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TITLE: Ex-Situ Anaerobic Bioremediation for Soil Contaminated with Total Petroleum Hydrocarbons

NAME: Mr. Ye Hlaing Win

THIS THESIS HAS BEEN ACCEPTED BY

_____ **THESIS ADVISOR**

(Mr. Suchat Leungprasert, Ph.D.)

_____ **THESIS CO-ADVISOR**

(Assistant Professor Sanya Sirivithayapakorn, Ph.D.)

_____ **DEPARTMENT HEAD**

(Associate Professor Chart Chiemchaisri, D.Eng.)

APPROVED BY THE GRADUATE SCHOOL ON _____

_____ **DEAN**

(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

EX-SITU ANAEROBIC BIOREMEDIATION FOR SOIL CONTAMINATED WITH TOTAL PETROLEUM HYDROCARBONS

YE HLAING WIN

A Thesis Submitted in Fulfillment of
the Requirement for the Degree of
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The soil contaminated with total petroleum hydrocarbons (TPHs) is generally found in several industrial areas. TPHs contamination leads to environmental concerns due to its hazardous characteristics and the cancer risk of its component. As a result, TPHs should be removed from the contaminated soil. To determine the feasibility of the biodegradation of TPHs under anaerobic condition, the contaminated soils with TPHs were added to 2-L lab-scale reactors in which the temperature was controlled by using a water bath incubator. During anaerobic bioremediation, TPHs could transform to be CH_4 and CO_2. To prove this strategy, the contaminated soil temperature was varied from 30^{o}C to 40 ^{o}C and to 50 ^{o}C every 15 days. The percentage of CO_2 and CH_4 production was about 34.8% and 2.94% for non-sterilization and 19.2%, and 0% for sterilization, respectively. After 45 days operation, TPHs were reduced by 49.49% for no sterilization soil samples and 6.62% for sterilization soil samples. To determine the effects of temperature on anaerobic bioremediation, the 1.2 kg of contaminated soil was added to the reactor with temperature control under 30^{o}C, 40 ^{o}C, and 50 ^{o}C for sterilization, non-sterilization, and nutrients ratio of C/N/P of 100:2:0.2. The result showed that for non-sterilized soil samples the maximum production of CH_4 was 12.9%, 14%, and 13.9% under 30^{o}C, 40 ^{o}C, and 50 ^{o}C for non-sterilization, respectively. The CO_2 production was also found in all temperature operation. It was found that as temperature increased, the CO_2 production was increased. Also, the reduction of TPHs in 30^{o}C, 40 ^{o}C, and 50 ^{o}C was 36.74 %, 35.38%, and 28.21% for non-sterilization, respectively.

_____ _____ ___ /_____ /___

Student's Signature Thesis Advisor's Signature

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Ye Hlaing Win

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LIST OF ABBREVIATIONS

TPH	=	Total Petroleum Hydrocarbons
HCs	=	Hydrocarbons
VOCs	=	Volatile Organic Compounds
SVOCs	=	Semi Volatile Organic Compounds
PCBs	=	Polychlorinated biphenyls
BTEX	=	benzene, toluene, ethylbenzene, and xylenes
PAH	=	poly-aromatic hydrocarbons
GC	=	gas chromatography
FID	=	flame ionization detector
SB	=	Slurry Bioreactors
GRO	=	Gasoline Range Organics
DRO	=	Diesel Range Organics
EPA	=	Environmental Protection Agency
PHC	=	Petroleum Hydrocarbon
ASTM	=	American Society for Testing and Materials
MC	=	Moisture Content
VS	=	Volatile Solid
TOC	=	Total Organic Carbon
COD	=	Chemical Oxygen Demand

EX-SITU ANAEROBIC BIOREMEDIATION FOR SOIL CONTAMINATED WITH TOTAL PETROLEUM HYDROCARBONS

INTRODUCTION

Anaerobic conditions in soil affect plant productivity as well as organics matter and nutrients. Water availability and temperature are primary factors on the amount of available carbon for respiration with its rate in anaerobic conditions in soil.

Anaerobic microorganisms can also degrade aliphatic hydrocarbons (HCs). The principal step of anaerobic alkane degradation, as well as anaerobic degradation of aromatic HCs, is the carboxylation of substrate molecules (Vasu, 1977; So and Young 1999). Various substances can act as carbon donors, which is included in carboxylic group formed, namely: fumarate (when degrading toluene, xylene and alkanes), bicarbonate (when degrading naphthalene and alkanes) etc, (Young and Phelps, 2005).

Petroleum fuel spills which resulted from damage, stress, and corrosion of pipelines, transportation accidents, leakage of storage tanks and various other industrial and mining activities are classified as hazardous waste (Bartha and Bossert, 1984) and are considered as the most frequent organic pollutants of terrestrial and aquatic ecosystems (Bossert ,1984, Margesin and Schinnur, 1997).

To understand the different technologies applied in bioremediation of petroleum contamination, it is necessary to be introduced to the physicochemical, hydrological and microbiological factors that control bioremediation of the contaminant (Ismail, 2005). Aromatic hydrocarbons one or two ring compounds degrade readily, higher molecular weight compounds less readily. Alcohols, esters, nitrobenzenes and ethers degrade slowly, chlorinated hydrocarbons decreasing

degradability within increasing chlorine substitution – highly chlorinated compounds like PCBs and chlorinated solvents do not appreciably degrade aerobically, pesticides are not readily degraded. Few environmental conditions are required for the soil remediation (Keshav, 2010).

In Bioremediation, there are ex-situ bioremediation and in-situ bioremediation. Ex-situ bioremediation requires shorter time periods than in situ treatment because of the certainty about the uniformity of treatment, the ability of homogenize, screen, and continuously mixing in soil.

In ex situ involves the removal of the contaminated material to be treated. Some examples of bioremediation technologies are bioventing, land farming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

To remediate the soil which is contaminated with Total Petroleum Hydrocarbon, it has to be clean up by bioremediation process by ex-situ or in-situ. This research is ex-situ anaerobic bioremediation for soil contaminated with Total Petroleum Hydrocarbon (TPH). It will be focused on the Total Petroleum Hydrocarbon Residue oil which contaminate in the soil to be treated by ex-situ anaerobic bioremediation process.

OBJECTIVES

The main objectives are as following:

1. To determine the effects of temperatures on the TPH reduction under anaerobic condition for soil remediation.

2. To determine the optimum conditions of nutrients addition on TPH biodegradation.

Scope of Study

The scope of study included:

1. The soil was collected from the industrial sites (Chomburi Province), which soil has been contaminated with residue Total Petroleum Hydrocarbon (TPH).

2. The variation of the temperature in $30^{o}C$, $40^{o}C$, and $50^{o}C$

3. TPH contaminated soil with sterilization in $30^{o}C$, $40^{o}C$, and $50^{o}C$

4. TPH contaminated soil without sterilization and nutrients in $30^{o}C$, $40^{o}C$, and $50^{o}C$

5. Adding the nutrients (Nitrogen and Phosphorous) in TPH contaminated soil with the ratio TPH: N: P of 100:2:0.2.

6. The degradation rate was estimated by using CO_2, CH_4 production and TPH reduction information.

LITERATURE REVIEW

1. Fuel Contaminants (Total Petroleum Hydrocarbons, TPH)

Many of fuel contaminants are presented in this literature review. Fuel contaminants can be found include aircraft maintenance areas, landfills, leaking storage tanks, solvent degreasing areas, surface impoundments, and vehicle maintenance areas.

The compound of fuel such as Volatile Organic Compounds (VOCs) and Semi Volatile Organic Compounds (SVOCs) is halogenated or non-halogenated. The halogen bond and halogen itself require more treatment than non-halogenated compounds. Fuel contaminants are non-halogenated.

Data requirements for soil, sediment, and sludge are inherently variable in their physical and chemical characteristics. They are soil particle site distribution, soil homogeneity and isotropy, bulk density, particle density, soil permeability, soil moisture, and soil pH. Examples of oxidation-reduction types of chemical reactions include alkaline chlorination of cyanides, reduction of hexavalent chromium with sulfide under acidic conditions, aerobic oxidation of organic compounds into CO_2 and H_2O, or anaerobic decomposition of organic compounds into CO_2 and CH_4 (Walter, 1994). Humic content (organic fraction) is the decomposing part of the naturally occurring organic content of the soil. Simple hydrocarbons and petroleum fuels degradability decreases as molecular weight and degree of branching increase (Keshav, 2010). Aromatic hydrocarbons one or two ring compounds degrade readily, higher molecular weight compounds less readily. Alcohols, esters, nitrobenzenes and ethers degrade slowly, chlorinated hydrocarbons decreasing degradability within increasing chlorine substitution – highly chlorinated compounds like PCBs and chlorinated solvents do not appreciably degrade aerobically, Pesticides are not readily degraded. Few environmental conditions are required for the soil remediation, see

Table 1 (Keshav, 2010). Table 2 is described the physical properties of different hydrocarbons (Lyman, 1992).

Table 1 Environmental factors and optimum condition for microbial activity for soil bioremediation

Environmental Factor	Optimum Conditions	Condition required for microbial activity
Available soil moisture	25-85% water holding capacity	25-28% of water holding capacity
Oxygen	>0.2 mg/L DO, >10% air-filled pore space for aerobic Degradation	Aerobic, minimum air-filled pore space of 10%
Redox potential	Eh > 50 mill volts	
Nutrients	C:N:P= 120:10:1 molar ratio	N and P for microbial growth
Ph	6.5-8.0	5.5 to 8.5
Temperature	20-30 ºC	15-45ºC
Contaminants	Hydrocarbon 5-10% of dry weight of soil	Not too toxic
Heavy metals	700ppm	Total content 2000ppm

Source: Keshav, (2010)

Table 2 Physical Properties of Different Hydrocarbons

Compound	Boiling Point (^{O}C)	Vapor Pressure (mm Hg 20^{o}C)	Specific Gravity
	Alkanes		
C6 Hexane	69	120	0.66
C10 Decane	174	1.50	0.73
	Cycloalkanes		
C5 Cyclopentanne	50	260	0.75
C6 Cyclohexane	81	77	0.78
	Aromatic		
1st ring:			
Benzene	80	76	0.80
Toluene	111	22	0.87
o-Xylene	144	5	0.85
Ethyl Benzene	136	7	0.87
2nd ring:			
Naphthalene	218	< 1	1.15
Biphenyl	256	< 1	0.87
1-Naphthol	288	< 1	1.22

Source: Lyman, (1992)

2. Ex-Situ Remediation Technologies

In Ex-situ containment, there are land disposal technologies to contain or minimize contaminants impacts released around the dredging operation (Edson, 2007), see following table.

Table 3 Descriptions of Sediment Remediation Technologies

Name of Treatment	Ex-Situ Technologies
1. Biological Treatment	Bioslurry Containment Land Composting
2. Physical/Chemical Treatment	Chelation Oxidation Dechlorination Solidification/Stabilization Basic Extraction Sludge Solvent Extration Carver-Greenfield Process Soil Washing Containment Barriers
3. Thermal Destruction Technologies	Incineration Pyrolysis High-Pressure Oxidation

Source: Edson, (2007)

Ex-Situ Treatment and Extraction: it refers to remediation technologies that are applied off-place, based on chemical and physical properties of the contaminants. The costs of each of these remediation operations may be significant. In some cases, physical properties of contaminated sediments (such as particle size and soils/water composition) may require the application of one or more pretreatment technologies prior to the processing of the sediment through a treatment unit (Edson, 2007).

3. Anaerobic Microorganisms Capable Of Degrading Organic Pollutants

The roles of the groups of bacteria that make up anoxic/anaerobic consortia important in biodegradation are not fully understood (Suflita, 1988). Anaerobic bioremediation methods have received little attention in the past. Anaerobic bioremediation has been shown to have considerable potential for remediation of soils and subsurface environments (Boopathy, 2003). Many investigators showed the effectiveness of various anaerobic consortia in degrading BTEX and poly-aromatic hydrocarbons (PAH) (Breguard, 1996).

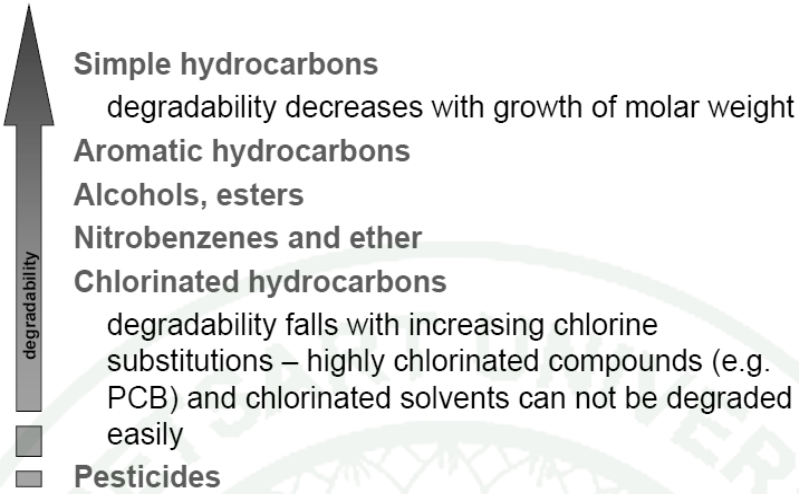


Figure 1 Relative capability of biodegradation

Source: Norris, (1994)

4. Research for Ex-SITU Anaerobic Bioremediation Treatment

The two main categories of ex-situ bioremediation are treatment in the solid-phase and the slurry-phase. Solid-phase bioremediation fosters contaminant biodegradation in dewatered soil, meaning that the weight of the overlying soil is borne by solid-to-solid contacts between soil particles or aggregates of soil particles, such that the contaminated soil can be treated in piles (David, 2003). In solid-phase bioremediation, contaminated soil is aerated in an above-ground treatment cell to promote aerobic contaminant biodegradation (David, 2003). Solid-phase bioremediation can take the form of land farming or biopiles. In contrast with solid-phase bioremediation, slurry-phase reactors treat soil particles or aggregates of soil particles suspended in water. Slurry-phase bioremediation (usually called bioslurry treatment) refers to the decontamination of contaminated soil or sludge by mixing in water to form aqueous slurry, which is then mixed and aerated in a tank or a lagoon to achieve contaminant biodegradation (U. S. EPA, 1990b).

