ผลของการทำงานของเชื้อเมทาโนโทรฟต่อการย่อยสลายไธโอเบนคาร์บที่ปนเปื้อนในดิน

นางสาว สุธารัตน์ หมื่นมี

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EFFECT OF METHANOTROPHIC ACTIVITY ON BIODEGRADATION OF THIOBENCARB IN CONTAMINATED SOIL

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สุธารัตน์ หมื่นมี: ผลของการทำงานของเชื้อเมทาโนโทรฟต่อการย่อยสลายไธโอเบนคาร์บที่ปนเปื้อนในดิน (EFFECT OF METHANOTROPHIC ACTIVITY ON BIODEGRADATION OF THIOBENCARB IN CONTAMINATED SOIL) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.วิไล เจียมไชยศรี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร. ชาติ เจียมไชยศรี, 101 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของการทำงานของเชื้อเมทาโนโทรฟต่อการย่อยสลายของสารไธโอเบนคาร์บ และผลกระทบของสภาวะแวดล้อมต่อการย่อยสลายไธโอเบนคาร์บที่ปนเปื้อนในดิน โดยการทดลองเปรียบเทียบอัตราการการย่อยสลายขอบไธโอเบนคาร์บระหว่างดินที่มีการทำงานของเชื้อเมทาโนโทรฟกับดินที่ไม่มีการทำงานของเชื้อเมทาโนโทรฟภายใต้สภาวะแวดล้อมต่างๆ จากการศึกษาพบว่าดินที่มีการทำงานของเชื้อเมทาโนโทรฟจะมีอัตราการย่อยสลายของสารไธโอเบนคาร์บมากกว่าดินที่ไม่มีการทำงานของเชื้อเมทาโนโทรฟในทุกๆ สภาวะแวดล้อม โดยปฏิกิริยาการย่อยสลายเป็นปฏิกิริยาอันดับหนึ่ง เมื่อเพิ่มความเข้มข้นของสารไธโอเบนคาร์บจาก 2.10 ถึง 14.87 ไมโครกรัมต่อกรัมดิน มีส่วนกระตุ้นให้อัตรามีเทนออกซิเดชั่นสูงขึ้น นอกจากนี้การเพิ่มความเข้มข้นของสารไธโอเบนคาร์บยังมีผลทำให้อัตราการย่อยสลายสารไธโอเบนคาร์บสูงขึ้นด้วย แสดงให้เห็นว่านอกจากจุลินทรีย์ตามธรรมชาติแล้ว เชื้อเมทาโนโทรฟมีส่วนเกี่ยวข้องในการย่อยสลายสารไธโอเบนคาร์บในดินด้วย โดยที่ความเข้มข้น 14.87 ไมโครกรัมต่อกรัมดิน จะมีอัตราการย่อยสลายและครึ่งชีวิตเท่ากับ 0.801 ไมโครกรัมต่อกรัมต่อวัน และ 9.24 วัน ตามลำดับ การเพิ่มความเข้มข้นของปุ๋ยไนเตรทระหว่าง 22.83 – 96.30 ไมโครกรัมไนเตรทต่อกรัมดิน มีผลต่อการลดลงของอัตรามีเทนออกซิเดชั่นและอัตราการย่อยสลายของสารไธโอเบนคาร์บในดิน ความเข้มข้นของปุ๋ยยูเรียที่ 22.83 ไมโครกรัมแอมโมเนียมต่อกรัมดิน มีอัตราการย่อยสลายและมีอัตรามีเทนออกซิเดชั่นสูงสุด ขณะที่ปริมาณความชื้นร้อยละ 20, อุณหภูมิ 38 – 40 องศาเซลเซียส และ ความเข้มข้นของมีเทนร้อยละ 10 (ปริมาตรต่อปริมาตร) เป็นสภาวะที่ทำให้เกิดอัตราการย่อยสลายและอัตรามีเทนออกซิเดชั่นสูงสุด กรณีการใส่ปุ๋ยไนเตรทมากกว่า 96 ไมโครกรัมต่อกรัมดินยับยั้งการย่อยสลายสารไธโอเบนคาร์บ

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต สุธารัตน์ หมื่นมี

ปีการศึกษา 2553 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก วิไล

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม ชาติ

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SUTHARAT MUENMEE: EFFECT OF METHANOTROPHIC ACTIVITY ON BIODEGRADATION OF THIOBENCARB IN CONTAMINATED SOIL. THESIS ADVISOR : ASSOCIATE PROFESSOR WILAI CHIEMCHAISRI, D.Tech.Sc., THESIS CO-ADVISOR : ASSOCIATE PROFESSOR CHART CHIEMCHAISRI, D.Eng., 101 pp.

The aim of this study was to investigate the effects of methanotrophic activity on biodegradation of thiobencarb in contaminated soil and environmental factor on thiobencarb degradation. Various environmental conditions were investigated the degradation rate of methanotrophs-rich soils were compared with that of methanotrophs-poor soils at all environmental conditions. The results indicated that methanotrophs-rich soils had the degradation rate of thiobencarb higher than methanotroph poor-soils had at all environmental conditions, and the degradation rate of thiobencarb was the first order reaction. Increasing thiobencarb concentrations from 2.10 to 14.87 μg per g soils resulted in increasing methane oxidation rate, and increasing thiobencarb degradation rate implying that not only several microorganisms in soil can degrade thiobencarb, but methanotrophs also involve in degradation of thiobencarb in soil. The maximum degradation rate and minimum half-live, at 14.87 μg THB per g, were 0.801 μg THB per g soil.day and 9.24 days, respectively. Addition nitrate fertilizer from 22.83 to 96.30 μg NO_3^- per g result in decrease methane oxidation rate and thiobencarb degradation rate. The maximum degradation rate and methane oxidation rate were found at 22.58 μg NH_4^+ per g. The optimal moisture content, temperature and methane content for thiobencarb degradation were 20% MC, 38-40 °C, 10 % (v/v), respectively. Increasing nitrate content $\geq$ 96 μg NO_3^- per g could inhibit degradation of thiobencarb.

Field of Study : Environmental Management Student's Signature Sutharat Muenmee

Academic Year :2010 Advisor's Signature W. Chiemchaisri

Co-Advisor's Signature Chart Chiemchaisri

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CONTENTS

CONTENTS (continued)

CONTENTS (continued)

CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

ABBREVIATIONS

ai	=	active ingredient
CPR	=	carbon dioxide production rate
◦C	=	degree celsius
d	=	day
DHA	=	dehydrogenase activity
g	=	gram
h	=	hour
k	=	rate constant
l	=	liter
MC	=	moisture content
MOR	=	methane oxidation rate
m^3	=	cubic meter
THB	=	Thiobencarb
OUR	=	oxygen uptake rate
µg	=	micro gram
µl	=	micro liter

CHAPTER I

INTRODUCTION

1.1 Background

Weeds, which are major agricultural pests that can devastate a crop if not properly be controlled or managed, can be eliminated quickly with one or more application of an appropriate herbicide. However, its application can produce adverse effects in aquatic ecosystems nearby agricultural fields to both fauna and flora. There are two types of herbicides: a pre-emerge and a post-emerge herbicide, which in term of soil pollution the pre-emerge herbicide is a high potential contaminant because it is directly sprayed on the soils. Thiobencarb ($C_{12}H_{16}ClNOS$; S-[(4-chlorophenyl)methyl] N,N-diethylcarbamothioate) is a pre-emerge herbicide widely used for controlling grasses, sedge and broadleaf weeds in rice fields. It is a popular herbicide and world widely applied in rice fields since 1970 (Ishikawa, 1981), for example; the total of 154.25 and 503.17 tons of thiobencarb were applied in California in 1999 and 2006 (Environmental Fate and Effects Division Office of Pesticide Programs, 2009) respectively; the annual imported thiobencarb in Thailand in 1997 and 2007 were 32.56 to 293.04 tons respectively, of which the average of thiobencarb imported was 158.36 tons/year. Thiobencarb poses a high risk of acute effects to fish and aquatic invertebrates in certain high-exposure situations; 48-hour LC_{50} of carp, loach, grass carp, eel, black silver carp and macro branch shrimp were 1.93, 2.54, 1.51, 0.89, 2.45 and 3.47 mg/l respectively (Wang *et al*., 1992). It has been found to accumulate in some aquatic organisms such as carp, eel, black silver carp and loach, and the BCFs were 48.5, 17, 624.5 and 22.2, respectively (Wang *et al*., 1992). Thiobencarb gave the adverse effect on embryos of *Oryzias latipes* at blastula with EC_{50} of 3.6 mg/l, while the putative no observable effect concentration (NOEC) was 1.0 mg/l (Villalobos *et al*, 2000). After thiobencarb applied to rice field, it was predominantly distributed between water (34%) and soil (43%) and <1% was dispersed in air and absorbed in vegetation (Ross and Sava, 1986). Microbial activities (hydrolysis and oxidation) in sediment and water are generally the most important determinants of its fate in the field, although microorganisms responsible for herbicide degradation in field soils are affected by oxygen concentration, temperature, moisture, bioavailability of herbicide, soil depth, and the microbial

response to the herbicide. Sunlight is also important in its degradation according to thiobencarb is applied at time of great light intensity. Thiobencarb is stable to degradation by hydrolysis especially under anaerobic aquatic conditions, with a calculated half-life in sediment of 5.4 years (Nakamura *et al*. 1977). It is degraded more slowly under flooded condition (half-life 100 days) than under dry aerobic condition (half-life 45 days). More than 20 degradation products of thiobencarb were detected form soil both under aerobic and flooded conditions. Bound residues and CO_2 were the primary products in proportions of 23-42% and 42-47%, respectively (Ishikawa *et al*., 1976), and aqueous residues did not exceed 4.5% in soil metabolism studies.

Several studies have reported that methanotrophs, which oxidize methane as their sole carbon source and energy, are found mostly in the soils where methane commonly generates such as rice fields, wetland and landfills. Their activities greatly reduce methane emission rate from those sources (Conrad, 2007). In rice fields, methanotrophs are more sensitive to various agricultural pesticides such as 2,4-D, dimethenamid, isoproturon and butachlor (Boeckx *et al*., 1998; Prieme and Ekelund, 2001, Mohandty *et al.*, 2004) subsequently increase in methane emission. However, in bioremediation, it is known that methane monooxygenase from methanotrophs can co-metabolize many chlorinated organic compounds such as trichloroethylene, mono-, di-chloroethane and chloroform (Wilson and Wilson, 1985 and Hamamura *et al*., 1997). For pesticides, previous studies reported that no inhibitory effect of atrazine and metolachlor on methane oxidation rate (Seghers *et al*., 2003). Although thiobencarb is world widely used in rice fields for a long time, however, there is rare investigation for biodegradability of thiobencarb by indigenous methanotrophs and the effect of environmental condition on its degradability.

1.2 Objective

- To investigate the effect of methanotrophic activity on biodegradation of thiobencarb and effects of environmental factors on thiobencarb degradation which were herbicide content, methane concentration, moisture content, temperature, ammonia nitrogen content and nitrate nitrogen content.

1.3 Scope of study

- The experiments were conducted via batch experiments in laboratory. The soil samples were collected from rice fields in Saraburi province.
- Commercial thiobencarb was used in all experiments.
- The parameters were determined: methane oxidation rate (MOR), oxygen uptake rate (OUR), carbon dioxide production rate (CPR), degradation rate of herbicide, ammonium content, nitrite content, nitrate content and dehydrogenase.
- The environmental factors were studied : herbicide content, methane content, moisture content, temperature, ammonium nitrogen content and nitrate nitrogen content.

CHAPTER II

LITERATURE REVIEW

2.1 Weed Control in Paddy Field

Weeds, are major problem in rice production in Thailand, vary according to the method of cultivation including planting (upland rice, dry-seeded rice, deepwater rice, wet-seeded and transplanted rice). The level and type of weed infestation varies with different methods of planting, then weed control is needed for all types of rice cultivation in order to increase rice production. There have many methods to control weed such as selection method of planting rice, selection of rice cultivars, purity of rice seeds, land preparation, time of planting, rate of planting, water management, hand weeding, and chemical control, however chemical control offer the most practical, effective and economical way of reducing weed competition, crop losses and production losses in rice (Datta, 1981). In Thailand controlling weeds in paddy fields by chemicals especially herbicide trend to increase every year (Fig 2-1).

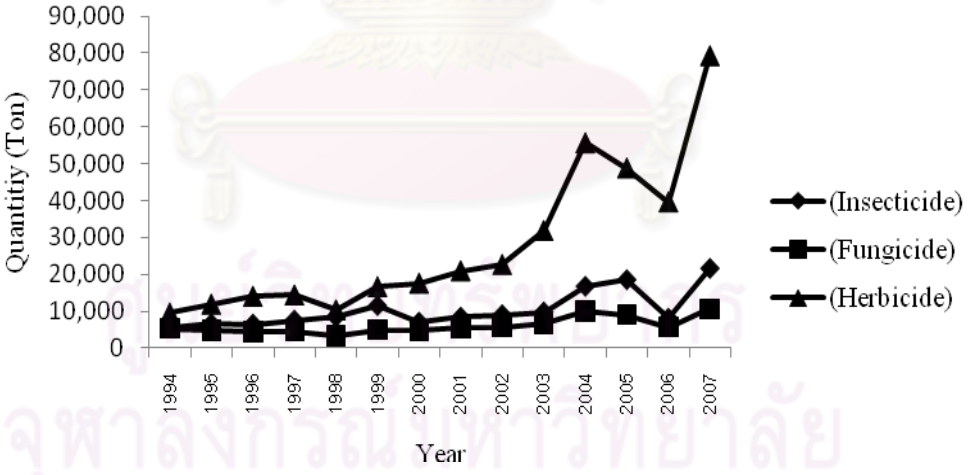


Figure 2-1 Herbicide imported to Thailand during 1994-2004

(Department of Agricultural, 2005).

Many herbicides are recommended for weed control by Thai Department of Agriculture (Table 2-1), but all are effective only on particular types of weeds and must be applied at specific times (Vongsaroj, 1987).

Table 2-1 Herbicides recommended in Thailand

Name of herbicide	Application Rate (g ai / rai)
2,4 - D	160
Butachlor	160
Fenoxaprop-p-ethyl	12-15
Methyl-furan chlorimuron	1+1
Pretilachlor	160
Bispyribac sodium	4-4.2
Propanil/butachlor	120
Propanil/thiobencarb	320
Thiobencarb	320
Oxadiazon	160

Source: Rice Department, 2009

For transplanted rice, the most common herbicides used are thiobencarb, bifenox, butachlor, oxadiazon, and bensulfuron methyl, applied 5-8 days after transplanting. The same chemicals can be used in wet-seeded rice 8-10 days after seeding. The effectiveness of herbicides depends on a number of other factors based on timing of applications, such as land preparation in the case of upland rice and dry seeded rice, and the water level in transplanted rice and wet seeded rice. The type of sprayer is also important.

Herbicides can be grouped by activity, application, chemical family, mode of action, or type of vegetation controlled. Base on its application, herbicide is separated into two main groups: pre-emerge herbicide and post-emerge herbicide, but the pre-emerge herbicide has a high potential to contaminate in soil because it directly spray on soil.

2.2 Thiobencarb

Thiobencarb (*S* -4-chlorobenzyl diethylthiocarbamate) (Fig 2-2) is widely used for controlling grasses, sedge and broadleaf weeds in paddy field. Thiobencarb is the top five of pre-emerge herbicide which imported to Thailand; the annual imported of thiobencarb in Thailand in 1997 and 2007 were 32.56 to 293.04 tons, which the average of thiobencarb imported was 158.36 tons/year. Thiobencarb belongs to the

carbamothioate family, which includes butylate, cycloate, diallate, EPTC, molinate, pebulate, triallate, and vernolate. They are derivatives of carbamic acid with one of the oxygen atoms replaced by a sulfur atom, or with other substitutions (Casida et al. 1974). Formulations include a liquid and a granular. Thiobencarb is a granular/flake herbicide or an emulsifiable concentrate herbicide, applied pre-plant, pre-flood directly to the soil, or post-flooded, post-emergence to drained fields. The average rate of thiobencarb used on rice was 3.2 kg ai/ha. Important physical and chemical properties of thiobencarb are given in Table 2-2.

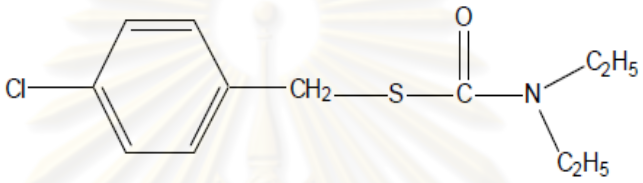


Figure 2-2 Thiobencarb chemical structures.

2.2.1 Mode of action

When applied thiobencarb is absorbed by roots and shoots of grass seedlings, and then translocates upward in the apoplast to locations where it inhibits cell division and enlargement (Jordan and Codney, 1987). Thiobencarb is a systematic, pre-emergence herbicide that acts by inhibiting shoots of emerging seedlings (U.S.EPA, 1997). Thiobencarb works best when applied after rice seedlings are at the 3-6 leaf stage and when applied prior to weed emergence (Ampong-Nyarko and Datta, 1991). Post emergent applications should be applied after the 1.5-leaf stage of rice to obtain the best weed kill without damage to rice (Ampong-Nyarko and Datta, 1991).

2.2.2 Environmental Fate

2.2.2.1. Fate in Air

Since thiobencarb has a relatively low small Henry's law constant (Table 2-2), thiobencarb has a faint tendency to partition into the atmosphere and low escaping tendency from aqueous solution, then the concentration is low in air over time. Thiobencarb has a relatively low vapor pressure and thus volatilization is a minor form of transport. Evaporation percentages of 0.90 and 0.10 have been reported a few days after application to rice water (Ross and Sava, 1986).

2.2.2.2. Fate in Water

Thiobencarb is stable to degradation by hydrolysis and is stable under anaerobic aquatic conditions, with a calculated half-life in sediment of 5.4 years (Cheng, 1996). Thiobencarb concentration in the water remained relatively constant for 2 to 8 days after application at 4.48 kg/ha, but dropped rapidly to 0.056 μg/g at 16 days after application and to 0.008 μg/g (8ppb) at 32 days after application (Ross and Sava, 1986). Thiobencarb degraded faster in water or on a glass surface or silica gel surface than on soil surface (Cheng, 1976).

Ferrando et al. (1992) studied the persistence of thiobencarb in the aquatic environment. They particularly measured the persistence of the pesticide in natural water, from Albufera Lake (Spain), and in experimental water from their laboratory. They conducted their experiment in 20 L glass aquaria (UV transmittance) containing a fixed amount of thiobencarb in 15 L of each medium, using 0.2 ppm for thiobencarb. They found thiobencarb to be very stable in both environments, and half-lives were 74.7 and 247.66 hours in natural and experimental medium, respectively. They indicated that microbial breakdown of thiobencarb in natural water may be speeding up the degradation process in this medium.

2.2.2.3. Fate in soil

The K_{ow} of thiobencarb is relatively high, the water solubility relatively low (Table 2-2) and thus tends to partition to soil. The result of Warnock (1975) who studied mobility of thiobencarb in four typical soils (two soils: Stockton adobe and Louisiana silty clay loam; Norwalk silty clay loam; Oakley sandy loam, from California) by using thin-layer chromatography techniques indicated thiobencarb moved very little in the soil, then thiobencarb has not been found below the 0-5 cm layer of soil. Mobility generally decreased with increasing clay content, organic matter content, and cation exchange capacity (U.S. EPA, 1997).

Table 2-2 Physical and chemical properties of Thiobencarb

Properties	Value
Molecular formula	$C_{12}H_{16}ClNOS$
Molecular weight	257.8
Physical State	Granular/Emulsifiable
Boiling point	127 – 131˚ C (0.12 mm Hg)
Vapor Pressure	1.5 x 10^{-5} torr @ 20˚C
Specific gravity	1.15
Water solubility	30 ppm
Henry's law constant	2.7 x 10^{-7} atm m^3/mol
$K_{O C}$	1380
Kow	2630
Hydrolysis $t_{1/2}$	>100 days
Photolysis in water (pH 7)	190 days @ 25˚C
Photolysis in soil	168 days
Aquatic half-life $t_{1/2}$	8.7 days
Aerobic soil metabolism $t_{1/2}$	40 days
Anaerobic soil metabolism $t_{1/2}$	300 days
Field dissipation half-life $t_{1/2}$	21 days

Source: U.S.EPA, 2000.

After being applied to rice fields, Thiobencarb is more likely to be found in the soil than in the floodwater (Ishikawa *et al.* 1976, 1977; Ross and

Sava, 1986). The partition of thiobencarb associated with soil was approximately 10 times more when applied pre-flooded to soil than when applied to standing water, as thiobencarb has more time to bind to soil prior to flooding. Therefore, sensitized aqueous photolysis is more significant as dissipation route when thiobencarb is applied to water than when it is applied to dry soil (Chen, 2002).

Chaiyanboon (1997) studied on persistence of thiobencarb herbicide in paddy field of Thailand that was carried out in split plot. Thiobencarb 8% G was applied at 7 days after transplanting, and the rate of application in the main plots were at the followings; no application, 320 g a.i./rai (recommended dose) and 640 g a.i./rai. It was found that thiobencarb residues decreased rapidly during 0-7 days. After 7 days until harvest, the residues decreased slowly in all sample. At 42 days, very small amounts of residues were detected in water and nondetected in rice plants. However, residues were still found in soil. At harvest, residues in soil were 38 ppb in no application plot, 137 ppb in 320 g a.i./rai plot and 128 ppb in 640 g a.i./rai plot.

2.2.3 Toxicity

Thiobencarb generally has been shown to be of low acute toxicity. It is slightly toxic by the oral and dermal route and has been classified, by EPA, in category III for these effects. It is practically non-toxic by the inhalation route and for eye irritation, and has been placed, by EPA, in Toxicity Category IV (the lowest of four categories) for these effects. Thiobencarb also tested negative for mutagenicity. Thiobencarb is toxic to rats at dietary concentrations of 100 ppm. It causes decreased body weight gains, food consumption and food efficiency, as well as increased blood urea nitrogen at this concentration. At a dietary concentration greater than 100ppm thiobencarb can impair reproduction in some birds (U.S. EPA, 2000). Based on U.S. EPA's classification thiobencarb is moderately toxic to fish, highly toxic to aquatic invertebrates, and acutely toxic to marine estuarine fish and mollusks. Fish are exposed to thiobencarb which is detrimental to the survival and reproduction of fresh water invertebrates at aqueous concentrations greater than ten parts per million. It can adversely affect the growth of juvenile fish at a concentration of 150 ppm.

Wang et al. (1992) conducted a study bioconcentration and excretion of thiobencarb in fish, clam, and shrimp. For the test, organisms were placed in a 500-L tank that received clean water amended with thiobencarb. The aqueous solution was renewed every two days. Uptake and depuration tests were continued for thirty days. Depuration tests were performed by removing the test organisms, three days after the uptake test. It was reported that more than half of the herbicide residue in carp, tilapia, loach, clam and black silvercarp was excreted within five days in clean water. After thirty days, only a slight residue was found; 19.1, 2.8, 3.1, 10.2, and 629 ppb for Carp, tilapia, loach, clam, and black silver carp, respectively.

2.2.4 Factors affecting herbicide persistence and concentration in the soil

There have many factors affecting herbicide persistence and concentration in the soil as shown in Fig 2-3: photodecomposition, volatilization, leaching, soil adsorption, plant uptake and metabolism, and microbial decomposition. Each factor is described as follows:

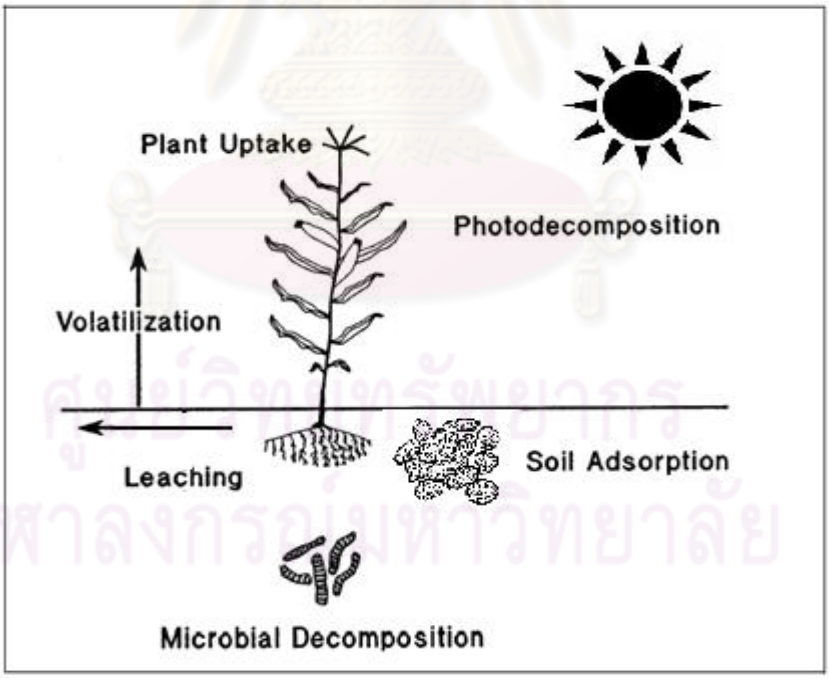


Figure 2-3 Factors affecting persistence of herbicides applied to crops (Devlin *et al*., 1992)**.**

2.2.4.1 Photodecomposition

Photodecomposition, which is the degradation of herbicide by sunlight, occurs in the atmosphere, in surface water and on various solid surfaces such as soil and plants. In the photodecomposition process, the energy from sunlight is absorbed by the herbicide molecule that causes chemical reaction, which result in herbicide inactivation. For the example, butachlor as thin film on glass photodecomposed rapidly under UV light; its half-life was found to be 1.5 hours (Chen and Chen, 1979). Cheng (1976) who conducted photodegradation study with [RING - 14C] thiobencarb in water, on a glass plate, and on soil thin layer chromatography (TLC) plate found thiobencarb readily degraded when exposed to either sunlight or short wavelength ultraviolet light (254 nm). It was degraded much faster though by ultraviolet light than by sunlight. The half-life of thiobencarb exposed to ultraviolet light was reported as 1, 1.5, 2, and 20 days on glass surface, in water, on silica gel TLC plate, and on soil TLC plate, respectively. The sulfoxide (2), 4-chlorobenzyl alcohol (3), 4-chlorobenzaldehyde (4) and 4-chlorobenzoic acid (5), were identified as the photodegradation product of thiobencarb (Chen, 1976; Ishikawa et al., 1976) (Fig 2-4), and thiobencarb sulfoxide was also identified as a photodecomposition product of thiobencarb when this unstable sulfoxide was reduced to thiobencarb by irradiation with UV light (Ishikawa et al., 1981). Dinitroaniline herbicides are readily degraded by sunlight when they left on the soil surface. However, soil in corporation will reduce or eliminate photodecomposition (Devlin *et al*., 1992).

