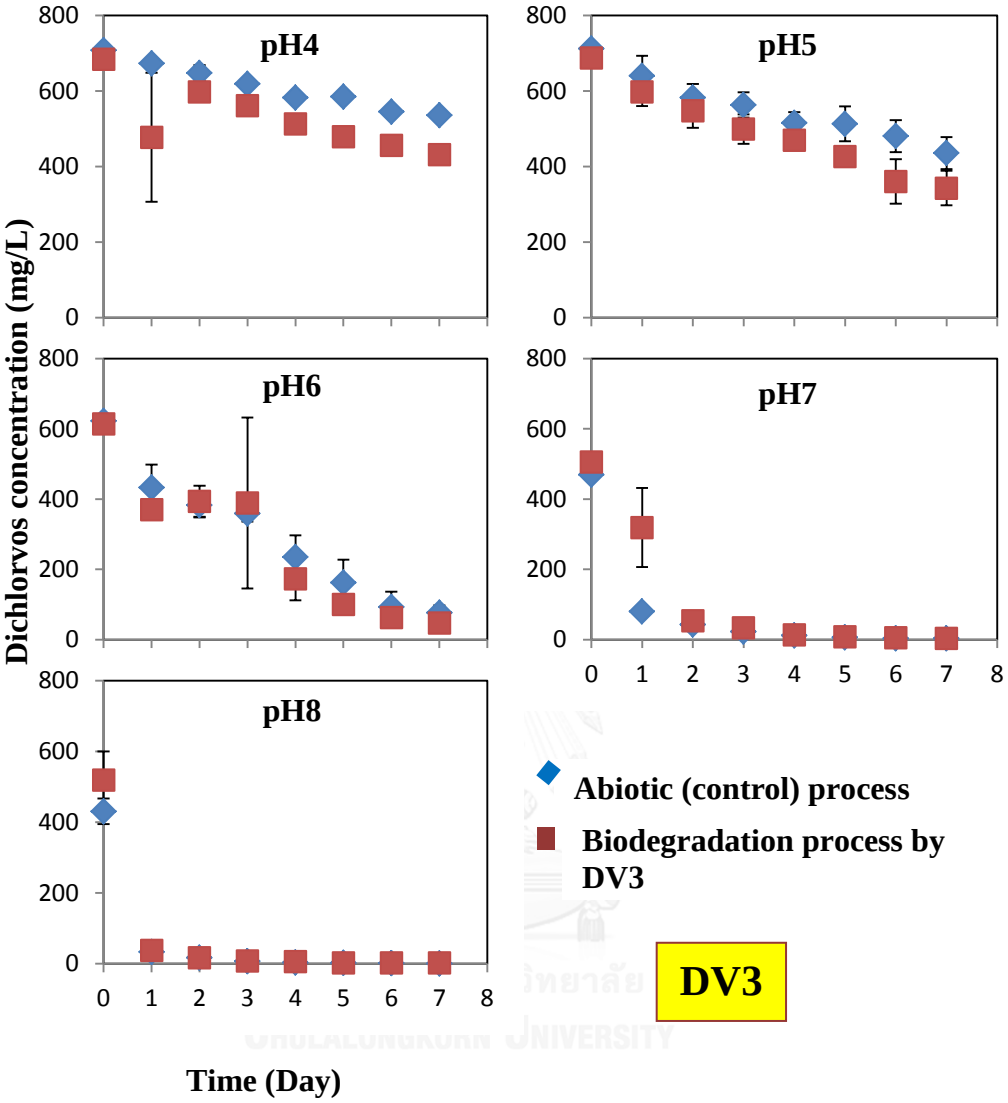


Figure 4.23 Dichlorvos degradation by abiotic and biodegradation processes (strain DV3) under different pHs

Table 4.16 (raw data presented in Appendix C) shows the degradation rates of dichlorvos by abiotic and biodegradation processes. All biodegradation treatments followed the first order kinetics with the rates of 0.06, 0.1, 0.4, 0.7 and 1.0 (1/d) for pH 4, 5, 6, 7 and 8, respectively. The degradation rates increased with the increasing of pHs.

Table 4.15 Dichlorvos removal rates and dichlorvos removal percentages of abiotic and biodegradation processes (strains DV1, DV2 and DV3)

Bacterial isolated strain	pH	Abiotic (control) process		Biodegradation process		Biodegradation alone
		Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal rate (mg/L/d)	Dichlorvos removal (%)	Dichlorvos removal (%)
DV1	pH4	24.56	24.31	26.47	28.65	**4.34**
	pH5	39.53	38.86	63.06	58.97	**20.11**
	pH6	78.04	87.72	85.02	91.85	**4.13**
	pH7	66.55	99.30	76.31	99.33	**N.A.**
	pH8	61.41	99.87	78.07	99.28	**N.A.**
DV2	pH4	24.56	24.31	46.05	41.37	**17.06**
	pH5	39.53	38.86	54.64	53.28	**14.42**
	pH6	78.04	87.72	103.83	94.46	**6.74**
	pH7	66.55	99.30	111.84	99.43	**N.A.**
	pH8	61.41	99.87	101.61	99.84	**N.A.**
DV3	pH4	24.56	24.31	36.05	36.92	**12.61**
	pH5	39.53	38.86	49.23	50.11	**11.25**
	pH6	78.04	87.72	81.04	92.36	**4.64**
	pH7	66.55	99.30	71.768	99.26	**N.A.**
	pH8	61.41	99.87	73.72	99.48	**N.A.**

N.A. = not applicable

Table 4.16 Dichlorvos degradation rates of abiotic and biodegradation processes (strains DV1, DV2 and DV3)

Bacterial isolated strain	pH	Abiotic (control) process			Biodegradation process		
		Dichlorvos degradation kinetic equation*	k (1/d)	R^2	Dichlorvos degradation kinetic equation*	k (1/d)	R^2
DV1	pH4	y = -0.04x + 6.55	0.04	0.98	y = -0.05x + 6.49	0.05	0.96
	pH5	y = -0.06x + 6.53	0.06	0.97	y = -0.12x + 6.58	0.12	0.98
	pH6	y = -0.30x + 6.53	0.30	0.96	y = -0.38x + 6.66	0.38	0.98
	pH7	y = -0.67x + 5.38	0.67	0.94	y = -0.67x + 5.52	0.67	0.94
	pH8	y = -0.80x + 4.86	0.80	0.91	y = -1.18x + 5.58	1.18	0.91
DV2	pH4	y = -0.04x + 6.55	0.04	0.98	y = -0.07x + 6.60	0.07	0.95
	pH5	y = -0.06x + 6.53	0.06	0.97	y = -0.09x + 6.48	0.09	0.94
	pH6	y = -0.30x + 6.53	0.30	0.96	y = -0.40x + 6.62	0.40	0.98
	pH7	y = -0.67x + 5.38	0.67	0.94	y = -0.78x + 5.80	0.78	0.92
	pH8	y = -0.80x + 4.86	0.80	0.91	y = -1.13x + 5.61	1.13	0.92
DV3	pH4	y = -0.04x + 6.55	0.04	0.98	y = -0.07x + 6.52	0.07	0.99
	pH5	y = -0.06x + 6.53	0.06	0.97	y = -0.10x + 6.51	0.10	0.99
	pH6	y = -0.30x + 6.53	0.30	0.96	y = -0.38x + 6.58	0.38	0.93
	pH7	y = -0.67x + 5.38	0.67	0.94	y = -0.68x + 5.70	0.68	0.95
	pH8	y = -0.80x + 4.86	0.80	0.91	y = -0.81x + 5.06	0.81	0.87

***** y = ln (dichlorvos concentration)
x= time (day)

The results from this experiment were compatible to the conclusion by Lamoreaux and Newland (1978). They mentioned that 70% of total dichlorvos degradation in the non-sterile soil (with added bacteria) due to chemical mechanisms (such as hydrolysis, adsorption and other reactions of abiotic degradation) and 30% from bacteriological mechanism. Therefore, the treatment with pesticide-degrading isolates was able to improve the pesticide degradation. pH of the environment should be considered because it played an importance role on dichlorvos hydrolysis. Different pHs did not only affect hydrolysis of dichlorvos but it also influenced degradation by bacteria. Xiao-Hua et al. (2006) indicated that *Ochrobactrum* sp. strain DDV-1 could degrade dichlorvos of up to 34.5% at pH 6 and reached the highest degradation efficiency at pH 7.0. So, pH 7 was selected to be an optimum pH for the previous work. Ning et al. (2012) isolated *Flavobacterium* sp. strain YD4 which could degrade dichlorvos at pH ranging from 5.5 to 7 with an optimum pH of 6. Based on the earlier studies and this work, it implied that during the biodegradation process, dichlorvos quickly decreased because of chemical hydrolysis. pH was a major factor on dichlorvos degradation. The degrading microbes isolated from diverse environment preferred different pH resulting in the various optimum pH for the biodegradation.

The microbial cultures obtaining from this study could be applied for dichlorvos degradation in the future. The cultures are likely to well utilize in acidic environment (pH of 4-5). Nonetheless, the pilot experiment should be performed to confirm the ability before the real practice.

3. Comparison of dichlorvos degradation by mixed cultures and pure isolates under different pHs

The growth between mixed cultures and isolated strains DV1, DV2, and DV3 with different pHs was presented in Figure 4.24 (raw data presented in Appendix C). In overall, the growths of the cultures were similar. But at pH 4 and pH 5, the mixed cultures had minimal higher growth rate followed by DV2, DV3 and DV1 respectively. Figure 4.25 (raw data presented in Appendix C) presents the dichlorvos degradation between mixed cultures and isolated strains. Under acidic pHs (pH 4 – pH 5), the growth rates of the mixed cultures was relatively greater than the individual isolates because the variety of bacterial species in the mixed cultures leaded to more survivability in stress (acidic) condition and growth than pure cultures. This agrees with Kumar et al. (2014) who examined the toxicity of silver nanoparticles on bacterial isolates and consortium. Base on cell viability evaluation, it was found that the bacterial viability of the mixed cultures was greater compared to the individual isolates.

It was observed that the higher pH resulted in the more difference in the dichlorvos degradability. At pHs of 6-8, it is clear that the pure isolates performed better than the mixed cultures. This indicated that there were sufficient carbon sources

(yeast extract and dichlorvos) subsequencing in similar bacterial growth in both mixed and pure cultures. Among the mixed cultures, some microbes may be only the dichlorvos-tolerant cultures (no dichlorvos degradability). It could imply that the number of dichlorvos-degrading cultures in the mixed cultures may be lower than those of the pure isolates resulting in less degradation efficiency. The results was similar to the study by Siripattanakul-Ratpukdi et al. (2015) who reported that *Pseudomonas plecoglossicida* strain PF1, *Pseudomonas aeruginosa* strain PF2, and *P. aeruginosa* strain PF3 had more efficiency in profenofos removal more than the consortium.