4.1. Slurry Bioreactors

Slurry bioreactor technology is an engineered complex that generally comprises four parts: installations for polluted soil handling and conditioning, the bioreactor battery itself, installations for treated soil handling and disposal, and ancillary equipment for treatment of process by-streams (Eweis, 1998). The SB can be classified as batch, semi-continuous, and continuous from the operation point of view.

The most common operational mode is batch. Another useful classification relies on the main electron acceptor used in the biodegradation process: aerobic (molecular oxygen), anoxic (nitrate and some metal cations), anaerobic (sulfate-reducing, methanogenic, fermentation) (Robles, 2008), see Table 4, and mixed or combined electron acceptors (Robles, 2008).

Table 4 Anaerobic Slurry Bioreactors

Matrix	Contaminant	Remarks	Removal
Soil	2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)	With anaerobic activated sludge as the microbial inocula and sludge cakes as the primary substrates	86%
Soil	Chlorpyrifos	Sequencing batch mode (anoxic-aerobic-anoxic)	91%
Soil	Hexahydro-1,2,3-trinitro-1,3,5-triazine (RDX)	With supplementation of municipal anaerobic sludge as an exogenous source of microorganisms	60%
Sediment	PAH Acenaphthene	Addition sulfate as an electron acceptor enhanced PAH degradation	79%
Soil	αβδ and γ-hexachlorocyclohexane	Bioaugmented with a aerobic slu ge	100%
Soil	2-sec- util-2,6-dinitrofenol (DINOSEB)	Pilot scale	51%
Soil	2,4-dichl rophenoxy-acetic acid	Sulfate reducing conditions with addition of sucrose	48%

Source: Robles, (2008)

4.2. Anaerobic Bioreactors (Gaspack systems)

Professionally manufactured anaerobic bioreactors are expensive to operate. During the anaerobic culturing, all oxygen must be excluded from the system (Prescott et al., 1990). The anaerobic jar is evacuated at least three times and refilled each time with nitrogen or a nitrogen-carbon-dioxide mixture (Prescott, 1990), figure 2.

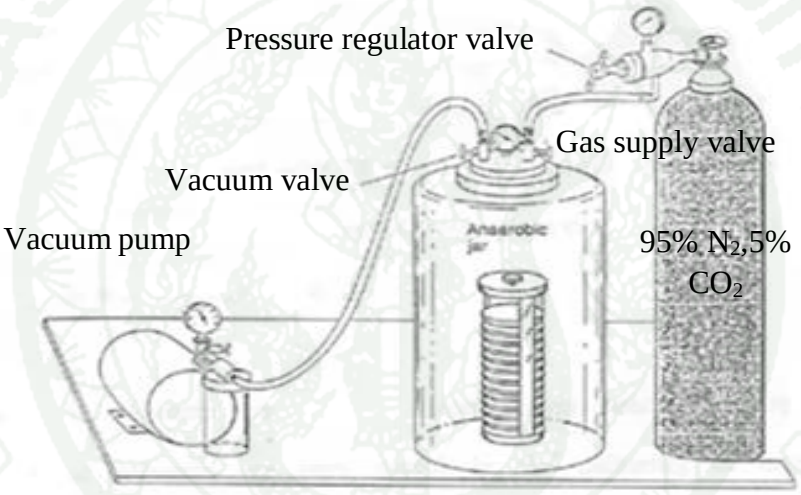


Figure 2 Vacuum and gas displacement method of anaerobic culture

Source: Prescott, (1990)

Oxygen may also be eliminated or be removing air with a vacuum pump and flushing out residual oxygen with nitrogen gas. Often CO_2 as well as nitrogen is added to the chamber since many anaerobes require a small amount of CO_2 for best growth, see figure 3 (Prescott, 1990).

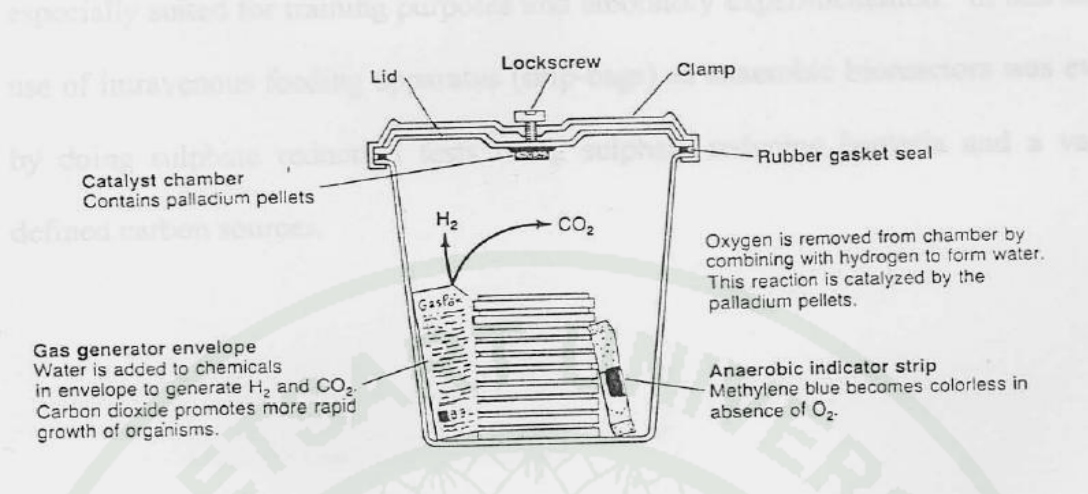


Figure 3 The GasPak anaerobic system

Source: Prescott, (1990)

One of the most popular ways of culturing small numbers of anaerobes is through the use of a GasPak Jar, figure 3 (Prescott, 1990). In this procedure, the environment is made anaerobic by using hydrogen and a palladium catalyst to remove oxygen through the formation of water.

5. Research for Biodegradability: Nutrients, Temperature and Soil Amendments for Total Petroleum Hydrocarbons (TPH)

Heavy Petroleum products such as lubricating oils and fuel oils generally contain a higher proportion of less soluble, higher molecular weight petroleum constituents that are biodegraded at a slower rate than more soluble, higher fraction petroleum compounds such as gasoline (US EPA, 2004). The Carbon: Nitrogen: Phosphorous ratios necessary to enhance biodegradation fall in the range of 100: 10: 1 to 100: 1: 0.5, depending on the constituents and bacteria involved in the biodegradation process (US EPA, 2004). The influence of C: N: P ratio on PAH degradation is that a molar C/N/P ratio of 100/10/1 is a generally used and

recommended ratio for soil remediation (Hoeppel and Hinchee, 1994). The C/N/P ratio of the soil slurry was modified by the addition of NH_4Cl as N source and K_2HPO_4 as P source in accordance to the PAH concentration as C source (Natalie et al, 2004). The average elemental content of a typical microbial cell growing conditions has been determined experimentally to be 50% (w/w) C, 14% (w/w) N, and 3% (w/w) P by dry weight, i.e. a bacterial biomass C/N/P ratio of 50/14/3 expressed in milligrams (Vidali, 2001). The PAH degradation rates of Sphingomonas and Mycobacterium strains in soil slurry were maximal when C_{PAH}/N/P concentrations approached a ratio of ca. 120/14/3 (mg) or 100/10/1 (molar) (Natalie, 2004). Biostimulation with nitrogen and phosphorous nutrient amendments to achieve C_{TPH}:N:P molar ratio of 100:9:1, and $CaCO_3$ amendment at 2,000 mg/Kg for maintaining neutral pH, and periodic 10-days tilling, reduced total petroleum hydrocarbons (TPH) concentrations by up to 64% over a 60 days period (Wongjae, 2010).

For Temperature, oxygen uptake and bacteria growth rate are directly affected by temperature. From 10^oC to 45^oC, the rate of microbial activity doubles for every 10^oC rise in temperature (US EPA, 2004). The method (US EPA 3540C, 1996) is a procedure for extracting nonvolatile and semi volatile organic compounds from solids such as soil, oil and grease etc. The soil sample is mixed with anhydrous sodium sulfate, placed in an extraction thimble or between two plugs of glass wool, and extracted using an appropriate solvent in a Soxhlet extractor. This method (US EPA 3540C, 1996) will be used for analyzing the Total Petroleum Hydrocarbon of soil contaminated with TPH from the Chomburi Industrial Province soils.

TPH analytical method is EPA method 8015 modified. This method reports the concentration of purgeable and extractable hydrocarbons; these are sometimes referred to as gasoline and diesel range organics, GRO and DRO, respectively, because the boiling point ranges of the hydrocarbon correspond to gasoline (C6 to C10-12) and diesel fuel (C8-12 To C24-26). Purgeable hydrocarbons are measured by

purge-and-trap gas chromatography (GC) analysis using a flame ionization detector (FID), while the extractable hydrocarbons are extracted and concentrated prior to analysis by GC/FID. The results are Total Petroleum Hydrocarbons. Table 5 includes the results of two types of analyses on these soil samples: 1) EPA Method 418.1, a spectrophotometric/infrared method that measures total PHC (Petroleum Hydrocarbon) content, and 2) Modified ASTM Method D3328 (similar to EPA Method 8100), a gas chromatographic method that also measures total PHC content. At the time of the analyses, Method 418.1 was required for analysis of soil contaminated with fuel oil type PHCs. Modified ASTM Method D3328 was performed because it is generally considered to be less weathering prone to interference, and it provides a more accurate representation of actual hydrocarbon concentrations. This method also provides an indication as to the type of PHC and degree of weathering.

Table 5 Hydrocarbon Content of Soil Samples

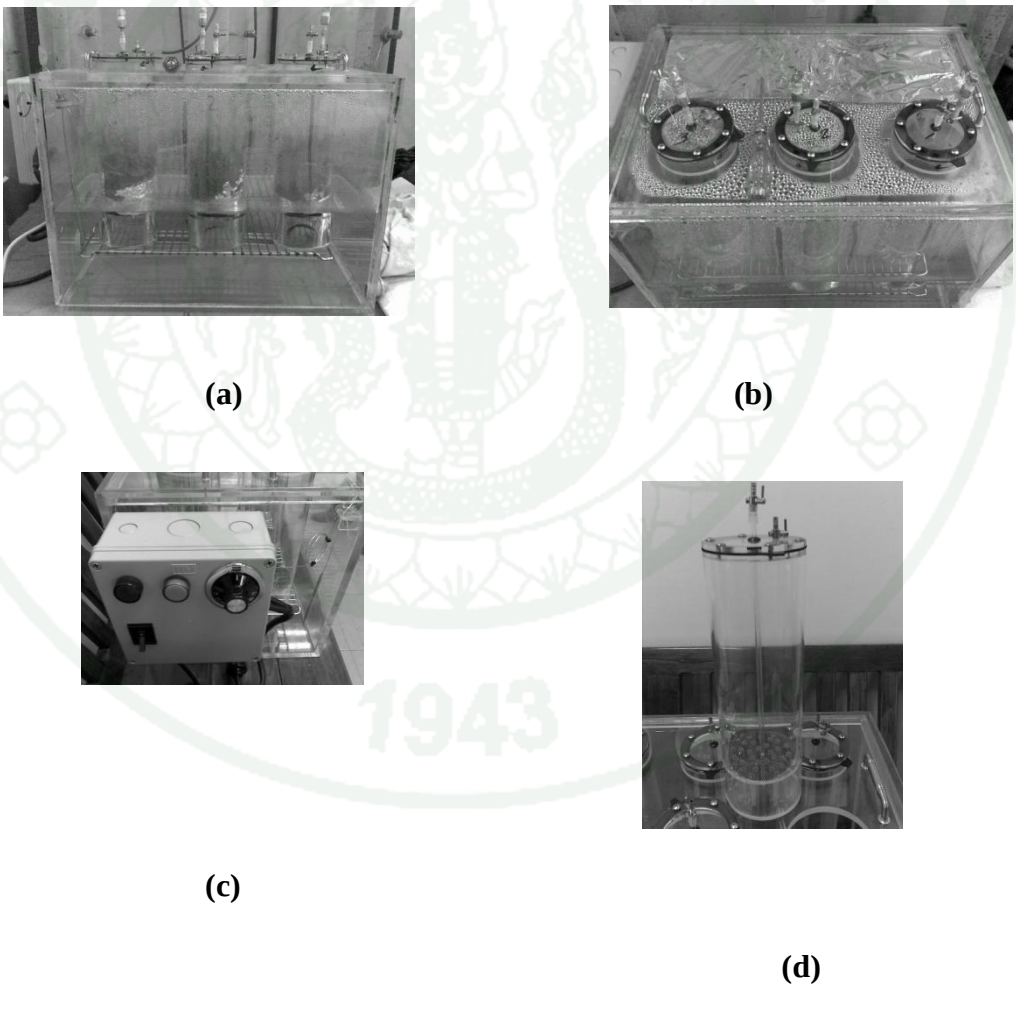
Sample ID	Sample Depth (feet below ground surface)	Total Petroleum Hydrocarbon Content (ppm)	Analytical Method
TSS1-S1	5-11	6,200	EPA 418.1
		3,800	Modified ASTM D3328
TSS1-S2	11-13	11,000	EPA 418.1
		14,000 (duplicate)	EPA 418.1
		4,500	Modified ASTM D3328
TSS2-S1	5-7	3,400	Modified ASTM D3328
		6,500	EPA 418.1
		6,300 (duplicate)	EPA 418.1
TSS4-S1	5-7	1,400	EPA 418.1
		520	Modified ASTM D3328
TSS4-S2	7-12	1,600	EPA 418.1
		850	Modified ASTM D3328
TSS5-S1	5-7	190	EPA 418.1
		67 (duplicate)	EPA 418.1
		26	Modified ASTM

Source: US EPA, (2004)

MATERIAL AND METHOD

1. Materials for Bioreactor

Water bath incubator has 2 Liters bioreactor bottles by six replications, two 0.4 cm diameter gas sampling portholes, rubber cork, and on/off controlling Temperature operation, see figure 4 as following.