2.2.4.2 Volatilization

Volatilization is the process whereby a herbicide change form to gaseous form. Herbicides vary widely in volatility and loss to the atmosphere as a gas. The volatilization of herbicides depends on temperature; the herbicide volatilization increase as the temperature rises, and their vapor pressure. The herbicide is easily volatile when its vapor pressure is low. Moreover, volatility also depends on water solubility, formulation, adsorption-desorption, soil texture and moisture content. Volatility loss is greater when herbicides are applied to a moist soil surface than a dry soil surface. In laboratory tests, the loss of butachlor by volatilization from a 0.05 M $CaCl_2$ solution was demonstrated to be 4.5% at 21.5˚C,

and 30% at 40˚C (Chen and Chen, 1979). Crosby *et al.* (1972) reported that volatilization of thiobencarb would be a significant route of loss for thiobencarb under field condition. Usually, very little volatilization occurs once the herbicide is mixed into the soil either by mechanical incorporation or rainfall (Devlin *et al*., 1992).

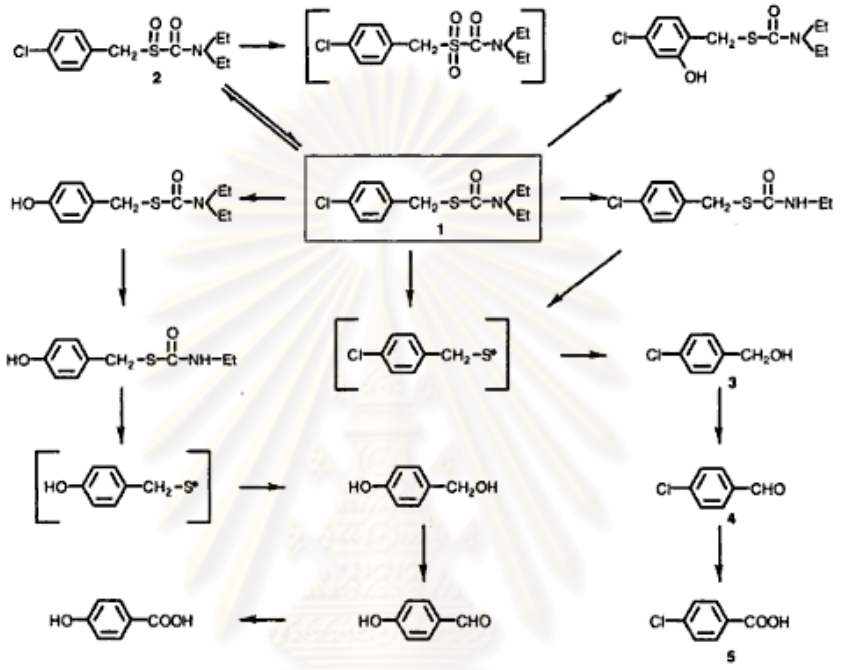


Figure 2-4 Possible photodegradation pathway of thiobencarb (Robert, 1998).

2.2.4.3 Leaching

Leaching that is the movement of herbicides through soil by water is greater in coarse textured soil (sandy loams, etc.) than in finer-textured soils (clay loams, etc.), and is limited by lack of rainfall and by compaction layers. The directions of leaching depend on water movement, but usually refer to the downward movement as water percolates down through the soil following precipitation of irrigation. Thiobencarb may undergo surface runoff, with subsequent transport to rivers and lakes, after being applied to rice paddy fields as an herbicide (Shiraishi *et al.*, 1988; Iizuka *et al.*, 1985). About 2 percent of the total thiobencarb application was removed from a field through surface runoff (Iizuka *et al.*, 1985).

2.2.4.4 Soil adsorption

Adsorption is the attraction or adhesion of molecules or ions to the surface of soil particles. Normally, soils high in clay, organic matter, or both have a grater potential for carryover because of increased binding of the herbicide to soil particles, with a corresponding decrease in leaching and loss through volatilization. A soil high in organic matter content will generally require a higher herbicide rate than a soil with less organic matter, and adsorbed herbicide molecules are unavailable for biological, physical, and chemical processes until released from the soil into the soil solution or vapor phase. Thiobencarb is not very mobile and tends to bind to soil organic matter, and doesn't desorb (U.S. EPA, 1997). Braverman *et al*. (1990) studied adsorption-desorption and degradation of thiobencarb on three Florida soils (Pahokee muck, Everglades muck, and Immokalee sand). They found that thiobencarb adsorption on soil was correlated with soil organic carbon. The adsorption values (ml g^{-1}) per unit of organic carbon were 1195, 765, and 539 for Immokalee sand, Pahokee muck, and Everglades muck, respectively. The Pahokee muck, Everglades muck, and Immokalee sand adsorbed 97, 94, and 56% of the 14C-thiobencarb out of solution respectively. Desorption (Kf) values were in the order Pahokee muck > Everglades > Immokalee sand and these were inversely proportional to the percent removal from soil (desorbed).

2.2.4.5 Plant uptake and metabolism

Herbicides from soils may be absorbed by plant roots, and plant foliage may intercept foliar-applied chemicals. Crops and plants tolerant to a herbicide often metabolize the chemical into non-toxic substances, and differential metabolism is often the basis for herbicide selectivity such as atrazine is absorbed and metabolized (detoxify) by corn and sorghum. Thiobencarb absorption and translocation were rapid in rice plants, barnyardgrass, broadleaved wild amaranth, smartweed, and lambsquarters (Nakamura *et al*., 1977). Thiobencarb was found to be widely distributed within the plants and did not accumulate at any location. The translocation was more rapid and extensive in barnyardgrass than rice plant, which the previous showed downward movement of the herbicide while rice did not. Thiobencarb, which was rapidly degraded after uptake by roots, was present at less

than 7 percent in rice and barnyardgrass with up to five times as much metabolite in roots than in leaf or stem.

2.2.4.6 Microbial decomposition

The most important pathway responsible for the breakdown of herbicide is degradation processes by soil microorganisms in soil. The microorganisms in soil, include algae, fungi, actinomcetes, and bacteria, not only consume the herbicide molecules and utilize them as a source of energy and nutrients for growth and reproduction either aerobic or anaerobic condition, but microorganisms can also degrade herbicides by a process called co-metabolism, which occurs when the organic herbicide is not used by the microorganisms for growth, is metabolized in conjunction with another substrate use for growth. The population levels and activity of microorganisms in soil depend on food supply, temperature, soil properties, oxygen, and herbicide properties. The optimum temperature for microbial activity generally is 80-90˚F, and when soil temperatures decrease, soil microbial activity declines. Usually, a warm, well-aerated, fertile soil with a near-neutral pH is most favorable for microbial growth and, hence, for herbicide breakdown. Biodegradation is the major mechanism of breakdown in soil (WSSA, 1989) and it occurs more rapidly in soils that have been acclimated to its use (Moon and Kuwatsuka, 1984). It was degraded faster in a soil incubated at 25 or 35^{o}C under moist conditions (10 kPa) than dry conditions (100kPa) (Braverman *et al*., 1990). Optimal conditions for thiobencarb degradation in culture *(Aspergillus niger* van Tieghem) were at pH 5.5 and 30°C (Torra-Reventos *et al*., 2004). For thiobencarb, the major products of degradation were deethylthiobencarb (4), thiocarb sulfoxide (2), 4-chlorobenzoic (8), 2-hydroxythiobencarb (9) and 4-chlorobenzyl methyl sulfone (24). Minor products were 4-chlorobenzyl methyl sulfoxide (6) and 4-chlorobenzyl alcohol (7) (Roberts, 1998) (Fig 2-5).

Nakamura *et al*. (1977) compared the degradation rates of 14C-thiobencarb in three soils under upland, oxidative flooded, and reductive flooded conditions. They found faster degradation of the herbicide occurring under upland condition in all three soils and to a lesser extent under oxidative flooded conditions. The slowest degradation occurred under reductive flooded condition. The half-life

period was calculated to be around 20, 50, and 200 days under upland, oxidative flooded, and reductive flooded conditions, respectively.

Gregory *et al*. (2006) found the thiobencarb concentration in the aerobic microcosm decreased sharply between day 5 and 20 on the soil, and then more slowly between day 20 and 45. The initial rapid degradation suggested a high rate of pesticide-microbe interaction, which decreased as thiobencarb was consumed. This rapid degradation may be a consequence of soil microbes being localized in discrete populations in the soil rather than evenly distributed throughout the soil, allowing high rates of degradation (Grundmann, 2004).

However, there have been reported the adverse effect of pesticides on soil microorganisms such as Kumar *et al*. (2006) carried out experiments under field condition, and simultaneous laboratory experiments were carried out to underpin the field observation, they found the soil respiration, the nitrification potential was slightly affected by certain pesticides especially soon after application (molinate and thiobencarb). The other pesticides (chlorpyrifos, benzofenap and clomazone) did not have an inhibitory effect on the nitrification at the recommended rate. Pesticides when applied in mixtures or together with high salinity levels were found to show greater toxicity to organisms.

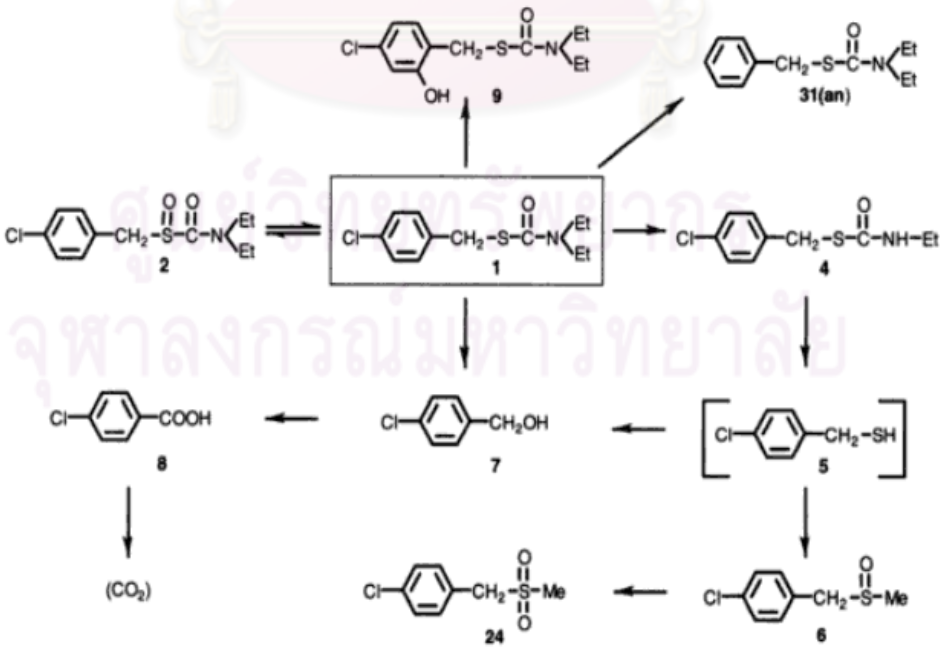


Figure 2-5 Possible degradation pathways thiobencarb in soil (Robert, 1998)

2.3 Degradation rate of herbicide

In several studies it had been observed that the degradation rate is proportional to the concentration of herbicides in soil, then degradation fitted the simple first-order kinetic (eq 1):

$$-dc/dt = kc \quad \text{————} (1)$$

where c is the concentration, t is time, and k is the rate constant. However, in some case the degradation rate was better described by a hyperbolic expression; Hamaker (1972) suggested that generally the degradation reactions in soil, which at low concentrations seem to be of the first order, are probably hyperbolic.

The degradation rate of herbicides in soils is determined by complex physicochemical and biological processes that are affected by two main groups of factors: the inherent stability of the compounds, which is related to the molecular structure, and the properties of the soil environment (Saltzman and Yaron, 1986). The nature of the substituent in the meta-position affected degradation, which decreased in the order NO_2 > CH_3-CO > Cl > CH_3-O > H, as same as the relative acidity of the compounds, and postional effects of the ring substituents showed that the degradation of para-substituents was faster than the degradation rate of compounds substituted in the meta-postion. The major soil properties known for their effect on herbicide degradation rate are moisture content, aeration, temperature, pH, and the amount and nature of the colloidal organic and mineral factions. These factors affect both the soil microbial population and the chemical transformation processes. For example, the presence of soil organic matter increases herbicide degradation that can explained by the increased microbial activity associated with the soil component, and the moisture content and temperature influence herbicide transformations because they have a direct effect on the microbial activity.

2.4 Methanotrophs

Methanotrophs, which are a subset of a physiological group of gram-negative bacteria known as methylotrophs, are obligately aerobic and utilize methane as their sole carbon sources and energy. Methanotrophs can be separated into two main groups based on their phylogenetic, affiliation, intracellular membrane arrangement, carbon assimilation pathway, and phospholipid fatty acid (PLFA) composition

(Hanson and Hanson, 1996; Bowman, 2000). Type I methanotrophs belong to the gamma-proteobacteria, assimilate the formaldehyde produced from the oxidation of methane by using the ribulose monophosphate pathway and contain predominantly 16-carbon fatty acids, while Type II methanotrophs, belonging to the alfa-proteobacteria, utilize the serine pathway for formaldehyde assimilation and contain predominantly 18-carbon fatty acids (Hanson and Hanson, 1996). Type II methanotrophs commonly have an ability to fix N_2, whereas N_2 fixation is absent in known Type I methanotrophs. Previous work has also suggested a difference between Type I and Type II methanotrophs with respect to O_2 metabolism, with Type II methanotrophs growing preferentially at lower O_2 concentrations (Amaral and Knowles, 1995).

2.4.1 Ecology of methanotrophs

Normally, aerobic methanotrophs can be found in the most situations where methane, which is continually generated in anaerobic environment, diffuses into aerobic environments, e.g., soils, rice paddies, landfill, surface layers of sediments, and natural waters.

2.4.1.1 Landfills

In the fact that landfills are estimated to produce 30 to 70 Tg of methane to the atmosphere per year (Hanson and Hanson, 1996), very high rates of methane oxidation (up to 45 g or 3 mol m^{-2} day^{-1}) have been observed in landfill cover soils (Whalen *et al.*, 1990). Type I methanotrophs can grow on soil surface of landfill cover soils where concentration of CH_4 is low, while Type II methanotrophs can grow on deep landfill cover soils where concentration of CH_4 is high and concentration of O_2 is low (Hanson and Hanson, 1996).

2.4.1.2 Rice fields

It has been reported rice fields are structured ecosystems and contain various habitats that methanogens and methanotrophs can occur (Conrad, 2007). The noticeable habitats are as follows (Fig 2-6):

- The bulk soil, which is generally anoxic, is a suitable habitat for anaerobic methanogens, but not for aerobic methanotrops; this habitat is limited by supply of degradable organic matter and its degradation products.

- Organic plant debris, such as rice straw or dead roots, which is also anoxic and reduced, but is not limited in substrate, then this is also a suitable habitat for methanogens.

- Rice roots; since O_2 can locally be released from roots, this habitat is partially oxic, and there are also rich in organic substrate by root exudation and decay; both anaerobic methanogens and aerobic methanotrophs can live.

- The shallow oxic surface layer of the flooded soil; this habitat is suitable for aerobic methanotrophs, but not for anaerobic methanogens.

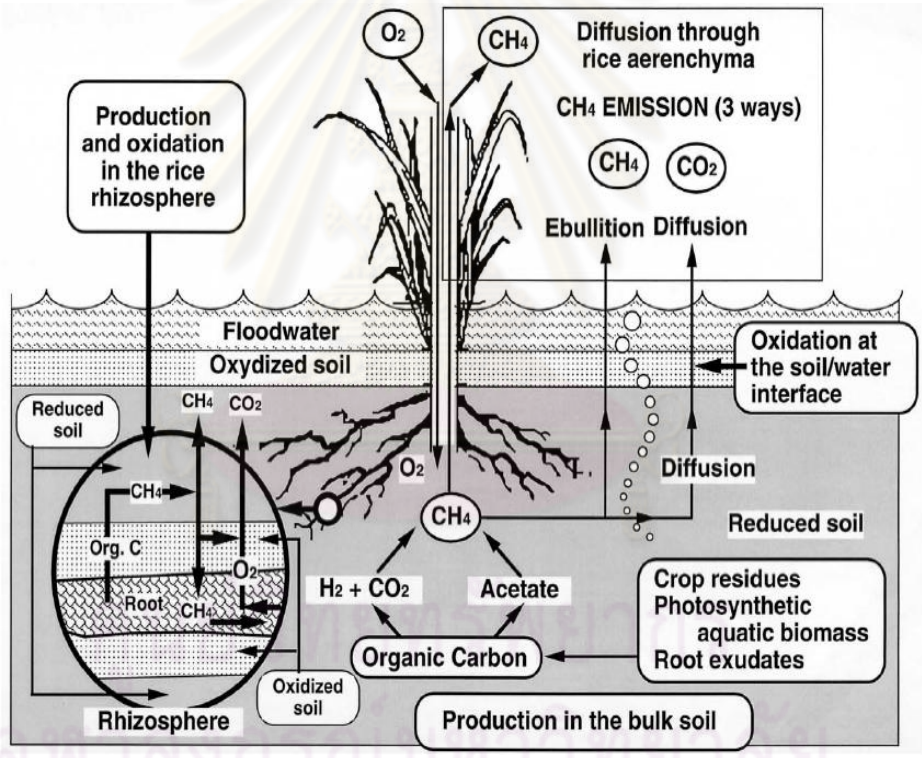


Figure 2-6 Prodution, consumption and transfer of methane to the atmosphere in ricefield (Le Mer and Roger, 2001).

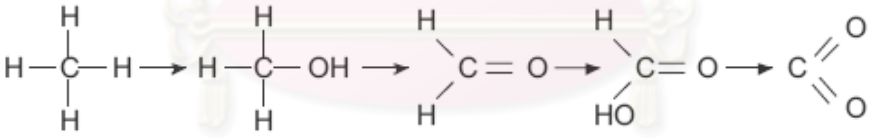
Methane is produced in the anaerobic zones of submerged soils by methanogens and is oxidised into CO_2 by methanotrophs in the aerobic zones. It has been estimated that methane production in flooded rice paddies was approximately 575 Tg year $^{-1}$ but that the amount escaping to the atmosphere was only

about 100 Tg year^{-1} (Reeburgh, 1993) or 60 to more than 90 % of methane produced in the anaerobic zones of wetlands is reoxidised in their aerobic zones (rhizosphere and oxidised soil-water interface) (Le Mer and Roger, 2001).

Macalady *et al*. (2002) who studied population dynamics of Type I and II methanotrophic bacteria in rice soils based on PLFA biomarker analyses found fluctuations of methanotroph population occurred primarily within the top 0–2 cm of soil, where methanotroph cells increased by a factor of 3–5 over the flooded rice-growing season, and their result indicated that rice roots and rhizospheres were less important than the soil–water interface for supporting methanotroph growth. Both Type I and Type II methanotrophs were abundant throughout the year but occupy different niches, with Type I methanotrophs more important for methane oxidation in drained fields and when conditions are changing rapidly, and Type II methanotrophs more important during the flooded period, when methane availability is high.

2.4.2 Methanotrophic activity

Methanotropic activities result from the natural ability of microorganisms to utilize methane as a carbon and energy source. Four stages in the oxidation of methane are distinguished (Murell, 1992):



Methane Methanol Formaldehyde Formate Carbon dioxide

The first reaction, catalyzed by methane monooxygenase enzyme, is followed by the stages with actions of methanol dehydrogenase, formaldehyde dehydrogenase and formate-dehydrogenase, respectively.

Methane monooxygenases (MMOs) are the enzymes that catalyze the oxidation of methane. Two distinct type of MMOs are known to exist in different cellular locations in methanotrophs are a membrane-bound particulate form (pMMO) and a cytoplasm soluble form (sMMO). pMMO has a narrow substrate specificity such as alkenes and alkanes up to pentane. sMMO, which can co-oxidize a wide range of hydrocarbons (alkanes, alkenes and aromatic compound) and chlorinated

hydrocarbons, have great potential as biocatalysts for bioremediation and biocatalysis. Wymore *et al*. (2007), who investigated biodegradation of trichloroethene (TCE) and *cis*-dichloroethene (DCE) by using a novel suite of assays including enzyme activity probes designed for the soluble methane monooxygenase (sMMO) enzyme, found that TCE and DCE could degraded in an aquifer via cometabolism of indigenous methanotrophs.

2.4.3 Dehydrogenase activity (DHA)

Biological oxidation of organic compounds is generally a dehydrogenation process, and there are many dehydrogenases (enzymes catalyzing dehydrogenation), which are highly specific. The dehydrogenase process may be presented as follows:

$$XH_2 + A \rightarrow X + AH_2 \quad \text{———(2)}$$

Where XH_2 is an organic compound (hydrogen donor) and A is a hydrogen acceptor. The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of soil organic matter as they transfer hydrogen from substrates to acceptors (Tabatabai, 1982). Therefore, the result of the assays for dehydrogenase activity in soil has often been used to achieve an index of the total soil microbial activity. The DHA is considered one of the better indicators of microbial activity because dehydrogenase only occurs within living cells, unlike other enzymes, which can occur in an extracellular state. Any assay of soil microbial activity may be influenced by numerous conditions such as substrate concentration, O_2 concentration, moisture content, temperature, nutrient availability and pH. For the examples, DHA is higher in anaerobically or flooded incubated soil than aerobically incubated soils (Trevors, 1984; Subhani *et al*., 2001), incubation at 37 °C increased dehydrogenase activity over that occurring lower temperature (Subhani et al., 2001), and ammonium sulfate at concentrations ranging from 40-120 µg/g had no inhibitory effect on DHA, however, at 160 and 200 µg/g, activity was reduced (Trevors, 1984). Soil capacity for methane oxidation is frequently studied in order to elucidate its dependence on fertilization (mineral and organic), environmental conditions, soil management, *etc*. (Hutsch, 1998; Stepniewski and Zygmunt, 2000). The highest stimulation of soil dehydrogenase occurred in the period of rapid depletion of CH_4 and O_2 and

simultaneous intensive CO_2 (Brzezinska *et al*., 2004). Therefore, the effects of environmental conditions are important.

2.4.4 The factors affecting on methanotrophic activity

2.4.4.1 Type of pesticide

Rice needs some chemicals to increase their productivity. A number of recent reports indicate that methnotrophs may be more sensitive to various agricultural management practices and heavy metal (Topp, 1993; Priem and Ekelund, 2001; Monhanty et al., 2004). Topp (1993) proved the inhibitory effect of the herbicide bromoxynil and the insecticide methomyl on the oxidation of low methane concentrations. Other studies also found an inhibitory effect of the herbicides 2,4-D, dimethenamid, isoproturon and butachlor (Prieme and Ekulund, 2001, Mohandty *et al*, 2004), and Mohanty *et al*. (2004) demonstrated that butachlor, even at very low concentrations, can affect CH_4 production and its oxidation in flooded rice soil, although, the result of Min *et al.* (2002) who studied the effects of butachlor on microbial enzyme activity in paddy soil showed after application of butachlor with concentrations of 5.5, 11.0, and 22.0 µg/g dried soil, the application of butachlor enhanced the activity of dehydrogenase, and the soil dehydrogenase showed the highest activity on the after application of 22.0 µg/g dried soil of butachlor. In contrast, previous study reported that no inhibitory effect of atrazine and metolachlor on the methane oxidation rate (Seghers *et al*., 2003).

2.4.4.2 Nitrogen source

Like any other organisms, methanotrophic bacteria require nitrogen as a nutrient for biomass formation. Lack of sufficient nitrogen may result in inactivation or dormancy of methanotrophs, which is overcome by addition of fertilizer.