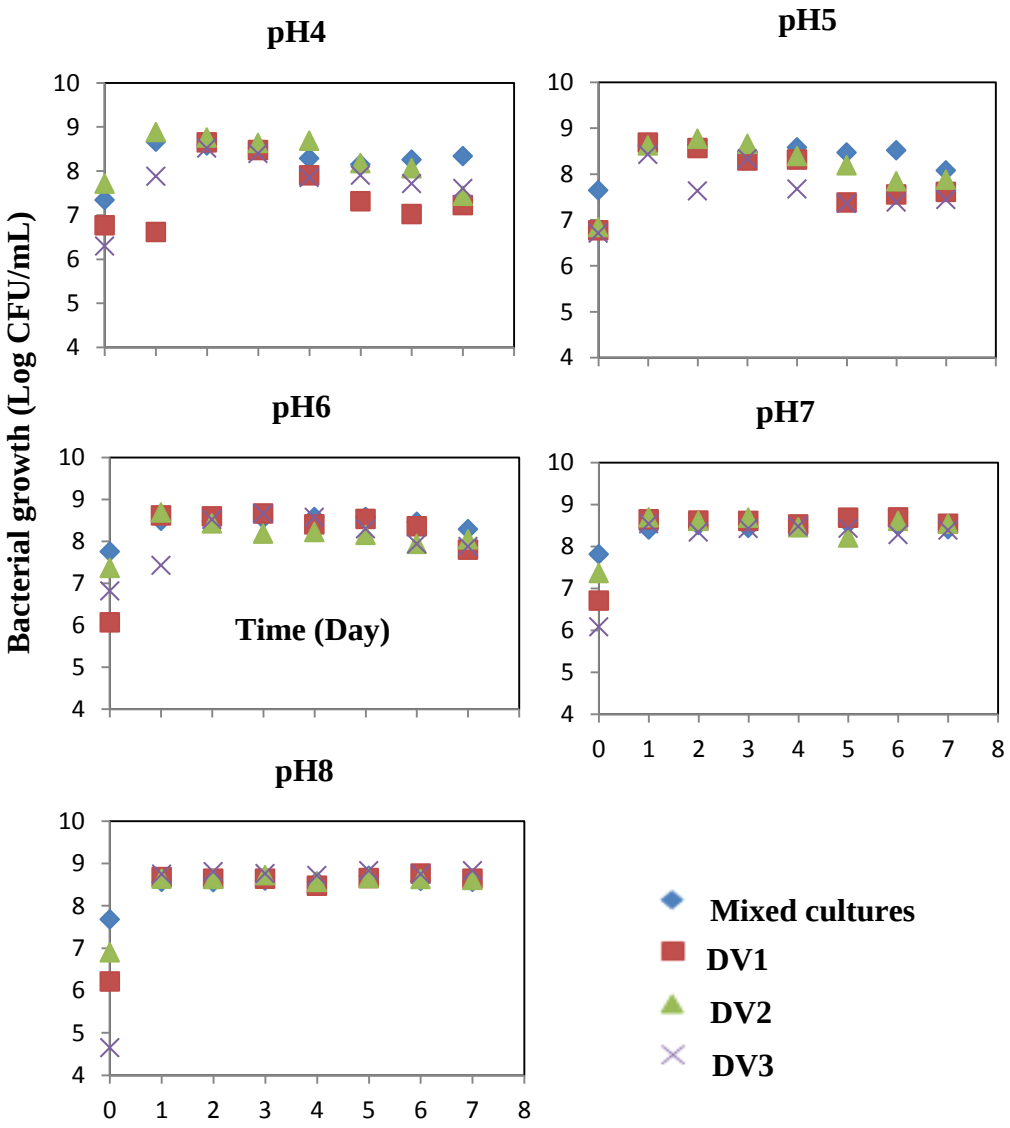


Figure 4.24 Bacterial growth comparison between mixed cultures and isolated strains DV1,DV2 and DV3 under pHs of 4, 5, 6, 7 and 8

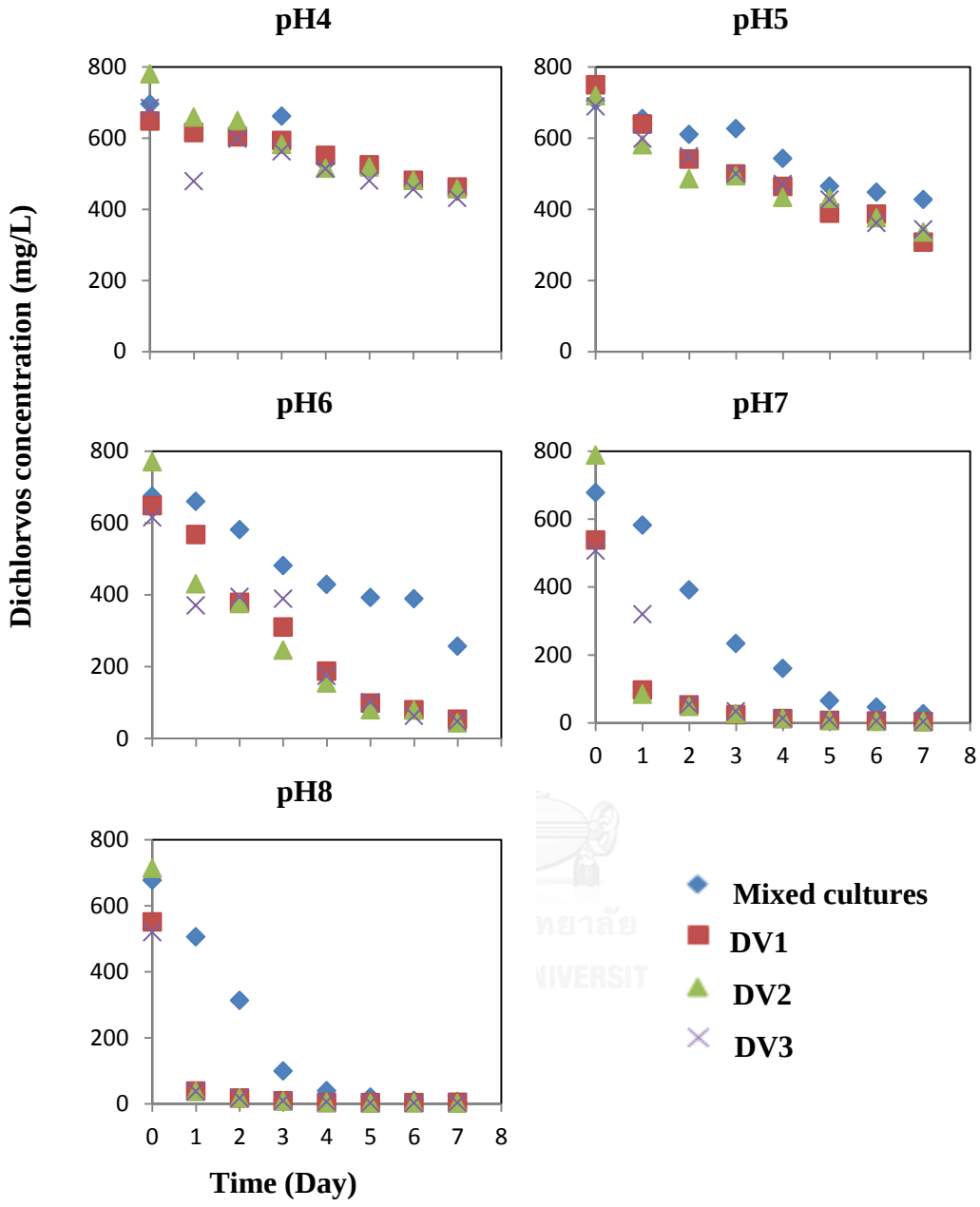


Figure 4.25 Degradation ability comparisons between bacterial mixed cultures and isolated strains DV1, DV2 and DV3 under pHs of 4, 5, 6, 7 and 8

4.2.4 Metabolic intermediate monitoring

The chemical structure of dichlorvos ($C_4H_7Cl_2O_4P$) was shown in Figure 4.26. The same compounds during dichlorvos degradation in all cultures under abiotic and biodegradation processes by isolated strains DV1, DV2 and DV3 were detected. Figure 4.27 demonstrated peaks and retention times of the compounds. Peak of dichlorvos presented 16.58 min while the compounds corresponded to the peaks of 6.23, 12.67 and 13.157-min retention time, respectively. Based on mass spectrum analysis, the mass spectrum of the peaks at 6.23, 12.67 and 13.157-min retention time were trimethyl phosphate (TEP) ($C_3H_9O_4P$), triethyl phosphate (TMP) ($C_6H_{15}O_4P$) **a**nd 2-chloroethyl dimethyl phosphate (2CDP) ($C_4H_8ClO_4P$) as shown in Figures 4.28, 4.29 and 4.30, respectively.

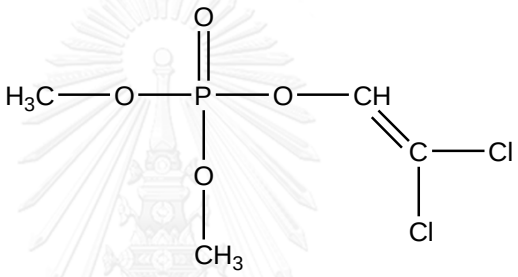


Figure 4.26 Chemical structure of dichlorvos

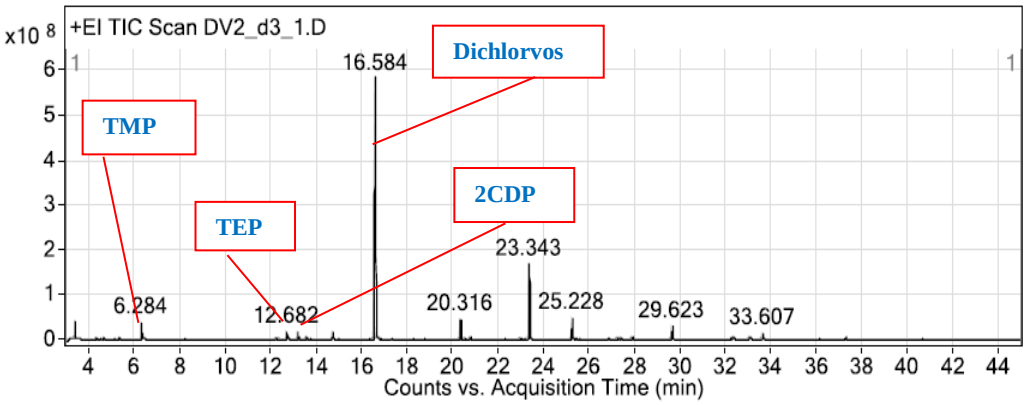


Figure 4.27 Chromatogram of metabolites occurred during dichlorvos degradation by abiotic and biodegradation process