(a) **(b)**

(c)

(d)

Figure 4 Enhanced Anaerobic Bioreactor (a, b, c, and d)

2. Ex-Situ Anaerobic Bioreactor Specification

The six anaerobic bioreactor bottles are constructed by heat resistance plastic fiber glass bottles. They will be duplicated in six bioreactors, which height is 30 cm and their diameter is 10 cm. The amount of each bioreactor bottle can hold 2 L. The size of the anaerobic bioreactors is enough for 1.2 kg soil contaminated with total petroleum hydrocarbon, which height is 19 cm of each 2 L bioreactor bottle. The ratios of nutrients by hydrocarbons and varying temperature with positive control and negative control are essential components in this technique. The size of the incubator has to be enough for six replications of 2L bioreactor bottle, and its length is 50 cm, its width is 40 cm with base height 7 cm, and its height is 30 cm.

There is an outlet port for CO_2 and CH_4 gases. The two gas counters are placed and connected to two outlet ports of the two reactors by 0.4 cm diameter pipe to collect and absorb the Carbon dioxide and Methane. In this thesis research, we use the incubator for three aerobic bioreactor bottles to enhance the bioremediation by varying temperature in soil by sterilization for reactor number 1, soil with no sterilization and nutrients for reactor number 2, and soil with nutrients such as nitrogen and phosphorous for reactor number 3. For the size of the incubator, its height is 35 cm with its base support, width is 35 cm, and length is 50 cm, see figure 5 and 6.

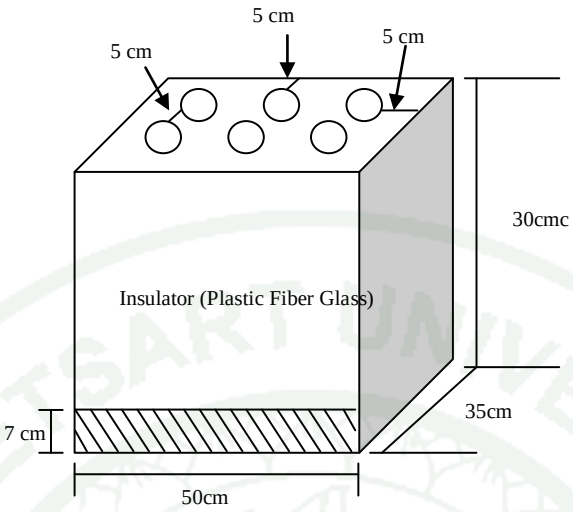


Figure 5 Incubator

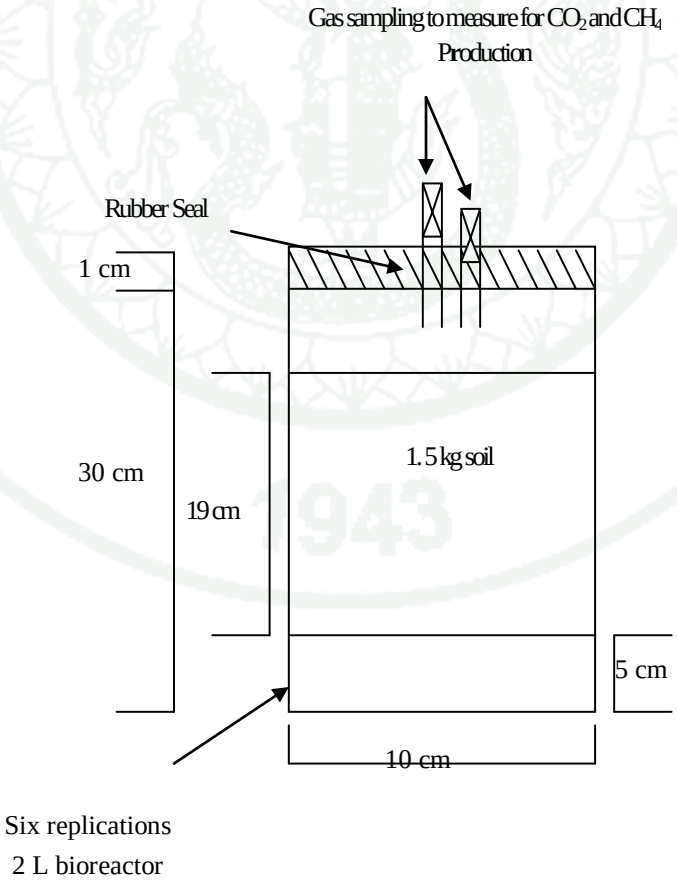


Figure 6 Ex-Situ Anaerobic Bioreactor Bottle

3. Chemical Reagents, Materials and Equipments for CO_2 and CH_4 production, for residue TPH measurement in Soils, and for Nitrogen and phosphorous

Materials and Equipments for CO_2 and CH_4 Production for measurement as following:

1. Hamilton syringe (1001 RN 1.0 ml)
2. Gas Chromotography 6890

Materials and Equipments use for Total Petroleum Hydrocarbon extraction from the soil sample for measurement of TPH reduction as following:

1. Thermometer
2. Soxhlet Extractor
3. Oven (105^{o}C)
4. Autoclave
5. Dessicator
6. Rotavapor R-114
7. weight meter
8. Parafilm
9. Syringe Plastic (1 ml)
10. volumetric flask 250 ml

Chemicals for TPH measurement

1. Acetone
2. Hexane

Chemicals for Nitrogen and Phosphorous measurement

1. H_2O_2 (30%)
2. Lithium Sulphate
3. Selenium Powder
4. H_2SO_4 (36N)
5. Sodium Citrate
6. NaOH
7. Sodium Hypochlorite Solution, 5% available Cl^-
8. Sodium Nitroprusside
9. Sodium Salicylate
10. Sodium Tartrate
11. Ammonium Sulphate
12. Ammonium Molybdate
13. Atimony Sodium Tartrate
14. Sulphuric Acid
15. Ascorbic Acid, 1%
16. KH_2PO_4

4. Methodology

FIRST PHASE

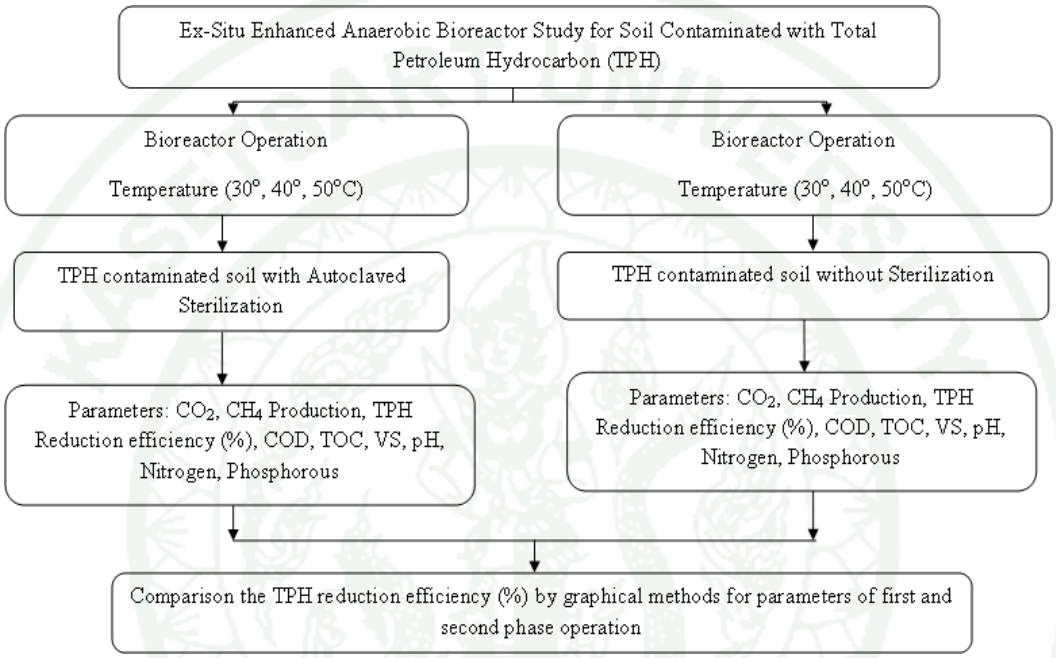


Figure 7 First Phase Ex-Situ Anaerobic Bioreactor Study for Soil Contaminated with Total Petroleum Hydrocarbon (TPH) experimental plan

Second Phase

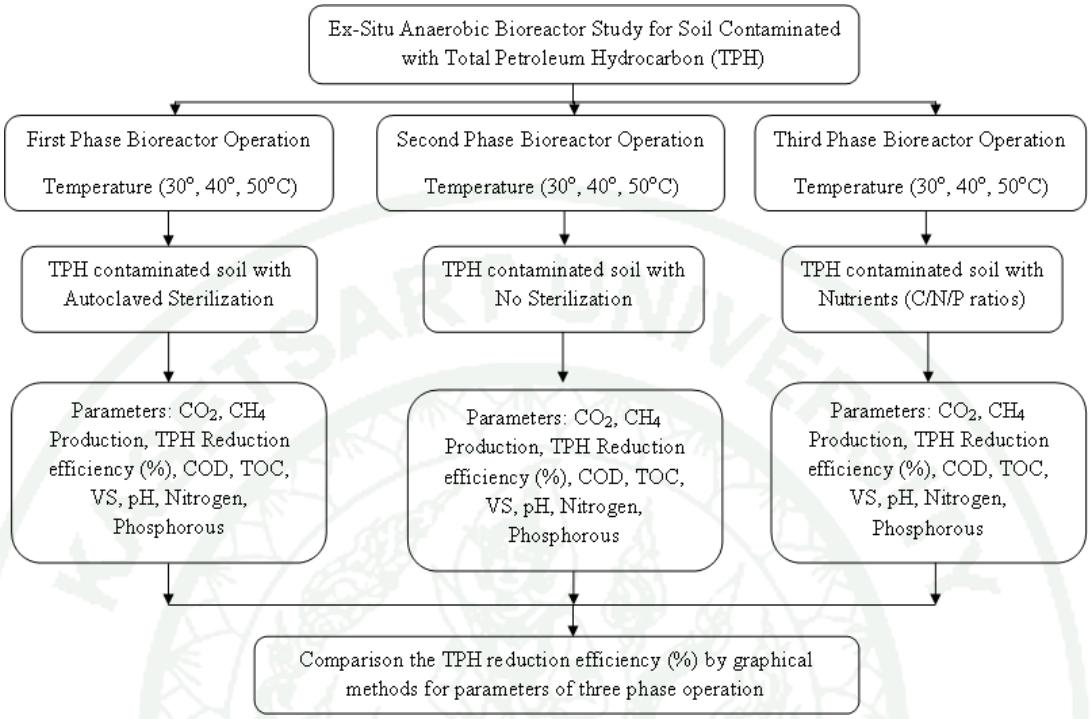


Figure 8 Second Phase Ex-Situ Anaerobic Bioreactor Study for Soil Contaminatedwith Total Petroleum Hydrocarbon (TPH) experimental plan

5. Ex-Situ Anaerobic Bioreactor Test for CO_2, CH_4 Production and TPH reduction for TPH contaminated soil with sterilization, TPH contaminated soil without sterilization and nutrients, and TPH contaminated Soil with nutrients

The Total Petroleum Hydrocarbon contaminated soils were be collected from Chonburi industrial areas. When using surface soil at 5 cm depth; rocks, stones, leaves, debris were removed for uniformity of the soil. For soil with autoclaving, heat is known to be inactive enzymes secreted by soil microorganisms (Tibbett, 2007) and one common biocidal treatment is autoclaving (Anderson and Magdoff, 2005). Autoclaving uses moist heat and pressure to kill soil organisms and typically results in a decrease in microbial biomass and microbially mediated metabolism, such as enzyme activity (Tiwari, 1988). Autoclaving was successful at killing the active microbial biomass, as measured by substrate induced respiration, in all soils (Carter, 2007). Soils selected for autoclaving were placed in heat resistance plastic bag. Each bag contained (1.2 kg) soil only. Three bags of each soil (1.2 kg) were autoclaved for this thesis research. Bags were sealed with rubber ring and autoclaved at 121^{o}C (103 kPa) for 30 min on 2 consecutive days (Bennett, 2003). Soils were incubated sealed at 22^{o}C for 24 hr between autoclave treatments (Carter, 2007). Volatile Solid (VS) and Total Petroleum Hydrocarbon (TPH) analysis will be conducted on TPH contaminated soils with nutrients, TPH contaminated soil without nutrients, and autoclaved sterilization soils contaminated with TPH for this thesis research by anaerobic bioreactor bottles.