Plants such as rice need some fertilizer to increase their productivity. Urea is the dominant form of N fertilizer applied to soil, the rate of application of urea in rice field is 1.60-1.92 kg/ ha or 0.74-0.88 kg N/ ha., then more NH_4^+ may be provided as substrate. Methanotrophs can oxidize NH_4^+ to NH_2OH via methane monooxygenase, although, NH_4^+ does not apparently support growth in

methanotrophs (Bedard and Knowles, 1989); methane oxidation rate may decrease because methanotrophs may oxidize NH_4^+ instead of CH_4, but maximum rates of NH_4^+ oxidation are considerably lower than in ammonia oxidizers, and the affinity for NH_4^+ is generally lower than in ammonia oxidizers. The population size of methanotroph was inversely related to the NH_4^+-N content of the soil, and the trend of population size followed the order bare soil > bulk > rhizosphere (Dubey and Singh, 2001). Reay and Netwell (2004) studied the effect of inorganic nitrogen addition on methane oxidation rate, and their data showed the methane oxidation rate of the soil contained high nitrogen content was lower than the soil contained low nitrogen content in both high and low methane concentration. Additions of inorganic nitrogen to soil with significant potential for CH_4 oxidation resulted in differential reduction in CH_4 capacity depending on N species added; small NO_3^- additions causing by far the greatest reductions in CH_4 oxidation, compared to the result of addition NH_4^+ and NO_2^- (Reay and Nedwell, 2004). Addition NH_4^+ and NO_3^- more than 30 mg/kg, methane oxidation rate decreased (Table 2-3), and the high concentration of NO_3^- may inhibit nitrification process that effect on accumulation of NO_2^- which can inhibit methanotrophs activity (Chiemchaisri, 2000).

Table 2-3 Effect of N-nutrients addition on methane oxidation activities

NH_4^+-N		NO_3-N	
Concentration (µg /g dry soil)	Methane oxidation activities (μgCH_4 /g dry soil. h)	Concentration (µg /g dry soil)	Methane oxidation activities (μgCH_4 /g dry soil. h)
57	7.71	1.5	7.71
87	5.06	31.5	5.78
107	5.05	51.5	4.91
127	4.92	70.5	4.82
157	4.91	101.5	3.34

Source: Chiemchaisri (2000)

However, recent rice microcosm studies (Bodelier and Frenzel, 1999; Bodelier *et al*., 2000) have clearly shown that N fertilization increases methane oxidation in densely rooted rice soil. N limitation of methane oxidation is compatible with the idea that methanotrophs in rhizosphere soil face intense plant and microbial competition for nitrogen, and in bulk soil (both rice and forest soil),

nitrogen fertilizer also seems to stimulate Type I methanotrophs, while Type II methanotrophs are inhibited (Mohanty *et al*., 2006). Chan and Parkin (2001) found at low CH_4 concentration, CH_4 oxidation rate were negatively correlated with the nitrogen contents of the soil, thus indicating an adverse effect of the nitrogen status on methanotrophic activity. On the other hand, at high CH_4 concentration were positively correlated to the nitrogen status of the soil. These results indicate that nitrogen fertilization has a differential effect on CH_4 oxidation, which is dependent on the resident methanotrophic populations, how they react on nitrogen addition, and concentration of CH_4.

2.4.4.3 Moisture content

Bacteria need (typically) foods that are high in moisture content to grow. Some bacteria can survive when there is little water, but they will not be able to grow very well. Striegel *et al*. (1992) who studied consumption of atmospheric methane by desert soil reported that the methanotrophs are limited by the available moisture and are able to recover within a few hours after precipitation, and their results also imply that the methanotrophs in environment can survive periods of drying in an inactive state and can recover their ability to oxidize methane rapidly when moisture becomes available. Moisture content between 14 to 20 % was favorable for methane oxidation rate. The methane oxidation rate was dropped to almost zero at moisture content below 5 %, and higher moisture content (more than 18%) could not produce higher oxidation that could be due to low diffusion transport of gases (Pokhrel, 1998).

In rice field, field drainage, which is conventionally applied for the aeration soil in the rice cultivation during midseason and also during the end of the growing season, inhibits methane production while, at the same time, depletes existing methane through aerobic oxidation by methanotrophs (Sass *et al*., 1992). Soil submersion allows the development of the methanogenic activity and reduces methanotrophic activity by reducing the size of the oxidised zones. The population size in flooded rice was smaller than in the dryland rice, and in dryland and flooded rice, population size of methanotrophs followed the order rhizosphere > bulk> bare

soil (Dubey and Singh, 2001). Therefore, water management in rice field affects CH_4 emission through their effects on methanogenesis, methanotrophy and CH_4 transfer.

2.4.4.4 Temperature

It is known that temperature plays an important role in processes which take place in the soil, including chemical reactions and biological interactions (Saleh-Lakha *et al*.**,** 2005). The activity of any chemical reaction increases with temperature, approximately doubling for every increase of 10 ˚**C**, and the rate of enzyme-catalyzed reaction increase as the temperature increase until some high temperature is reached at which the rate begins to decrease because of enzyme inactivation, then the most soil enzymes are inactivated (denatured) at temperature between 60 to 70 ˚C (Tabatabai, 1982). The different soils demonstrate different methane oxidation responses with respect to temperatures that show populations of methanotrophs in nature can adapt to different temperatures. Most methanotrophs available in pure culture are mesophiles, although *Methylococcus capsulatus* Bath and related strains are capable of growth at temperatures up to 50 °C (Hanson and Hanson, 1996). The optimum temperature for methane oxidation was 25 to 35˚C (Bender and Conrad**,** 1995). The oxidation rate was slowed down at lower temperature more rapidly (Pokhrel, 1998) and the oxidation rates were 10 to 38% of the maximum rate observed at the optimum temperature according to the result of Dunfield *et al*. (1993).

However, high temperature that favorable for oxidation also caused decrease in moisture content of topsoil, which reduce methane oxidation rate.

2.4.4.5 Methane content

Methanotrophs can utilize methane as their sole carbon source and energy, then populations or activity of methanotrophs are related with the methane content. The highest methane oxidation rate has been observed in soils where methanogenesis is or has been effective and where methene concentration is or has been much higher than in the atmosphere (Le Mer and Roger, 2001), and the threshold concentrations at which methane was not oxidized (as low as 0.04 to 0.06 nM) were much lower for aerobic forest and grassland soils than for soils with

subsurface sources of methane, like landfill soils, rice field and lakes, where the threshold values were in the range of 50 to 150 nM (Whalen *et al*, 1990). At high methane mixing ratios (1,000ppmv or higher), the number of methanotrophs correlated with the methane oxidation rate, while at oxic soils exposed only to atmospheric concentration of methane (2 ppmv or lower), there was not correlation between the distribution of the numbers of methanotrophs (Bender and Conrad, 1995), and population of methanotrophs was correlated with the methane content in soil when methane composed up to 50% by volume of the gas (Hanson, and Wattenburg, 1991; Topp and Hanson, 199; Bender and Conrad, 1995; Hanson and Hanson, 1996). Moreover, the supply of CH_4 resulted in an increase of both respiration (as measured by O_2 uptake and CO_2 production) and dehydrogenase activity when compared with the soil that incubated without methane injection (Brzezinska *et al*., 2004).

In rice field, methane is not produced immediately when the soil are flooded. The delay of methane production depends on many factors such as pH, temperature, substrate availability, etc. The methane productions in alkaline and calcareous soils at 25-30 °C were started after flooding an hours. The methane production in neutral soils was delayed two to three weeks after flooding while in acid soils, the delay could be as long as five or more weeks (Neue, 1993). Le Mer and Roger (2001) reported that three CH_4 emission peaks may occur during the crop cycle. The first peak, is observed shortly after submersion, is attributed to the decomposition of the easily mineralizable organic matter; it is usually observed in soil where organic manure was applied (Watanabe *et al*., 1995). A second peak is usually observed during the reproducing phase of the crop and seems to be related to an increased rhizospheric exudation. The incorporation into the soil of the photosynthetic aquatic biomass by weeding and by the activity of the soil fauna also provides organic matter to the soil that may contribute to the anaerobic fermentation process at this stage. A third peak, is observed at the end of the crop cycle, could result from an input of organic matter due to root exfoliation and plant senescence.

Tadeuchi *et al.* (2001) used scaling technique to estimate the methane emission from paddy fields of the central Plain of Thailand, and found the values of methane flux are 38-620 $mg/m^2/day$. The different methane emission is

influenced by the variation of chemical characteristics of soils and field management system in cultivated area especially water management; a dryland rice emitted less CH_4 than a flooded rice (Trolldenier, 1995). The emission-to-oxidation ratio was 0.66 under flooded conditions, but increased remarkably to 2.79 during the initial 5 days after drainage. CH_4 oxidation exceeded CH_4 production around 10 days after drainage (Le Mer and Roger, 2001).

CHAPTER III

METHODOLOGY

3.1 Rich-methanotrophic soil preparation

Soil sample was collected from the top 15 cm depth of a rice field located in Sao Hai district, Saraburi province. They were air-dried, and sieved through 2 mm screening, respectively. Their principal physical and chemical properties were determined. The 6 kg of the fine soil samples were added with water to provide 10-15 % of moisture content and filled in the lysimeter (Fig 3-1), a synthetic gas (60:40 of CH_4 to CO_2) was purged at the bottom of the lysimeter; the flow rate was 1 ml per min, and the lysimeter was under operated room temperature (28-30˚C) for 2 months, then, the soils were tested for methane oxidation rate as described in section 3.3 prior to be employed in each experiment.

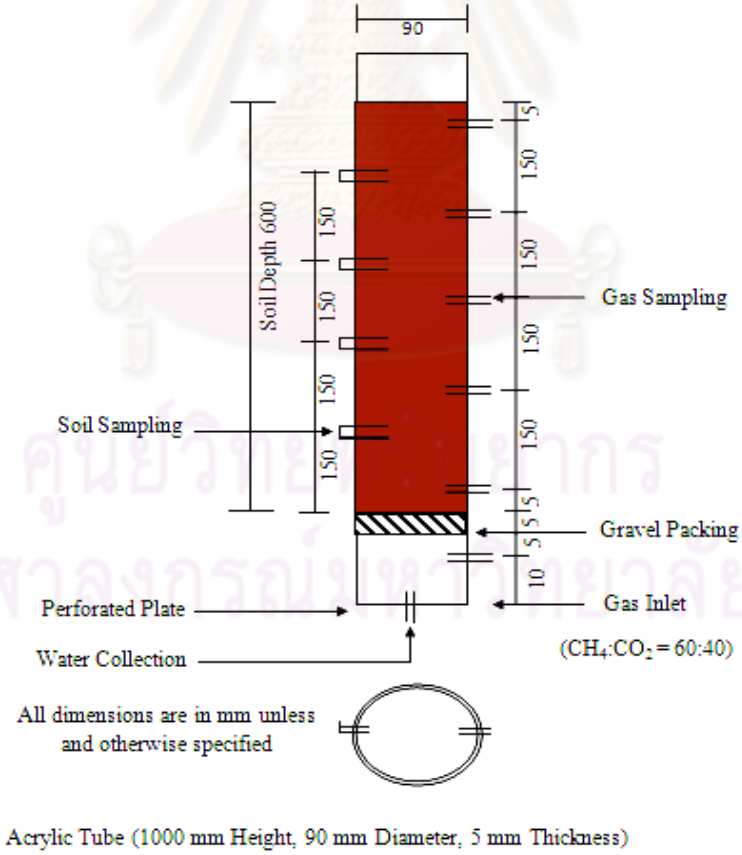


Figure 3-1 Soil lysimeter.

Table 3-1 Characteristics of raw soil used in the lysimeter

Parameter	Value
Density	1220 kg/m^3
Porosity	53.96%
Texture	Clay loam
Phosphorus	18.25 mg/kg of soil
Total Kjeldahl Nitrogen	1006 mg/kg of soil
Total organic carbon	1.30%

3.2 Effects of environmental factors on methanotrophic activities

3.2.1 Herbicide concentrations

Thiobencarb, dissolved in distilled water was added to the soils taken from lysimeter to provide concentrations of 2.1, 4.58, 8.23 and 14.78 µg/g with 10% moisture content based on practical application rate in rice field (Chaiyanboon, 1997) and unamended soils receiving only distilled water were served as the controls. Each 67 g of the THB amended and unamended soils were placed in the 500-ml of 64 serum bottles and sealed with rubber stoppers and aluminum caps for each condition. Then, methane was injected into the bottles to provide 10% (v/v) initial methane concentration in the headspaces. The head space gases were monitored and when methane was dropped to 5%, the bottles were opened and re-aerated, and methane was re-injected. Parallel sets of the soils were conducted without methane as the controls. All bottles were incubated under room temperature (28-29˚C) for two months and two bottles of each set were opened by weekly basis. The soils were examined for available inorganic nitrogen (NH_4^+, NO_2^- and NO_3^-), methane oxidation rate (MOR), oxygen uptake rate (OUR), carbon dioxide production rate (CPR), soil DHA and THB residues in soil. Organic-N was determined at the initial and the end of experimental period. All experiments were conducted in duplicate.

3.2.2 Effect of nitrate content

THB content, which gave the highest THB degradation rate from experiment 3.2.1; 14.78 µg/g, was selected in this experiment. 12 kg of THB

contaminated soils were prepared. The different amounts of $Ca(NO_3)_2$ fertilizer were dissolved in distilled water and each solution was mixed with 3000 g of the soils to obtain the nitrate content of 0.52, 27.00, 52.86, 81.43 and 96.30 µg NO_3^-/g soil. . At each nitrate content, the soils was separated in the 500-ml of serum bottles, then sealed with rubber stoppers and aluminum cap (Fig. 3-2). Then, pure methane was injected into each bottle to provide 10% (v/v) initial methane concentration in the headspace. The head space gases were monitored. When headspace methane was dropped to 5%, the bottles was opened and re-aerated, and methane was re-injected. Parallel sets were conducted under absent methane as the controls. The soil of analyses were similar to the experiment 3.2.1.

3.2.3 Effects of urea content, moisture content, methane content and temperature

The procedure of soil preparation was as same as the experiment 3.2.2 for studying effects of urea content, moisture content, methane content, and temperature. The urea fertilizer was used instead of nitrate fertilizer. The different urea content were prepared to obtain 0.58, 22.83, 50.94, 81.38, 100.12 NH_4^+µg/g soil. . The following steps of gas and soil analyses were similar to the experiment 3.2.2. For moisture content study, different volumes of distilled water were added to the soils to provide soil water content of 5, 10, 20, 40 %. It was noted that the initial NH_4^+ and NO_3^- contents of the soils were 7.90 NH_4^+µg/g soil and 1.82 NO_3^-µg/g soil. Gas and soil analyses were similar to the experiment 3.2.1. For methane content study, various volumes of methane were injected as 0, 4.5, 23, and 45 ml into the set of bottles equivalent 0(control), 1%, 5%, 10% (v/v) initial methane concentration in the headspaces. The initial NH_4^+ and NO_3^- contents of the soil were 0.48 NH_4^+µg/g soil and 2.79 NO_3^-µg/g soil. The head space gases were monitored and when methane dropped to 0.5, 2.5, 7.5% (v/v), respectively, the bottles were opened and re-aerated, and methane was re-injected. All of the serum bottles were kept at room temperature, and the step of analysis as same as the experiment 3.2.1. For temperature study, the serum bottles were incubated under different temperatures of 20, 30, 40 ˚C. The initial NH_4^+ and NO_3^- contents of the soils were 0.26 NH_4^+µg/g soil and 1.82 NO_3^-µg/g soil.

3.3 Microbial activity in the soils.

3.3.1 Determination of methanotrophic activity and soil respiration

A three gram of the tested soil sample was placed in the 25 ml serum bottle and sealed with rubber stopper and aluminum cap (Fig. 3-3). Methane was injected into the bottle to provide 10% (v/v) methane concentration in the headspace. Then, the bottle was incubated at room temperature (27-29˚C). At 0, 3, 6, 12, 24 hours, the 300 µl-head space gas was analyzed by gas chromatograph (GC, Agilent-6890 ; TCD detector; carrier gas: helium with the flow rate of 21.1 ml/min; column: stainless steel, ID 6.35 mm, 1.8 m length; supporting material, activated molecular sieve; injection temperature, 50˚C; oven temperature, 35˚C; detector temperature: 180 ˚C). Changes of methane, oxygen and carbon dioxide concentrations with time were plotted to determine methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) (Chiemchaisri *et al*., 2001) by using general linear model analysis using Microsoft excel 2007.

3.3.2 Deydrogenase activity

The biological oxidation of organic compounds is generally dehydrogenation: The assay of DHA in soil is commonly used to represent heterotrophic activity. Soil dehydrogenase assay in test soils were determined via TTC (2,3,5-triphenyl tetrazolium chloride) reduction by colorimetric method according to Tabatabai (1982).

3.4 Parameter Analyses

3.4.1 Thiobencarb extraction and determination

The method of Chiang *et al*. (2001) was modified to extract thiobencarb from soil. The soil sample (20 g) was mixed with anhydrous sodium sulphate and extracted with a 100 ml of acetone, and agitated for 3 hours on a wrist action shaker. An aliquot of the extract was filtered through Whatman No.1 filter paper into 250-mL round-bottom flasks, then the filter paper was rinsed with a 10 ml of acetone, the extraction was concentrated below 40 ˚C by evaporator, and adjusted to 2 ml final volume with hexane. The extract was analyzed with gas chromatograph mass spectrometer (Shimadzu GC2010; carrier gas, helium with a flow rate of 1.5

ml/min; column: RTX-35MS, ID 0.25 mm, 30 m length; ion source temperature: 230˚C; oven temperature, 80˚C). The percent recovery was 85.

3.4.2 Available nitrogen

Ammonium-nitrogen (NH_4^+-N) and nitrite-nitrogen (NO_2^- -N) were extracted by 2 M KCl and analyzed by colorimetric method (Black, 1965). Nitrate-nitrogen (NO_3^- -N) was extracted by 0.5M K_2SO_4 and analyzed by colorimetric method (Black, 1965).

Figure 3-2 The 500-ml serum bottles utilized in each batch experiment.

Figure 3-3 The 25-ml serum bottles utilized for methane oxidation rate.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Effect of thiobencarb concentration

4.1.1 Available nitrogen

The trends of organic nitrogen contents in both methanotroph-rich (test) and poor soils (control) were similar by increasing with increase in THB content (Table 4-1) according to the fact that THB is classified as organic nitrogen by chemical structure. Organic-N after 56 days of incubation in both sets were significantly decreased with increase in THB concentration. In the controls, the results demonstrated that THB did not give negative effect on ammonification and nitrification at all concentrations (2.10 to 14.78 µg/g) as shown by decreasing in organic nitrogen (Table 4-1) and increasing in ammonium contents followed by nitrite and nitrate contents, respectively (Fig 4-1). Consideration in the tests, by the fact that methanotrophs utilize nitrate as nitrogen source to support their growth (Hanson and Hanson, 1996), thus nitrate contents in all test sets were lower than the controls implying that methanotrophs could grow in these experimental conditions as supported by the increasing MOR (Fig 4-2) which is discussed in the next section. It can be summarized that additional THB could enhance ammonification subsequently in stimulation of nitrification. Methanotrophs could gain nitrate from nitrification for their methane oxidation.

Table 4-1 Organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Thiobencarb concentration (µg/g)	Organic-N of control (µg-N/g)			Organic-N of test (µg-N/g)		
	Day 1	Day 56	ΔN	Day 1	Day 56	ΔN
0	1000.47	985.21	-15.26	1000.47	986.58	-13.89
2.1	1000.86	977.57	-23.29	1000.86	980.89	-19.97
4.58	1001.01	926.70	-74.31	1001.01	929.64	-71.37
8.23	1001.37	881.47	-119.90	1001.37	874.52	-126.85
14.78	1001.44	853.65	-147.79	1001.44	851.22	-150.22

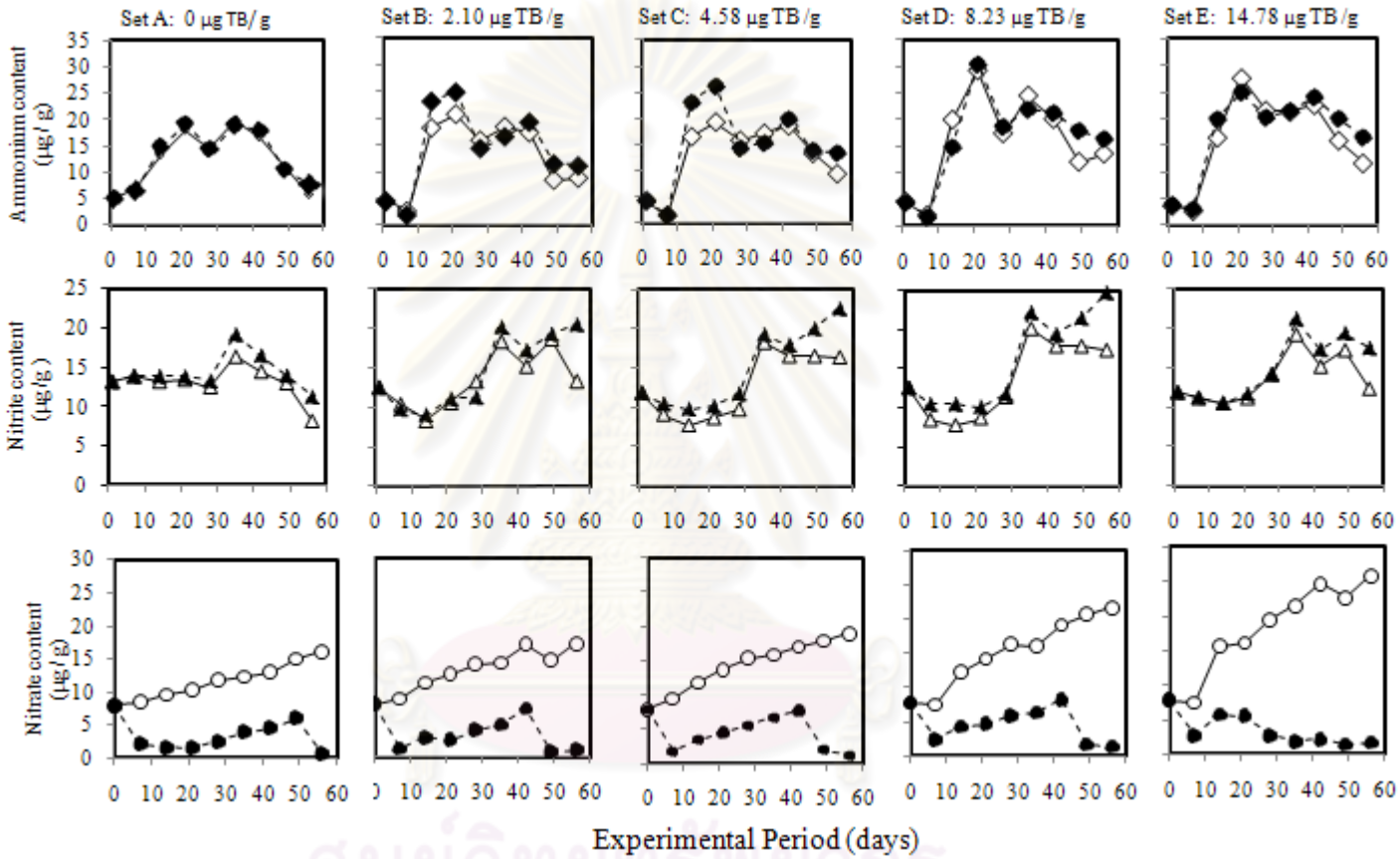


Figure 4-1 Effect of thiobencarb concentration on nitrogen available of both poor-methanotrophs soil (white legends) and rich-methanotrophs soil (black legends). Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil; moisture content: 10% (w/w); temp: 27-30 ˚C; methane content: 10% (v/v).

4.1.2 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

Normally, MOR can be described degree of methanotrophic activity, which in this experiment, it had shown that MOR of the controls were lower than that of the tests at all thiobencarb concentrations indicating that methanotrophs could grow in the environment having THB up to about 15 µg/g. The MOR tended to increase with increase in THB concentrations indicating that methanotrophic activity was stimulated by THB application at these concentrations. The lag phase of growth took first 14 days after incubation, and highest methane oxidation rates had appeared during days 20-40 in all conditions after that, the decreased MOR might be due to imbalance of nitrite and nitrate contents (King and Schnell, 1994). Moreover, the results showed that increase in THB content can indirectly stimulate methanotrophic activity as resulted from more nitrate available in higher THB content (Fig 4-1) as described before. The OUR and CPR of the tests were higher than the controls according to methanotrophic activity, and the OUR of the controls increased with increased THB concentration indicated that aerobic heterotrophs could use THB as their carbon source.

4.1.3 Thiobencarb (THB) degradation rate

Degradability of THB among the tests and controls were compared using reaction rate constant and half-life of thiobencarb (Table 4-2). It was found that the degradation rate of thiobencarb was the first order with respect of THB concentration. Increasing THB concentrations resulted in increasing degradation rate, but reducing the half-life in both experimental sets. However, degradation rate and half-life of the tests were significantly different from the controls particularly at 8.23 and 14.78 µg/g soil ($P < 0.05$). In the test, the concentration of THB residual in soil tended to decrease, while the MOR tend to increase with time at all concentration of THB (Fig 4-3) implying that the higher degradation rate in the tests resulted from methanotrophic activity. For the controls, thiobencarb degradation related to the DHA which is discussed in the next section.