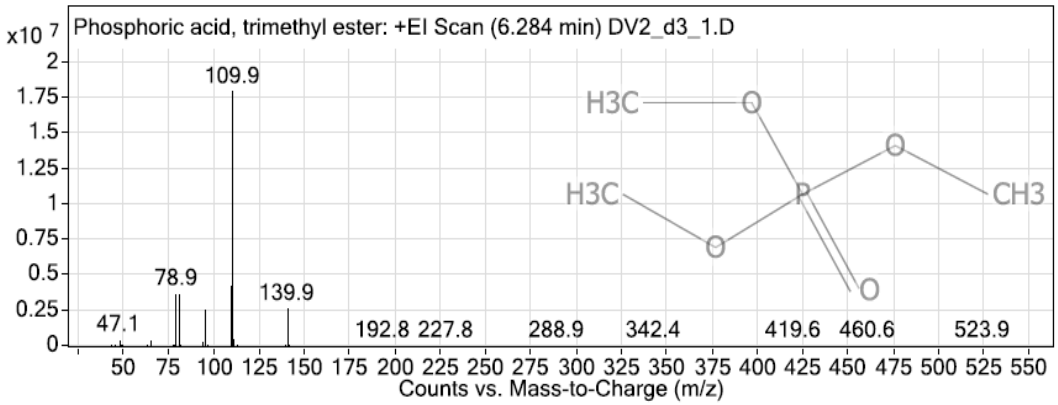


Figure 4.28 Mass spectrum of trimethyl phosphate (TMP)

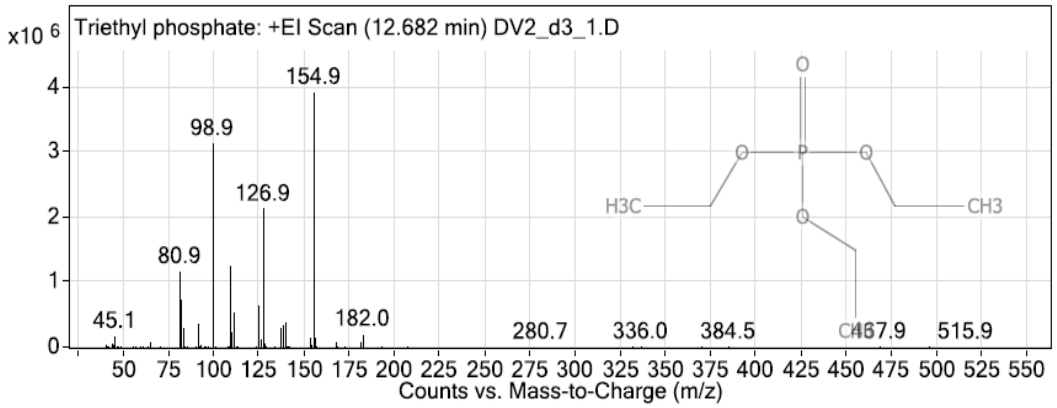


Figure 4.29 Mass spectrum of triethyl phosphate (TEP)

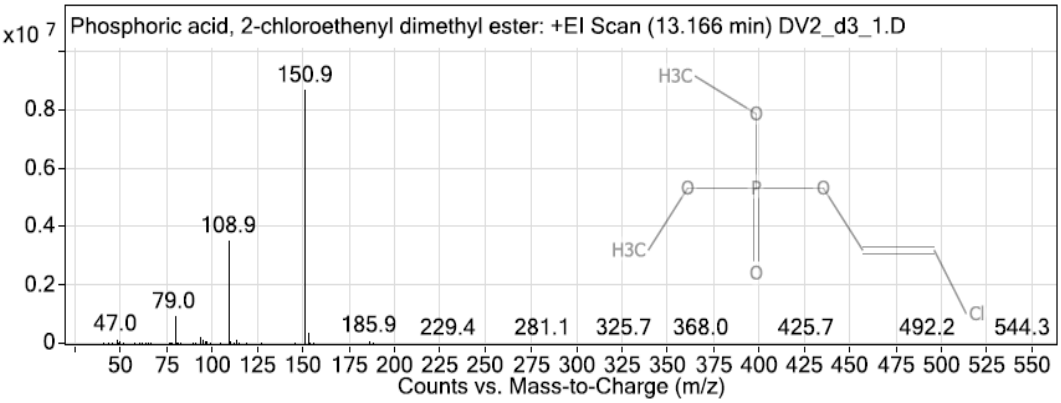


Figure 4.30 Mass spectrum of 2-chloroethyl dimethyl phosphate (2CDP)

The metabolic intermediates occurred during dichlorvos degradation and dichlorvos degradation pathway were investigated and reported by previous studies. Booth et al. (2007) found that hydrolysis is the primary route of dichlorvos metabolism which related with the reaction of the phosphoryl group of dichlorvos by water. Dimethyl phosphate and dichloroacetaldehyde were the products from this reaction before further metabolized to dichloroethanol, dichloroacetic acid and ethyl dichloroacetate (Chemical evaluation and research institute [CERI] 2007). However, there is limited knowledge on dichlorvos biodegradation mechanism. The study by Liberman and Alexander (1983) reported that dichloroethanol, dichloroacetate and ethyl dichloroacetate were detected during dichlorvos degradation by the treatment with bacterial cells (*Pseudomonas aeroginosa*) while the compounds were absent in the treatment without cells. They proposed pathway of dichlorvos as follows. Dichlorvos could be turned into dichloroacetaldehyde and dimethyl phosphate by non-enzymatic hydrolytic cleavage. Dimethyl phosphate could further convert to monomethyl phosphate and finally inorganic phosphate was formed. In case of dichloroacetaldehyde, it converted to either dichloroethanol or dicloroacetic acid. After that, ethyl dichloro acetate was formed by esterification between dichloroacetic acid and ethanol which was generated by dehalogenation from dichloroethanol. Biodegradation pathway of dichlorvos was shown in Figure 4.31.

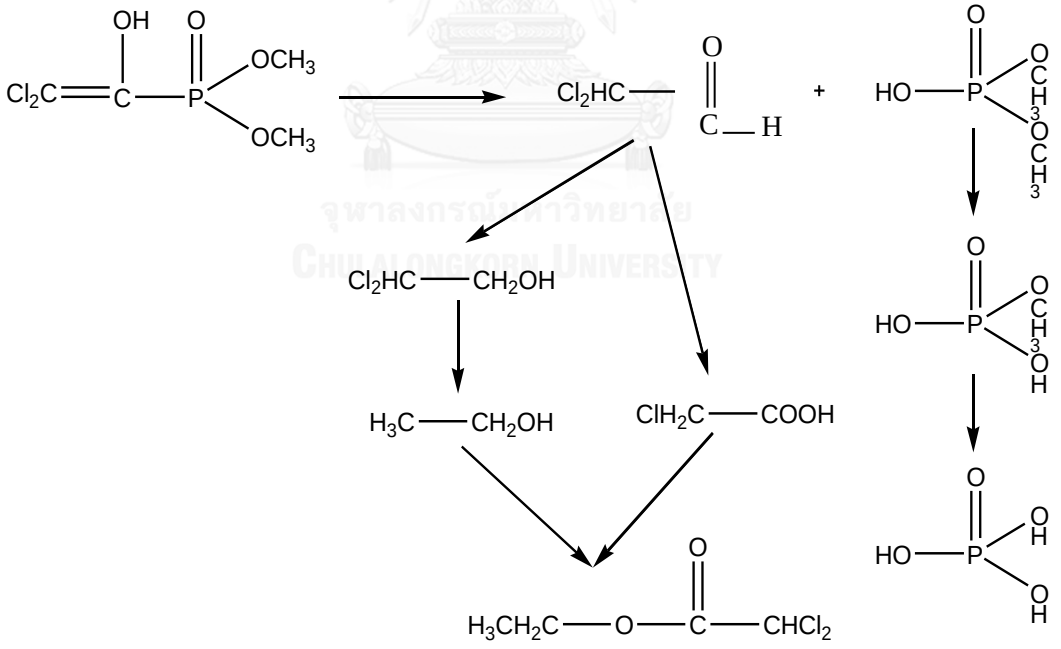


Figure 4.31 Biodegradation pathway of dichlorvos (Lieberman and Alexander 1983)

Based on the result found in present study, during dichlorvos degradation by both abiotic and biodegradation processes, three compounds including 2CDP, TEP and TMP had been detected. All compounds detected in present study were different from the compounds in metabolic pathway previously reported. Additionally, in our study the commercial product of dichlorvos was used, so it was possible to have impurity or reactant residues in the medium. Therefore, it may not be able to clearly summarize whether 2CDP, TEP and TMP were metabolites occurred during dichlorvos degradation. The complete dichlorvos degradation pathway should be further performed.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

5.1 Conclusion

5.1.1 Dichlorvos degradation by mixed cultures

1. Influence of bacterial medium on dichlorvos degradation

Dichlorvos-degrading consortium was enriched from contaminated soil. The consortium could utilize dichlorvos as either a sole carbon source or with an additional source. However, the growth and removal percentage of dichlorvos (7.7 to 8.8 logCFU/mL and 87.50%, respectively) of the consortium cultivated in medium with dichlorvos and yeast extract were better than ones with dichlorvos (5.9 to 7.2 logCFU/mL and 59.09%, respectively).

2. Influence of dichlorvos concentration

The consortium was efficient in dichlorvos degradation. Dichlorvos removal of 54.48- 82.99 and 73.73-87.75% was observed in the abiotic and biodegradation (with abiotic degradation) processes, respectively. The biodegradation process alone could decrease dichlorvos of up to19.25 mg/L/d. The results from the present study could indicate that abiotic process by hydrolysis reaction played a significant role on dichlorvos degradation. However, at high dichlorvos concentrations (more than 400 mg/L), the removal by microorganisms also promoted the dichlorvos degradation.

3. Influence of pH

pH was important on dichlorvos degradation. For abiotic hydrolysis study (control), at acidic conditions (pH 4-6), the degradations were relatively low compared to those at the higher pHs (pH 7-8). The removal percentages of dichlorvos of 20-60% were found in acidic pH (pH 4- 6) whereas the tests at pH 7 and pH 8 could remove dichlorvos by over 90%. The difference of dichlorvos degradation by abiotic and biodegradation processes can be clearly seen at acidic pH (pH 4-6). Biodegradation (abiotic and biotic reactions) had higher efficiency in dichlorvos degradation compared to abiotic process. Biodegradation (alone) could improve the dichlorvos removal of up to 14.94, 11.98 and 7.19% at pH 4, 5 and 6, respectively.

5.1.2 Dichlorvos degradation by pure cultures

1. Isolation of dichlorvos degrading pure cultures

Three isolates (designed as DV1, DV2 and DV3) which performed highest efficiencies in dichlorvos degradation were selected. Based on the 16S rDNA

characterization, DV1, DV2 and DV3 were identified as *Klebsiella* sp., *Enterobacter* sp. and *Klebsiella pneumonia*, respectively.

2. Influence of dichlorvos concentration

All isolates were able to grow in dichlorvos concentrations of 50, 100, 200, 400 and 800 mg/L. Initial dichlorvos concentration affected dichlorvos degradation. In abiotic process, the removal percentages of higher than 75% were observed under dichlorvos concentrations ranging from 50 to 400 mg/L while the removal of 55% was observed during the test at dichlorvos concentration of 800 mg/L. For biodegradation process by DV1, DV2 and DV3, the removal percentages of higher than 81% occurred even at high initial concentration. It could indicate that biodegradation process improved the dichlorvos degradation, especially at high concentration (800 mg/L). Biodegradation (alone) could increase the removal percentages of up to 25%, 33% and 28% by DV1, DV2 and DV3, respectively. In overall, DV2 performed dichlorvos degradation better than DV3, DV1 and the consortium, respectively.