In the first phase experimental thesis, TPH contaminated soil were sterilized and not sterilized by ex-situ anaerobic bioremediation process with continuous running variable temperatures; 30^{o}C, 40^{o}C, and 50^{o}C for all six bioreactor bottles (2L of volume in each) respectively for 45 days period. The 250 milliliters of distill water will add daily into the reactor.

In the second phase experimental thesis, soil contaminated with total petroleum hydrocarbon were conducted with nutrients (C/N/P, 100:2:0.2 molar ratios) and temperature changing to increase of every 15 days in each of 30^{o}C, 40^{o}C, and 50^{o}C for 30 days for three bioreactors with nutrients for positive control and another two reactors without nutrients for negative control by autoclaved, biocidal sterilized soil and no sterilized soil, which soils were contaminated with total petroleum hydrocarbon (TPH). Every five days, water for moisture was provided only once in the reactor bottles for TPH contaminated soils. In some literature review of Soil contaminated with TPH, Biostimulation with nitrogen and phosphorous nutrient amendments to achieve C_{TPH}:N:P molar ratio of 100:9:1, and $CaCO_3$ amendment at 2,000 mg/Kg for maintaining neutral pH, and periodic 10-days tilling, reduced total petroleum hydrocarbons (TPH) concentrations by up to 64% over a 60 days period (Chang, 2010), and the Carbon: Nitrogen: Phosphorous ratios necessary to enhance biodegradation fall in the range of 100: 10: 1 to 100: 1: 0.5, depending on the constituents and bacteria involved in the biodegradation process (US EPA, 2004). Water needed for the soil will be porosity (n=30%), initial saturation (S=20%), and desired water content (60%) (Massachusetts Institute of Technology Open Course Ware). For the moisture contents, each 250 ml of distilled water was mixed in the TPH contaminated soil without sterilization and nutrients; and with nutrients to enhance the anaerobic bioremediation process in temperatures: 30^{o}C, 40^{o}C, and 50^{o}C.

Thus, water will be needed 250 ml for (30) days period for 1.2 kg soil contaminated with total petroleum hydrocarbons (TPH). The ratios of nutrients are C_{TPH}:N:P molar ratio of 100:2:0.2, which will be used in this research, for reactor bottle number 3 (2 L volume of each bottle).

CO_2 and CH_4 production from soil contaminated with Total Petroleum Hydrocarbon (TPH) were analyzed by using a gas chromatograph (GC 6890 series) equipped with a thermal Conductivity detector (Carrier gas flow rate: 65ml/min He, Injection port temperature: 105^{o}C back inlet, Column: Packed column CTR I, Oven

Temperature: 35^{o}C-ambient isothermal, Run time: 10 min, Detector Temperature: 150^{o}C front TCD, Reference flow: 65 ml/min He, Makeup flow: 2 ml/min He).

The determination of the TPH from contaminate soil was done by Soxhlet Extration (US EPA, 1996) and Rotavapor R-114. Five grams of contaminated soils with petroleum hydrocarbon were collected and mixed with anhydrous sodium sulfate (five grams), placed in an extraction thimble. For the extraction solvents, all solvents must be pesticide quality or equivalent (Acetone/Hexane; 1:1, v/v). The 150 ml acetone: hexane at the ratio of 1:1 will be used as a solvent.

The correlation of the CO_2 and CH_4 Production, temperature, nutrients, and the efficiency of the degradation of the TPH by time will be determined by using graphical result.

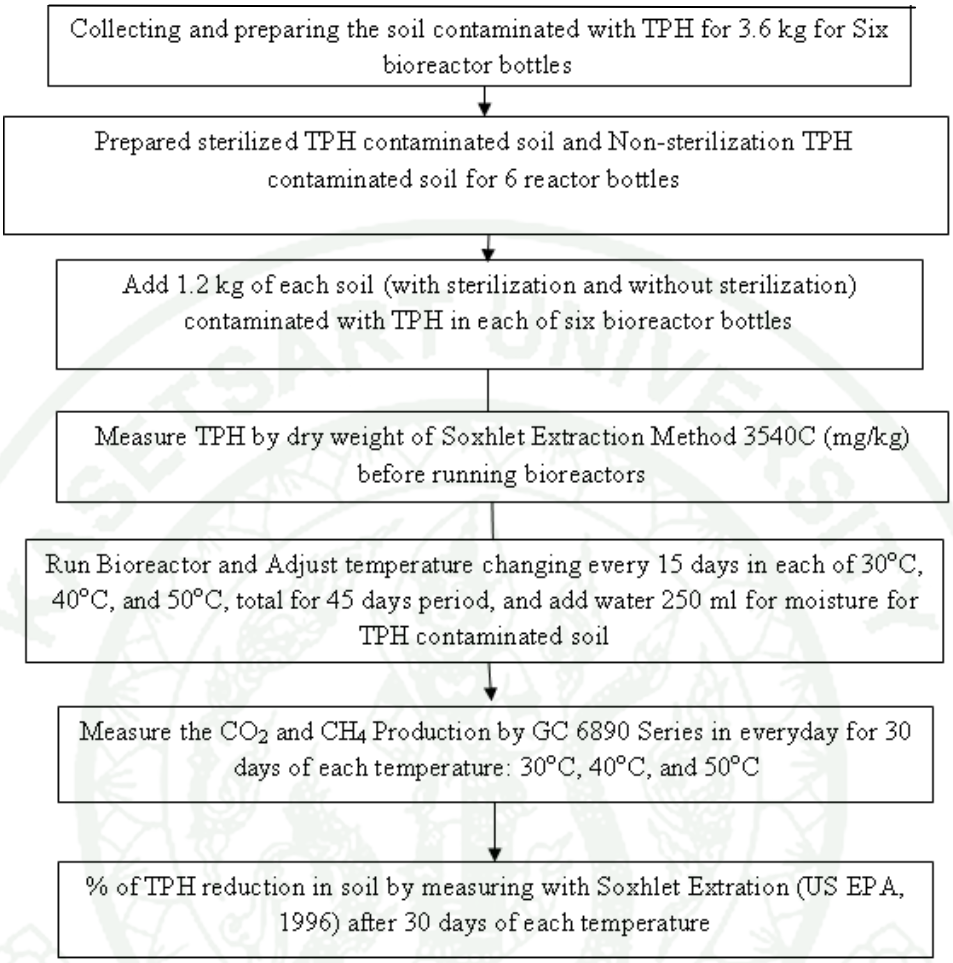


Figure 9 First Phase Experiment of Ex-Situ Anaerobic Bioreactor Research Method for optimum temperature of the TPH Reduction

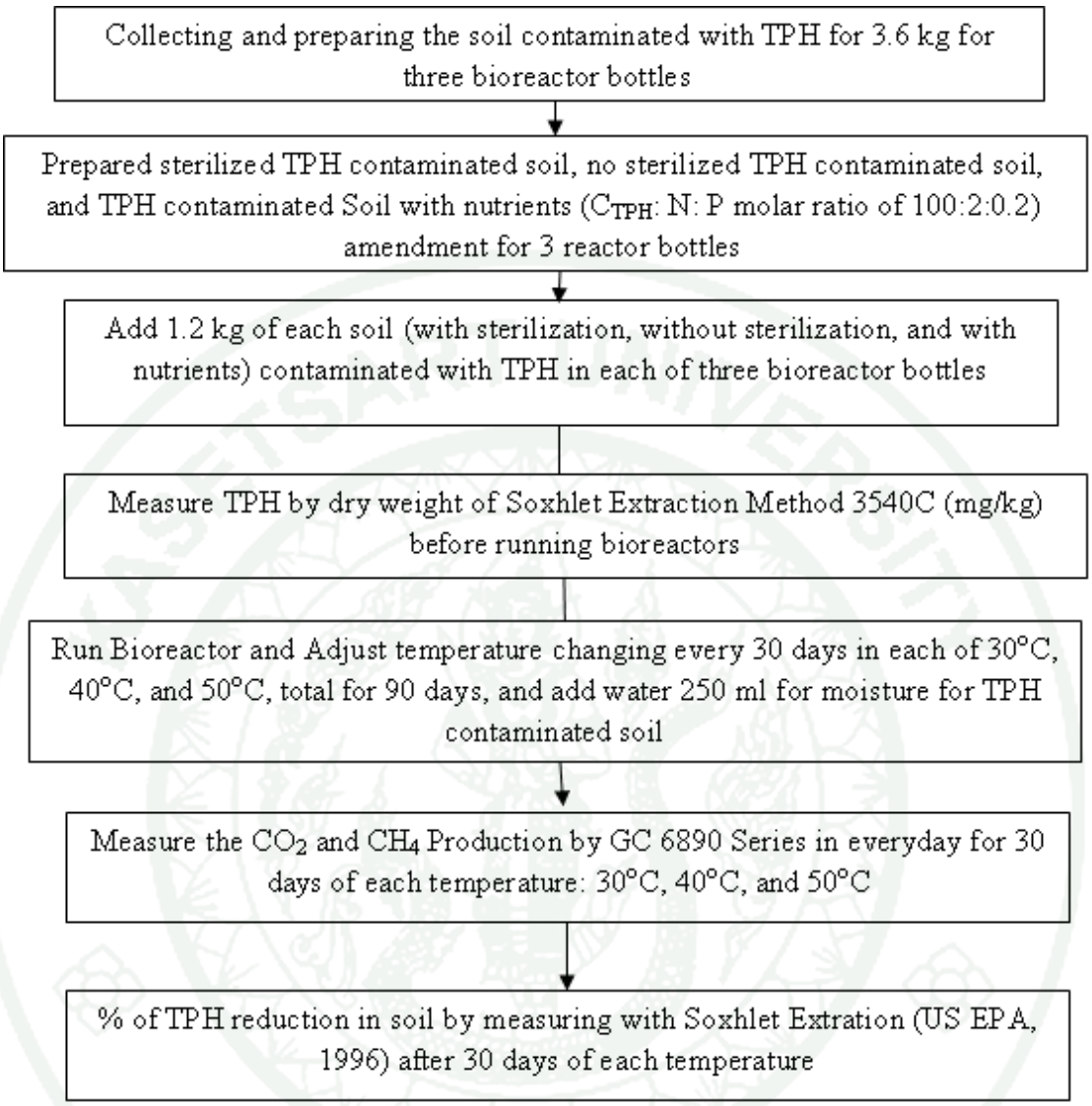


Figure 10 Second Phase Experiment of Ex-Situ Anaerobic Bioreactor Research Method for optimum temperature of the TPH Reduction

5.1 Conditions on CO_2 and CH_4 Production by Gas Chromatography Method (GC 6890 Series, Agilent)

- Carrier gas flow rate: 65ml/min He,
- Injection port temperature: 105^{o}C back inlet,
- Column: Packed column CTR I,
- Oven Temperature: 35^{o}C-ambient isothermal,
- Run time: 10 min,
- Detector Temperature: 150^{o}C front TCD,
- Reference flow: 65 ml/min He,
- Makeup flow: 2 ml/min He
- Read the total Area for CO_2 and CH_4 production from GC 6890 Series Chromatogram

6. Analysis on TPH extraction method By Soxhlet Extraction

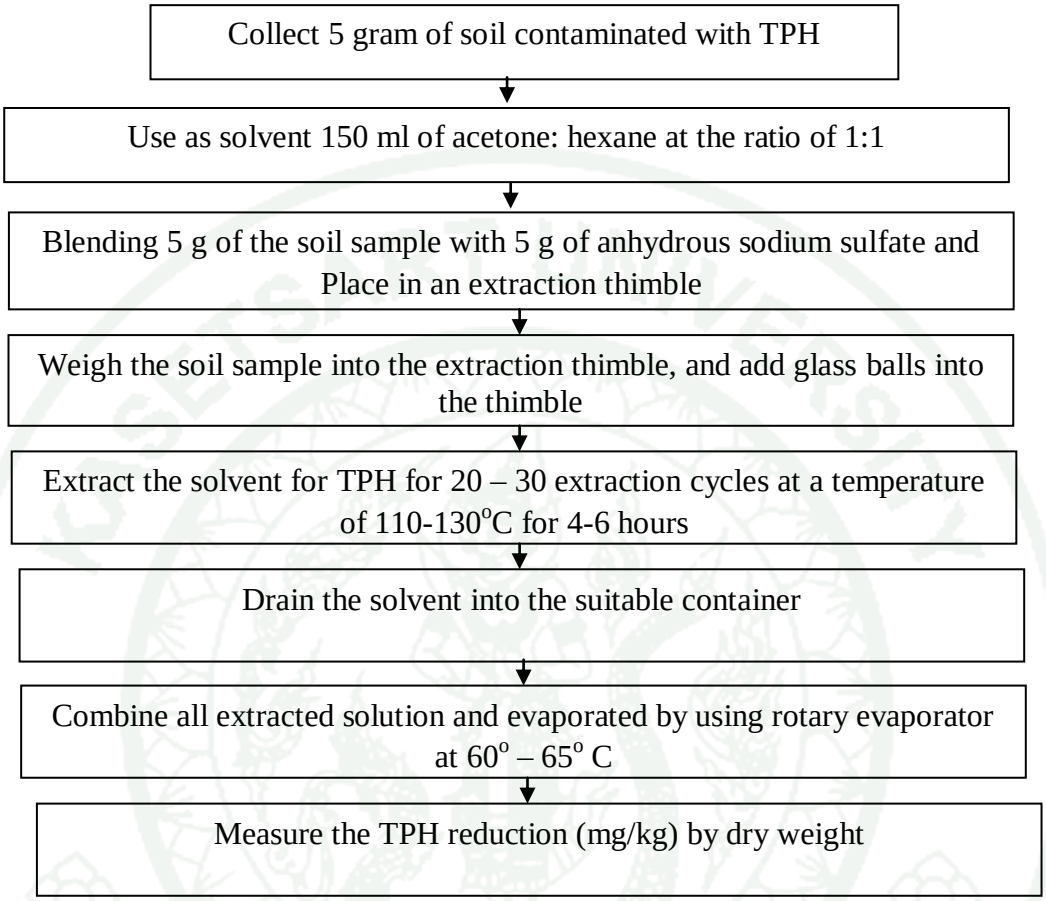


Figure 11 Residue (TPH) oil extraction method

Source: US EPA, (1996)