The major degradation products of both controls and tests were similar (Table 4-3), they were diethylthiocabamate (50-70%), (dichloromethyl)sulfonyl]

methyl (10-15%), p-nitrotolyl(dichloromethyl) sulfone (10-15%), and 1-chloro-4-[(chlorophenyl)thio] (2-5%). The types of minor degradation products of the test were more than the controls of which comparison to previous study (Ishikawa *et al*., 1976), only major product, diethylthiocabamate, was similar. It can be explained that the difference in degradation products from previous studies resulted different microorganism types, soil environmental conditions, etc.

Table 4-2 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t^{½}$

Thiobencarb concentration (µg/g soil)	Rate constant (K,day^{-1})		r^2		$t^{½}$ (day)	
	Control	Test	Control	Test	Control	Test
2.10	0.042	0.049	0.95	0.98	16.50	14.14
4.58	0.052	0.062	0.86	0.97	13.40	11.18
8.23	**0.053**	**0.067**	0.94	0.98	13.08	10.08
14.78	**0.056**	**0.075**	0.92	0.94	12.38	9.24

Table 4-3 List of products of microbial activities

Controls	**Tests**	**Ishikawa (1976)**
	Major compounds	
Diethylthiocabamate	Diethylthiocabamate	Diethylthiobencarb
Dichloromethyl-sulfonyl-methyl	Dichloromethyl- sulfonyl methyl	Thiocarb sulfoxide
p-Nitrotolyl-dichloromethyl-sulfone	p-Nitrotolyl-dichloromethyl-sulfone	2-Hydroxythiobencarb
1-Chloro-4-chlorophenyl-thio	1-Chloro-4-chlorophenyl-thio	4-Chlorobenzoic
		4-Chlorobenzyl- methyl-sulfone
	Minor compounds	
2-Chlorophenyl-methoxy	2-Chlorophenyl-methoxy	4-Chlorobenzyl alcohol
	Methyl 3,5-di-O-4-chlorobenzyl	4-Chlorobenzyl- methyl-sulfoxide
	Trichloro-chlorophenyl-methoxy	

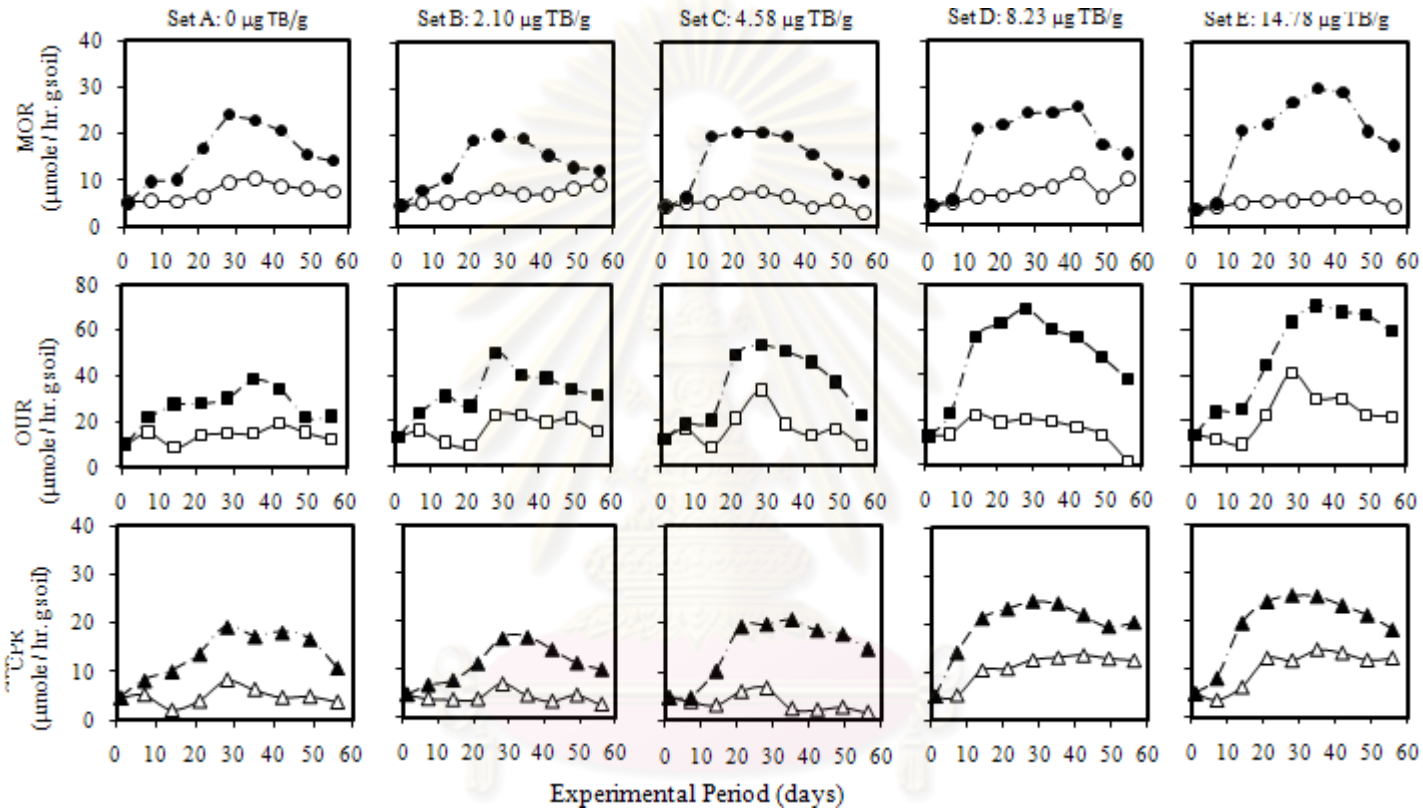


Figure 4-2 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment. Black legends: tests (with CH_4); white legends: control (without CH_4). Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature: 27-30 ˚C; methane content: 10% (v/v).

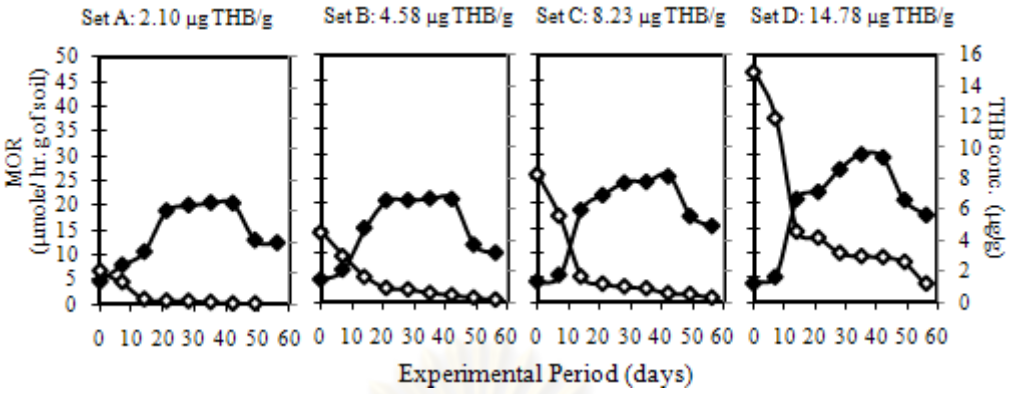


Figure 4-3 Correlation between methane oxidation rate (MOR) and THB concentration in tests soil. Black legends: MOR; white legends: THB concentration. Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature: 27-30 ˚C; methane content: 10% (v/v).

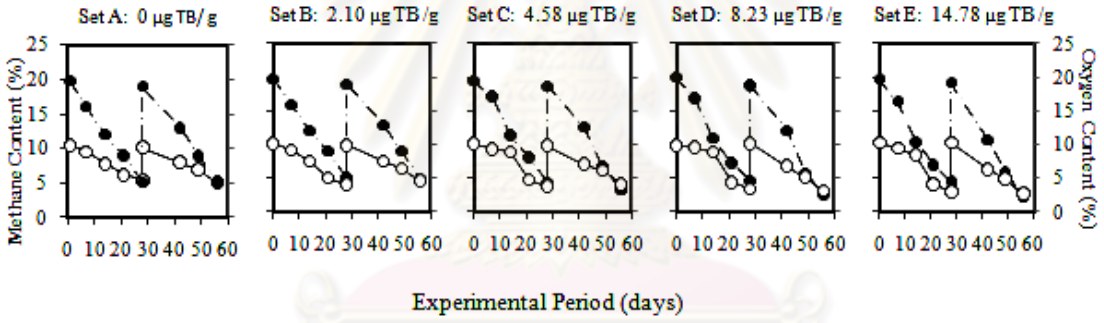


Figure 4-4 Methane (○) and oxygen (●) available in the tests. Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature: 27-30 ˚C; methane content: 10% (v/v).

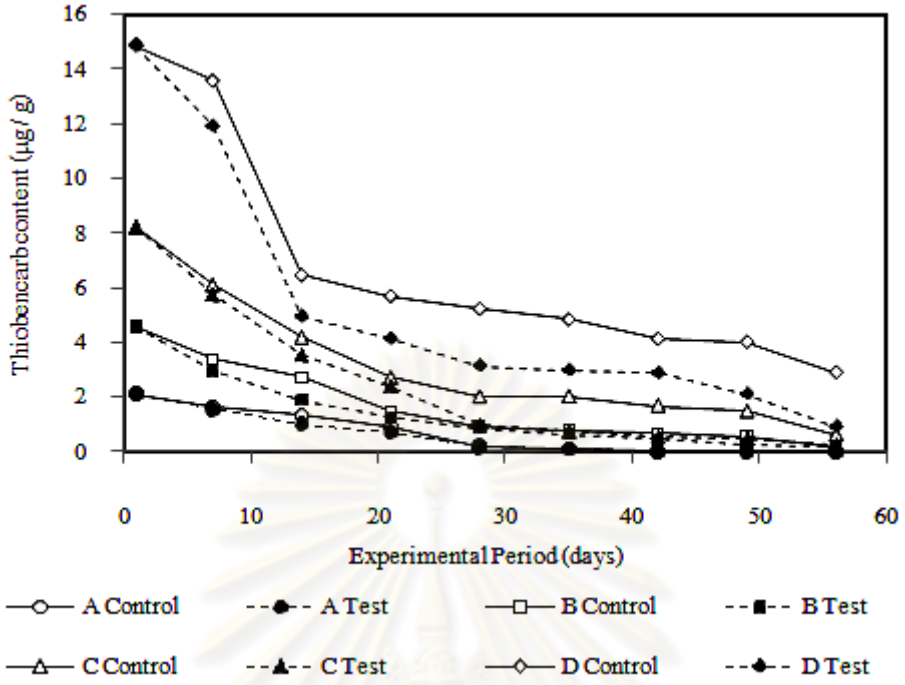


Figure 4-5 Content of thiobencarb residues of the controls and tests with time. Set A: 2.10 µg/g; Set B: 4.58 µg/g; Set C: 8.23 µg/g; Set D: 14.78 µg/g. Black legends: tests (with CH_4); white legends: control (without CH_4). Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil;
moisture content: 10% (w/w); temperature: 27-30 ˚C;
methane content: 10% (v/v).

4.1.4 Dehydrogenase activity (DHA)

Both in the tests and controls, the trends of DHA increased with increased THB concentrations during 40 days of experimental period, after that DHA were decreased with time (Fig 4-6). In the tests, DHA were higher than the controls at all thiobencarb concentrations due to the fact that methanotrophs having dehydrogenase enzymes to catalyze intermediate substrates: methanol, formate, and formaldehyde which their amounts directly corresponded to the methanotrophic populations. In the controls, the results indicated that DHA of heterotrophs was activated by application of THB, which suggested that they used THB as carbon source. These results confirmed the result of Gregory *et al.* (2006) who reported that both aerobic and anaerobic microorganism could degrade THB in soil. Decreasing of DHA after 40 days of incubation resulted from decreasing THB substrates as shown in Fig 4-3.

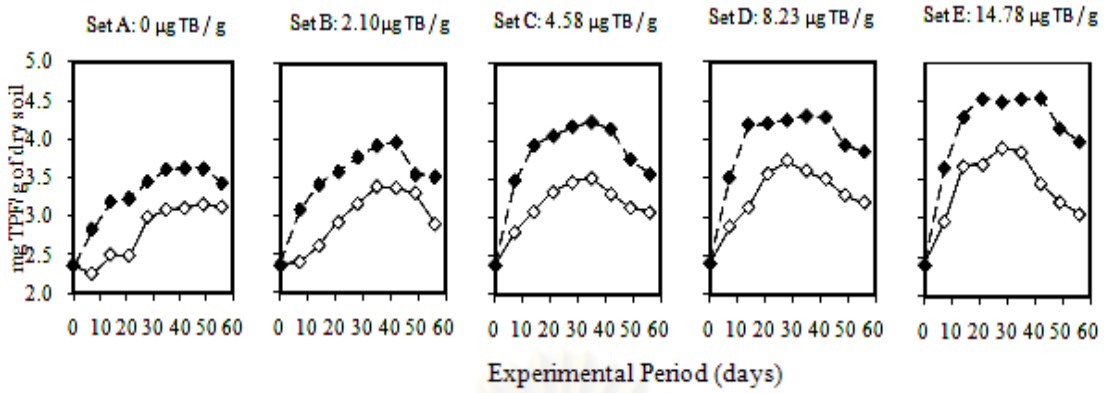


Figure 4-6 DHA of both the controls and the tests. Black legends: tests (with CH_4); white legends: control (without CH_4). Initial NH_4^+ content: 4.444 NH_4^+µg/g soil; initial NO_3^- contents : 7.878 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature : 27-30 ˚C; methane content: 10% (v/v).

4.2 To study the effect of nitrate amendment

4.2.1 Available nitrogen

Fig 4-7 showed the trends of NH_4^+, NO_2^- and NO_3^-contents in controls and tests. Change of NH_4^+ contents were found in tests and control resulting from ammonium oxidation to nitrite, and also utilization by soil microorganisms. It was found that the trend of NO_2^- in controls and tests were similar, and NO_2^- content tended to increase with increase in NO_3^- concentration at concentrations of 27.00-81.43 µg NO_3^-/g. As a result, addition nitrate fertilizer could induce accumulation of NO_2^- , which is very toxic to plant and microorganism in soil. NO_3^- contents of controls were higher than the tests at the concentration of 27.00-81.43 µg NO_3^-/g. The NO_3^- content in controls after adding 27.00-81.43 µg NO_3^-/g were slightly increase while the NO_3^- content in tests decreased. This result suggested that methanotrophs could survive and grow at these conditions, which was confirmed with MOR in the next section. However, at concentration of 100 µg NO_3^-/g, the NH_4^+, NO_2^- and NO_3^- content did not change both control and test indicated that nitrification was completely inhibited by addition of nitrate at 96.30 µg NO_3^-/g.

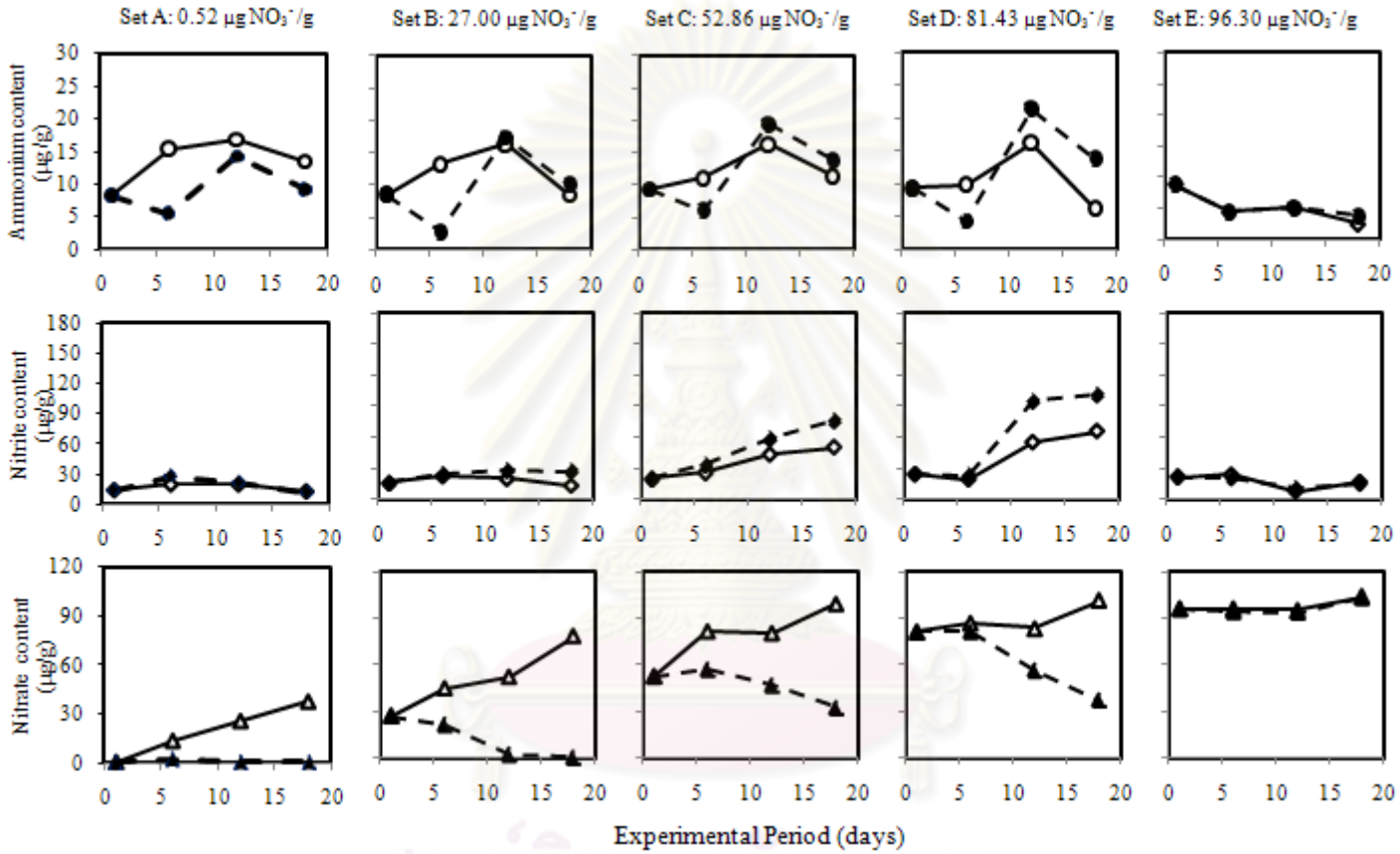


Figure 4-7 Effect of nitrate content on nitrogen available of both poor-methanotrophs soil (white legends) and rich-methanotrophs soil (black legends). THB: 15.32 µg/g; initial NH_4^+: 8.783; moisture content: 10 % (w/w); temperature : 27-30 ˚C; methane content: 10% (v/v).

4.2.2 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

In tests, addition of nitrate fertilizers 27-96 µg NO_3^-/g soil resulted in reduction of MOR, OUR, and CPR (Fig 4-8). The MOR decreased was an impact of toxicity of accumulated nitrite. The large amount of nitrate in soil could reduce nitrite oxidation rate by mass action effect, while nitrite was also continuously produced in nitrification (Chiemchaisri et al., 2001). It can summarize that nitrate addition has an indirect effect on MOR by nitrite accumulation. It was noted that, addition nitrate content at 96.30 µg NO_3^-/g completely inhibited MOR, while OUR and CPR could be measured indicating that some aerobic heterotrophs had activities at this condition.

4.2.3 Thiobencarb biodegradation rate

THB degradation rates in soils having 0.52 and 27 µg NO_3^-/g soil of the tests were significantly higher than the controls resulting from the activity of methanotrophic bacteria (table 4-4). However, increasing in nitrate contents from 53 - 96 µg NO_3^-/g soil decreased degradation rates subsequently increased half-life both controls and tests resulted from decreasing in nitrite oxidation (Fig 4-7) and methane oxidation (Fig 4-8). Addition nitrate induced nitrite accumulation commonly known as microbial toxicity in soil (Dunfield and Knowles, 1995).

Table 4-4 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t_{½}$

Nitrate content (µg NO_3^-/g dried soil)	Rate (K, day^{-1})		r^2		$t_{½}$ (day)	
	Control	Test	Control	Test	Control	Test
0.52	0.042	0.064	0.99	0.99	16.500	10.828
27.00	0.043	0.057	0.93	0.97	16.116	12.158
52.86	0.022	0.024	0.90	0.91	31.500	28.875
81.43	0.013	0.015	0.93	0.96	49.500	46.200
96.30	0.004	0.004	0.94	0.83	173.250	173.250

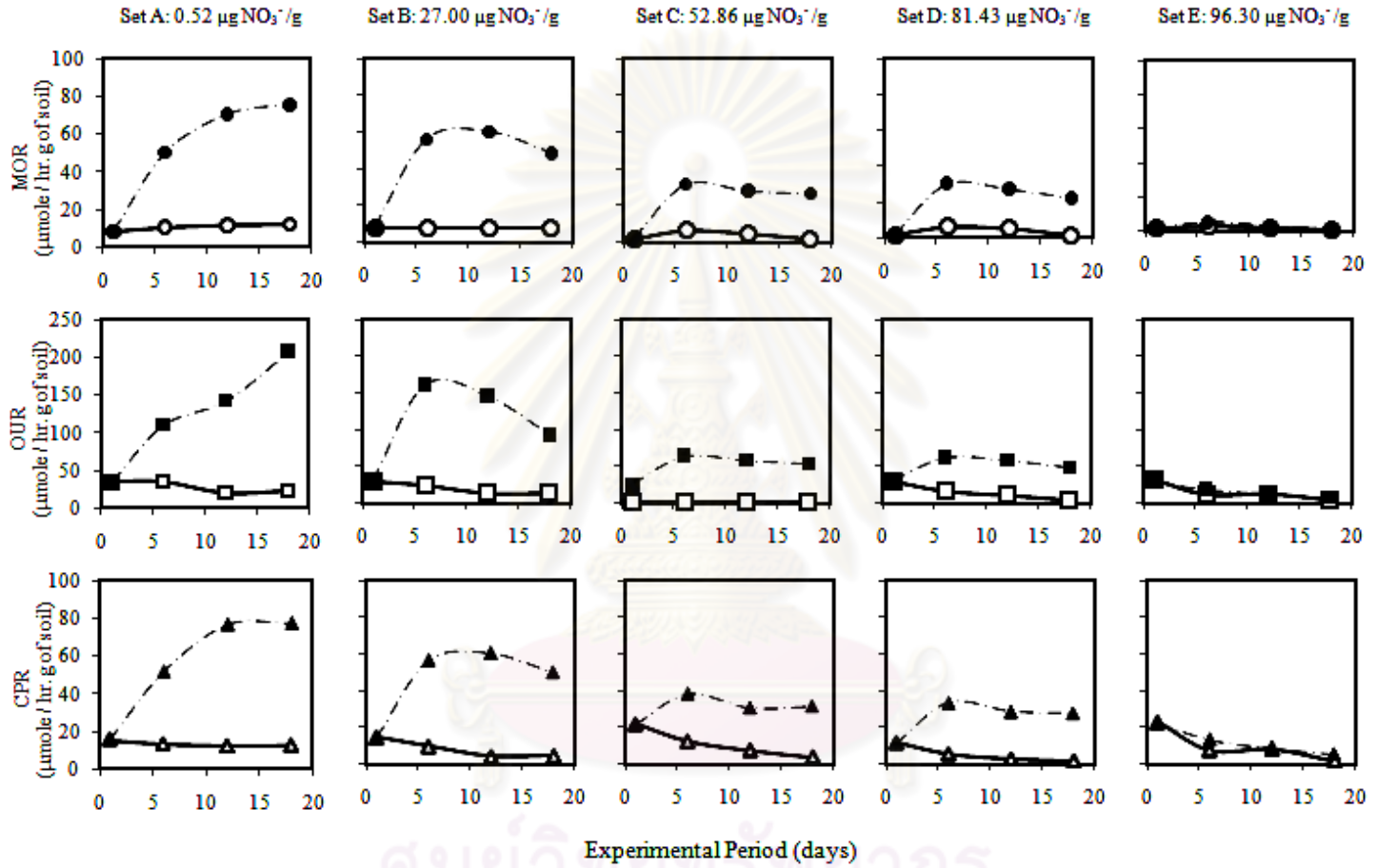


Figure 4-8 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment.

Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 15.32 µg/g; initial NH_4^+: 8.783; moisture content: 10 % (w/w); temperature : 27-30 ˚C; methane content: 10% (v/v).

4.2.4 Dehydrogenase activity (DHA)

The controls, DHA had increased with increasing nitrate contents upto 96.30 μg NO_3^-/g soil. The DHA were not different between controls and tests at 96.30 μg NO_3^-/g (Fig 4-9). In tests, DHA were decreased with increase in nitrate content according to decreased methanotrophic activities. The DHA in methane oxidation include methanol dehydrogenase, formaldehyde dehydrogenase, formate dehydrogenase which may affect by increase in nitrate content, supporting that methanotrophic activity was inhibited subsequently in the reduction of MOR (Fig 4-7).