3. Influence of pH

The growth of three isolates was affected by pH. In acidic pHs (pH 4 -5), the growth of all isolates was obviously decreased. For abiotic process, it was found that pH affected dichlorvos degradation. Higher degradation was presented with the increasing of pH. Under pHs ranging from 4 to 6, biodegradation (abiotic and biotic reactions) could improve the degradation of dichlorvos. After subtracted by abiotic process, the removal percentages of 4–20, 6–17% and 4–12% by biodegradation (alone) for strains DV1, DV2 and DV3 respectively was investigated.

4. Metabolic intermediates monitoring

Three compounds detected along with the dichlorvos degradation included 2CDP, TEP and TMP. These compounds presented in both abiotic and biodegradation processes.

5. Recommendation for managing dichlorvos contamination problem

Based on the results from this study, abiotic hydrolysis was effective for dichlorvos removal from aquatic environment. Biodegradation process by either mixed cultures or pure cultures was able to enhance the removal of dichlorvos. However, biological process was likely to be suitable for the contaminated soil since dichlorvos degradation by hydrolysis was less than that in water. Biological process was an important role on the degradation.

5.2 Recommendations for future work

1. Other environmental factors influencing the dichlorvos degradation, such as temperature and oxygen concentration should be conducted.
2. Dichlorvos-degrading isolates should be applied with other organophosphate pesticides.
3. Complete degradation pathway of dichlorvos should be performed.
4. In practice, since the isolated cultures obtained from present study were potential pathogens, so closed system have to be used.

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APPENDIX

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) $NaH_2PO_4.2H_2O$ 20.9565 g
2) $Na_2HPO_4.7H_2O$ 31.00g

Minimal salt medium (MSM)

1)	$NaHPO_412H_2O$	14.678	g
2)	KH_2PO_4	3	g
3)	NaCl	0.5	g
4)	NH_4Cl	2	g
5)	$MgSO_4.7H_2O$	0.513	g
6)	Yeast extract	0.1	%
7)	Profenofos	200	mg/L

Procedure

For phosphate buffer, monosodium phosphate dihydrate and disodium phosphate heptahydrate were dissolved in 250 mL of distilled water.

For MSM medium, the chemical compositions of MSM medium as shown above were dissolved in distilled water to the final volume of 1,000 mL with 10 mM of phosphate buffer at pH 6.8. After that, MSM solution was sterile by autoclave at 121 °C for 15 min. Then, waiting for cooling the solution before supplemented with dichlorvos solution at final concentration of 200 mg/L by filtrate through the sterile filter 0.22 µm.

For MSM agar medium using for bacterial growth evaluation and bacterial isolation, 1.5% of agar was mixed in MSM liquid medium before autoclaving without 0.1% of yeast extract. After autoclaving, MSM agar medium was poured into sterilized plastic plates.

APPENDIX B
CHEMICAL ANALYSIS

1. Standard curve of dichlorvos

1.1 Dichlorvos preparation

The commercial grade of dichlorvos (50% w/v) which was purchased from Supernox Pratoothong, Thailand was used to prepare a standard curve. First, dichlorvos 50% w/v was dissolved in methanol to the final concentration of 50,000 mg/L. Then, the solution of dichlorvos was diluted in hexane until obtain the other concentrations including 0.1, 1, 5, 10, 20, 30, 40, 50, 100, 500 and 800 mg/L. After that dichlorvos solution at various concentrations were clean up by filtrating through a 0.2-µm sterile syringe filter to GC vial. The concentration of dichlorvos was analyzed by using GC-ECD, and a standard curve was plotted by the relationship between dichlorvos concentration and area as seen in Figure B.1 and the data from GC-ECD were shown in Table B.1.

Table B.1 The data from GC-ECD using for standard curve

Concentration	Area	Average
0.1	43.51346	43.368905
	43.22435	
1	124.31268	123.17571
	122.03874	
5	382.61789	374.538585
	366.45928	
10	654.73437	653.837565
	652.94076	
20	1166.24888	1169.51497
	1172.78105	
30	1777.16253	1753.11244
	1729.06235	
40	2326.18947	2347.41556
	2368.64165	
50	2821.86365	2789.99594
	2758.12823	
100	5393.99419	5429.88614
	5465.77809	
500	43692.52195	44393.576535
	45094.63112	

Concentration	Area	Average
800	65218.10432	64895.33033
	64572.55634	

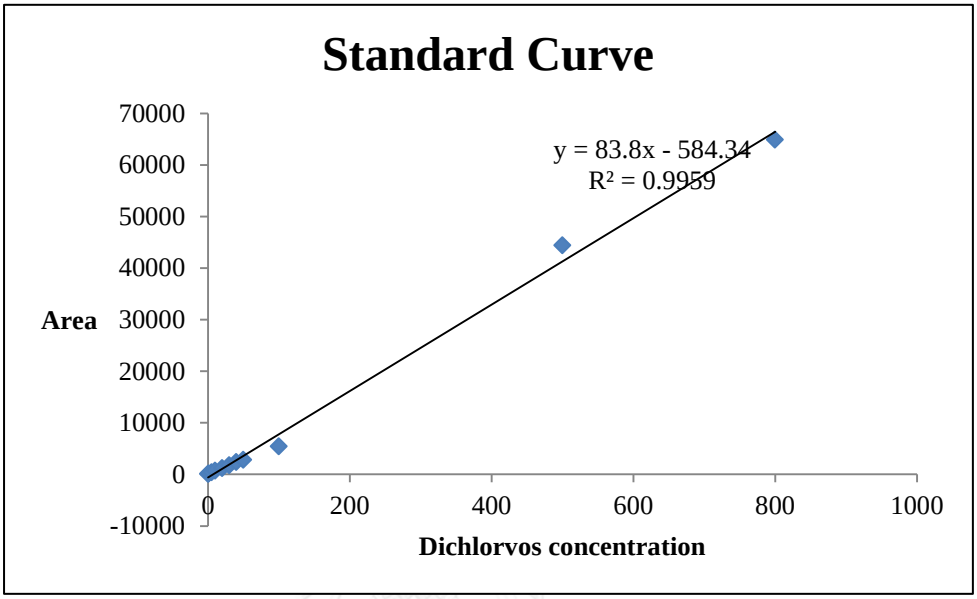

Figure B.1 Standard curve of dichlorvos

Dichlorvos could be detected by GC-ECD at detection time of 3.9 min. Chromatogram of dichlorvos analyzed by GC-ECD was shown in Figure B.2.

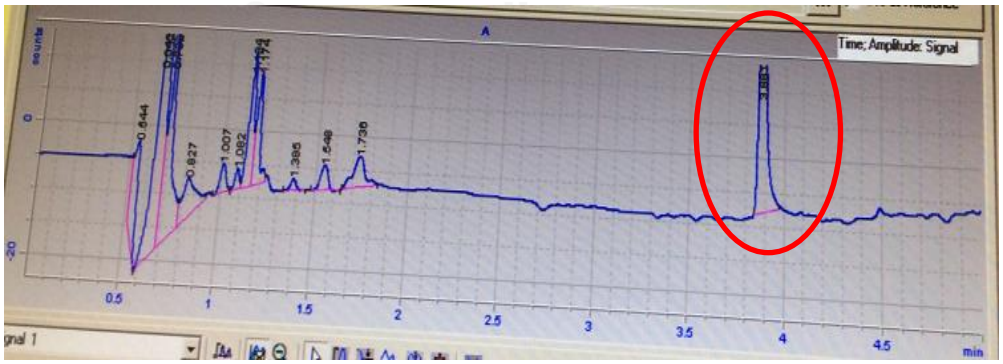

Figure B.2 Chromatogram of dichlorvos

2. Dichlorvos solution preparation

A stock solution was prepared from a commercial product of dichlorvos (50% w/v) by dissolving in methanol to the final concentration of 100,000 mg/L. This solution was diluted to 50,000 mg/L in hexane before diluted again in distilled water to the final concentrate of 10,000 mg/L. The stock solution was kept at 4 °C in an amber glass for preventing the photo degradation.

3. Recovery check of dichlorvos

For recovery checking, dichlorvos solution of 10,000 mg/L which was previously kept as stock solution was diluted to concentration of 0.1, 10, 20, 50, 100, 200, 400 and 800 mg/L in MSM medium to simulate the media cultivation. 0.5 mL of dichlorvos in a liquid MSM medium at different concentration placed into a 1.5-mL centrifuge tube and then the mixture between n-hexane and 0.01% of acetic acid was added. The tube was mixed at 2,500 rpm for 10 min by vortex mixer. After solution separated, supernatant (upper layer) was collected and cleaned up by filtrating through a sterile syringe filter 0.2 µm before transfer to a GC vial. The recovery percentages of dichlorvos which was analyzed by GC-ECD were shown in TableB.2.

Table B.2 Recovery check of dichlorvos

Dichlorvos Concentration	**% recovery**	**Average of % recovery**
0.1	-	-
	-	
	-	
10	97.94	95.65%
	89.53	
	99.47	
20	85.95	87.12%
	87.78	
	87.63	
50	80.78	82.11%
	79.16	
	86.38	
100	86.09	86.90%
	84.08	
	90.52	

Dichlorvos Concentration	% recovery	Average of % recovery
200	84.83	83.57%
	87.04	
	78.84	
400	94.51	95.23%
	99.76	
	91.42	
800	74.75	70.07%
	67.42	
	68.04	

*Detection limit of dichlorvos was 0.1 mg/L

APPENDIX C

RAW DATA

1. Result of preliminary study: Bacterial growth of mixed-culture at different condition

Table C.1 Bacterial growth of mixed culture at day 0 to day 7

Day	Condition						
	MSM medium with yeast extract			MSM medium without yeast extract		
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	5.5E+06	5.6E+06	5.55E+06	9.3E+05	8.5E+05	8.9E+05
1	6.0E+08	6.6E+08	6.3E+08	1.53E+07	1.68E+07	1.61E+07
2	6.3E+08	6.9E+08	6.6E+08	5.5E+06	4.4E+06	4.95E+06
3	6.0E+08	4.4E+08	5.2E+08	6.0E+06	7.6E+06	6.8E+06
4	2.16E+08	5.2E+08	3.68E+08	6.8E+06	1.1E+07	8.9E+06
5	7.1E+07	6.6E+07	6.85E+07	1.54E+07	3.07E+07	2.31E+07
6	6.5E+07	9.3E+07	7.9E+07	7.8E+06	7.6E+06	7.7E+06
7	6.4E+07	2.0E+07	4.2E+07	6.9E+06	7.1E+06	7.0E+06

2. Result of preliminary study: Dichlorvos degradation by bacterial mixed-culture at different condition

Table C.2 Dichlorvos residual of mixed culture at day 0 to day 7

Condition / Day	MSM medium with yeast extract			MSM medium without yeast extract		
	No. 1 (mg/L)	**No. 2 (mg/L)**	**Average (mg/L)**	**No. 1 (mg/L)**	**No. 2 (mg/L)**	**Average (mg/L)**
0	137.85	153.22	145.53	157.91	160.59	159.25
1	120.24	113.61	116.92	130.65	134.04	132.34
2	87.10	85.10	86.10	120.53	119.30	119.92
3	68.50	69.65	69.08	108.25	110.83	109.54
4	45.66	46.67	46.16	96.83	94.43	95.63
5	25.52	31.15	28.34	72.37	75.85	74.11
6	22.72	24.17	23.44	72.97	71.20	72.09
7	17.26	19.14	18.20	64.92	65.38	65.15