7. Analysis of Total Organic Carbon in Soils [TOC-V_{CSH} (SSM-5000A)]

In this research, TOC-V_{CSH} (SSM-5000A) has been used to measure TOC following its manual standard:

Total Carbon = Total Organic Carbon + Inorganic Carbon

TOC = TC – IC

Where SSM-5000 A analyzes TC and IC to find TOC

8. Analysis and Procedure for Total Nitrogen and phosphorous in Soils

For the digestion for total nitrogen (and Phosphorus), prepare the reagents: hydrogen peroxide (30%), Lithium sulphate, Selenium powder, and Sulphuric acid (Concentrated solution: 36N) [Cataldo, 1975]. The procedure as following:

Procedure

1. Weight about 0.2±0.001 g ground soil into a digestion tube (min 75 ml size). Record the weight, W (g).
2. Add 4.4 ml digestion mixture to each tube.
3. Digest at 360^{O}C for 2 hr. The solution must be colorless and any remaining solids white. If color can still be seen, heat for one hour.
4. Allow to cool.
5. Add about 50 ml water and mix well until no more sediment dissolves. Allow to cool.
6. Volumetrically make up to 100 ml with water and mix well.

7. Allow to settle so that a clear solution can be taken for analysis.
8. Determine the nitrogen and phosphorus in the digests. Make up the working standards with the addition of 2.5 ml digested digestion blank.

8.1 Colorimetric Determination of Ammonium

Reagents

- Sodium citrate
- Sodium hydroxide
- Sodium hypochlorite solution, 5% available Cl^-
- Sodium nitroprusside
- Sodium salicylate
- Sodium tartrate

Reagent N1: dissolve 34 g sodium salicylate, 25 g sodium citrate and 25 g sodium tartrate together in about 70 ml water. Add 0.12g sodium nitroprusside and when dissolved make up to 1000 ml with water. Mix well.

Reagent N2: dissolve 30g sodium hydroxide in about 750 ml water. Allow to cool, add 10 ml sodium hypochlorite solution and make up to 1000 ml with water. Mix well.

Standards

- Dry about 7 g ammonium sulphate at 105^{O}C for 2 hr. Cool in a desiccators.
- Dissolve 4.714 g dry ammonium sulphate in water and make up to 1000 ml in a volumetric flask. This is a 1000 µg/ml NH_4^+-N stock solution
- Pipette 50 ml of the 1000 µg/ml NH_4^+-N solution into a 500 ml volumetric flask and make up to the mark with water. This is a 100 µg/ml N solution.
- Pipette 0, 5, 10, 15, 20 and 25 ml of the 1000 µg/ml NH_4^+-N solution into labeled 100 ml volumetric flask.

Procedure

- Using a micropipette, transfer 0.100 ml of each standard and sample into suitably marked test tubes.
- Add 5.00 ml of reagent N1 to each test tube, mix well and leave for 15 minutes.
- Add 5.00 ml of reagent N2 to each test tube, mix well and leave for 1 hr for full color development. The color is stable for the day only.
- Read each standard and sample absorbance at 655 nm.

Calculation

Plot a graph of absorbance against standard concentration. Determine solution concentrations for the sample and the blanks. Subtract the mean blank value from the sample; this gives a value for corrected concentration, C.

$$\text{Nitrogen (\%)} = \{(C \times V)/ W\} \times 0.0001$$

Where

C = corrected concentration (µg/ml)

V = final digest or extract volume (ml)

W = weight of sample (g)

7.2 Colorimetric determination of Phosphorus

Reagents

- Ammonium molybdate
- Antimony sodium tartrate
- Sulphuric acid, conc.
- Ascorbic acid, 1 %: dissolve 1 g ascorbic acid in 100 ml water, make a fresh solution every day.
- Molybdate reagent: dissolve 4.3 g ammonium molybdate in 400 ml water in a 1000 ml measuring cylinder. Dissolve 0.4 g antimony sodium tartrate in 400 ml water, then add to the measuring cylinder.

Standards

- Dry about 7 g KH_2PO_4 at 105^OC for 2 hr. Cool in a dessicator.
- Dissolve 4.394 g dry KH_2PO_4 in water and make up to 1000 ml in a volumetric flask. This is a 1000 µg/ml P stock solution
- Pipette 10 ml of the 1000 µg/ml P solution into a 500 ml volumetric flask and make up to the mark with water (or with the appropriate solution as required for a given determination). This is a 20 µg/ml solution.
- Pipette 0, 5, 10, 15, 20 and 25 ml of the 20 µg/ml P solution into labeled 100 ml volumetric flasks. Make up to the mark with water and mix well. There are working standards and contain 0, 1, 2, 3, 4 and 5 µg/ml P in suitable background solution.

Procedure

1.Pipette 1 ml standard or sample into a test tube.
2.Add 4.0 ml ascorbic acid solution
3.Add 3.0 ml molybdate reagent and mix well
4.Leve for 1 hr for the color to develop fully.
5.Read the standard and sample absorbances at 880 nm.

Calculation

Plot a graph of absorbance against standard concentration. Determine solution concentrations for each unknown and the blanks.

$$C\ (P\ \mu g/ml) = P_{sample} - P_{mean\ blank}$$

9. Preliminary Measurement of TPH Concentrations, Moisture Content, Volatile Solid, TOC, Nitrogen, Phosphorous, and pH

The EPA method (US EPA, 1996) is a procedure for extracting nonvolatile and semi volatile organic compounds from solids such as soil, oil and grease etc.
The preliminary results of the concentration of Total Petroleum Hydrocarbons and analytical methods are described in the following tables. See table 6 as following for soil parameters: concentrations and analytical methods;

Table 6 Soil Parameters (Preliminary Results)

Parameters	Concentrations	Analytical Method
TPH	50,000 mg/kg soil	US EPA(3540C)
Mositure Content	28.75 %	US EPA (1684)
TOC	6.795%	TOC-V_{CSH} (SSM-5000A)
Volatile Solid	6.18 %	US EPA (1684)
COD	186,761 mg/kg soil	Titration standard method
Ph	6.71	Standard Method (McKeague,1978a)
Nitrogen	786.52 mg TKN/kg soil	Standard Method
Phosphorous	3,793.10 mg TKp/kg Soil	Standard Method

RESULT AND DISCUSSION

1. The Effects of Temperature on TPH reduction

Petroleum was a complex mixture of hydrocarbons. Hydrocarbons within the saturate fraction include n-alkanes, branched alkanes, and cycloalkanes (naphthenes) (Ronald, 1981). The n-alkanes were generally considered the most readily degraded components in a petroleum mixture (Davies, 1968). Biodegradation of n-alkanes with molecular weights up to n-C_{44} had been demonstrated (Haines, 1974). Further degradation of the carboxylic acid proceeds β-oxidation with the subsequent formation two-carbon-unit shorter fatty acids and acetyl coenzyme A, with eventual liberation of CO_2 (Ronald, 1981), and also fatty acids, some of which are toxic, had been found to accumulate during hydrocarbon degradation (Bartha, 1973). Highly branched isoprenoid alkanes had also been found to undergo omega oxidation, with formation of dicarboxylic acids as the major degradative pathway (McKenna, 1971). Cycloalkanes were particularly resistant to microbial attack (Norris, 1976). Complex alicyclic compounds, such as hopanes (tripentacyclic compounds), are among the most persistent components of petroleum spillages in the environment (Atlas, 1981).

In this thesis research, there are two phases: first phase is about the TPH contaminated soil with sterilization and without sterilization for continuous operating to increase temperature every (15) days in 30^{o}C, 40^{o}C, and 50^{o}C for total 45 days period; and second phase is about the TPH contaminated soil with sterilization, without sterilization, and with nutrients for 30^{o}C about 30 days period, for 40^{o}C about 30 days period, and for 50^{o}C about 30 days period. When comparing the results of the TPH reduction efficiency, first phase experiment for TPH reduction efficiencies are more than second phase experiment. First phase experiment for TPH contaminated Soil by anaerobic bioremediation at different temperatures started from 30^{o}C to 40^{o}C and to 50^{o}C to degrade hydrocarbons. This is because TPH contaminated soil of

second phase experiment by anaerobic bioremediation was changed to replace with another TPH contaminated soil after finishing at different temperature in 30^{o}C, 40^{o}C, and 50^{o}C after each of (30) days.

2. Reduction of TPH at Temperature of 30^{o}C, 40^{o}C, and 50^{o}C for 30 day operation time for First Phase Experiment

Within the range of 10^{o}C and 45^{o}C, the rate of microbial activity typically doubles for every 10^{o}C rise in temperature (US EPA, 2004). For the First Phase experiment for the TPH Contaminated soil with Sterilization and without Sterilization, temperature started from 30^{o}C to 40^{o}C and to 50^{o}C with continuous heating by no changing to replace with another TPH contaminated soil again and the results of average reduction efficiency of TPH contaminated soil without sterilization for methane production showed 2.943±0.955% in 30^{o}C, and for CO_2 production is 34.82±6.07%, see appendix table 6 and 7; and figure 12, 13, and 14. For 30^{o}C, 40^{o}C, and 50^{o}C with Sterilization, the methane production is 0%; see appendix table 11, and for CO_2 production is 19.2025±4.66%, see appendix table 12; and figure 15, 16, and 17. Thus, after increasing in 10^{o}C of each (15) days period, the degradation rate of CH_4 and CO_2 production are increased.

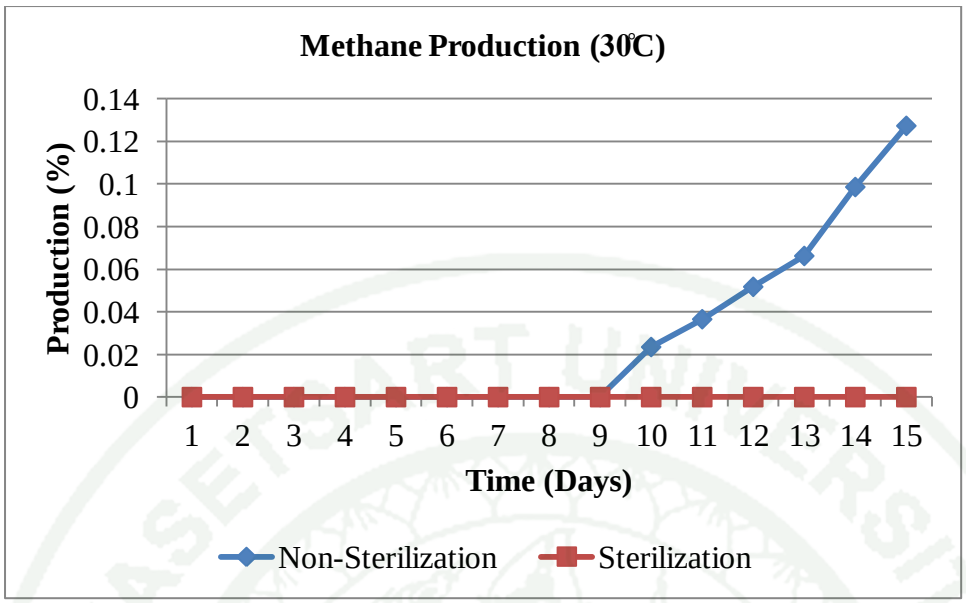


Figure 12 Methane Production by TPH Soil (30°C)

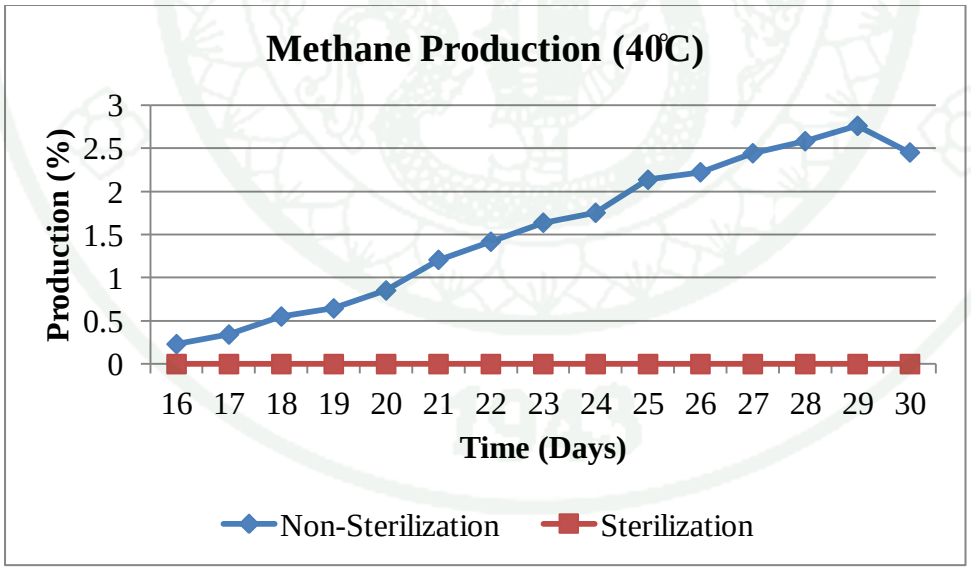


Figure 13 Methane Production by TPH Soil (40°C)

The maximum production of TPH contaminated soil without sterilization for methane production after (15) days showed around 0.12% in 30^{o}C, and for CO_2 production is around 30% in 30^{o}C, see figure 12, and 15. Then, continuously running for 40^{o}C of (15) days period without sterilization of TPH contaminated soil, maximum methane production is around 3%, see figure 13, and maximum CO_2 production is around 35%; see figure 16. For continuously increasing in 50^{o}C of (15) days period without sterilization of TPH contaminated soil, maximum methane production is 10%, see figure 14, and maximum CO_2 production is 60%; see figure 17.