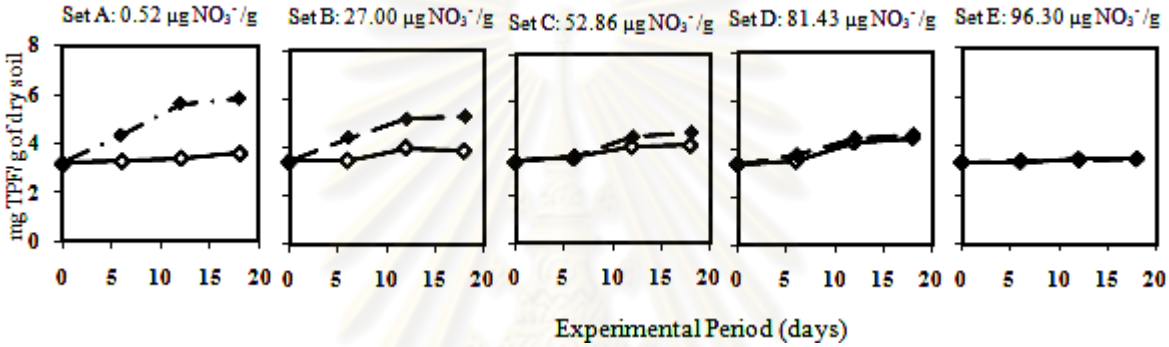


Figure 4-9 DHA of the controls and the tests. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 15.32 μg/g; initial NH_4^+: 8.783; moisture content: 10 % (w/w); temperature: 27-30 ˚C; methane content: 10% (v/v).

4.3 Effect of ammonium amendment

4.3.1 Available nitrogen

In the controls, it was found that addition of urea resulted in increased ammonium content at initial time. After that, the ammonium content had tended to decrease with time while nitrite and nitrate content had tended to increase at all concentration (Fig 4-10), indicated that urea addition at these concentrations (0.58-100.12 μg NH_4^+/g soil) stimulated nitrification. Previous laboratory studies had shown that the rate of nitrification in agricultural soils after ammonium sulfate added depends on the soil properties; increase nitrification rate with increase soil water potential from -1500 to -33 k Pa (Malhi and McGill, 1981). The inhibitory effect of ammonium on nitrification occurs at concentrations of > 300 mg of N/g (Nishio and Fujimoto, 1990). In the tests, the trends of ammonium, nitrite and nitrate contents were almost similar to the controls, except ammonium content ≤ 22.83 μg NH_4^+/g soil. Disappearance of nitrate resulted from

methanotrophic utilization. These results indicated that addition of NH_4^+ content ≤ 25 μg NH_4^+/g soil could promote methanotrophic activity according to optimum nitrate available for methane oxidation. Higher available were found in the test soils when urea were applied ≥ 50 μg NH_4^+/g soil suggesting that methanotrophic activities were reduced as increased ammonium contents.

4.3.2 Effect of ammonium content on methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

In tests, methane oxidation rate had been reduced as increase ammonium contents at all concentrations, however methanotrophs could survive after exposing ammonium upto concentrations 100.12 μg NH_4^+/g soil (Fig 4-11). The highest MOR in tests were observed after addition NH_4^+ at 22.83 μg/g, indicated that optimum addition of ammonium could stimulate methanotrophic growth and activity, correlating with decreased nitrate (Fig 4-10). However, by the fact that NH_4^+ acted as a competitive inhibitor of methane, MOR reduced with increase in NH_4^+ concentration more than 25 μg NH_4^+/g. Moreover, addition of NH_4^+ induced higher NO_2^- accumulation in which nitrite can affect microbial metabolism generally and formate DHA specifically for methanotroph (Schnell and King, 1994). It could be summarized that the optimum of NH_4^+concent for methanotrophic activity was 25 NH_4^+μg/g. When methane content was high but ammonium content was limited, low competitive inhibition of methanotrophs was reported. According to carbon source (as methane) is readily available, methanotrophs may become nitrogen limited (Schimel, 2000).

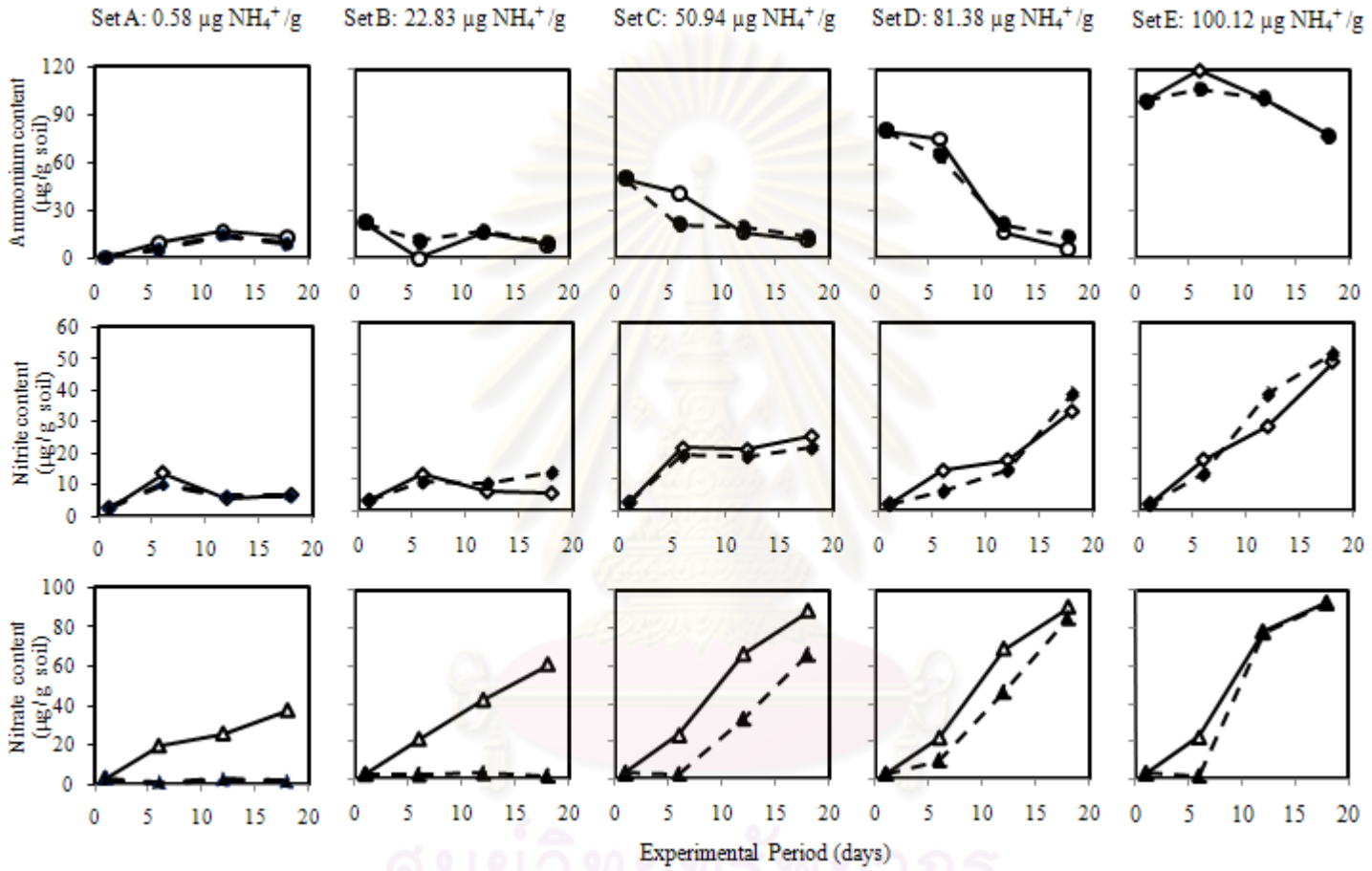


Figure 4-10 Effect of variations ammonium content on nitrogen available of both poor-methanotrophs soil (white legends) and rich-methanotrophs soil (black legends). THB: 15.49 µg/g; initial NO_3^-: 2.90 µg NO_3^-/g; moisture content: 10 % (w/w); temperature : 27-30 ˚C; methane content: 10% (v/v).

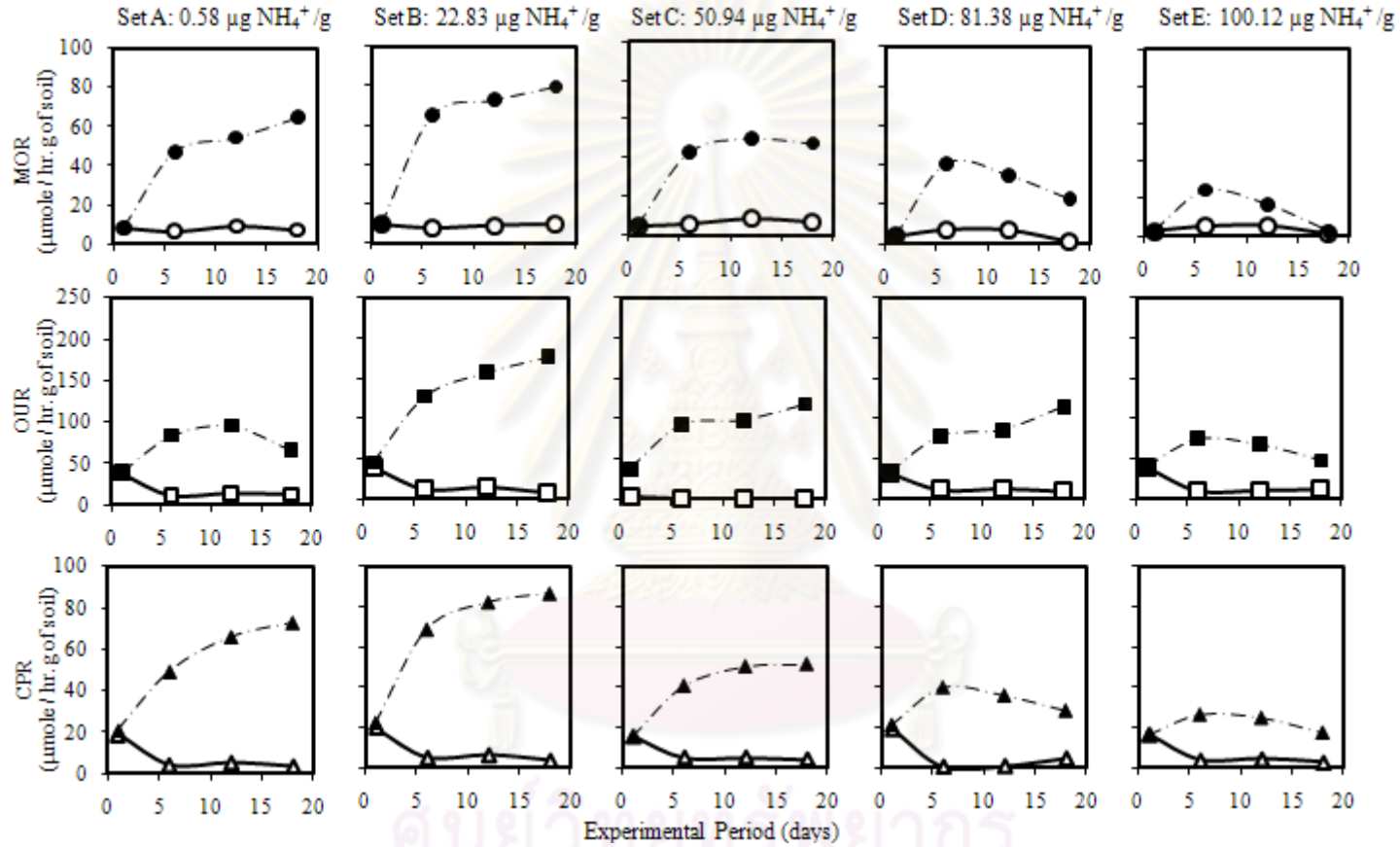


Figure 4-11 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 15.49 µg/g; initial NO_3^-: 2.90 µg NO_3^-/g; moisture content: 10 % (w/w); temperature : 27-30 ˚C; methane content: 10% (v/v).

4.3.3 Thiobencarb degradation rate

In the controls, increasing NH_4^+ contents (0.58 - 81.38 µg NH_4^+/g) could increase degradation rates and reduce half-life (Table 4-5) of THB in the soil as resulted from the activity of nitrifying bacteria and heterotrophs in soil, supported by increasing nitrification (Fig4-10) and DHA (Fig 4-12) which was explained in the next section. Consideration in the tests, increasing NH_4^+ content to ≤ 22.83 µg NH_4^+/g could increase degradation rate and reduce half-life of THB. However, in the test, increasing NH_4^+ content between 51 to 100 µg NH_4^+/g could reduce degradation rates and increase half-life. This resulted from ammonium of methanotrophic bacteria as seen by MOR reduction (Fig 4-11). Therefore, the THB degradation rates of tests were slightly different from the controls for > 50.94 µg NH_4^+/g

Table 4-5 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t_{½}$

Ammonium content ($\mu g\ NH_4^+$/g dried soil)	Rate (K, day^{-1})		r^2		$t_{½}$ (day)	
	Control	Test	Control	Test	Control	Test
0.58	0.048	0.069	0.93	0.93	14.438	10.043
22.83	0.059	0.088	0.94	0.97	11.746	7.875
50.94	0.063	0.079	0.88	0.92	11.000	8.772
81.38	0.051	0.054	0.82	0.87	13.588	12.833
100.12	0.046	0.045	0.83	0.84	15.065	15.400

4.3.4 Dehydrogenase activity (DHA)

In the controls, DHA had increased with increase in NH_4^+ concentrations, while DHA in the tests were decreased when NH_4^+ concentration > 20 µg NH_4^+/g (Fig 4-12) according to formate dehydrogenase of methanotrophs was inhibited (Schnell and King, 1994). This result confirmed that methanotrophic bacteria were inhibited by added NH_4^+ fertilizer > 22.83 µg/g, whereas the growth of nitrifying microbes and heterotrophs

were stimulated according to increasing dehydrogenase activity, NO_2^- and NO_3^- contents in the controls.

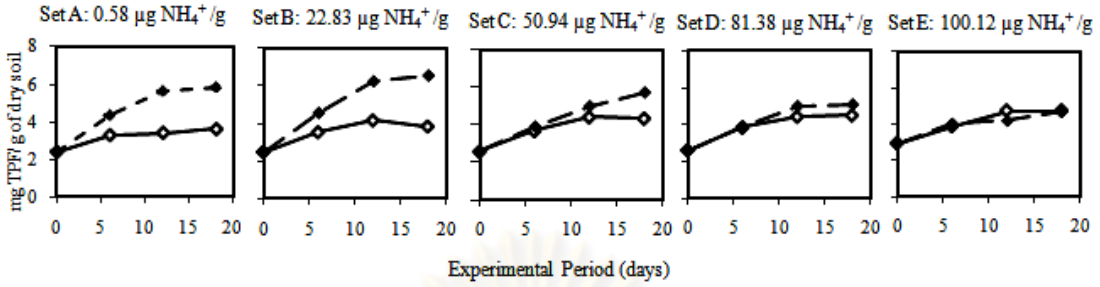


Fig 4-12 DHA of the controls and the tests. Black legends: tests (with CH_4); white legends: controls (without CH_4).THB: 15.49 µg/g; initial NO_3^-: 2.90 µg NO_3^-/g; moisture content: 10 % (w/w); temperature : 27-30 ˚C;
methane content: 10% (v/v).

4.4 Effect of moisture content

4.4.1 Available nitrogen

In control, the NH_4^+ content tended to increase at 5 - 20 % moisture contents while NO_2^- and NO_3^-content increased corresponding to changing ammonium content manifested that the nitrification process occur 5 – 20 % moisture contents (Fig 4-13). Further increase of soil moisture content to 40% inhibited nitrification process, which demonstrated by no NO_3^- production. In tests, the trend of NH_4^+ and NO_2^- were similar to the controls, but NO_3^- content was significant lower than the controls at all moisture contents resulted from methanotrophic utilization.

4.4.2 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

Figure 4-14 demonstrated relationship between MOR and soil moisture content. It was found that MOR increased with increase in soil moisture content upto 20%, with the further increasing (40 % moisture content), MOR decreased with increase in moisture content due the fact that high soil moisture can limit oxygen and methane diffusion between head space and soil (Pokhrel, 1998). The trends of CPR and OUR also corresponded to the MOR both tests and controls. The trends of OUR and CPR slightly increased indicating that the activity of nitrification required low oxygen demand.

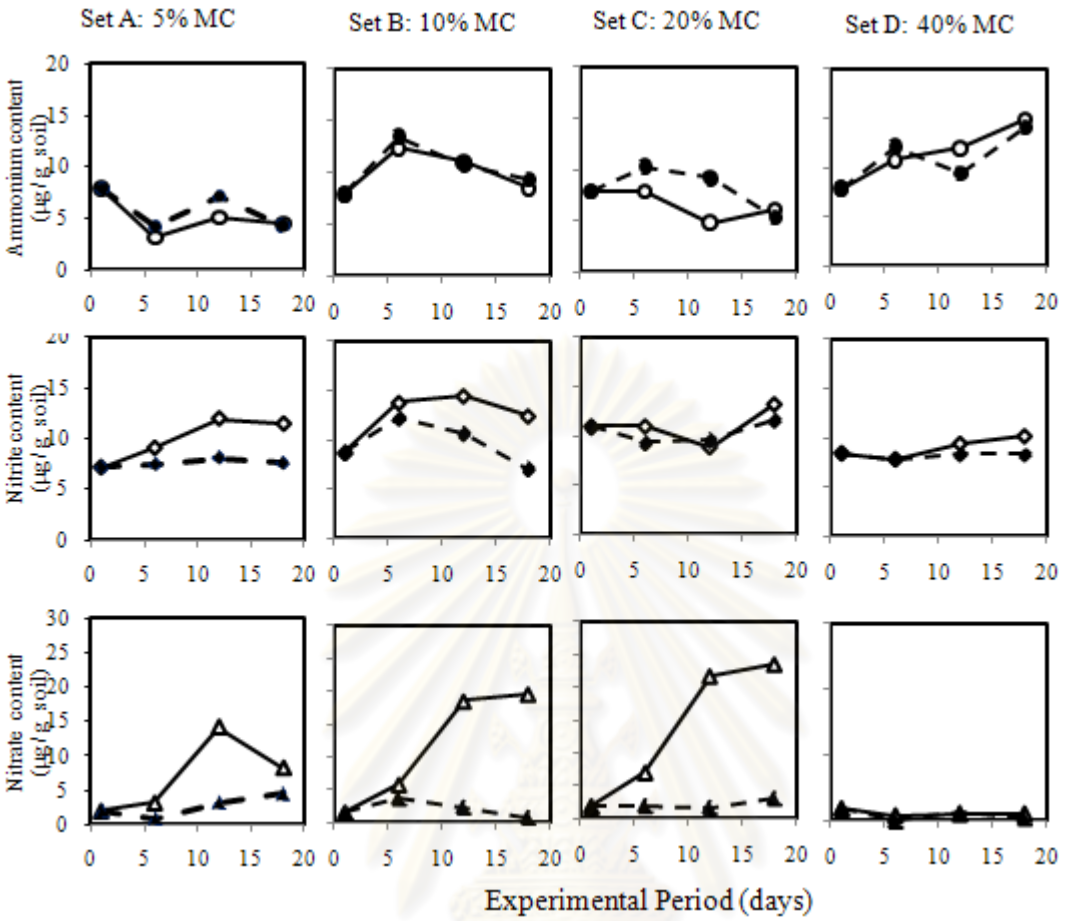


Figure 4-13 Effect of moisture content on nitrogen available of both poor-methanotrophs soil (white legends) and rich-methanotrophs soil (black legends). THB: 15.62 µg/g; NH_4^+ content: 7.90 NH_4^+µg/g soil; NO_3^- contents: 1.82 NO_3^-µg/g soil; temperature : 27-30 ˚C methane content: 10% (v/v).

4.4.3 Thiobencarb degradation rate

The THB degradation rate and the half-life both controls and tests were shown in Table 4-6, and the degradation rate and half life were maximum at moisture content 20%. The results were corresponding to the MOR, OUR and CPR as show in 4.4.2. However, the THB degradation rates of the tests were significantly higher than the controls at moisture contents between 10 and 20 % ($P < 0.05$). It suggested that the optimum moisture content for THB degradation was 20% moisture content. Increasing moisture content to 40%, thiobencarb was degraded by facultative or anaerobic microorganism supported by reducing in OUR (Fig 4-14).

Table 4-6 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t^{1/2}$

Moisture content (%, w/w)	Rate (K, day^{-1})		r^2		$t_{1/2}$	
	Control	Test	Control	Test	Control	Test
5	0.013	0.016	0.95	0.96	53.308	43.313
10	0.04	0.068	0.98	0.99	17.325	10.191
20	0.048	0.078	0.83	0.99	14.438	8.885
40	0.018	0.039	0.96	0.88	38.500	17.769

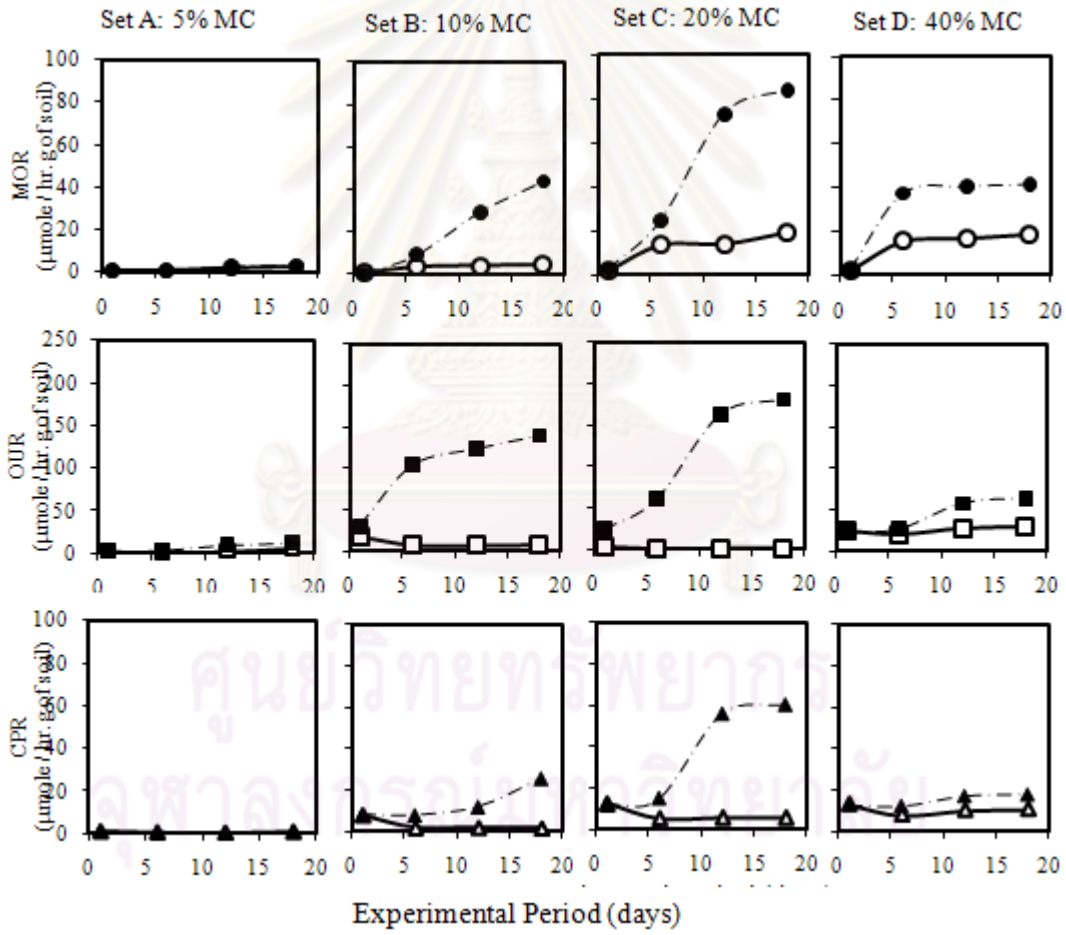


Figure 4-14 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 15.62 µg/g. NH_4^+ content: 7.90 NH_4^+µg/g soil; NO_3^- contents: 1.82 NO_3^-µg/g soil; temperature : 27-30 ˚C; methane content: 10% (v/v).

4.4.4 Dehydrogenase activity (DHA)

In the tests and controls, DHA were increased with increasing moisture contents upto 20 % according to high moisture could bring soluble organic matters into solution, which might be enhance bacterial growth (Subhani *et al*., 2001). Future increasing, DHA of the test was reduced according to lack of O_2 and CH_4 diffusion for methanotrophic growth, while DHA of the control slightly increased due to the fact that the most dehydrogenase enzyme are of anaerobic origin (Subhani *et al*., 2001). It also indicating that heterotrophic bacteria can survive in this condition. The DHA of the tests were higher than the control at 5 - 40%, at the maximum was at 10 %.

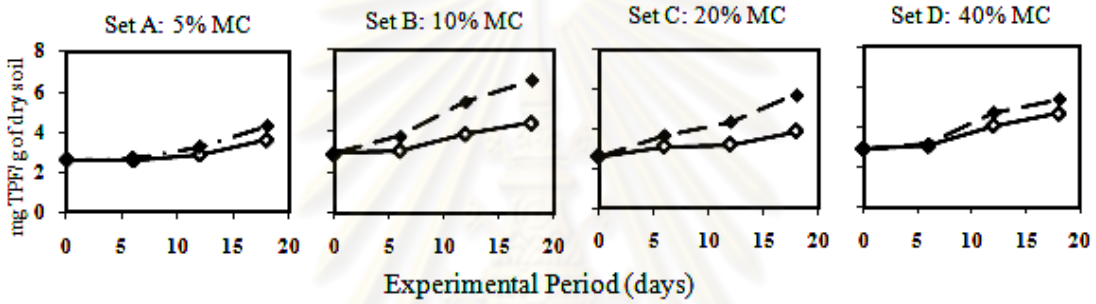


Figure 4-15 DHA of both the controls and the tests. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 15.62 µg/g. NH_4^+ content: 7.90 NH_4^+µg/g soil; NO_3^- contents: 1.82 NO_3^-µg/g soil; temperature : 27-30 ˚C; methane content: 10% (v/v).