3. Result of dichlorvos degradation by bacterial mixed-culture

3.1 Bacterial growth of mixed-culture at different dichlorvos concentration: Influence of dichlorvos concentration

Table C.3 Bacterial growth of mixed culture at day 0 to day 7

Day	Bacterial growth of mixed culture at different dichlorvos concentration											
	50 mg/L				100 mg/L				200 mg/L			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)
0	3.7E+07	5.7E+07	6.5E+07	5.3E+07	2.5E+08	2.3E+08	1.4E+08	2.1E+08	5.7E+07	4.8E+07	5.6E+07	5.4E+07
1	4.5E+08	4.0E+08	4.2E+08	4.2E+08	1.2E+09	2.2E+09	2.9E+09	2.1E+09	4.3E+09	2.3E+09	2.4E+09	3.0E+09
2	1.33E+10	1.43E+10	1.8E+10	1.52E+10	1.33E+10	1.56E+10	1.61E+10	1.5E+10	1.35E+10	1.27E+10	1.54E+10	1.4E+10
3	4.2E+08	4.1E+08	3.7E+08	4.0E+08	2.3E+09	1.7E+09	2.1E+09	2.0E+09	1.2E+09	1.8E+09	1.8E+09	1.6E+09
4	4.3E+08	5.1E+08	1.7E+08	3.7E+08	2.9E+09	1.2E+09	1.8E+09	2.0E+09	1.9E+09	-	1.1E+09	1.5E+09
5	2.0E+08	6.9E+08	2.8E+08	3.9E+08	2.0E+09	2.7E+09	1.0E+09	1.9E+09	1.7E+09	1.6E+09	1.0E+09	1.4E+09
6	5.3E+08	3.8E+08	4.0E+08	4.4E+08	7.4E+08	5.6E+08	4.1E+08	5.7E+08	5.5E+08	7.1E+08	1.9E+08	4.8E+08
7	3.4E+08	3.1E+08	1.9E+08	2.8E+08	3.8E+08	3.1E+08	4.1E+08	3.7E+08	5.6E+08	3.1E+08	2.3E+08	3.7E+08

Table C.3 Bacterial growth of mixed culture at day 0 to day 7 (cont.)

Day	Bacterial growth of mixed culture at different dichlorvos concentration							
	400 mg/L				800 mg/L			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)
0	5.4E+07	5.6E+07	5.8E+07	5.6E+07	2.9E+07	3.2E+07	3.3E+07	3.1E+07
1	7.5E+09	4.4E+09	3.3E+09	5.1E+09	3.2E+09	7.2E+09	1.5E+09	4.0E+09
2	1.17E+10	-	-	1.17E+10	-	-	-	-
3	1.0E+09	1.2E+09	1.9E+09	1.4E+09	-		-	-
4	1.9E+09	2.5E+09	-	2.2E+09	2.0E+08	2.7E+08	2.0E+08	2.2E+08
5	1.7E+09	1.2E+09	1.8E+09	1.6E+09	2.7E+08	2.1E+08	1.6E+08	1.7E+08
6	1.0E+09	1.2E+09	1.6E+09	1.3E+09	2.9E+08	1.9E+08	3.9E+08	2.9E+08
7	5.1E+08	8.7E+08	8.2E+08	7.3E+08	2.1E+08	2.4E+08	1.6E+08	2.0E+08

3.2 Dichlorvos degradation by bacterial mixed-culture at different dichlorvos concentration

Table C.4 Dichlorvos residual of abiotic process (control) at day 0 to day 7

Dichlorvos concentration	Dichlorvos degradation by abiotic process (Control)							
	Day0	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**
50 mg/L	38.57	33.29	27.64	24.07	18.91	14.05	13.93	7.05
100 mg/L	76.85	66.42	56.24	56.08	43.91	32.97	26.10	15.68
200 mg/L	264.13	133.36	160.50	60.62	103.29	83.58	58.76	44.93
400 mg/L	382.89	351.25	465.56	239.26	235.06	155.57	125.93	92.96
800 mg/L	550.82	516.91	611.27	499.89	479.05	430.16	350.25	250.72

Table C.5 Dichlorvos residual of biodegradation process by mixed-culture at day 0 to day 7

Day	Dichlorvos degradation by mixed culture at different dichlorvos concentration											
	50 mg/L				100 mg/L				200 mg/L			
	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)
0	46.93	46.27	46.04	46.41	95.45	87.87	90.90	91.41	186.89	168.49	188.07	181.15
1	35.68	36.34	34.78	35.60	70.69	65.17	68.11	67.99	119.81	132.20	123.96	125.32
2	30.59	30.34	32.52	31.15	66.53	62.23	64.97	64.58	125.03	130.89	131.97	129.30
3	30.73	30.42	28.99	30.05	64.33	41.18	41.12	48.87	74.50	66.01	123.57	88.03
4	20.20	20.99	20.77	20.66	49.81	42.68	43.22	45.24	87.75	85.70	82.22	85.22
5	18.04	13.28	15.57	15.63	35.30	31.55	31.74	32.86	67.64	59.14	54.74	60.51
6	11.25	10.41	10.41	10.69	26.49	22.19	22.56	23.75	48.31	49.70	41.24	46.41
7	7.34	4.84	4.88	5.69	19.13	15.69	16.12	16.98	26.85	33.39	21.25	27.16

Table C.5 Dichlorvos residual of biodegradation process by mixed-culture at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by mixed culture at different dichlorvos concentration							
	400 mg/L				800 mg/L			
	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)
0	399.38	389.78	390.79	393.31	598.44	575.60	581.16	585.06
1	358.84	283.88	268.98	303.90	477.01	512.34	499.84	496.40
2	247.70	277.01	280.91	268.54	543.40	553.73	530.06	542.39
3	181.36	163.38	210.31	185.02	444.84	466.77	432.38	448.00
4	172.40	163.59	168.17	168.05	426.36	441.42	390.19	419.33
5	129.98	133.19	122.79	128.66	359.67	347.66	286.87	331.40
6	92.28	95.73	86.75	91.59	252.42	235.69	198.15	228.75
7	60.90	69.07	58.81	62.93	131.53	166.70	162.76	153.67

3.3 Bacterial growth of mixed-culture at different pH: Influence of pH

Table C.6 Bacterial growth of mixed culture at day 0 to day 7 (dichlorvos concentration = 800 mg/L)

Day	Bacterial growth of mixed culture at different pH											
	pH4				pH5				pH6			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)
0	2.4E+07	2.6E+07	1.7E+07	2.2E+07	4.1E+07	4.1E+07	5.1E+07	4.4E+07	5.1E+07	6.2E+07	5.6E+07	5.6E+07
1	4.6E+08	4.7E+8	4.5E+08	4.6E+08	5.0E+08	4.1E+08	3.7E+08	4.3E+08	2.7E+08	3.7E+08	2.8E+08	3.1E+08
2	4.3E+08	3.9E+08	3.3E+08	3.8E+08	6.0E+08	4.0E+08	3.4E+08	4.5E+08	3.7E+08	1.6E+08	4.7E+08	3.3E+08
3	2.7E+08	2.6E+08	5.5E+08	3.6E+08	4.7E+08	2.4E+08	2.3E+08	3.1E+08	3.4E+08	3.1E+08	3.9E+08	3.5E+08
4	1.9E+08	1.4E+08	2.8E+08	2.0E+08	4.0E+08	4.4E+08	3.1E+08	3.8E+08	3.9E+08	2.9E+08	4.7E+08	3.8E+08
5	1.2E+08	1.7E+08	1.4E+08	1.4E+08	3.1E+08	4.0E+08	2.0E+08	3.0E+08	4.5E+08	2.6E+08	4.4E+08	3.8E+08
6	1.4E+08	1.9E+08	2.2E+08	1.8E+08	3.2E+08	5.0E+08	2.2E+08	3.5E+08	2.8E+08	2.8E+08	3.0E+08	2.9E+08
7	2.1E+08	1.5E+08	3.2E+08	2.3E+08	1.2E+08	1.1E+08	1.3E+08	1.2E+08	2.7E+08	1.4E+08	1.9E+08	2.0E+08

Table C.6 Bacterial growth of mixed culture at day 0 to day 7 **(dichlorvos concentration = 800 mg/L) (cont.)**

Day	Bacterial growth of mixed culture at different pH								
	pH7				pH8				
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	No. 3 (CFU/mL)	Average (CFU/mL)
0	6.0E+07	6.3E+07	7.0E+07	6.4E+07	4.5E+07	4.9E+07	4.9E+07	4.8E+07
1	2.2E+08	2.9E+08	2.4E+08	2.5E+08	3.5E+08	4.2E+08	3.4E+08	3.7E+08
2	2.8E+08	4.1E+08	2.8E+08	3.2E+08	2.3E+08	4.2E+08	4.8E+08	3.8E+08
3	2.5E+08	3.6E+08	2.2E+08	2.8E+08	4.2E+08	3.7E+08	4.0E+08	4.0E+08
4	2.2E+08	3.8E+08	2.9E+08	3.0E+08	4.0E+08	3.8E+08	3.5E+08	3.8 E+08
5	3.2E+08	4.6E+08	2.9E+08	3.6E+08	5.4E+08	4.8E+08	5.1E+08	5.1E+08
6	3. 4E+08	3.8E+08	2.3E+08	3.2E+08	3.9E+08	3.8E+08	3.8E+08	3.8E+08
7	2.8E+08	2.3E+08	2.6E+08	2.6E+08	4.4E+08	2.9E+08	3.8E+08	3.7E+08

3.4 Dichlorvos degradation by bacterial mixed-culture at different pH

Table C.7 Dichlorvos residual of abiotic process (control) at day 0 to day 7

Day	Dichlorvos degradation by abiotic process (control) at different pH											
	pH4				pH5				pH6			
	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)
0	693.76	712.97	740.40	715.71	701.95	718.16	715.44	711.85	665.62	679.13	672.21	672.32
1	625.99	622.88	622.95	623.94	617.86	629.96	642.08	629.96	658.53	662.99	644.04	655.19
2	626.21	557.02	603.51	595.58	562.12	593.04	606.20	587.12	532.11	577.67	556.08	555.29
3	714.66	711.99	701.87	709.51	663.34	629.60	668.71	653.88	543.57	568.12	500.15	537.28
4	584.33	554.53	557.85	565.57	629.17	638.52	621.50	629.73	510.02	503.15	489.05	500.74
5	614.25	616.23	593.79	608.09	588.27	603.78	570.82	587.62	428.67	413.14	394.65	412.15
6	571.45	553.39	554.55	559.80	535.44	551.63	513.67	533.58	424.07	423.82	465.92	437.93
7	615.60	545.03	549.20	569.94	540.81	510.22	497.29	516.11	295.62	324.90	290.93	303.82