The methane production of TPH contaminated with sterilization after (15) days is 0% in 30^{o}C, see figure 12, and the maximum CO_2 production is around 25% in 30^{o}C, see figure 15. Then, continuing to operate (15) day period by increasing in 40^{o}C of sterilization of TPH contaminated soil, there is no methane production, and the maximum CO_2 production is around 20%, see figure 13 and 16. Then, continuing to increase of 50^{o}C for (15) days period for the sterilized TPH contaminated soil, there is no methane production, and the maximum CO_2 production is around 30%, see figure 14 and 17. However, the CO_2 production was slightly increased when temperature was increased. Compared to the sterilization contaminated soil, it was found that there is no CH_4 production occurred although the temperature was increased. This was indicated that microorganisms were very important to play an important role for the organic substance degradation. The anaerobic conditions occurred from the water addition to the contaminated soil samples as indicated by zero percent of oxygen within the reactors.

Thus, some CO_2 production occurred until no more CO_2 production, and also the rate of CO_2 production is more in non-sterilized TPH contaminated soil than sterilized TPH contaminated soil.

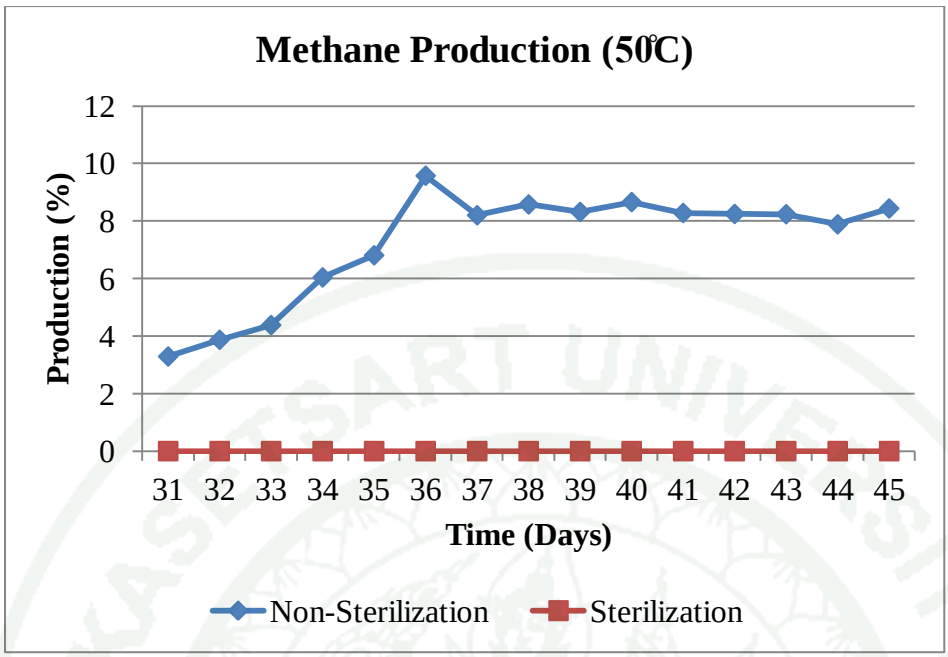


Figure 14 Methane Production by TPH Soil (50°C)

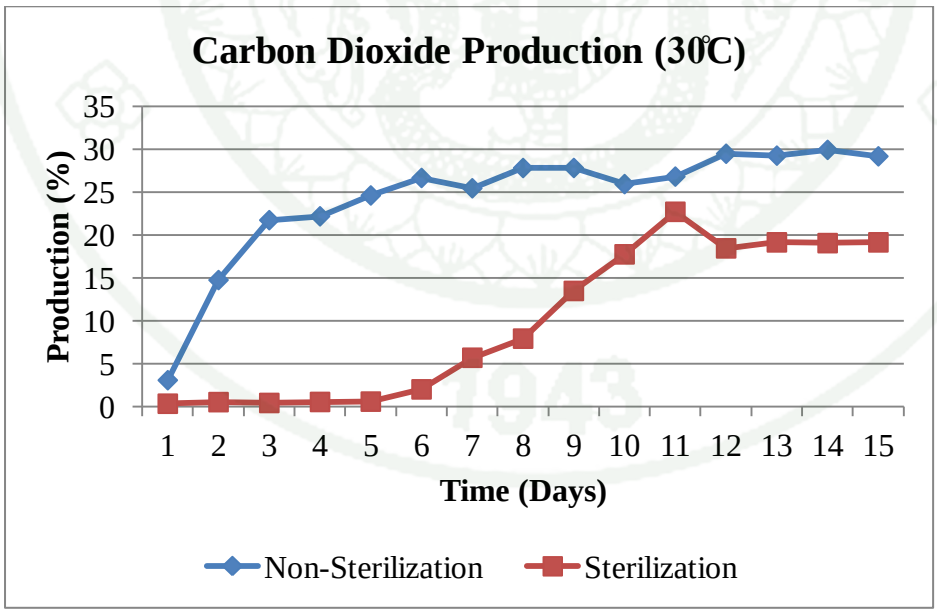


Figure 15 Carbon Dioxide Production by TPH Soil (30°C)

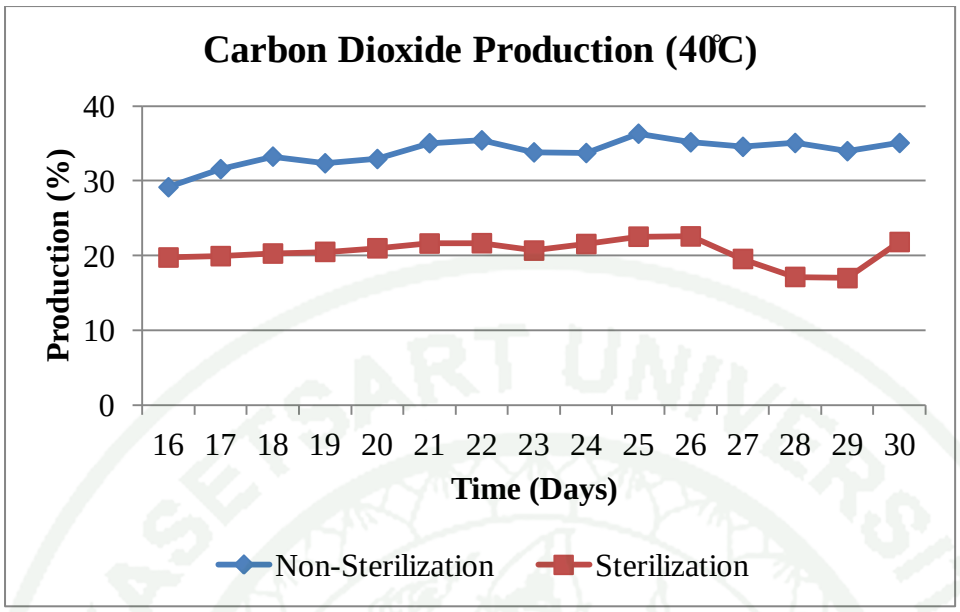


Figure 16 Carbon Dioxide Production by TPH Soil (40°C)

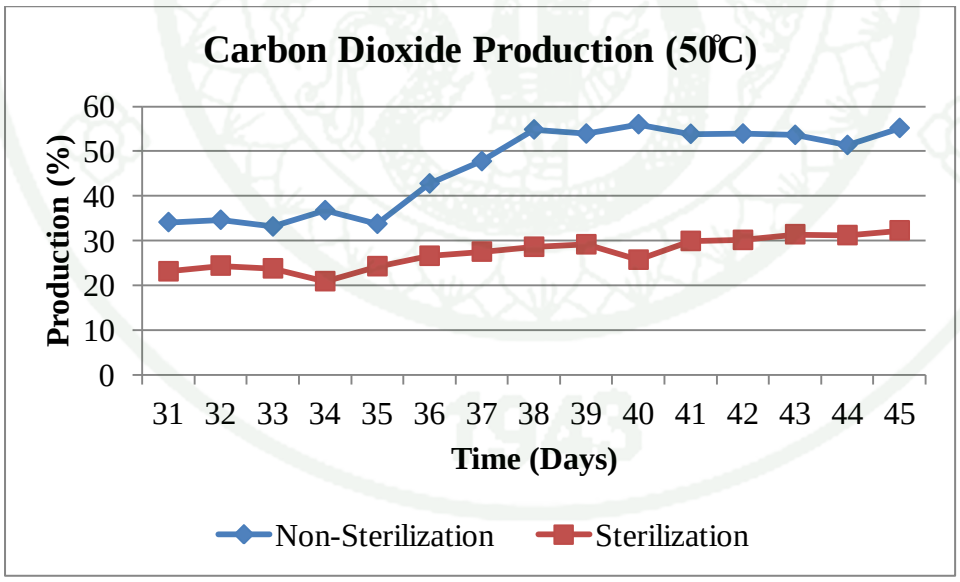


Figure 17 Carbon Dioxide Production by TPH Soil (50°C)

The increasing different temperature in 30^{o}C, 40^{o}C, and 50^{o}C has significant effects on the anaerobic bioremediation for soil contaminated with total petroleum hydrocarbons. Therefore, the TPH concentration of soil in the anaerobic reactor bottles was gradually decreased. The TPH contaminated soils have been autoclaved or sterilized before operating temperature in 30^{o}C, 40^{o}C, and 50^{o}C. Thus, there are no more methane production in sterilized TPH contaminated soil reactor bottles see figure 12, 13, and 14. In the sterilization of TPH contaminated soil, the CO_2 production was also started to increase after 5 days period during 30^{o}C until 50^{o}C, see figure 15, 16, and 17, because the small concentration of CO_2 came from the remaining in the contaminated soil before the operation since the removal of CO_2 before the addition of contaminated soil in the reactor was not performed.

Initial TPH concentration of the soil is 50,000 mg/kg after making average concentration of the collected soil samples to be manually digested, homogenized and uniformed TPH contaminated soil. The reduction efficiencies of the sterilized TPH contaminated soil should have been nearly 0%, but it has shown the average TPH reduction was around 6.62%, see table 7 and figure 18. For the non-sterilization, the % average TPH reduction efficiency is 49.49%, see table 7. During Ex-Situ Anaerobic bioremediation process by increasing temperature, the reactor number 5 has been leaked by water from incubator, and it has increased moisture contents inside the bioreactor bottle to produce more CO_2. Thus, some of TPH concentrations have been reduced in reactor 5. The measurement by analytical method for TPH concentrations in soil has been varied though making homogenized TPH contaminated soil by manual. Thus, the reduction of the TPH contaminated soil with sterilization could have been very high concentrations for initial and after conditions. So, it is acceptable that no more TPH reduction is in sterilized TPH contaminated soils for first phase experiment.