4.5 Effect of temperature

4.5.1 Available nitrogen

At 20◦C, NO_3^- content of controls were the lowest, which explained by inactivated enzyme. The highest of NO_3^- content was observed in temperature during 27 and 30◦C (Fig 4-16). With increase of temperature to 40 ◦C, NO_3^- content was lower when compared with the NO_3^- content of soil incubated at 30◦C. This result is similar to the result of Bhaskar and Charyulu (2005) who reported that the optimum temperature for growth of Nitrosomonas and Nitrobacter bacteria is 30 ◦C. The trends of NH_4^+ and NO_2^- contents of controls and tests were similar, but NO_3^- content of tests was lower than controls at all temperature due to methanotrophic utilization, which was confirmed with the increase of MOR (Fig 4-17).

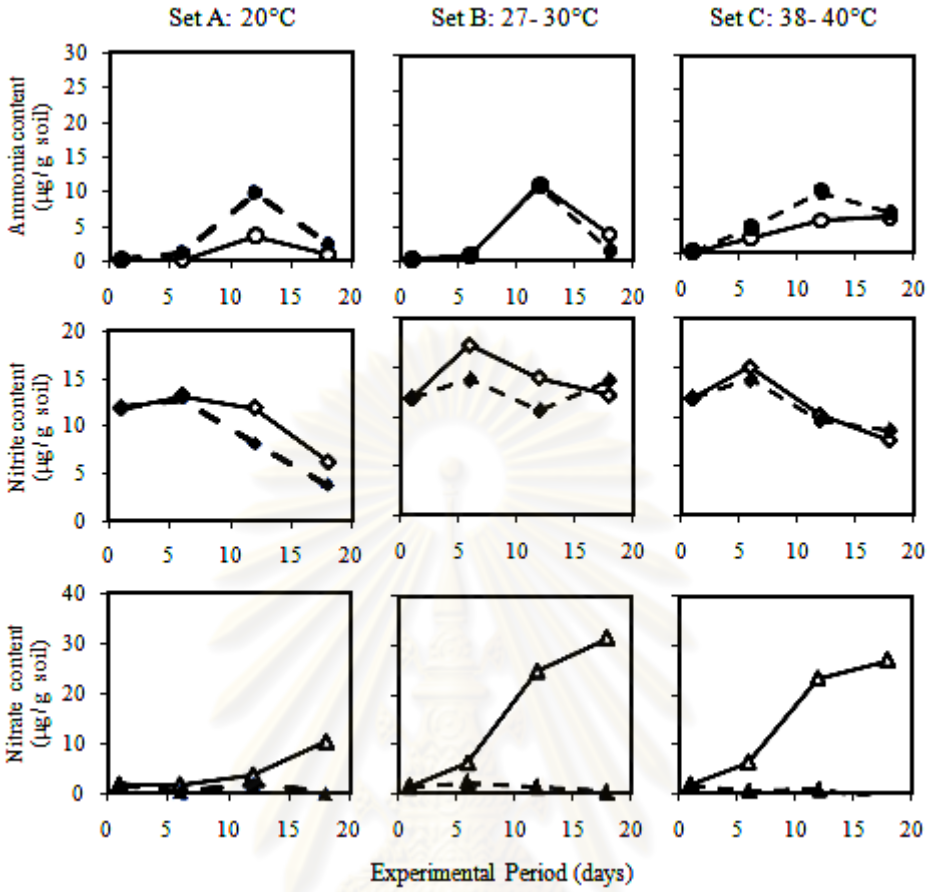


Figure 4-16 Effect of temperature on nitrogen available of both poor-methanotrophs soil (white legends) and rich-methanotrophs soil (black legends). THB: 14.91 µg/g; NH_4^+ content: 0.26 NH_4^+µg/g soil; NO_3^- contents : 1.82 NO_3^-µg/g soil; moisture content: 10% (w/w); methane content: 10% (v/v).

4.5.2 Effect of temperature on methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

MOR of controls were slightly observed at all temperature implying that methanotrophic bacteria could survive at these temperature (20, 27-30, 38-40◦C), although carbon source was equal during incubating time. In tests, MOR tended to increase with rising temperature implying that methanotrophic activity was stimulated with increasing temperature from 20 ◦C upto 40◦C, and the highest MOR occurred between 38 and 40◦C. The amounts of OUR and CPR of the tests were higher than the controls according to methanotrophic activity, and the OUR of the controls obviously

increased with rising temperature upto 40°C indicated that aerobic heterotrophs were also stimulated their activity by catalyzing their enzyme.

4.5.3 Thiobencarb biodegradation rate

The trends of THB degradation rate of controls and tests were similar. The degradation rates were maximum and the half-lives were minimum at 40°C (Table 4-7), but that of controls were lower than of the tests at all temperatures (20-40°C) resulting from methanotrophs activity supporting by increasing MOR (Fig4-16). For the controls, the THB degradation rate increased with increasing temperature. The degradation products were similar to the experiment 4.1.

Table 4-7 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t_{1/2}$.

Temperature	Rate (K, day^{-1})		r^2		$t_{1/2}$ (day)	
(°C)	Control	Test	Control	Test	Control	Test
20	0.015	0.018	0.94	0.99	46.200	38.500
27-30	0.041	0.06	0.99	0.96	16.902	11.550
38-40	0.071	0.084	0.97	0.98	9.761	8.250

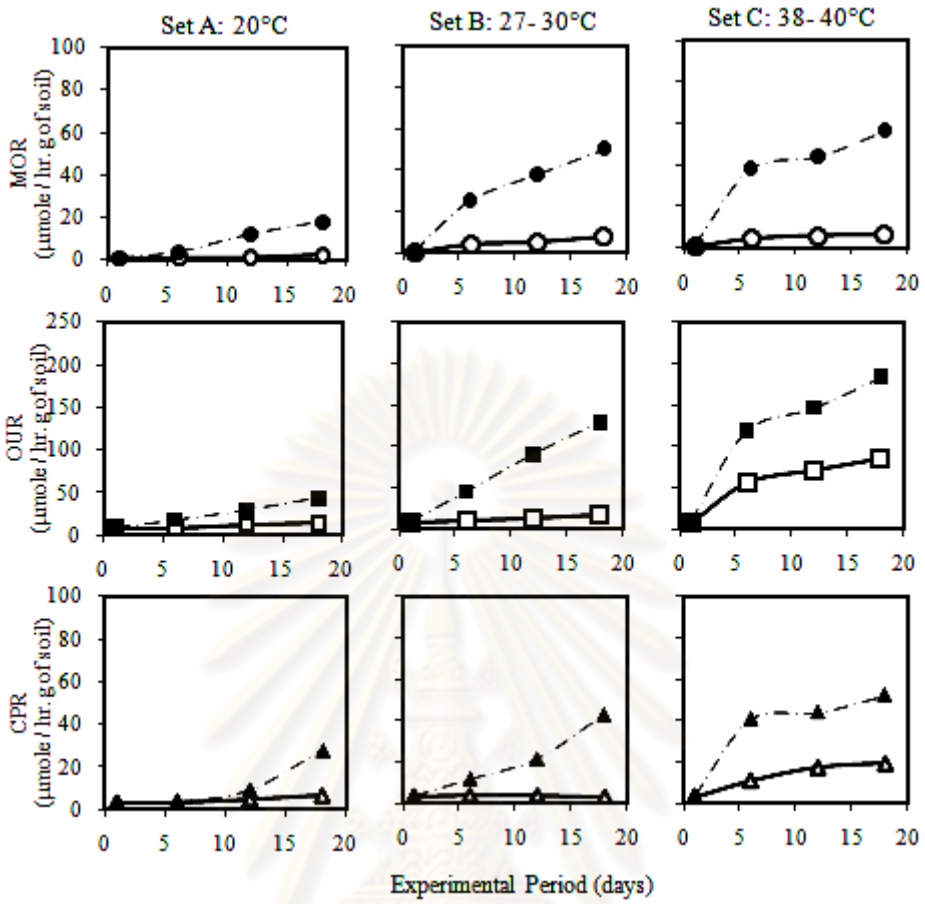


Figure 4-17 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 14.91 µg/g; initial NH_4^+ content: 0.26 NH_4^+µg/g soil; initial NO_3^- content : 1.82 NO_3^-µg/g soil; moisture content: 10% (w/w); methane content: 10% (v/v).

4.5.4 Dehydrogenase activity (DHA)

Both in the controls and tests, the trends of DHA increased with increased temperature (20-40◦C) (Fig 4-18) implying that increasing temperature upto 40◦C slightly stimulated the activity of heterotrophic bacteria. The DHA of tests were higher than the controls resulting from methanotrophic activity. It could be summarized that increasing temperature to 40◦C could stimulate several microbial activities in soils. Despite the rate of enzyme-catalyzed reaction increase as the temperature increase, most soil enzymes are inactivated (denatured) when temperature is rise to 60 - 70 ˚C (Tabatabai,1982).

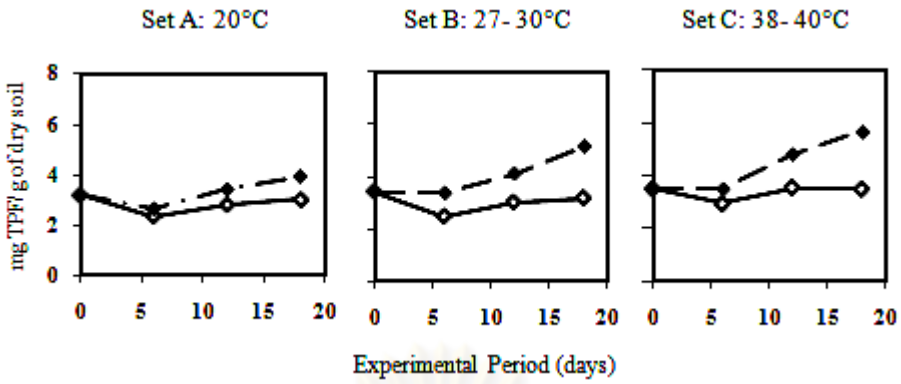


Figure 4-18 DHA of both the controls and the tests. Black legends: tests (with CH_4); white legends: controls (without CH_4). THB: 14.91 μg/g; NH_4^+ content: 0.26 NH_4^+μg/g soil; NO_3^- contents: 1.82 NO_3^-μg/g soil; moisture content: 10% (w/w); methane content: 10% (v/v).

4.6 Methane content

4.6.1 Available nitrogen

Previous study reported that methane acted as competitive inhibitor of the process that transform NH_4+ to NO_2^- by co-metabolism of nitrifying bacteria (Bedard and Knowles, 1989). In contrast, the result of this study demonstrated that NO_2^- contents of all methane content were slightly different indicating that methane content did not inhibit process transformation of transform NH_4+ to NO_2^-. NO_3^- content obviously decreased with increasing in methane content implying that NO_3^- was utilized for methanotrophic growth supporting by MOR (Fig 4-20).

4.6.2 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production (CPR)

MOR tended to increase with rising CH_4 concentration due to the fact that methanotrophic bacteria utilize methane as their soil carbon source. The highest MOR occurred in the present of 10 % methane content. The trends of OUR and CPR were corresponding to the trend of MOR. The CPR was also increased according to product related with substrate.

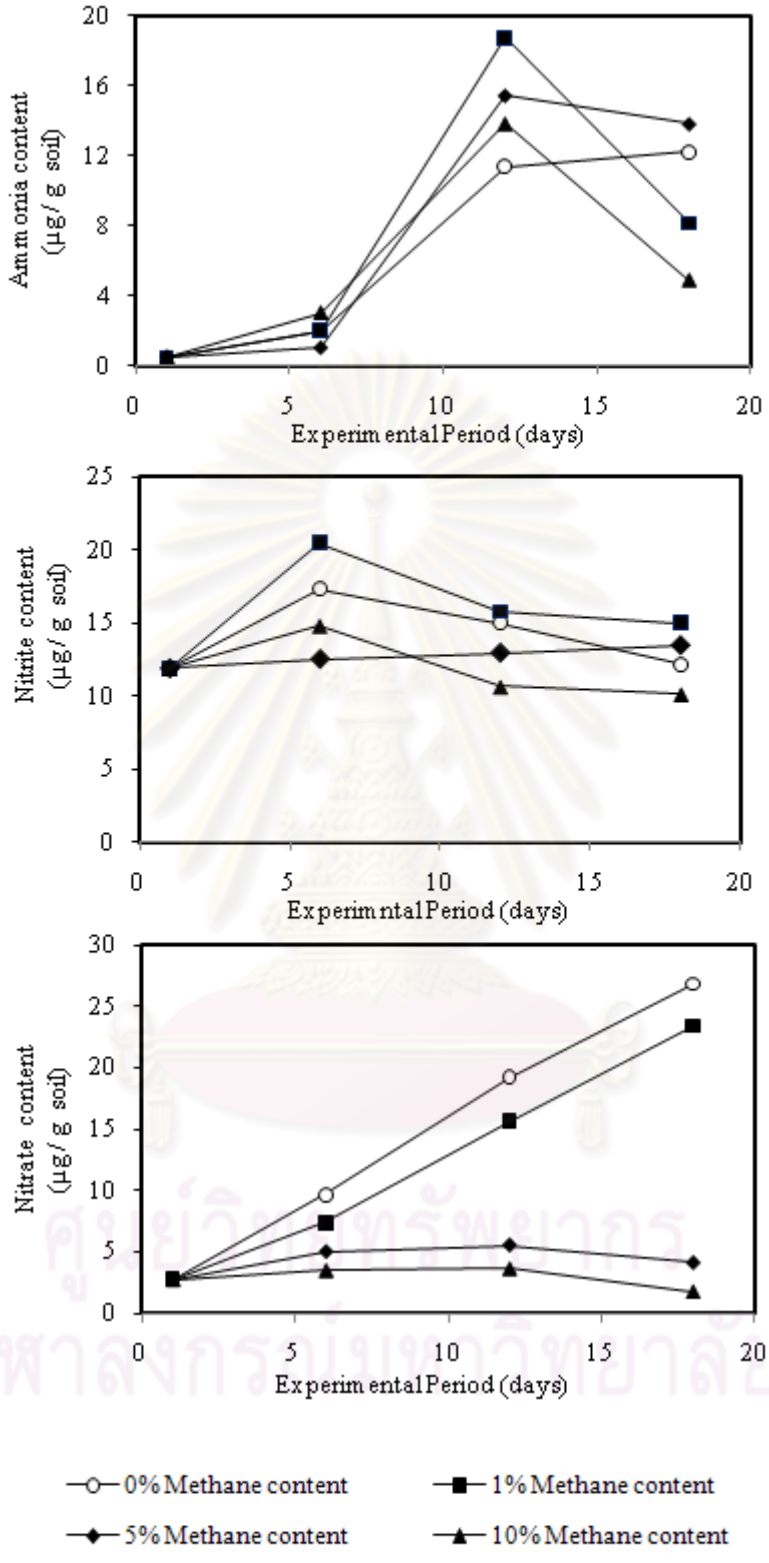


Figure 4-19 Effect of methane content on nitrogen available. THB: 14.91 µg/g; initial NH_4^+ content: 0.481 NH_4^+µg/g soil; initial NO_3^- contents : 2.793 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature : 27-30 ˚C.

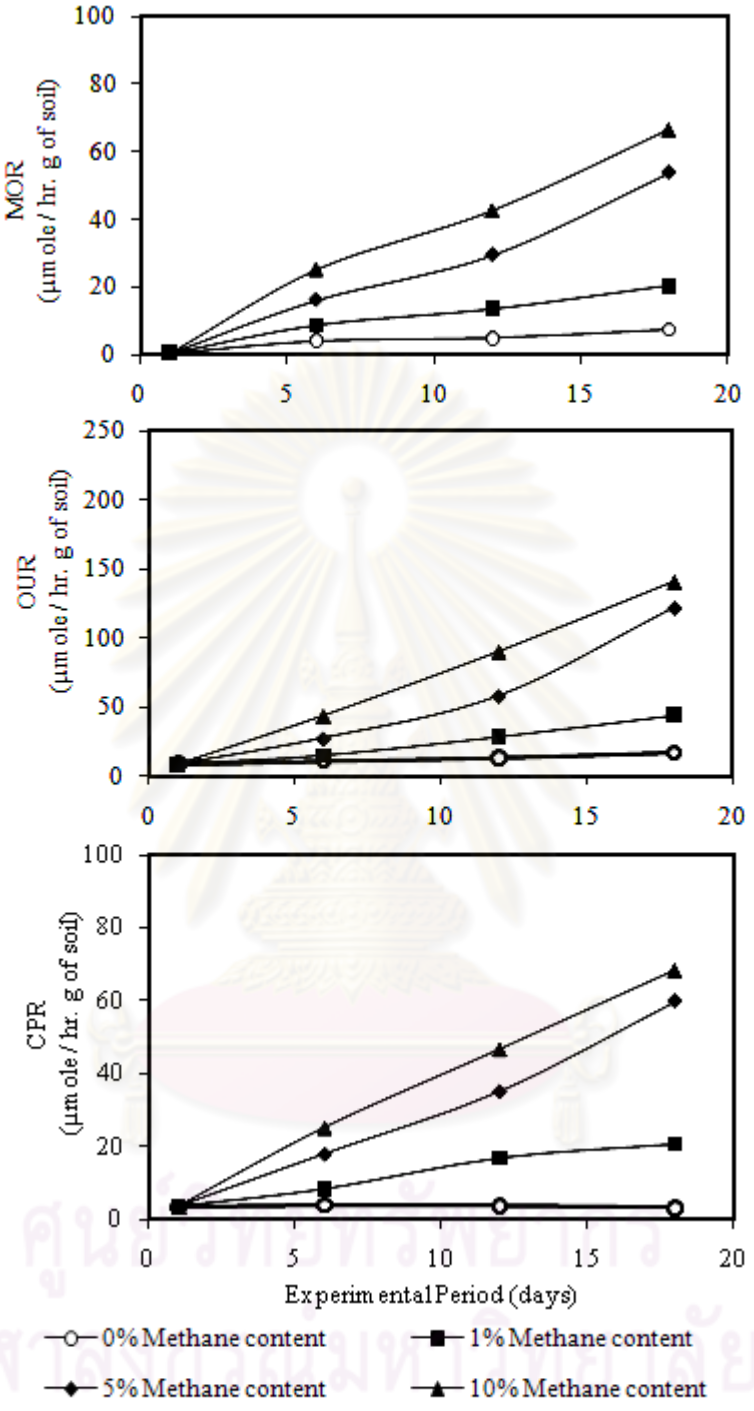


Figure 4-20 Methane oxidation rate (MOR), oxygen uptake rate (OUR) and carbon dioxide production rate (CPR) in the experiment. THB: 14.91 µg/g; initial NH_4^+ content: 0.481 NH_4^+µg/g soil; initial NO_3^- contents : 2.793 NO_3^-µg/g soil; moisture content: 10% (w/w); temperature : 27-30 ˚C.

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4.6.3 Thiobencarb (THB) degradation rate

The THB degradation rates were increased and the half-lives were reduced with increasing in methane content resulting from the activity of methanotrophic bacteria, supporting by increasing MOR (Fig 4-20). Previous studies have reported that one rice field had relatively high flux and the other two had relatively low fluxes, depending on the chemical composition of the soils (Le Mer and Roger, 2001). In addition, vary of methane emission is emitted at the same filed, depending on the environmental conditions. As a result, thiobencarb was more rapidly degrade at the field soil where high methane was emitted than low methane. The degradation products were similar to the experiment 4.1.

Table 4-8 Kinetic constant of degradation of thiobencarb (*k*), determination coefficient (r^2) and $t_{½}$

Methane content (%, v/v)	Rate K (day^{-1})	r^2	$t_{½}$ (day)
0	0.043	0.95	16.116
1	0.046	0.99	15.065
5	0.055	0.99	12.600
10	0.063	0.98	11.000

4.6.4 Dehydrogenase activity (DHA)

The figure demonstrated that the trend of DHA increased with increasing in methane concentration according to increase in methanotrophic activity (Fig 4-21). As a result, methane content could stimulate heterotrophic bacteria especially methanotrophic bacteria.

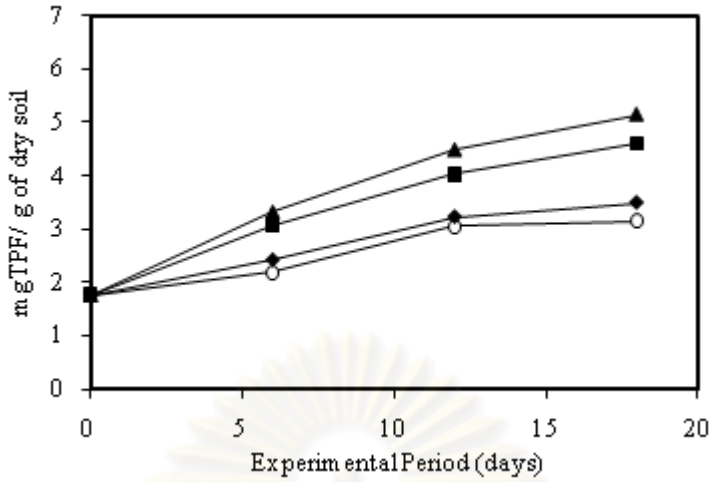

Figure 4-21 The effect of methane content on DHA. THB: 14.91 μg/g; initial NH_4^+ content: 0.481 NH_4^+μg/g soil; initial NO_3^- content: 2.793 NO_3^-μg/g soil; moisture content: 10% (w/w); temperature : 27-30 ˚C.

4.7 Relevancy to rice field

Consideration, in the cultivate practice; the condition of rice field is separated to four condition during a crop cycle. The first condition of wet-seeded rice, THB is applied pre-flooded after seeding 5 - 7 days, of which the soil moisture content is around 10 - 20 %, but methane content is low. The second condition, field is flooded, then the soil moisture content is more than 40 %. Methane content is high. The third conditions, the fields are drained before and after fertilizing 1-2 days, then the soil moisture content is around 10 - 20%. 30 - 50 μg NH_4^+ of urea fertilizer is applied, and the methane content is still high. The forth conditions, when flowering is complete and the grains have grown, water is drained from the rice field before harvest 7 - 14 days in order to let the rice grains mature, then the soil moisture content is around 10 – 20 %. The methane content is still high.

According to cultivate practice, the maximum degradation rate of THB may be high possible occurring at the third conditions; the water is drained and urea fertilizer is applied. Based on methane available of which promotion of methanotrophic activity, the degradation rates of THB of the first, second, and fourth conditions may be approximate 58 % 49 %, and 80% of the maximum degradation rate of the third conditions, respectively (Appendix A). Besides, the degradation rate of THB depends on season; the degradation rates of THB in winter and rain may be 21 % and 71 % of the THB degradation rate in summer.

On the other hand, the maximum degradation rate of THB occurred; the efficiency of controlling weed in rice field is reduced. The herbicide is applied again to control weed in rice field. As a result, the cost of rice production and THB residue will be increased

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Effect of thiobencarb content

Additional THB could enhance ammonification subsequently in stimulation of nitrification. Methanotrophs could gain nitrate from nitrification for their methane oxidation and thiobencarb co-metabolism consequently in degradation rates of thiobencarb in methanotrophs-rich soils were higher than methanotrophs-poor soils. Increasing thiobencarb concentrations upto 15 µg TB/g resulted in increasing degradation rate and reducing half-life of thiobencarb. In absent methane, DHA of heterotrophs was activated by application of thiobencarb. The degradation rate of THB in soil with/without methane was first order with respect of thiobencarb concentrations.

5.1.2 Effect of nitrate content

Addition nitrate fertilizer (27.00-96.30 µg NO3-/g) could induce accumulation of nitrite, which is very toxic to microorganism in soil in, and addition nitrate content ≥ 96.30 µg NO3-/g completely inhibited nitrification. Nitrate addition posed adverse effect on methane oxidation according to nitrite accumulation. As a result, increasing nitrate content resulted in decreasing degradation rate and increasing half-life of thiobencarb of methanotrophs-rich soils.

5.1.3 Effect of ammonium content

Ammonium fertilizer (0.58-100.12 µg NH_4^+/g) could stimulate nitrification. Addition ammonium concentration of ≤ 25 µg NH_4^+/g could stimulate methanotrophic activity, with further increasing ammonium concentration, NH_4^+ acted as competitive inhibitor for methanotrophs. Increasing ammonium content (0.58-81.83 µg NH_4^+/g) increased degradation rate and reduced half-life of THB in methanotrophs-poor soils, while increasing NH_4^+ content ≤ 25 µg NH_4^+/g also increased degradation rate and reduced half-life in methanotrophs-rich soils. DHA of methanotroph-rich soil was decreased according to formate dehydrogenase of methanotrophs was inhibited.