Table C.7 Dichlorvos residual of abiotic process (control) at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by abiotic process (control) at different pH							
	pH7				pH8			
	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	No. 3 (mg/L)	Average (mg/L)
0	674.74	670.93	669.56	671.75	648.87	658.91	684.37	664.05
1	565.46	596.79	593.36	585.20	495.69	477.43	493.97	489.03
2	369.84	383.17	376.57	376.53	190.56	242.32	235.41	222.76
3	252.21	216.07	233.72	234.00	103.27	83.44	85.01	90.57
4	109.21	100.40	110.63	106.74	36.78	34.47	35.25	35.50
5	84.80	82.52	79.88	82.40	18.47	15.77	16.74	17.00
6	47.28	53.65	51.81	50.91	8.02	7.62	9.80	8.48
7	20.58	18.11	18.49	19.06	4.41	4.38	3.86	4.22

Table C.8 Dichlorvos residual of biodegradation process by mixed-culture at day 0 to day 7

Day	Dichlorvos degradation by mixed culture at different pH											
	pH4				pH5				pH6			
	No. 1 (mg/L)	**No. 2 (mg/L)**	**No. 3 (mg/L)**	**Average (mg/L)**	**No. 1 (mg/L)**	**No. 2 (mg/L)**	**No. 3 (mg/L)**	**Average (mg/L)**	**No. 1 (mg/L)**	**No. 2 (mg/L)**	**No. 3 (mg/L)**	**Average (mg/L)**
0	691.77	700.78	692.71	695.08	673.67	718.40	720.36	704.14	629.97	711.15	681.00	674.04
1	616.73	632.01	658.46	635.73	649.51	660.94	650.99	653.81	630.52	685.30	662.22	659.35
2	624.07	631.41	624.38	626.62	599.15	609.66	618.38	609.06	546.07	604.85	591.51	580.81
3	641.44	680.33	660.35	660.71	605.43	633.81	638.10	625.78	491.16	491.34	458.99	480.49
4	526.81	561.88	496.09	528.26	513.81	523.77	588.46	542.02	454.83	464.92	365.14	428.30
5	530.72	539.22	470.45	513.46	485.66	451.16	454.48	463.76	377.90	405.07	392.45	391.81
6	465.22	493.87	473.30	477.46	429.22	446.43	464.61	446.75	405.55	385.02	374.63	388.40
7	438.12	472.56	438.22	449.63	419.85	416.22	442.43	426.16	246.75	269.42	252.12	256.10

4. Result of selecting the best three of dichlorvos degrading isolates which performed the highest in dichlorvos degradation ability

Table C.9 Initial cell number and final cell number of bacterial isolates

Strains	Initial cell			Final cell		
	No. 1 (CFU/mL)	**No. 2 (CFU/mL)**	**Average (CFU/mL)**	**No. 1 (CFU/mL)**	**No. 2 (CFU/mL)**	**Average (CFU/mL)**
A1	9.0E+05	4.0E+05	6.5E+05	9.0E+07	1.3E+08	1.1E+08
A2	3.7E+07	1.3E+07	2.5E+07	3.4E+08	3.5E+08	3.45E+08
A3	1.6E+07	2.3E+07	1.95E+07	1.1E+08	1.2E+08	1.15E+08
A4	2.7E+07	3.7E+07	3.2E+07	8.0E+07	8.0E+07	8.0E+07
A5	8.0E+06	7.0E+06	7.5E+06	1.9E+08	2.2E+08	2.05E+08
A6	3.5E+07	2.5E+07	3.0E+07	9.0E+07	8.0E+07	8.5E+07
A7	5.7E+07	4.3E+07	5.0E+07	6.5E+07	5.5E+07	6.0E+07
A8	2.1E+07	3.1E+07	2.6E+07	1.3E+08	1.2E+08	1.25E+08
A9	1.2E+07	2.1E+07	1.65E+07	3.5E+08	3.9E+08	3.7E+08

Table C.10 Initial dichlorvos concentration and final dichlorvos concentration cultured with bacterial isolates

Strains	Initial dichlorvos concentration			Final dichlorvos concentration		
	No. 1 (mg/L)	**No. 2 (mg/L)**	**Average (mg/L)**	**No. 1 (mg/L)**	**No. 2 (mg/L)**	**Average (mg/L)**
Control (no cell)	336.74	325.77	331.26	61.87	72.13	67.00
A1	371.70	485.60	428.65	62.59	50.37	56.48
A2	389.10	390.87	389.99	33.56	35.09	34.33
A3	406.13	358.00	382.07	57.97	42.14	50.05
A4	421.98	390.89	406.44	49.82	65.16	57.49
A5	369.70	396.58	383.14	49.87	44.51	47.19
A6	374.80	380.88	377.84	38.76	33.12	35.94
A7	421.46	479.63	450.55	43.17	42.91	43.04
A8	362.58	410.07	386.33	61.16	47.62	54.39
A9	459.05	328.46	393.75	33.30	33.64	33.47

5. Result of dichlorvos degradation by bacterial isolates <u>DV1: Influence of dichlorvos concentration</u>

Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.11 Bacterial growth of DV1 at day 0 to day 7

Day	Bacterial growth of DV1 at different dichlorvos concentration											
	50 mg/L			100 mg/L			200 mg/L					
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)			
0	8.5E+07	8.0E+07	8.2E+07	6.2E+07	5.6E+07	5.9E+07	4.1E+07	3.9E+07	4.0E+07			
1	4.8E+08	6.2E+08	5.5E+08	6.0E+08	6.5E+08	6.2E+08	4.1E+08	4.0E+08	4.0E+08			
2	5.0E+08	5.4E+08	5.2E+08	6.9E+08	5.3E+08	6.1E+08	1.8E+08	6.5E+08	4.1E+08			
3	4.2E+08	7.6E+08	5.9E+08	5.6E+08	3.5E+08	4.5E+08	6.6E+08	4.4E+08	5.5E+08			
4	3.7E+08	5.8E+08	4.7E+08	7.6E+08	4.9E+08	6.2E+08	3.7E+08	4.6E+08	4.1E+08			
5	6.3E+08	7.1E+08	6.7E+08	6.7E+08	7.9E+08	7.3E+08	4.3E+08	6.2E+08	5.2E+08			
6	5.3E+08	5.7E+08	5.5E+08	2.6E+08	3.6E+08	3.1E+08	4.6E+08	2.1E+08	3.3E+08			
7	5.6E+08	5.1E+08	5.3E+08	5.3E+08	2.8E+08	4.0E+08	3.1E+08	2.5E+08	2.8E+08			

Table C.11 Bacterial growth of DV1 at day 0 to day 7 (cont.)

Day	Bacterial growth of DV1 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	1.0E+07	1.6E+07	1.3E+07	2.6E+05	2.4E+05	2.5E+05
1	5.5E+08	5.7E+08	5.6E+08	5.7E+08	4.9E+08	5.3E+08
2	4.4E+08	6.0E+08	5.2E+08	2.5E+08	3.2E+08	2.8E+08
3	4.5E+08	5.2E+08	4.8E+08	2.6E+08	3.4E+08	3.0E+08
4	6.0E+08	6.0E+08	6.0E+08	2.2E+08	2.5E+08	2.3E+08
5	6.0E+08	4.0E+08	5.0E+08	2.3E+08	3.1E+08	2.7E+08
6	4.7E+07	4.3E+07	4.5E+07	1.1E+08	2.0E+08	1.5E+08
7	4.8E+07	4.3E+07	4.5E+07	6.7E+07	5.5E+07	6.1E+07

DV2: Influence of dichlorvos concentration

Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.12 Bacterial growth of DV2 at day 0 to day 7

Day	Bacterial growth of DV2 at different dichlorvos concentration									
	50 mg/L			100 mg/L			200 mg/L			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	3.1E+07	1.0E+07	2.0E+07	1.5E+07	1.7E+07	1.6E+07	2.9E+07	3.0E+07	2.9E+07
1	6.0E+08	6.0E+08	6.0E+08	3.6E+08	4.7E+08	4.1E+08	4.0E+08	4.7E+08	4.3E+08
2	3.6E+08	3.8E+08	3.7E+08	4.7E+08	5.5E+08	5.1E+08	3.5E+08	3.7E+08	3.6E+08
3	2.9E+08	2.4E+08	2.6E+08	2.1E+08	2.5E+08	2.3E+08	2.6E+08	4.1E+08	3.3E+08
4	1.5E+08	1.6E+08	1.5E+08	9.0E+07	1.5E+08	1.2E+08	1.1E+08	1.3E+08	1.2E+08
5	2.6E+07	6.1E+07	4.3E+07	1.0E+08	4.3E+07	7.1E+07	3.1E+07	2.9E+07	3.0E+07
6	2.7E+07	4.4E+07	3.5E+07	1.7E+07	3.3E+07	2.5E+07	2.3E+07	1.1E+07	1.7E+07
7	2.3E+07	2.4E+07	2.3E+07	2.2E+07	1.3E+07	1.7E+07	6.0E+07	7.0E+07	6.5E+07

Table C.12 Bacterial growth of DV2 at day 0 to day 7 (cont.)

Day	Bacterial growth of DV1 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	1.1E+07	1.2E+07	1.1E+07	1.1E+07	9.0E+06	1.0E+07
1	4.0E+08	4.8E+08	4.4E+08	2.1E+08	1.2E+08	1.6E+08
2	3.7E+08	3.2E+08	3.4E+08	3.2E+07	3.5E+07	3.3E+07
3	3.6E+08	3.7E+08	3.6E+08	6.0E+07	2.4E+07	4.2E+07
4	2.0E+08	2.8E+08	2.4E+08	3.0E+07	3.6E+07	3.3E+07
5	3.4E+07	4.3E+07	3.8E+07	2.4E+07	2.4E+07	2.4E+07
6	1.8E+07	2.8E+07	2.3E+07	1.3E+07	1.5E+07	1.4E+07
7	4.2E+07	2.3E+07	3.2E+07	4.2E+06	3.2E+07	1.8E+07

DV3: Influence of dichlorvos concentration

Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.13 Bacterial growth of DV3 at day 0 to day 7

Day	Bacterial growth of DV3 at different dichlorvos concentration									
	50 mg/L			100 mg/L			200 mg/L			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	2.3E+07	1.5E+07	1.9E+07	2.9E+07	4.9E+07	3.9E+07	4.7E+07	5.7E+07	5.2E+07
1	7.2E+08	7.2E+08	7.2E+08	7.4E+08	8.4E+08	7.9E+08	8.0E+08	8.6E+08	8.3E+08
2	6.5E+08	6.9E+08	6.7E+08	1.0E+09	1.1E+09	1.1E+09	7.9E+08	8.0E+08	7.9E+08
3	7.0E+08	8.0E+08	7.5E+08	1.4E+09	9.0E+08	1.1E+09	1.0E+09	1.2E+09	1.1E+09
4	7.1E+08	7.9E+08	7.5E+08	1.2E+09	1.5E+09	1.3E+09	1.5E+09	9.3E+08	1.2E+09
5	9.2E+08	5.7E+08	7.4E+08	7.8E+08	9.5E+08	8.6E+08	3.4E+08	4.9E+08	4.1E+08
6	7.6E+08	7.5E+08	7.5E+08	8.5E+08	6.3E+08	7.4E+08	3.2E+07	3.9E+07	3.5E+07
7	5.3E+08	5.8E+08	5.5E+08	6.0E+08	8.4E+08	7.2E+08	4.2E+07	1.4E+07	2.8E+07

Table C.13 Bacterial growth of DV3 at day 0 to day 7 (cont.)