Table 7 First Phase Experiment of TPH Reduction Efficiency (%)

Average TPH Reduction Efficiency (%)			
TPH Contaminated Soil without Sterilization			
	Initial (Average Concentration)	After (Average Concentration)	% Reduction
Reactor 1, 2, and 3	50,000 mg/kg	25,250.82 mg/kg	49.49
TPH Contaminated Soil with Sterilization			
	Initial (Average Concentration)	After (Average Concentration)	% Reduction
Reactor 4 and 6 (Reactor 5 excluded)	50,000 mg/kg	46,689.55 mg/kg	6.62%

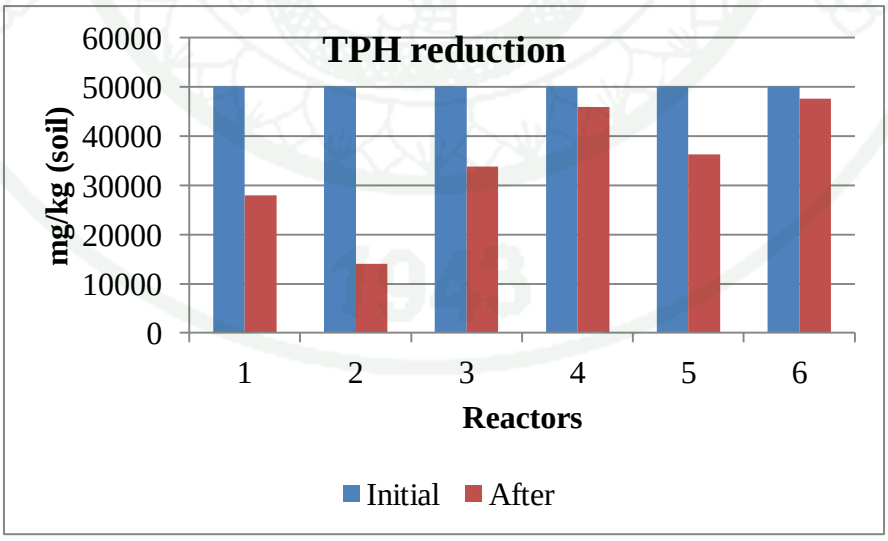


Figure 18 TPH Reduction for all reactors (1, 2, and 3 for Non-Sterilization; 4, 5, and 6 for Sterilization) for First Phase experiment

2.1 The percent variable of Moisture Content, Volatile Solids, Total Organic Carbon, COD, and pH for First Phase Experiment

In the reactor 1, 2, and 3 without sterilization; 250 ml distilled water has been added daily in the soil contaminated with total petroleum hydrocarbons, in which different operating temperature of 30°C, 40°C, and 50°C for 45 days period. After 45 days period, 52.73%, 59.65%, and 52.03% moisture contents have left in the non-sterilized TPH contaminated soil of reactor 1, 2, and 3, see appendix table 14. In reactor 4, 5, and 6 with sterilization TPH contaminated soil; moisture contents were 51.092%, and 45.899%, see appendix table 14. Initial moisture content before water added was 28.75%, see table 6. Thus, average moisture contents in the soil contaminated with TPH effected increasing anaerobic biodegradation, see table 8.

The initial amount of volatile solids and TOC had been around 6% and 7%, see table 6. After operating with variable increasing temperature, average remaining of volatile solids had been around 3.311% for non-sterilization and 3.869% for sterilization, see table 8, average remaining of TOC had been around 3.32% for non-sterilization and 3.26% for sterilization, see table 8. Thus, TOC percent has been changed by decreasing. The initial COD concentration was 186,761 mg/kg soil, see table 6, and the remaining COD percent for reactor 1, 2, and 3 without sterilization had been around 12% of initial amount, see table 8, which was decreased after operating with increasing temperature. The average remaining of COD percent for reactor 4, 5, and 6 with sterilization had been around 11% of initial amount, see table 8. The initial pH value for soil contaminated with total petroleum hydrocarbons was 6.71, see table 6. After operating, the pH value for reactor 1, 2, and 3 without sterilization was average of 6.96, see table 8, and also the pH value for reactor 4, 5, and 6 with sterilization was average of 6.87, see table 8. Therefore, the pH value of initial and after was not much changed, see table (8).

When using sterilization for soil contaminants, the microorganisms could have been completely killed and also moisture contents were important in the soil contaminants for degradation. Also, the volatilization of TPH components was found about 6.62% due to high temperature operation, see table 7. In addition, the TPH reduction about 42.87% might be occurred due to anaerobic microbial degradation, see table 7. This should be confirmed by determining the microbial population in the contaminated soil before and after operation. Thus, the percent values of VS, TOC, and COD could be different from each other between sterilized and non-sterilized TPH contaminated soils; see table (8). If no moisture contents and no microorganisms are in TPH contaminated soils, the percent values of VS, TOC, and COD could not be different.

Table 8 MC, VS, TOC, COD, and pH values

	Average Remaining				
Experiment Condition	Moisture Content	Volatile Solids	Total Organic Carbons	COD	pH
TPH contaminated soil without sterilization	54.803%	3.311%	3.32%	12.04%	6.96
TPH Contaminated Soil With Sterilization	48.49%	3.869%	3.26%	10.89%	6.87

3. The Reduction of TPH at Temperature of 30^{o}C for 30 days operation time for Second Phase Experiment

Total petroleum hydrocarbon could be biodegraded under a wide range of temperatures and moisture content during anaerobic condition. The biodegradation of TPH was found to be faster at 25^{o}C than at 5^{o}C (ZoBell, 1964). Very low rates of hydrocarbon utilization were found at low water temperature (Gunkel, 1968). The effects of temperature are interactive with other factors, such as the quality of the hydrocarbon mixture and the composition of the microbial community (Atlas, 1981). For the below figure 19, the maximum methane production in 30^{o}C of no sterilization TPH contaminated soil and nutrient added TPH contaminated soil had increased steadily to 8% in reactor 2 and 1% in reactor 3 until (30) days period, starting from 2nd day and 13th day, and there was no methane production in sterilized TPH contaminated soil. In figure 20, the maximum CO_2 was produced to 40% in reactor 2 and 35% in reactor 3 from 2nd day in no-sterilized TPH contaminated soil and nutrient added TPH contaminated soil until (30) days period in 30^{o}C, and no more CO_2 production in sterilized TPH contaminated soil in 30^{o}C.

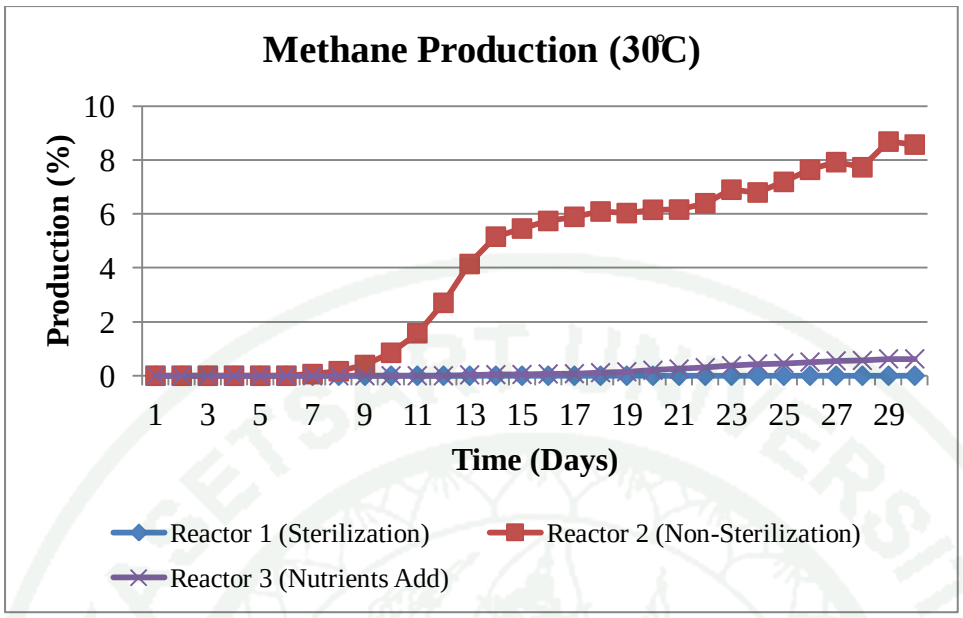


Figure 19 Methane Production (%) for TPH Soil in 2nd Phase Experiment in 30°C

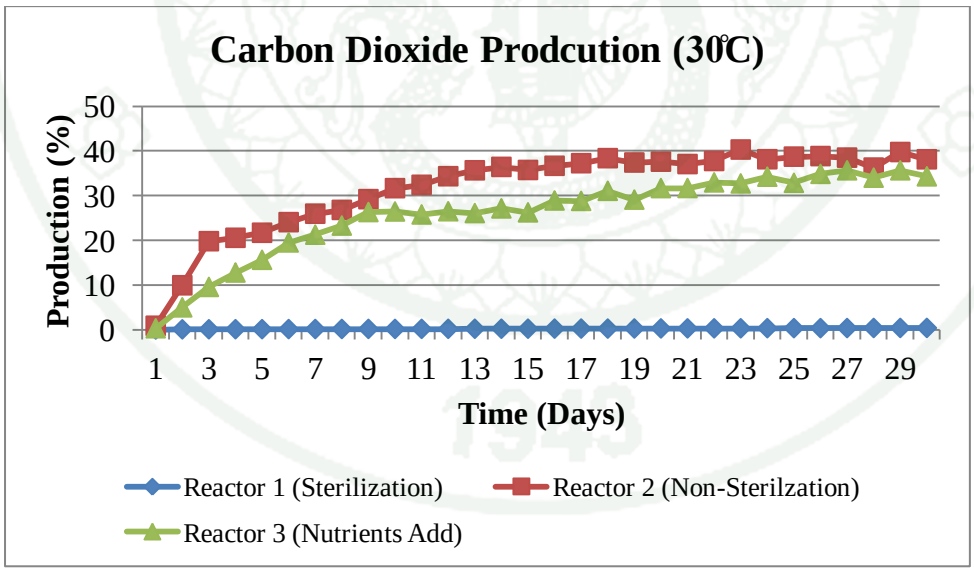


Figure 20 Carbon Dioxide Production (%) for TPH Soil in 2nd Phase Experiment in 30°C

3.1 The Reduction of TPH at Temperature of 40^{o}C for 30 days operation time

In figure 21 for 40^{o}C, maximum methane production in 40^{o}C started to increase 10% in no sterilization TPH contaminated soil reactor 2 and 5% in nutrient added TPH contaminated soil reactor 3, and was no more methane production in sterilized TPH contaminated soil reactor 1 until (30) days period. For CO_2 production in 40^{o}C, see figure 22; it increased steadily from 2nd day by 60% and 50% in reactor 2 and 3, and was almost zero percent production in reactor (1).

The maximum methane production for non-sterilization and nutirents added TPH contaminated soils were 14.01% and 9.07%; and no methane production in sterilized TPH contaminated soils, see appendix table 16. Thus, methane production only occurred in non-sterilization and nutrients added TPH contaminated soils. After increasing at different temperature, the rate of CH_4 production is also increased. For the maximum CO_2 production of non-sterilizaiton and nutrients added TPH cotaminated soils, it had prodcued 48.03% and 44.79%, see appendix table 16. When temperature was increased, the rate of CO_2 production was doubled.

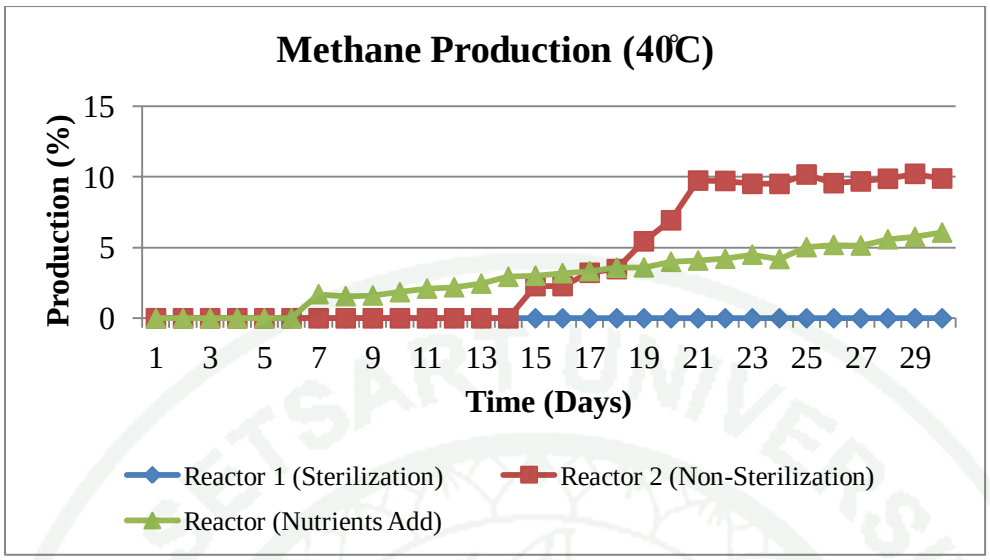


Figure 21 Methane Production (%) for TPH Soil in 2nd Phase Experiment in 40°C

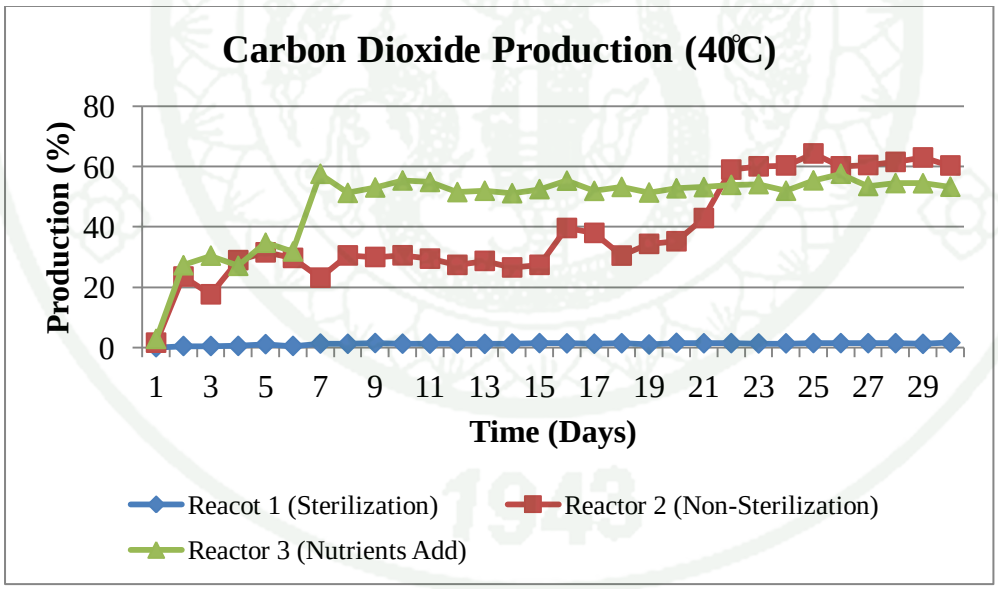


Figure 22 Carbon Dioxide Production (%) for TPH Soil in 2nd Phase Experiment in 40°C

3.2 The Reduction of TPH at Temperature of 50^{o}C for 30 days operation time

In the figure 23 in 50^{o}C, the maximum methane production only occurred around 10% in no-sterilized TPH contaminated soil reactor 2 and nutrients added TPH contaminated soil reactor 3, and there was no CH_4 production in reactor 1. There were more CO_2 production in no sterilization TPH contaminated soil Reactor 2 and nutrient added TPH contaminated soil reactor 3 than in sterilized TPH contaminated soil reactor 1, see figure 24.