5.1.4 Effect of moisture content

The optimum of moisture content for nitrification and methane oxidation rate was 20%, and increasing in moisture content ≥ 20% reduced methane oxidation rate and inhibited nitrification resulting from low O2 diffusion. The maximum degradation rate and the minimum half-life were observed at 20 % moisture content in methanotrophic-rich soil. In contrast, the DHA was stimulated by increasing in moisture content.

5.1.5 Effect of temperature

Rising temperature from 20 °C upto 30°C increased nitrification, and the maximum methane oxidation rate was observed at 40°C. Increasing temperature to 40°C could stimulate several microbial activities in soil, then the maximum THB degradation rate and THB half-live were observed at 40°C.

5.1.6 Effect of methane content

The concentration of methane did not disturb ammonification and nitrification, while increasing in methane content (1 - 10 %) increased MOR and DHA of heterotroph. The degradation rates were increased and the half-lives were reduced with increasing in methane content. The maximum THB degradation rat was observed at 10% of methane content.

5.1.7 Summary

THB can be degraded by several microorganisms in soil. Methanotrophic bacteria may involve in degradation of THB in soil. The concentration of THB can not applied more than recommendation rate (~5 µg THB/g soil), although addition THB can stimulate methanotrophic activity, according to the toxicity of THB to the growth of rice. Small NO3- additions causing by far the greater reduction in MOR compared to the result of addition NH4+. Addition urea fertilizer at recommendation rate (~20 NH4+µg/g soil) can stimulate methanotrophic activity and reduce half-live of THB in soil. The degradation rate of THB in oxidative soil is higher than reductive soil. Temperature is the most important factor to induce THB degradation in soil; the degradation of THB on summer is the highest. The optimum condition, which gave

maximum degradation rate obtained from this study: the concentration of thiobencarb was 15 µg/g, no addition nitrate fertilizer, ammonium content was 23 µg NH4+/g, moisture content was 20 %, temperature was 38 - 40° C, and 10 % of methane content.

Table 5.1 The degradation rate of all conditions

Condition		THB degradation rate (K, day^{-1})		Remarks
		Control (without CH_4)	Test (with CH_4)	
THB content (µg THB/g soil)	2.1	0.042	0.049	Initial NH_4^+ content: 4.44 NH_4^+µg/g soil; initial NO_3^- contents : 7.88 NO_3^-µg/g soil; moisture content: 10% (w/w); Temp: 27-30 ° C; methane content: 10% (v/v).
	4.58	0.052	0.062	
	8.23	0.053	0.067	
	14.78	**0.056**	**0.075**	
NO_3^- content (NO_3^-µg/g soil)	0.52	0.042	**0.064**	THB conc.: 15.32 µg/g; initial NH_4^+: 8.783; moisture content: 10 % (w/w); Temp: 27-30 ° C; methane content: 10% (v/v).
	27.00	**0.043**	0.057	
	52.86	0.022	0.024	
	81.43	0.013	0.015	
	96.30	0.004	0.004	
NH_4^+ content (NH_4^+µg/g soil)	0.58	0.048	0.069	THB conc.: 15.49 µg/g; initial NO_3^-: 2.90 µg NO_3^-/g; moisture content: 10 % (w/w); Temp: 27-30 ° C ; methane content: 10% (v/v).
	22.83	0.059	**0.088**	
	50.94	**0.063**	0.079	
	82.38	0.051	0.054	
	100.12	0.046	0.045	
Moisture content (%, w/w)	5%	0.013	0.016	THB conc.: 15.62 µg/g; NH_4^+ content: 7.90 NH_4^+µg/g soil; NO_3^- contents: 1.82 NO_3^-µg/g soil; Temp: 27-30 ° C; methane content: 10% (v/v).
	10%	0.040	0.068	
	20%	**0.048**	**0.078**	
	40%	0.018	0.039	
Temperature (° C)	20	0.015	0.018	THB conc.: 14.91 µg/g; NH_4^+ content: 0.26 NH_4^+µg/g soil; NO_3^- content: 1.82 NO_3^-µg/g soil; moisture content: 10% (w/w); methane content: 10% (v/v).
	27-30	0.041	0.060	
	38-40	**0.071**	**0.084**	
Methane content (%, v/v)	0%	0.043	-	THB conc.: 14.91 µg/g; initial NH_4^+ content: 0.481 NH_4^+µg/g soil; initial NO_3^- content: 2.793 NO_3^-µg/g soil; moisture content: 10% (w/w); Temp: 27-30 ° C.
	1%	-	0.046	
	5%	-	0.055	
	10%	-	**0.063**	

5.2 **Recommendations for future study**

Pure culture of methanotrophic bactiral should be used to indicate that which process metanotrophic bacteria involve in THB degradation. According to more than two types of herbicides are applied in the real situation to increase the efficiency of the controlling weed in paddy field, THB should be mixed with another herbicide to study the effect of the mixed herbicide on microbial activities.

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APPENDICES

APPENDIX A

1. Calculation of Gas Concentration

1.1 Conversion Gas (%) to Gas (mole)

Experimental conditions:

Bottle volume: 25 ml, soil wet weight: 3 g, %WC = 10%,

Soil dry weight of soil = 3 g - (3 g x 0.10) = 2.7 g

Density of soil : 1.22 g/ml, soil volume (3g/(1.22g/ml)) = 2.46 ml

Air remaining 25-2.46 ml= 22.54 ml

Temperature: 30^{o}C

Assume the pressure inside the bottles equals to 1 atm

In closed system, it can calculate mole of gas from the formula:

$$PV = nRT$$

Where, P: partial pressure or %gas

R = 82.06 atm.ml/g.mol.K

K= 273+30=303 K

n= mole of gas

Then: Mole of gas = (PV/RT)

Mole of CO2 (mole) = (PV/RT)

= (A%)(22.54 ml)/(82.06 atm.ml.mol-1.K-1)(303K)

= 9.07 x 10^{-4}(A%)

Mole of O2 (mole) = (PV/RT)

= (B%)(22.54 ml)/(82.06 atm.ml.mol-1.K-1)(303K)

= 9.07 x 10^{-4} (B%)

Mole of CH4 (mole) = (PV/RT)

= (C%)(22.54 ml)/(82.06 atm.ml.mol-1.K-1)(303K)

= 9.07 x 10^{-4} (C%)

It can obtain the conversion factors for each gas component.

Mole of CO2 (µmole) = 9.07 x 10^{-4} x 10^{6}(A%) = 9.07 x 10^{2} / (A/100) = 9.07 (A)

Mass of O2 (µmole) = 9.07 x 10^{-4} x 10^{6} (B%) = 9.07 x 10^{2} / (B/100) = 9.07 (B)

Mass of CH4 (µmole) = 9.07 x 10^{-4} x 10^{6} (C%) = 9.07 x 10^{2} / (C/100) = 9.07 (C)

2. Calculation of Methane Oxidation Rate, Cabon Dioxide Production Rate and Oxygen Uptake Rate

Plot a graph between gas changes with time

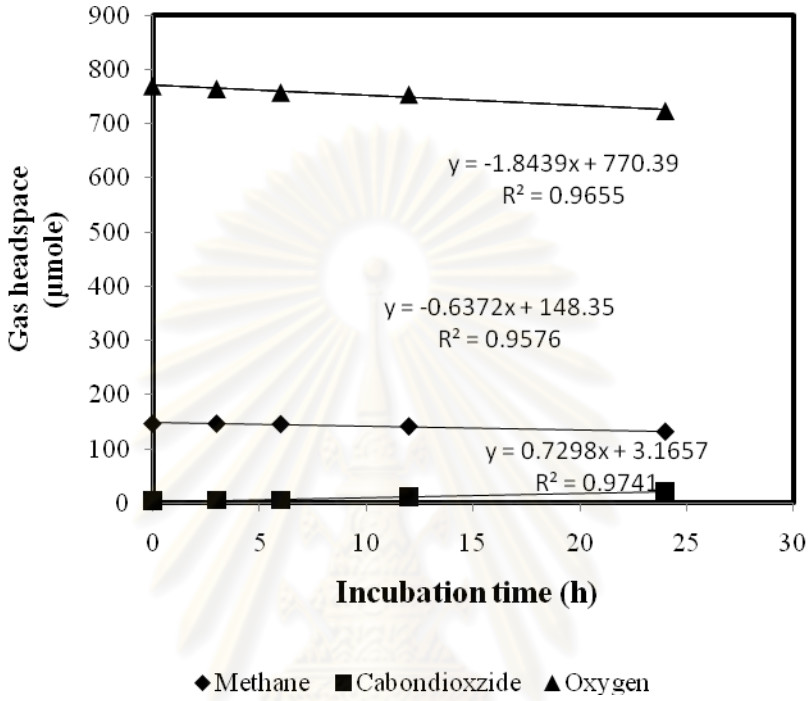

Figure A-1 Change of gas headspace with time

From a graph, it can obtain activity rate from a slope of curve,

Soil dry weight = 2.7 g

Methane oxidation rate = 0.6372 µmole/h
= 0.6372 µmole/ 2.7 g dry soil.h
= 0.24 µmole/ dry soil.h

Carbon dioxide prodution rate = 0.7298 µmole/h
= 0.7298 µmole/ 2.7 g dry soil.h
= 0.27 µmole/ dry soil.h

Oxygen uptake rate = 1.8439 µmole/h
= 1.8439 µmole/ 2.7 g dry soil.h
= 0.68 µmole/ dry soil.h

3. Calculation of Half-live

Plot graph between the changes of thiobencarb concentration with time in log scale

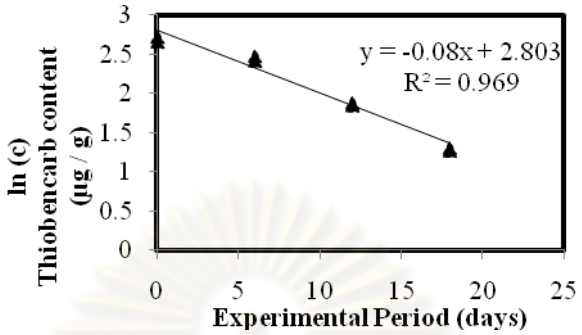

Figure A-2 Change of thiobencarb concentration with time

From a graph, it can obtain rate constant (k) from a slope of curve,

Half-live formula: $t^{½}$ = ln 2/k

= 0.693/0.08 day^{-1}

= 8.66 day

4. Calculation of Thiobencarb Concentration

Density of soil is 1220.17 kg/m^3

Recommended concentration is 1.67 ml/m^3

= 1.67 ml/m^3 / (1220.17 kg/m^3 x 10^3 g/kg)

= 1.37 x 10^{-6} ml/g wet soil

Assume wet soil obtain 80 % moisture content:

1 g of soil dry weight = 1 g of soil wet weight - (1 g x 0.8) = 0.2

Then: thiobencarb concentration: 1.37 x 10^{-6} ml/g wet soil / 0.2 g dry soil/g wet soil

= 6.85 x 10^{-6} ml/g dry soil

The solution of thiobencarb is 80% w/v EC: 100 ml of thiobencarb solution contain 80 g ai

Then: recommended thiobencarb active ingredient: 6.85 x 10^{-6} ml/g dry soil x 0.8

= 5.48 x 10^{-6} ml of ai/ g dry soil

= 5.48 x 10^{-3} µl of ai/ g dry soil

Thiobencarb density is 1179 g/l, then recommended thiobencarb active ingredient:

= 5.48 x 10^{-3} µl of ai/ g dry soil x 1179 g/l x 10^{-6} l/µl

= 6.46 g ai/g dry soil

5. To compare degradation rate during the crop cycle

For the first conditions,

Assume THB content = 15 μg/g soil

Moisture content = 10 %

Methane content = 1%

From Table 4-8, the degradation rate of THB is 0.046 day^{-1}

For the second conditions,

Assume THB content = 15 μg/g soil

Methane content = 10 %

Moisture content = 40 %

From Table 4-6, the degradation rate of THB is 0.039 day^{-1}

For the third conditions,

Assume THB content = 15 μg/g soil

Methane content = 10 %

Moisture content = 10 %

Ammonium content = 51 μg NH_4^+/g soil

From Table 4-5, the degradation rate of THB is 0.079 day^{-1}

For the forth conditions,

Assume THB content = 15 μg/g soil

Methane content = 10 %

Moisture content = 10 %

From Table 4-8, the degradation rate of THB is 0.063 day^{-1}

5.1 To compare the degradation rate between the first conditions and the third conditions

The % degradation rate of first periods = (0.046 x 100)/0.079

= 58 % of the third conditions

5.2 To compare the degradation rate between the second conditions and the third conditions

The % degradation rate of third periods = (0.039 x 100)/0.079

= 49 % of the third conditions

5.3 To compare the degradation rate between the forth conditions and the third conditions

The % degradation rate of third periods = (0.063 x 100)/0.079

= 80 % of the third conditions

APPENDIX B

Table B-1 The effect of thiobencarb content on ammonium available during experimental period

Day	Ammonium available (μgNH_4^+/g dried soil)									
	0 µgTB/g soil		2.10 µgTB/g soil		4.58 µgTB/g soil		8.23 µgTB/g soil		14.78 µgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	5.051	5.051	4.545	4.545	4.293	4.293	4.545	4.545	3.788	3.788
7	6.869	6.465	2.626	2.020	1.414	1.616	2.020	1.818	2.828	4.021
14	13.247	14.903	18.215	23.183	16.559	23.183	19.871	14.903	16.559	4.309
21	17.988	19.372	20.756	24.907	19.372	26.290	29.058	30.441	27.674	4.333
28	14.430	14.430	15.873	14.430	15.873	14.430	17.316	18.759	21.645	4.501
35	19.641	19.080	18.419	16.637	17.231	15.449	24.361	21.985	21.390	4.549
42	16.667	18.000	17.333	19.333	18.667	20.000	20.000	21.333	22.667	4.521
49	11.111	10.556	8.462	11.538	13.333	14.000	12.000	18.000	16.000	4.164
56	5.882	7.647	8.824	11.176	9.412	13.529	13.529	16.471	11.765	3.992

Table B-2 The effect of thiobencarb content on nitrite available during experimental period

Day	Nitrite available (μgNO_2^-/g dried soil)									
	0 µgTB/g soil		2.10 µgTB/g soil		4.58 µgTB/g soil		8.23 µgTB/g soil		14.78 µgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	13.236	13.236	11.843	11.843	12.539	12.539	11.843	11.843	12.539	12.539
7	13.932	13.932	11.146	11.146	10.449	9.753	9.056	10.449	8.359	10.449
14	13.236	13.932	10.449	10.449	8.359	9.056	7.663	9.753	7.663	10.449
21	13.468	13.864	11.111	11.616	10.606	11.111	8.586	10.101	8.586	10.101
28	12.525	13.333	14.141	14.141	13.333	11.313	9.697	11.717	11.313	11.717
35	16.354	19.240	19.240	21.164	18.278	20.202	18.278	19.240	20.202	22.126
42	14.483	16.552	15.172	17.241	15.172	17.241	16.552	17.931	17.931	19.310
49	13.000	14.000	17.241	19.310	18.621	19.310	16.552	20.000	17.931	21.379
56	8.205	11.282	12.308	17.436	13.333	20.513	16.410	22.564	17.436	24.615

Table B-3 The effect of thiobencarb content on nitrate available during experimental period

Day	Nitrate available (μgNO_3^-/g dried soil)									
	0 μgTB/g soil		2.10 μgTB/g soil		4.58 μgTB/g soil		8.23 μgTB/g soil		14.78 μgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	7.959	7.959	7.922	7.922	7.922	7.922	7.775	7.775	7.812	7.812
7	8.511	2.199	8.809	1.305	9.455	1.777	7.566	2.497	7.442	2.746
14	9.655	1.634	11.206	2.958	11.811	3.450	12.303	4.358	15.670	5.758
21	10.276	1.586	12.449	2.539	13.745	4.521	14.164	4.711	16.108	5.588
28	11.834	2.613	13.965	4.096	15.402	5.579	16.328	5.996	19.433	2.706
35	12.274	4.023	14.159	4.876	15.831	6.761	16.009	6.406	21.450	1.853
42	13.062	4.603	16.997	7.259	16.964	7.685	19.161	8.275	24.669	2.144
49	15.009	6.096	14.617	0.745	17.897	1.996	20.677	1.747	22.602	1.390
56	16.103	0.690	17.069	1.034	18.897	1.207	21.655	1.448	25.862	1.690

Table B-4 The effect of thiobencarb content on MOR during experimental period

Day	Methane oxidation rate (μmole / hr. g of soil)									
	0 μgTB/g soil		2.10 μgTB/g soil		4.58 μgTB/g soil		8.23 μgTB/g soil		14.78 μgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	5.375	5.375	4.650	4.650	4.438	4.438	4.063	4.063	3.856	3.856
7	5.604	8.449	5.331	7.900	5.079	6.461	4.660	5.400	4.243	5.162
14	5.435	10.152	5.461	10.563	5.461	14.981	6.139	18.692	5.417	21.014
21	6.692	16.870	6.467	18.743	7.238	20.613	6.222	21.713	5.514	22.431
28	9.594	19.400	7.050	19.873	7.769	20.662	7.590	24.248	5.789	27.007
35	10.456	22.979	7.032	20.368	7.914	21.016	8.354	24.444	6.167	30.063
42	8.706	20.664	7.194	20.266	4.252	20.891	11.067	25.563	6.539	29.440
49	8.129	15.488	8.456	12.896	5.692	11.565	6.039	17.421	6.444	20.808
56	7.638	14.259	9.352	12.361	3.056	9.907	9.907	15.417	4.444	17.755

Table B-5 The effect of thiobencarb content on OUR during experimental period

Day	Oxygen uptake rate (µmole / hr. g of soil)									
	0 µgTB/g soil		2.10 µgTB/g soil		4.58 µgTB/g soil		8.23 µgTB/g soil		14.78 µgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	10.128	10.128	12.997	12.997	12.210	12.210	12.598	12.598	13.734	13.734
7	15.246	21.684	15.826	23.340	16.502	18.645	13.523	22.802	12.008	23.685
14	8.673	27.592	10.540	31.037	8.618	20.299	22.153	56.418	9.540	25.024
21	14.099	27.949	9.350	26.564	21.152	49.281	18.755	62.690	22.732	44.329
28	15.097	30.462	22.657	50.101	33.527	53.697	20.159	69.067	40.785	63.387
35	14.910	38.638	22.549	40.388	18.529	51.007	18.984	60.251	29.699	70.630
42	19.129	34.578	19.563	39.058	13.682	46.118	16.697	56.422	29.457	67.857
49	15.265	21.979	21.246	33.944	16.550	37.131	13.077	47.993	22.694	66.548
56	12.245	22.337	15.289	31.540	9.074	22.847	1.451	38.055	21.817	59.282

Table B-6 The effect of thiobencarb content on CPR during experimental period

Day	Carbon dioxide production rate (µmole / hr. g of soil)									
	0 µgTB/g soil		2.10 µgTB/g soil		4.58 µgTB/g soil		8.23 µgTB/g soil		14.78 µgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	4.695	4.695	4.835	4.835	4.835	4.835	4.845	4.845	5.014	5.014
7	5.320	8.293	3.976	6.825	4.024	4.928	5.105	13.953	3.550	8.273
14	2.166	10.120	3.795	7.809	3.265	10.275	10.394	21.187	6.261	19.630
21	4.036	13.708	3.939	11.311	5.959	19.244	10.743	23.190	12.295	24.088
28	8.326	19.267	6.940	16.586	6.726	19.696	12.558	24.758	11.809	25.338
35	6.385	17.356	4.685	16.830	2.594	20.676	13.066	24.288	14.049	25.141
42	4.770	18.133	3.472	14.251	2.327	18.477	13.572	21.929	13.380	23.254
49	4.986	16.789	4.703	11.412	2.864	17.698	12.883	19.459	11.914	21.238
56	3.864	10.884	2.837	10.109	1.810	14.731	12.466	20.295	12.357	18.283

Table B-7 The effect of thiobencarb content on residual thiobencarb in soil during experimental period

Thiobencarb content	2.10 µgTB/g soil			4.58 µgTB/g soil			8.23 µgTB/g soil			14.78 µgTB/g soil		
day	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P
1	2.15	2.15	0.50	4.43	4.43	0.50	8.16	8.16	0.50	14.87	14.87	0.50
	2.05	2.05		4.73	4.73		8.30	8.30		14.69	14.69	
7	1.43	1.38	0.11	3.38	2.96	0.01	5.69	5.13	0.01	13.56	11.91	0.00
	1.54	1.33		3.34	3.02		5.78	5.02		13.56	11.87	
14	0.44	0.29	0.05	2.15	1.64	0.00	2.10	1.63	0.06	6.87	5.39	0.01
	0.50	0.29		2.18	1.60		2.31	1.63		7.29	4.95	
21	0.42	0.18	0.04	1.14	0.88	0.02	1.25	1.14	0.01	5.69	4.14	0.00
	0.36	0.18		1.21	0.92		1.25	1.15		5.69	4.14	
28	0.21	0.16	0.15	0.95	0.82	0.02	1.01	0.97	0.11	4.93	3.15	0.00
	0.33	0.16		0.97	0.82		1.05	0.97		4.93	3.15	
35	0.12	0.10	0.06	0.79	0.61	0.00	0.86	0.86	0.25	4.42	2.94	0.00
	0.12	0.09		0.79	0.59		0.90	0.86		4.42	2.94	
42				0.66	0.45	0.03	0.68	0.54	0.07	4.13	2.88	0.00
				0.65	0.39		0.76	0.54		4.13	2.88	
49				0.56	0.29	0.01	0.64	0.48	0.04	3.98	2.57	0.00
				0.51	0.22		0.68	0.48		3.98	2.57	
56				0.19	0.15	0.03	0.62	0.22	0.02	2.24	1.17	0.00
				0.20	0.15		0.62	0.26		2.24	1.17	

Table B-8 The effect of thiobencarb concentration on dehydrogenase activity during experimental period

Day	Dehydrogenase activity (mg TPF/ g of dry soil)									
	0 µgTB/g soil		2.10 µgTB/g soil		4.58 µgTB/g soil		8.23 µgTB/g soil		14.78 µgTB/g soil	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	2.355	2.355	2.764	2.764	2.781	2.781	2.798	2.798	2.814	2.814
7	2.402	3.021	2.456	3.664	3.004	3.837	3.069	3.861	3.167	4.021
14	2.499	3.190	2.625	3.846	3.142	3.948	3.124	4.194	3.617	4.309
21	2.487	3.232	2.908	3.894	3.341	4.068	3.377	4.206	3.641	4.333
28	2.691	3.323	3.521	4.026	3.407	4.188	3.473	4.254	3.671	4.501
35	3.088	3.497	3.599	4.056	3.695	4.303	3.599	4.303	3.557	4.549
42	3.105	3.807	3.700	4.039	3.527	4.265	3.617	4.289	3.456	4.521
49	3.152	3.694	2.890	3.557	3.069	3.646	3.521	3.992	3.396	4.164
56	3.123	3.410	2.783	3.527	2.914	3.569	3.367	3.843	3.242	3.992

Table B-9 The effect of nitrate on organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Nitrate content	Organic-N of control (µg-N/g)			Organic-N of test (µg-N/g)		
% (w/w)	Day 1	Day 18	ΔN	Day 1	Day 18	ΔN
0.52	1041.72	1012.17303	-29.547	1041.72	982.193991	-59.526
27.00	1041.444	1010.38542	-31.0586	1041.444	1008.70242	-32.7416
20	1023.95033	997.183009	-26.7673	1023.95033	994.658009	-29.2923
40	1023.95033	1019.32803	-4.62231	1023.95033	1011.75203	-12.1983
	1080.02133	1091.49209	11.47076	1080.02133	1090.22909	10.20776

Table B-10 The effect of nitrate content on ammonium available of soil during experimental period

Day	Ammonium available (μgNH_4^+/g dried soil)									
	0.52 (μgNO_3^-/g)		27.00 (μgNO_3^-/g)		50.94 (μgNO_3^-/g)		81.38 (μgNO_3^-/g)		96.30 (μgNO_3^-/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	8.280	8.280	8.556	8.556	9.383	9.383	9.383	9.383	8.312	8.312
6	15.455	5.520	13.247	2.760	11.039	6.072	9.935	4.416	4.416	4.416
12	16.835	14.284	16.325	17.345	16.325	19.386	16.325	21.426	5.102	5.102
18	13.468	9.259	8.418	10.101	11.364	13.889	6.313	13.889	2.525	3.788

Table B-11 The effect of nitrate content on nitrite available of soil during experimental period

Day	Nitrite available (μgNO_2^-/g dried soil)									
	0.52 (μgNO_3^-/g)		27.00 (μgNO_3^-/g)		52.86 (μgNO_3^-/g)		81.48 (μgNO_3^-/g)		96.30 (μgNO_3^-/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	13.932	13.932	16.022	16.022	20.202	20.202	24.382	24.382	21.595	21.595
6	19.505	27.865	22.989	24.382	25.775	33.438	19.505	22.989	23.685	20.899
12	19.505	21.595	20.202	27.865	50.157	58.516	55.033	94.741	7.663	10.449
18	12.676	11.884	13.636	27.273	55.051	75.758	65.657	101.010	16.667	15.657

Table B-12 The effect of nitrate content on nitrate available of soil during experimental period