Day	Bacterial growth of DV3 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	4.4E+07	3.8E+07	4.1E+07	1.9E+07	2.4E+07	2.1E+07
1	1.0E+09	1.3E+09	1.1E+09	5.7E+08	3.6E+08	4.6E+08
2	7.9E+08	7.9E+08	7.9E+08	5.9E+08	5.3E+08	5.6E+08
3	7.2E+08	7.5E+08	7.3E+08	2.2E+08	2.0E+08	2.1E+08
4	7.2E+08	7.0E+08	7.1E+08	8.0E+07	1.0E+08	9.0E+07
5	1.6E+08	1.5E+08	1.5E+08	2.7E+07	1.4E+07	2.0E+07
6	6.5E+07	6.0E+07	6.2E+07	1.8E+07	2.7E+07	2.2E+07
7	1.0E+07	1.1E+07	1.1E+07	1.0E+06	6.0E+05	8.0E+05

Dichlorvos degradation by bacterial isolated at different dichlorvos concentration

Table C.14 Dichlorvos degradation by abiotic process at day 0 to day 7

Day	Dichlorvos degradation by abiotic process (control) at different dichlorvos concentration									
	50 mg/L			100 mg/L			200 mg/L			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	50.64	55.17	52.90	91.88	106.24	99.06	233.04	279.98	256.51
1	33.19	31.77	32.48	49.21	68.09	58.65	170.00	172.51	171.25
2	41.06	31.10	36.08	65.36	71.60	68.48	139.02	217.68	178.35
3	30.53	26.33	28.43	39.58	39.74	39.67	90.12	106.81	98.47
4	30.24	22.74	26.49	33.59	41.11	37.35	62.13	76.20	69.16
5	15.00	21.00	18.00	29.41	33.18	31.29	53.65	59.96	56.81
6	15.34	11.83	13.59	21.90	31.22	26.56	44.67	51.40	48.03
7	12.92	11.90	12.41	25.26	23.77	24.51	38.90	37.57	38.24

Table C.14 Dichlorvos degradation by abiotic process at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by abiotic process (control) at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	348.73	407.70	378.21	546.49	614.54	580.51
1	420.20	507.97	464.08	563.79	696.28	630.04
2	342.94	407.40	375.17	495.55	553.51	524.53
3	167.16	322.30	244.73	399.75	476.90	438.33
4	131.12	210.10	170.61	298.90	345.32	322.11
5	105.49	128.48	116.99	300.08	319.91	309.99
6	81.42	109.74	95.58	273.10	306.08	289.59
7	82.12	92.86	87.49	242.63	276.24	259.44

DV1: Influence of dichlorvos concentration

Dichlorvos residual of biodegradation process by DV1 at different dichlorvos concentration

Table C.15 Dichlorvos degradation of DV1 at day 0 to day 7

Day	Dichlorvos degradation by DV1 at different dichlorvos concentration									
	50 mg/L			100 mg/L			200 mg/L			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	49.73	47.93	48.83	84.41	94.87	89.64	182.60	277.55	230.08
1	39.37	37.51	38.44	67.89	81.06	74.48	131.24	171.02	151.13
2	27.16	31.89	29.52	58.19	61.52	59.86	115.24	116.38	115.81
3	25.95	26.65	26.30	45.85	51.14	48.50	66.27	82.59	74.43
4	16.42	18.88	17.65	20.66	26.73	23.70	42.19	52.63	47.41
5	10.61	10.89	10.75	15.60	20.54	18.07	35.40	35.02	35.21
6	13.12	12.19	12.65	10.11	13.63	11.87	21.96	34.60	28.28
7	6.55	7.00	6.77	13.38	13.52	13.45	19.22	19.01	19.11

Table C.15 Dichlorvos degradation of DV1 at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by DV1 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	325.32	389.34	357.33	523.28	548.20	535.74
1	338.04	380.79	359.42	536.30	593.33	564.82
2	274.75	283.66	279.21	577.84	489.56	533.70
3	105.71	100.74	103.23	333.90	406.95	370.42
4	84.68	95.09	89.89	198.46	254.90	226.68
5	69.22	72.68	70.95	249.82	246.91	248.36
6	40.12	55.57	47.84	118.46	233.80	176.13
7	36.45	34.15	35.30	93.58	108.80	101.19

DV2: Influence of dichlorvos concentration

Dichlorvos residual of biodegradation process by DV2 at different dichlorvos concentration

Table C.16 Dichlorvos degradation of DV2 at day 0 to day 7

Day	Dichlorvos degradation by DV2 at different dichlorvos concentration									
	50 mg/L			100 mg/L			200 mg/L			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	65.27	42.13	53.70	95.70	108.84	102.27	214.25	280.28	247.27
1	37.86	41.03	39.44	83.93	78.94	81.44	191.75	264.27	228.01
2	30.55	27.83	29.19	43.32	57.81	50.57	98.69	127.31	113.00
3	21.28	21.39	21.33	34.57	33.90	34.24	56.53	44.92	50.73
4	11.88	10.43	11.16	17.66	22.18	19.92	38.08	43.19	40.64
5	7.95	7.33	7.64	10.90	14.15	12.53	23.50	35.71	29.60
6	9.53	6.31	7.92	9.63	12.72	11.17	23.07	26.38	24.72
7	6.06	6.42	6.24	7.57	8.75	8.16	12.83	15.72	14.27

Table C.16 Dichlorvos degradation of DV2 at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by DV2 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	377.36	435.63	406.49	582.44	566.52	574.48
1	356.10	350.46	353.28	507.82	561.05	534.44
2	318.26	329.38	323.82	437.20	442.75	439.98
3	123.29	208.06	165.68	259.00	388.30	323.65
4	82.10	90.31	86.20	167.80	254.99	211.40
5	55.21	66.71	60.96	165.56	147.43	156.50
6	41.32	53.45	47.39	70.13	101.57	85.85
7	31.72	40.52	36.12	58.47	64.90	61.69

DV3: Influence of dichlorvos concentration

Dichlorvos residual of biodegradation process by DV3 at different dichlorvos concentration

Table C.17 Dichlorvos degradation of DV3 at day 0 to day 7

Day	Dichlorvos degradation by DV3 at different dichlorvos concentration								
	50 mg/L			100 mg/L			200 mg/L		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	60.14	41.63	50.88	96.91	110.99	103.95	247.66	295.16	271.41
1	35.70	53.74	44.72	73.95	90.59	82.27	143.60	178.80	161.19
2	33.17	27.07	30.12	43.40	55.47	49.43	124.88	123.84	124.36
3	20.50	18.79	19.65	34.06	59.26	46.66	92.92	100.04	96.48
4	12.76	11.28	12.02	17.11	24.34	20.72	39.04	46.61	42.83
5	7.85	8.94	8.39	14.84	15.31	15.08	29.32	28.61	28.96
6	9.75	11.11	10.43	9.73	10.99	10.36	18.32	20.12	19.22
7	4.84	6.39	5.61	8.65	11.57	10.11	14.39	14.63	14.51

Table C.17 Dichlorvos degradation of DV3 at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by DV3 at different dichlorvos concentration					
	400 mg/L			800 mg/L		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	409.64	410.09	409.87	555.03	567.44	561.23
1	349.61	455.23	402.42	501.87	542.82	522.35
2	272.95	276.19	274.57	378.58	410.80	394.69
3	198.93	121.75	160.34	317.33	414.76	366.05
4	74.01	86.49	80.25	269.97	318.85	294.41
5	58.25	76.00	67.13	230.08	221.23	225.65
6	35.93	50.39	43.16	82.75	124.34	103.55
7	36.07	28.81	32.44	93.58	88.21	90.89

<u>DV1: Influence of pH</u> : Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.18 Bacterial growth of DV1 at day 0 to day 7

Day	Bacterial growth of DV1at different pH									
	pH4			pH5			pH6			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	7.0E+06	4.8E+06	5.9E+06	9.0E+06	2.8E+06	5.9E+06	8.0E+05	1.5E+06	1.1E+06
1	4.2E+06	4.1E+06	4.1E+06	5.6E+08	4.1E+08	4.8E+08	3.8E+08	4.3E+08	4.0E+08
2	4.0E+08	5.0E+08	4.5E+08	3.5E+08	3.9E+08	3.7E+08	4.0E+08	3.8E+08	3.9E+08
3	1.3E+08	4.7E+08	3.0E+08	1.8E+08	2.1E+08	1.9E+08	6.1E+08	2.9E+08	4.5E+08
4	1.9E+07	1.4E+08	7.9E+07	1.2E+08	2.9E+08	2.0E+08	1.4E+08	3.6E+08	2.5E+08
5	2.0E+07	2.1E+07	2.0E+07	1.8E+07	3.0E+07	2.4E+07	3.0E+08	3.7E+08	3.3E+08
6	1.1E+07	1.0E+07	1.0E+07	5.5E+07	1.7E+07	3.6E+07	5.0E+07	4.0E+08	2.2E+08
7	1.4E+07	2.0E+07	1.7E+07	4.0E+07	4.2E+07	4.1E+07	5.0E+07	7.4E+07	6.2E+07

Table C.18 Bacterial growth of DV1 at day 0 to day 7 (cont.)

Bacterial growth of DV1at different pH								
Day	400 mg/L			800 mg/L				
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)		
0	5.0E+06	5.0E+06	5.0E+06	1.8E+06	1.5E+06	1.6E+06		
1	4.0E+08	4.7E+08	4.3E+08	5.0E+08	4.5E+08	4.7E+08		
2	4.0E+08	4.2E+08	4.1E+08	4.2E+08	4.6E+08	4.4E+08		
3	5.0E+08	3.0E+08	4.0E+08	4.6E+08	4.2E+08	4.4E+08		
4	3.6E+08	3.0E+08	3.3E+08	2.9E+08	3.1E+08	3.0E+08		
5	5.8E+08	3.8E+08	4.8E+08	4.0E+08	5.0E+08	4.5E+08		
6	4.7E+08	5.0E+08	4.8E+08	5.5E+08	6.0E+08	5.7E+08		
7	3.0E+08	3.8E+08	3.4E+08	3.5E+08	5.2E+08	4.3E+08		

<u>DV2: Influence of pH</u> : Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.19 Bacterial growth of DV2 at day 0 to day 7

Day	Bacterial growth of DV2 at different pH									
	pH4			pH5			pH6			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	7.7E+07	2.7E+07	5.2E+07	9.0E+06	5.0E+06	7.0E+06	1.8E+07	2.8E+07	2.3E+07
1	5.3E+08	1.0E+09	7.6E+08	1.0E+08	7.4E+08	4.2E+08	3.3E+08	6.2E+08	4.7E+08
2	5.4E+08	6.0E+08	5.7E+08	5.8E+08	5.9E+08	5.8E+08	2.6E+08	2.7E+08	2.6E+08
3	2.6E+08	6.0E+08	4.3E+08	5.0E+08	4.0E+08	4.5E+08	2.5E+08	5.0E+07	1.5E+08
4	6.6E+07	9.0E+08	4.8E+08	2.0E+08	2.9E+08	2.4E+08	1.7E+08	1.6E+08	1.6E+08
5	9.0E+07	2.1E+08	1.5E+08	2.1E+08	1.0E+08	1.5E+08	6.0E+07	2.2E+08	1.4E+08
6	1.9E+08	4.0E+07	1.1E+08	5.9E+07	8.0E+07	6.9E+07	1.3E+08	4.2E+07	8.6E+07
7	3.6E+07	1.9E+07	2.7E+07	9.0E+07	6.0E+07	7.5E+07	1.4E+08	8.0E+07	1.1E+08

Table C.19 Bacterial growth of DV2 at day 0 to day 7 (cont.)

Day	Bacterial growth of DV2 at different pH							
	pH7			pH8				
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)		
0	1.9E+06	4.4E+07	2.3E+07	1.5E+07	9.0E+05	7.9E+06		
1	6.5E+08	3.2E+08	4.8E+08	5.0E+08	3.7E+08	4.3E+08		
2	7.0E+08	1.0E+08	4.0E+08	5.0E+08	3.6E+08	4.3E+08		
3	9.0E+08	6.4E+07	4.8E+08	4.7E+08	5.8E+08	5.2E+08		
4	3.7E+08	1.9E+08	2.8E+08	3.9E+08	3.5E+08	3.7E+08		
5	1.4E+08	1.8E+08	1.6E+08	4.5E+08	4.5E+08	4.5E+08		
6	5.0E+08	3.0E+08	4.0E+08	3.0E+08	5.6E+08	4.3E+08		
7	5.2E+08	1.6E+08	3.4E+08	5.7E+08	2.4E+08	4.0E+08		

DV3: Influence of pH : Bacterial growth of bacterial isolated at different dichlorvos concentration

Table C.20 Bacterial growth of DV3 at day 0 to day 7

Day	Bacterial growth of DV3 at different pH									
	pH4			pH5			pH6			
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	2.2E+06	1.8E+06	2.0E+06	4.4E+06	5.8E+06	5.1E+06	5.7E+06	7.2E+06	6.4E+06
1	8.0E+05	1.5E+08	7.5E+07	5.5E+07	4.8E+08	2.7E+08	5.2E+07	4.0E+05	2.6E+07
2	4.5E+08	2.1E+08	3.3E+08	5.5E+07	3.0E+07	4.2E+07	3.0E+08	3.5E+08	3.2E+08
3	3.0E+08	2.0E+08	2.5E+08	8.4E+07	3.5E+08	2.2E+08	3.5E+08	5.6E+08	4.5E+08
4	8.0E+07	6.1E+07	7.0E+07	8.0E+07	1.4E+07	4.7E+07	3.9E+08	3.5E+08	3.7E+08
5	8.0E+07	8.0E+07	8.0E+07	2.3E+07	2.3E+07	2.3E+07	1.9E+08	2.0E+08	1.9E+08
6	3.3E+07	7.0E+07	5.1E+07	2.8E+07	2.1E+07	2.4E+07	1.0E+08	7.0E+07	8.5E+07
7	4.1E+07	4.0E+07	4.0E+07	2.5E+07	3.0E+07	2.7E+07	7.9E+07	7.0E+07	7.4E+07

Table C.20 Bacterial growth of DV3 at day 0 to day 7 (cont.)

Day	Bacterial growth of DV3 at different pH					
	pH7			pH8		
	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)	No. 1 (CFU/mL)	No. 2 (CFU/mL)	Average (CFU/mL)
0	1.5E+06	9.0E+05	1.2E+06	5.0E+04	4.0E+04	4.5E+04
1	3.1E+08	3.7E+08	3.4E+08	6.3E+08	4.8E+08	5.5E+08
2	2.7E+08	1.6E+08	2.1E+08	7.0E+08	5.7E+08	6.3E+08
3	3.1E+08	2.2E+08	2.6E+08	7.0E+08	4.6E+08	5.8E+08
4	3.0E+08	3.0E+08	3.0E+08	4.9E+08	5.5E+08	5.2E+08
5	3.5E+08	1.9E+08	2.7E+08	5.5E+08	7.9E+08	6.7E+08
6	1.9E+08	1.9E+08	1.9E+08	6.1E+08	5.1E+08	5.6E+08
7	2.0E+08	2.8E+08	2.4E+08	7.6E+08	5.8E+08	6.7E+08

Dichlorvos degradation by bacterial isolated at pH

Table C.21 Dichlorvos degradation by abiotic process at day 0 to day 7

Day	Dichlorvos degradation by abiotic process (control) at different pH									
	pH4			pH5			pH6			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	719.36	695.47	707.42	710.74	713.61	712.18	613.79	631.65	622.72
1	664.29	682.40	673.34	685.59	593.59	639.59	488.90	376.94	432.92
2	665.48	629.17	647.32	613.88	550.60	582.24	413.35	352.53	382.94
3	617.37	620.78	619.07	591.78	533.53	562.65	378.66	338.68	358.67
4	576.15	589.42	582.78	540.32	491.39	515.86	288.27	182.25	235.26
5	592.99	576.13	584.56	552.78	472.72	512.75	218.66	105.75	162.21
6	536.60	553.44	545.02	517.05	443.80	480.43	130.18	55.92	93.05
7	546.90	524.04	535.47	472.40	398.51	435.45	94.45	58.41	76.43

Table C.21 Dichlorvos degradation by abiotic process at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by abiotic process (control) at different pH					
	pH7			pH8		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	479.66	458.55	469.11	461.66	399.26	430.46
1	84.26	76.36	80.31	34.04	31.90	32.97
2	47.25	37.75	42.50	14.95	18.17	16.56
3	25.59	20.82	23.21	7.13	7.06	7.09
4	12.47	11.06	11.76	3.16	3.06	3.11
5	6.59	6.81	6.70	2.76	2.11	2.44
6	3.84	3.55	3.70	2.03	1.57	1.80
7	4.34	2.19	3.27	0.37	0.77	0.57

DV1: Influence of pH

Dichlorvos residual of biodegradation process by DV1 at different pH

Table C.22 Dichlorvos degradation of DV1 at day 0 to day 7

Day	Dichlorvos degradation by DV1 at different pH									
	pH4			pH5			pH6			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	767.38	526.29	646.84	762.21	734.87	748.54	678.98	616.91	647.94
1	629.36	599.11	614.23	649.83	627.51	638.67	607.95	526.24	567.09
2	606.43	599.02	602.73	551.69	528.39	540.04	429.74	325.97	377.85
3	585.71	598.52	592.12	512.44	483.57	498.01	360.08	259.96	310.02
4	544.52	556.14	550.33	495.52	430.22	462.87	266.13	107.17	186.65
5	530.44	517.54	523.99	439.62	336.20	387.91	122.40	74.01	98.21
6	490.79	470.39	480.59	408.14	363.33	385.74	87.42	71.15	79.29
7	479.16	443.89	461.52	370.11	244.07	307.09	62.15	43.44	52.79

Table C.22 Dichlorvos degradation of DV1 at day 0 to day 7 (cont.)

Dichlorvos degradation by DV1 at different pH							
Day	pH7			pH8			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	521.73	553.81	537.77	541.06	559.87	550.46
1	107.82	84.69	96.26	36.00	39.38	37.69
2	50.78	53.52	52.15	16.57	18.05	17.31
3	26.65	21.79	24.22	9.06	8.25	8.65
4	14.27	10.33	12.30	3.61	2.57	3.09
5	6.60	7.73	7.16	4.90	2.03	3.46
6	5.80	4.70	5.25	4.19	2.46	3.33
7	3.70	3.49	3.60	3.83	4.05	3.94

DV2: Influence of pH

Dichlorvos residual of biodegradation process by DV2 at different pH

Table C.23 Dichlorvos degradation of DV2 at day 0 to day 7

Day	Dichlorvos degradation by DV2 at different pH									
	pH4			pH5			pH6			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	737.60	820.61	779.11	692.42	743.41	717.92	660.06	878.83	769.44
1	663.52	652.03	657.77	595.21	565.24	580.23	447.95	411.28	429.61
2	699.16	596.70	647.93	-	484.79	484.79	408.92	341.51	375.21
3	559.90	602.96	581.43	512.78	474.13	493.45	312.19	177.79	244.99
4	518.50	511.15	514.83	473.29	392.28	432.78	174.49	133.04	153.77
5	518.81	517.51	518.16	443.45	416.28	429.87	86.46	71.28	78.87
6	483.34	477.25	480.30	407.70	343.01	375.35	83.14	74.41	78.77
7	462.78	450.75	456.77	381.29	289.54	335.42	48.42	36.86	42.64

Table C.23 Dichlorvos degradation of DV2 at day 0 to day 7 (cont.)

Day	Dichlorvos degradation by DV2 at different pH					
	pH7			pH8		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	766.48	808.39	787.43	712.95	711.94	712.45
1	94.14	73.40	83.77	39.66	35.03	37.35
2	49.53	47.47	48.50	17.53	18.31	17.92
3	26.96	26.61	26.79	8.36	7.80	8.078
4	13.12	12.44	12.78	4.08	4.30	4.19
5	7.89	5.87	6.88	1.24	1.03	1.13
6	4.82	4.10	4.46	3.26	2.31	2.79
7	5.11	3.93	4.52	1.27	1.03	1.15

DV3: Influence of pH

Dichlorvos residual of biodegradation process by DV3 at different pH

Table C.24 Dichlorvos degradation of DV3 at day 0 to day 7

Dichlorvos degradation by DV3 at different pH									
Day	pH4			pH5			pH6		
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	680.80	686.40	683.60	696.90	678.45	687.68	637.48	591.07	614.27
1	625.01	329.93	477.47	630.65	564.79	597.72	369.18	370.54	369.86
2	583.12	611.27	597.19	587.13	508.34	547.73	431.62	354.95	393.28
3	562.18	560.98	561.58	532.50	465.58	499.04	177.71	599.69	388.70
4	516.43	509.76	513.09	481.28	458.94	470.11	225.79	120.10	172.94
5	484.29	474.31	479.30	438.86	414.90	426.88	106.57	94.29	100.43
6	472.61	439.11	455.86	411.52	309.27	360.40	73.95	51.04	62.49
7	434.29	428.17	431.23	382.42	303.72	343.07	53.86	40.05	46.96

Table C.24 Dichlorvos degradation of DV3 at day 0 to day 7 (DV3)

Dichlorvos degradation by DV2 at different pH							
Day	pH7			pH8			
	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)	No. 1 (mg/L)	No. 2 (mg/L)	Average (mg/L)
0	484.83	527.44	506.13	589.22	448.25	518.74
1	416.27	221.82	319.04	36.24	38.68	37.46
2	58.52	48.61	53.56	16.89	17.65	17.27
3	27.50	39.26	33.38	8.59	7.94	8.27
4	14.74	11.74	13.24	5.77	5.80	5.79
5	9.21	7.70	8.46	2.06	2.74	2.40
6	6.10	4.88	5.49	0.91	3.70	2.31
7	3.84	3.68	3.76	2.50	2.88	2.69

VITA

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