Day	Nitrate available (μgNO_3^-/g dried soil)									
	0.52 (μgNO_3^-/g)		27.00 (μgNO_3^-/g)		52.86 (μgNO_3^-/g)		81.43 (μgNO_3^-/g)		96.30 (μgNO_3^-/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	0.520	0.520	26.998	26.998	52.861	52.861	81.432	81.432	96.296	96.296
6	13.594	2.273	44.853	21.292	81.588	56.994	87.065	81.330	96.314	94.195
12	25.635	0.514	52.160	2.673	80.484	46.866	83.620	56.221	95.289	93.695
18	37.523	0.410	78.220	0.703	99.066	32.604	101.233	36.762	103.985	103.399

Table B-13 The effect of nitrate content on methane oxidation rate during experimental period

Day	Methane oxidation rate (µmole / hr. g of soil)									
	0.52 (μgNO_3^-/g)		27.00 (μgNO_3^-/g)		52.86 (μgNO_3^-/g)		81.43 (μgNO_3^-/g)		96.30 (μgNO_3^-/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	8.382	8.382	7.678	7.678	2.181	2.180	1.785	1.785	1.296	1.296
6	10.667	50.146	7.442	55.838	6.655	31.653	6.238	30.363	2.861	4.975
12	11.912	70.329	7.479	59.785	5.012	27.961	5.414	27.498	1.824	2.933
18	12.449	75.414	7.588	48.266	2.037	26.639	1.433	22.535	0.586	1.051

Table B-14 The effect of nitrate content on oxygen uptake rate during experimental period

Day	Oxygen uptake rate (µmole / hr. g of soil)									
	0.52 (μgNO_3^-/g)		27.00 (μgNO_3^-/g)		52.86 (μgNO_3^-/g)		81.43 (μgNO_3^-/g)		96.30 (μgNO_3^-/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	35.194	35.194	30.053	30.053	24.471	24.471	29.552	29.552	31.221	28.906
6	35.385	111.020	24.810	161.516	14.356	64.021	16.751	62.291	10.527	18.427
12	20.600	142.766	14.361	146.710	11.153	58.357	11.128	58.529	11.507	11.714
18	23.554	207.696	14.299	93.067	4.183	53.620	4.959	48.164	2.789	7.411

Table B-15 The effect of nitrate content on carbon production rate during experimental period

Day	Carbon production rate (µmole / hr. g of soil)									
	0.52 ($\mu gNO_3^-/g$)		27.00 ($\mu gNO_3^-/g$)		52.86 ($\mu gNO_3^-/g$)		81.43 ($\mu gNO_3^-/g$)		96.30 ($\mu gNO_3^-/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	15.694	15.694	14.609	14.609	11.561	11.561	21.492	21.492	22.706	22.706
6	13.312	51.522	9.746	56.369	5.309	33.500	12.183	38.568	7.656	13.402
12	12.138	76.562	4.214	60.232	2.661	28.664	7.252	30.782	8.369	8.519
18	12.465	77.376	4.593	49.811	1.523	27.801	3.607	31.662	2.029	5.390

Table B-16 The effect of nitrate content on dehydrogenase activity during experimental period

Day	Dehydrogenase activity (mg TPF/ g of dry soil)									
	0.52 ($\mu gNO_3^-/g$)		27.00 ($\mu gNO_3^-/g$)		52.86 ($\mu gNO_3^-/g$)		81.43 ($\mu gNO_3^-/g$)		96.30 ($\mu gNO_3^-/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	3.194	3.194	3.432	3.432	3.492	3.492	3.373	3.373	3.313	3.313
6	3.301	4.331	3.486	4.385	3.670	3.724	3.486	3.789	3.343	3.432
12	3.423	5.626	3.999	5.169	4.144	4.541	4.276	4.468	3.463	3.397
18	3.615	5.831	3.866	5.269	4.197	4.720	4.468	4.607	3.463	3.483

Table B-17 The effect of nitrate content on thiobencarb residual in soil during experimental period

Nitrate content	0.52 (μgNO_3^-/g dried soil)			27.00 (μgNO_3^-/g dried soil)			52.86 (μgNO_3^-/g dried soil)			81.43 (μgNO_3^-/g dried soil)			96.30 (μgNO_3^-/g dried soil)		
day	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P
1	15.34	15.34	0.5	15.48	15.48	0.50	15.85	16.02	0.50	15.34	15.34	0.50	15.34	15.34	0.50
	15.38	15.38		15.85	15.85		16.02	15.85		15.31	15.31		15.31	15.31	
6	13.03	12.72	0.04	15.33	13.80	0.03	15.92	15.34	0.13	15.00	14.86	0.41	15.34	15.24	0.21
	13.24	12.59		15.33	13.46		15.87	15.68		14.50	14.49		14.96	15.01	
12	10.16	8.76	0.02	10.31	8.97	0.04	13.64	13.55	0.24	13.94	13.64	0.12	14.15	14.28	0.48
	10.60	8.55		10.62	8.30		13.51	12.92		13.91	13.26		14.83	14.62	
18	7.59	5.46	0.03	7.49	6.79	0.04	11.06	10.06	0.07	12.10	11.90	0.20	14.35	14.25	0.46
	7.65	4.98		7.59	6.48		11.06	9.53		12.10	12.07		14.32	14.52	

Table B-18 The effect of ammonium content on organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Ammonium content	Organic-N of control (μg-N/g)			Organic-N of test (μg-N/g)		
% (w/w)	Day 1	Day 18	ΔN	Day 1	Day 18	ΔN
0.58	1138.034	1097.088	-40.946	1138.034	1073.519	-64.515
22.83	1132.282	1074.360	-57.922	1132.282	1044.899	-87.383
50.94	1137.182	1043.636	-93.546	1137.182	1013.333	-123.849
81.38	1139.740	1104.243	-35.498	1139.740	1180.000	40.260
100.12	1137.507	1104.243	-33.264	1137.507	1115.606	-21.901

Table B-19 The effect of ammonium content on ammonium available during experimental period

Day	Ammonium available ($\mu gNH_4^+/g$ dried soil)									
	0.58 ($\mu gNH_4^+/g$)		22.83 ($\mu gNH_4^+/g$)		50.94 ($\mu gNH_4^+/g$)		81.38 ($\mu gNH_4^+/g$)		100.12 ($\mu gNH_4^+/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	0.580	0.580	22.834	22.834	50.937	50.937	81.382	81.382	100.117	100.117
6	9.804	5.447	0.000	10.893	41.394	21.786	76.253	65.359	119.826	107.843
12	16.835	14.284	16.325	17.345	16.325	19.386	16.325	21.426	102.030	102.030
18	13.468	9.259	8.418	10.101	11.364	13.889	6.313	13.889	78.283	78.283

Table B-20 The effect of ammonium content on nitrite available during experimental period

Day	Nitrite available ($\mu gNO_2^-/g$ dried soil)									
	0.58 ($\mu gNH_4^+/g$)		22.83 ($\mu gNH_4^+/g$)		50.94 ($\mu gNH_4^+/g$)		81.38 ($\mu gNH_4^+/g$)		100.12 ($\mu gNH_4^+/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	2.676	2.676	3.191	3.191	2.676	2.676	2.162	2.162	2.162	2.162
6	13.743	10.140	11.684	9.368	20.176	17.860	20.691	14.000	21.463	16.831
12	5.765	6.537	6.022	8.853	22.235	22.750	16.316	18.117	26.867	37.161
18	6.794	6.279	5.507	12.456	26.610	20.434	37.161	32.014	47.455	50.029

Table B-21 The effect of ammonium content on nitrate available during experimental period

Nitrate available (μgNO_3^-/g dried soil)

Day	0.58 (μgNH_4^+/g)		22.83 (μgNH_4^+/g)		50.94 (μgNH_4^+/g)		81.38 (μgNH_4^+/g)		100.12 (μgNH_4^+/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	2.931	2.931	2.740	2.740	3.199	3.199	2.510	2.510	3.122	3.122
6	19.315	0.953	21.192	2.572	23.473	2.351	21.744	9.895	22.369	1.505
12	25.635	2.570	36.739	3.187	66.399	31.879	68.986	46.383	78.613	77.299
18	37.523	1.464	60.653	1.874	89.159	66.235	91.109	85.787	93.586	93.059

Table B-22 The effect of ammonium content on methane oxidation rate during experimental period

Methane oxidation rate (µmole / hr. g of soil)

Day	0.58 (μgNH_4^+/g)		22.83 (μgNH_4^+/g)		50.94 (μgNH_4^+/g)		81.38 (μgNH_4^+/g)		100.12 (μgNH_4^+/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	8.225	8.225	9.146	9.146	4.646	5.162	3.796	3.796	2.407	2.407
6	6.577	47.229	7.473	64.938	5.706	42.755	6.815	40.532	4.991	24.375
12	9.110	54.417	8.777	72.733	8.138	49.782	6.620	34.600	5.354	16.903
18	7.152	64.858	9.235	79.563	6.573	47.282	1.433	22.535	0.586	2.208

Table B-23 The effect of ammonium content on oxygen uptake rate during experimental period

Oxygen uptake rate (µmole / hr. g of soil)

Day	0.58 (μgNH_4^+/g)		22.83 (μgNH_4^+/g)		50.94 (μgNH_4^+/g)		81.38 (μgNH_4^+/g)		100.12 (μgNH_4^+/g)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	38.799	38.798	39.960	44.955	37.285	37.285	32.523	32.523	39.340	39.346
6	10.880	84.039	12.361	127.395	12.905	92.052	11.456	78.069	10.597	75.000
12	13.655	96.053	15.151	157.877	13.046	97.898	13.159	85.670	11.203	68.304
18	12.145	65.706	7.972	176.692	10.592	117.372	9.811	114.756	12.384	47.537

Table B-24 The effect of ammonium content on oxygen uptake rate during experimental period

Carbon dioxide production rate (µmole / hr. g of soil)

Day	0.58 ($\mu gNH_4^+/g$)		22.83 ($\mu gNH_4^+/g$)		50.94 ($\mu gNH_4^+/g$)		81.38 ($\mu gNH_4^+/g$)		100.12 ($\mu gNH_4^+/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	18.636	20.966	19.904	22.392	15.404	15.404	19.053	21.435	16.582	16.582
6	4.344	49.403	4.924	68.890	4.837	40.673	0.521	40.004	3.949	26.540
12	5.399	66.203	6.225	82.358	4.837	50.300	0.819	36.082	4.543	25.024
18	3.706	73.030	3.519	86.677	3.896	51.354	4.566	28.629	2.885	17.588

Table B-25 The effect of ammonium content on dehydrogenase activity during experimental period

Dehydrogenase activity (mg TPF/ g of dry soil)

Day	0.58 ($\mu gNH_4^+/g$)		22.83 ($\mu gNH_4^+/g$)		50.94 ($\mu gNH_4^+/g$)		81.38 ($\mu gNH_4^+/g$)		100.12 ($\mu gNH_4^+/g$)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
1	2.420	2.420	2.480	2.480	2.599	2.599	2.611	2.611	2.962	2.962
6	3.301	4.331	3.551	4.563	3.670	3.902	3.843	3.908	3.879	4.027
12	3.423	5.626	4.197	6.228	4.402	4.997	4.448	4.978	4.720	4.190
18	3.615	5.831	3.866	6.525	4.329	5.731	4.521	5.063	4.720	4.746

Table B-26 The effect of ammonium content on thiobencarb residual in soil during experimental period

Ammonium content	0.58 (μgNH_4^+/g dried soil)			22.83 (μgNH_4^+/g dried soil)			50.94 (μgNH_4^+/g dried soil)			81.38 (μgNH_4^+/g dried soil)			100.12 (μgNH_4^+/g dried soil)		
day	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P
1	15.48	15.48	0.50	15.48	15.48	0.50	16.43	16.43	0.50	15.00	15.00	0.50	15.00	15.00	0.50
	16.43	16.43		15.50	15.50		15.00	15.00		15.95	15.95		15.95	15.95	
6	14.62	13.10	0.02	14.48	12.38	0.03	13.71	12.38	0.03	15.71	14.52	0.42	14.33	14.29	0.23
	14.14	12.62		14.52	12.76		14.19	12.86		13.81	14.52		14.33	13.33	
12	11.19	9.95	0.03	9.14	6.57	0.04	11.43	9.24	0.05	12.86	11.90	0.36	12.76	12.76	0.36
	10.81	9.48		8.19	6.00		10.48	8.71		10.95	10.95		11.81	10.86	
18	6.57	5.05	0.04	6.05	3.76	0.01	5.24	3.95	0.01	5.95	6.10	0.31	7.38	6.05	0.44
	7.52	4.57		6.05	3.57		5.24	4.05		6.90	6.10		6.43	7.48	

Table B-27 The effect of moisture content on organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Moisture content	Organic-N of control (µg-N/g)			Organic-N of test (µg-N/g)		
% (w/w)	Day 1	Day 18	ΔN	Day 1	Day 18	ΔN
5	1114.662	1102.277	-12.385	1114.662	1102.543	-12.120
10	1118.377	1079.170	-39.207	1118.378	1058.775	-59.603
20	1144.228	1052.165	-92.063	1144.229	1023.567	-120.661
40	1108.609	1043.393	-65.216	1108.610	1014.795	-93.814

Table B-28 The effect of moisture on ammonium available of soil during experimental period

Day	Ammonium available (µgNH_4^+/g dried soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	1.842	1.842	7.895	7.895	7.895	7.895	7.895	7.895
6	3.158	4.211	12.368	13.421	7.895	10.263	10.789	12.105
12	5.102	7.142	10.968	10.713	4.846	9.183	11.989	9.438
18	4.519	4.253	8.506	9.304	6.114	5.316	14.886	14.088

Table B-29 The effect of moisture on nitrite available of soil during experimental period

Day	Nitrite available (µgNO_2^-/g dried soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	8.726	8.726	8.726	8.726	8.726	8.726	8.726	8.726
6	9.045	7.452	13.822	12.229	10.955	9.363	7.771	7.771
12	11.941	8.081	14.515	10.654	8.853	9.625	9.368	8.338
18	11.426	7.566	12.456	7.051	13.228	11.684	10.140	8.338

Table B-30 The effect of moisture on nitrate available of soil during experimental period

Day	Nitrate available (μgNO_3^-/g dried soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	1.818	1.818	1.818	1.818	1.818	1.818	1.818	1.818
6	3.168	0.808	5.839	3.758	7.054	1.849	0.808	0.010
12	14.157	3.188	18.576	2.320	21.733	1.610	0.979	1.184
18	8.245	4.509	19.663	0.703	23.528	3.033	1.101	0.457

Table B-31 The effect of moisture on methane oxidation rate during experimental period

Day	Methane oxidation rate (µmole / hr. g of soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	0.361	0.361	0.438	0.438	2.433	2.433	2.438	2.438
6	0.463	0.532	3.194	9.032	13.657	24.769	15.639	37.384
12	1.620	2.326	3.912	29.259	14.074	73.148	16.968	40.486
18	2.319	2.778	4.213	43.637	19.144	83.796	18.889	41.574

Table B-32 The effect of moisture on oxygen uptake rate during experimental period

Day	Oxygen uptake rate (µmole / hr. g of soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	2.671	2.671	30.053	30.053	24.471	24.471	23.836	23.836
6	1.667	3.958	7.569	103.646	16.671	61.227	19.191	27.303
12	2.650	9.801	6.817	123.561	18.403	162.431	26.934	57.645
18	6.424	11.558	7.859	139.363	18.750	180.741	28.785	63.542

Table B-33 The effect of moisture on carbon dioxide production rate during experimental period

Day	Carbon dioxide production rate (µmole / hr. g of soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	1.145	1.145	7.992	7.992	12.355	12.355	13.047	13.047
6	0.717	0.726	1.768	7.702	5.455	15.008	7.790	12.444
12	0.509	0.593	1.835	11.742	5.867	55.955	10.129	17.364
18	0.955	1.065	1.541	25.328	5.824	60.370	10.602	18.171

Table B-34 The effect of moisture on thiobencarb residual in soil during experimental period

Moisture content	5 %			10%			20%			40%		
day	Control	Test	P	Control	Test	P	Control	Test	P	Control	Test	P
1	15.53	15.53	0.50	15.53	15.53	0.50	15.53	15.53	0.50	15.53	15.53	0.50
	14.47	14.47		14.47	14.47		14.47	14.47		14.47	14.47	
6	14.21	14.47	0.30	13.16	11.42	0.01	14.95	11.42	0.03	14.21	13.89	0.23
	16.32	14.47		13.68	10.89		13.89	10.89		15.26	14.42	
12	13.16	12.58	0.14	11.16	6.58	0.02	13.00	6.05	0.01	14.89	12.42	0.28
	14.16	12.58		10.11	7.63		12.47	7.11		12.26	12.58	
18	12.16	12.63	0.24	7.37	4.95	0.01	6.16	4.05	0.04	10.89	8.89	0.03
	13.21	11.05		7.89	5.47		6.68	4.05		10.37	9.42	

Table B-35 The effect of moisture content on dehydrogenase activity during experimental period

Day	Dehydrogenase activity (mg TPF/ g of dry soil)							
	5% MC		10% MC		20% MC		40% MC	
	Control	Test	Control	Test	Control	Test	Control	Test
1	2.629	2.629	2.640	2.640	2.938	2.938	2.926	2.926
6	2.605	2.706	3.123	3.646	3.093	3.754	3.069	3.188
12	2.881	3.238	3.205	4.369	3.899	5.447	4.085	4.217
18	3.615	4.276	3.866	5.731	4.409	6.525	4.673	4.753

Table B-36 The effect of temperature on organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Temperature	Organic-N of control (µg-N/g)			Organic-N of test (µg-N/g)		
°C	Day 1	Day 18	ΔN	Day 1	Day 18	ΔN
20	1042.242	1031.697	-10.545	1042.242	1030.135	-12.107
30	1042.242	1012.121	-30.121	1042.242	982.412	-59.830
40	1042.242	978.507	-63.735	1042.242	945.675	-96.567

Table B-37 The effect of temperature on ammonium available of soil during experimental period

Day	Ammonium available (µgNH_4^+/g dried soil)					
	20 °C		27-30°C		38-40°C	
	Control	Test	Control	Test	Control	Test
1	0.258	0.258	0.258	0.258	0.258	0.258
6	0.258	1.289	0.773	0.773	2.320	3.866
12	3.645	9.893	11.194	10.934	4.946	9.372
18	1.041	2.603	3.905	1.562	5.467	6.248

Table B-38 The effect of temperature on nitrite available of soil during experimental period

Day	Nitrite available (μgNO_2^-/g dried soil)					
	20 °C		27-30°C		38-40°C	
	Control	Test	Control	Test	Control	Test
1	11.911	11.911	11.911	11.911	11.911	11.911
6	13.185	12.866	17.325	13.822	15.096	13.822
12	11.941	8.081	14.000	10.654	10.140	9.625
18	6.279	3.706	12.198	13.743	7.566	8.596

Table B-39 The effect of temperature on nitrate available of soil during experimental period

Day	Nitrate available (μgNO_3^-/g dried soil)					
	20 °C		27-30°C		38-40°C	
	Control	Test	Control	Test	Control	Test
1	1.818	1.818	1.818	1.818	1.818	1.818
6	1.979	0.505	6.293	2.348	6.358	0.722
12	3.901	1.985	24.833	1.445	23.359	1.002
18	10.404	0.214	31.620	0.366	27.071	1.679

Table B-40 The effect of temperature on methane oxidation rate during experimental period

Day	Methane oxidation rate (µmole / hr. g of soil)					
	20 °C		27-30°C		38-40°C	
	Control	Test	Control	Test	Control	Test
1	0.600	0.600	0.600	0.600	0.600	0.600
6	0.799	3.449	4.174	25.331	4.120	37.963
12	0.956	11.833	5.097	38.102	5.625	44.028
18	2.137	17.708	7.500	50.440	5.926	56.597

Table B-41 The effect of temperature on oxygen uptake rate during experimental period

Day	Oxygen uptake rate (µmole / hr. g of soil)					
	20 ◦C		27-30◦C		38-40◦C	
	Control	Test	Control	Test	Control	Test
1	8.854	8.854	8.854	8.854	8.854	8.854
6	9.178	17.894	11.296	45.051	56.192	118.866
12	12.234	29.433	13.600	90.023	70.891	146.644
18	14.167	43.368	17.257	128.762	84.375	184.028

Table B-42 The effect of temperature on carbon dioxide production rate during experimental period

Day	Carbon dioxide production rate (µmole / hr. g of soil)					
	20 ◦C		27-30◦C		38-40◦C	
	Control	Test	Control	Test	Control	Test
1	3.468	3.468	3.468	3.468	3.468	3.468
6	3.662	3.788	3.973	11.754	11.212	40.752
12	4.934	9.293	3.906	21.507	17.507	43.996
18	6.754	27.997	3.333	43.274	19.436	52.323

Table B-43 The effect of temperature on thiobencarb residual in soil during experimental period

Temperature	20 °C			30 °C			40 °C		
day	Control	Test	P	Control	Test	P	Control	Test	P
1	15.36	15.36	0.5	15.36	15.36	0.5	15.36	15.36	0.5
	14.45	14.45		14.45	14.45		14.45	14.45	
6	14.55	13.77	0.18	14.32	11.82	0.01	13.45	12.05	0.08
	15.45	14.68		13.86	11.59		12.55	11.36	
12	12.95	12.68	0.14	10.23	8.18	0.02	7.45	6.46	0.01
	13.41	12.68		10.23	8.41		7.59	6.64	
18	11.50	11.27	0.18	7.23	5.32	0.03	4.95	3.59	0.01
	12.23	11.27		7.68	5.45		4.77	3.73	

Table B-44 The effect of temperature on dehydrogenase activity during experimental period

Day	Dehydrogenase activity (mg TPF/ g of dry soil)					
	20 °C		27-30°C		38-40°C	
	Control	Test	Control	Test	Control	Test
1	3.194	3.194	3.432	3.432	3.492	3.492
6	2.361	2.658	2.480	3.373	2.956	3.492
12	2.821	3.417	3.020	4.111	3.542	4.806
18	3.020	3.926	3.192	5.136	3.496	5.665

Table B-45 The effect of methane content on organic nitrogen content of both poor (control) and rich-methanotrophs soil (test), ΔN: the nitrogen different value

Methane content % (v/v)	Organic-N (µg-N/g)		
	Day 1	Day 18	ΔN
0	1042.019	1009.652	-32.367
1	1042.019	1013.726	-28.293
5	1042.019	992.557	-49.462
10	1042.019	985.496	-56.523

Table B-46 The effect of methane content on dehydrogenase activity during experimental period

Day	Dehydrogenase activity (mg TPF/ g of dry soil)			
	Methane content, %(v/v)			
	0%	1%	5%	10%
1	1.765	1.765	1.765	1.765
6	2.182	2.420	3.075	3.313
12	3.053	3.218	4.032	4.475
18	3.152	3.483	4.607	5.136

Table B-47 The effect of methane content on nitrogen available of soil during experimental period

Day	Ammonium available (μgNH_4^+/g dried soil)				Nitrite available (μgNO_2^-/g dried soil)				Nitrate available (μgNO_3^-/g dried soil)			
	Methane content, %(v/v)				Methane content, %(v/v)				Methane content, %(v/v)			
	0%	1%	5%	10%	0%	1%	5%	10%	0%	1%	5%	10%
1	0.481	0.481	0.481	0.481	11.911	11.911	11.911	11.911	2.793	2.793	2.793	2.793
6	2.018	2.018	1.009	3.027	17.325	20.510	12.548	14.777	9.674	7.393	5.001	3.529
12	11.408	18.742	15.482	13.853	15.029	15.801	12.970	10.654	19.242	15.635	5.520	42.943
18	12.223	8.149	13.853	4.889	12.198	15.029	13.485	10.140	26.830	23.415	3.292	1.810

Table B-48 The effect of methane content on methane oxidation and soil respiration during experimental period

Day	Methane oxidation rate (µmole / hr. g of soil)				Oxygen uptake rate (µmole / hr. g of soil)				Carbon dioxide production rate (µmole / hr. g of soil)			
	Methane content, %(v/v)				Methane content, %(v/v)				Methane content, %(v/v)			
	0%	1%	5%	10%	0%	1%	5%	10%	0%	1%	5%	10%
1	0.600	0.600	0.600	0.600	8.854	8.854	8.854	8.854	3.468	3.468	3.468	3.468
6	4.174	8.852	16.282	25.331	11.296	15.137	27.757	43.894	3.973	8.290	17.991	25.222
12	5.097	13.597	29.611	42.731	13.600	28.796	58.098	90.023	3.906	16.860	35.215	46.759
18	7.500	20.463	53.843	66.644	17.257	44.514	121.69	140.336	3.333	20.705	59.949	68.527

Table B-49 The effect of methane content on thiobencarb degradation rate and half-live during experimental period

Methane content / day	0%	1%	5%	10%
1	16.13	16.12	16.12	16.12
	15.50	15.50	15.50	15.50
6	15.57	13.33	12.52	12.10
	14.76	12.86	11.57	11.19
12	10.86	9.95	9.05	8.43
	10.19	9.43	8.48	8.57
18	7.86	7.71	6.10	5.52
	8.05	6.86	6.33	5.14

BIOGRAPHY

Miss Sutharat Muenmee was born on 8th February, 1986 in Saraburi province, and she has an older sister. She finished her high school from Saraburi Witthayakhom School, Saraburi in 2004. After that, she studied in the major of Environmental Engineering in Faculty of Engineering at Kasetsart University. When she was student, her engineering project was about application of clean technology in food industry and plastic company. She got bachelor's degree of environmental engineering in March 2008.