Methane production was only occurred in the no-sterilized TPH contaminated soil reactor 2 and nutrients added TPH contaminated soil reactor 3. Therefore, the rate of hydrocarbon degradation was increased by increasing temperature from 30^{o}C to 40^{o}C and 50^{o}C. Thus, hydrocarbon degradation could be determined by CH_4 production, and CO_2 production.

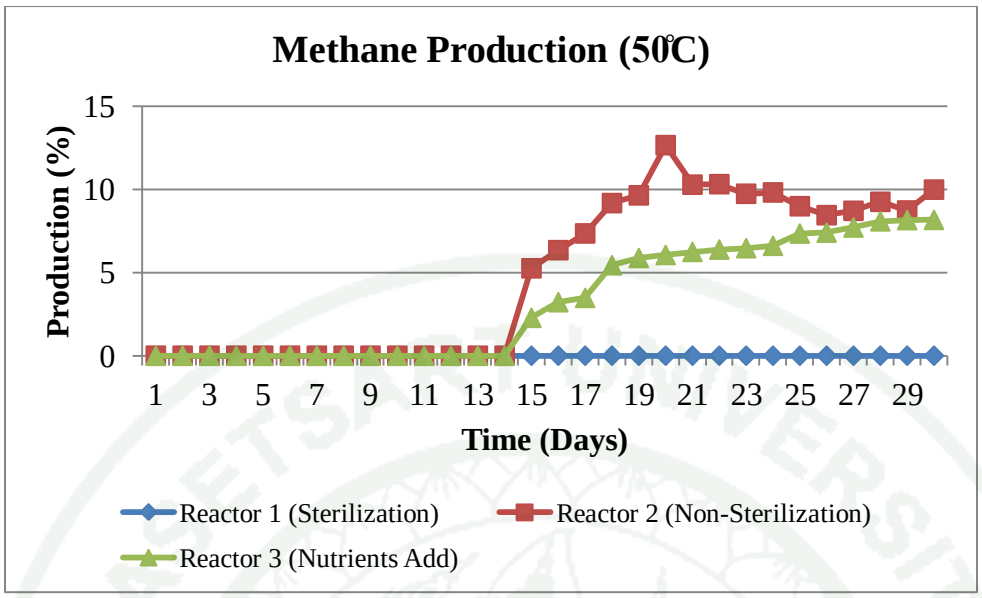


Figure 23 Methane Production (%) for TPH Soil in 2nd Phase Experiment in 50°C

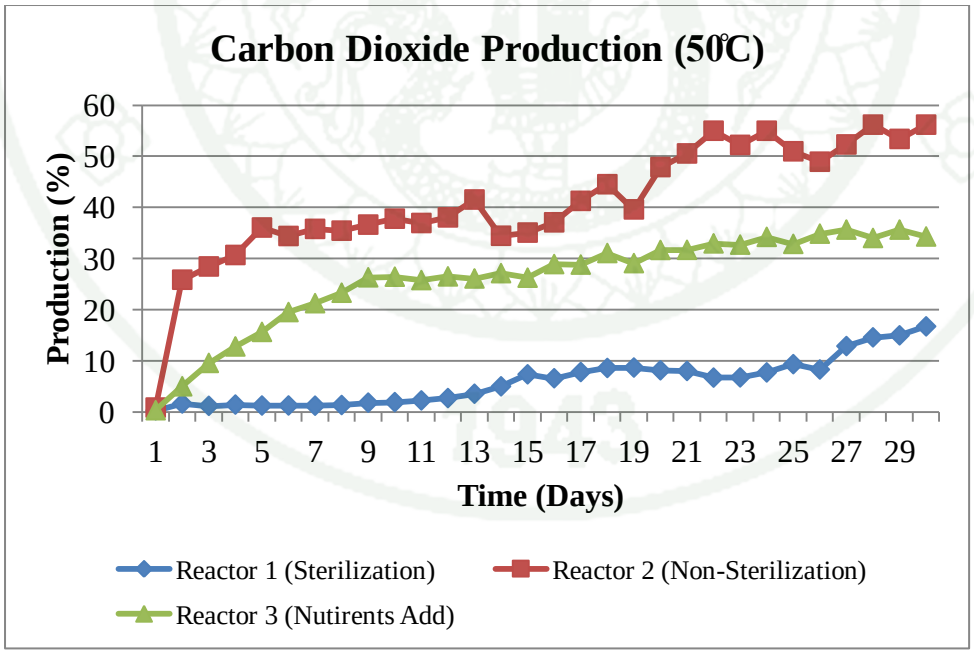


Figure 24 Carbon Dioxide Production (%) for TPH Soil in 2nd Phase Experiment in 50°C

3.3 TPH Reduction Efficiency (%) for TPH Contaminated Soil with Sterilization

In the figure 25, TPH reduction efficiency for contaminated soil with sterilization was not changed to decrease and was almost stable condition, in which the microorganism had been killed by autoclaved process. It could show to compare the other phases: without sterilization and nutrients; and with sterilization how the microorganism could degrade the total petroleum hydrocarbons by variables temperature control under 30^{o}C, 40^{o}C, and 50^{o}C.

Thus, in the table 9, the volatilization of TPH components were found about 9.09%, 5.61%, and 9.23% in all temperatures of 30^{o}C, 40^{o}C, and 50^{o}C duing the sterilization contaminated soil. This was occurred due to high temperature operation. Eevn though we used the sterilization in which one common biocidal treatment was autoclaving (Tibbett et al., 2000), but some remaining volatile contents had not been removed to perform. Thus, all microorganism could have been killed by sterilization.

Table 9 TPH Reduction Efficiency (%) for TPH Contaminated Soil with Sterilization

Temperature	TPH Initial	TPH Final	% Reduction
30^{o}C	44,000 mg/kg	40,000 mg/kg	9.09
40^{o}C	42,000 mg/kg	39,644 mg/kg	5.61
50^{o}C	39,000 mg/kg	35,402 mg/kg	9.23

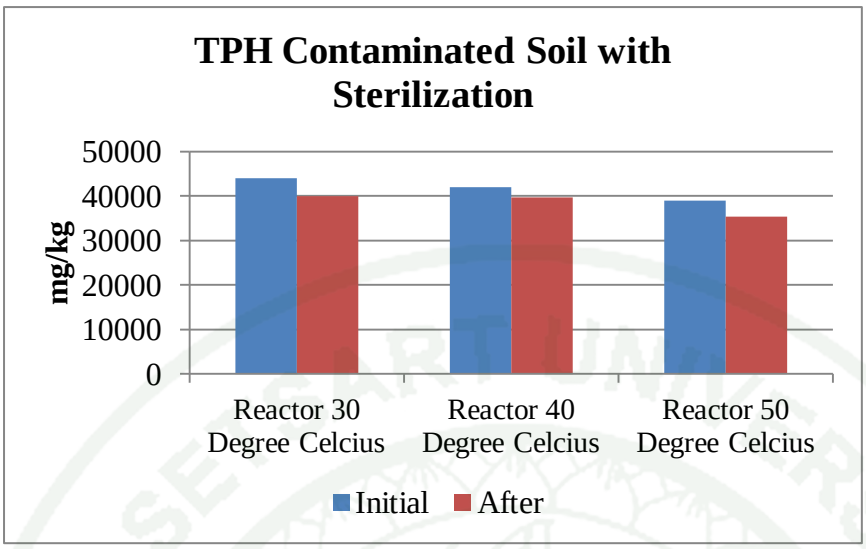


Figure 25 TPH Contaminated Soil with Sterilization

3.4 TPH Reduction Efficiency (%) for TPH Contaminated Soil without Sterilization

In the figure 26, it showed that TPH reduction efficiency for contaminated soil without sterilization was significantly reduced to around 37% in 30°C, 35% in 40°C, and 28% in 50°C. In the table 10, the reduction efficiency of TPH contaminated soil without sterilization was 36.74% in 30°C, 35.38% in 40°C, and 28.21% in 50°C. Thus, temperatures were assumed that every 10°C rise from 30°C to 40°C and 50°C, which doubled to increase the biodegradation rate (US EPA, 2004).

Table 10 TPH Reduction Efficiency (%) for TPH Contaminated Soil without Sterilization

Temperature	TPH Initial	TPH Final	% Reduction
30°C	44,000 mg/kg	27,833 mg/kg	36.74
40°C	42,000 mg/kg	27,140 mg/kg	35.38
50°C	39,000 mg/kg	28,000 mg/kg	28.21

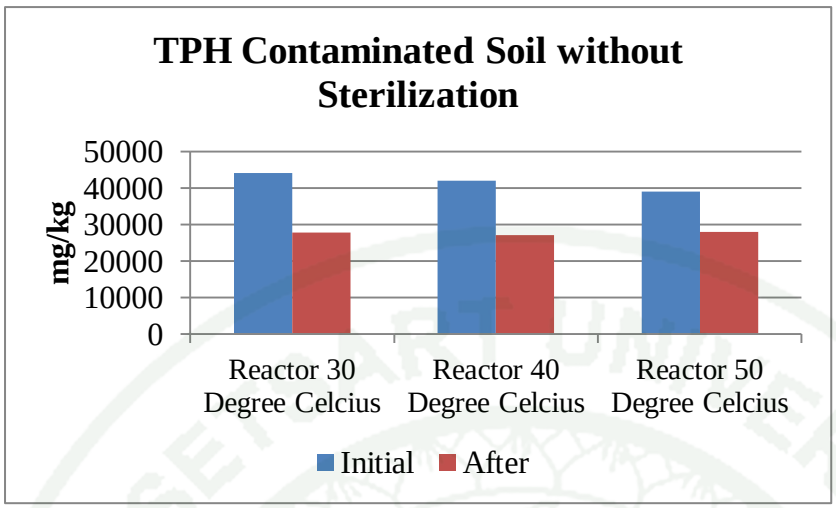


Figure 26 TPH Contaminated Soil without Sterilization

3.5 TPH Reduction Efficiency (%) for TPH Contaminated Soil with Nutrients

Nutrients of C/N/P ratio of 100:2.0:0.2 (Hutchinson, 2001) were used in this research for anaerobic bioremediation for TPH contaminated soil. In table 11, the average concentration of nitrogen were 1,765.43 (mgTKN/kg soil) in non-sterilization soil, 1,359 (mgTKN/kg soil) in sterilization soil, and 2,181.73 (mgTKN/kg soil) in nutrients added soil, see table 11, and also the concentration of phosphorous in non-sterilization, sterilization, and nutrients added soils were 2,015 (mgTKN/kg), 1,826.72 (mgTKN/kg), 3,009.95 (mgTKN/kg), see table 11. The nutrient added TPH contaminated soil had left much higher amount of concentration of nutrients after experiment in 30^{o}C, 40^{o}C, and 50^{o}C. Degrading petroleum hydrocarbons by adding nutrients (nitrogen and phosphorous in molar ratios) in TPH contaminated soil were 38.22% and 28.27% in 30^{o}C and 40^{o}C for anaerobic bioremediation process, see table 12. In 50^{o}C, some anaerobes were not able to degrade the TPH contaminants, and so the TPH reduction was only 11.37% in soil because of higher temperature, which considerably killed some microorganisms such as anaerobes.

Table 11 Nitrogen and Phosphorous concentration after experiment

Temper-ature	Nitrogen (mgTKN/kg soil)			Phosphorous (mgTKp/kg soil)		
	Non-Sterilizat-ion soil	Sterili-tionSoil	Nutrient soil	Non-Sterilization Soil	Sterilization Soil	Nutrien t soil
30^{o}C	1600.51	1274	2079.1	1803.8	1436.13	2343.16
40^{o}C	2727.27	2041	2077.9	2222.2	2160.49	3148.15
50^{o}C	968.52	762	2388.2	2019	2183.54	3538.55
Average	1765.43	1359	2181.73	2015	1826.72	3009.95

Table 12 TPH Reduction Efficiency (%) for TPH Contaminated Soil with Nutrients

Temperature	TPH Initial	TPH Final	% Reduction
30°C	44,000 mg/kg	27,184 mg/kg	38.22
40°C	42,000 mg/kg	30,128 mg/kg	28.27
50°C	39,000 mg/kg	34,564 mg/kg	11.37

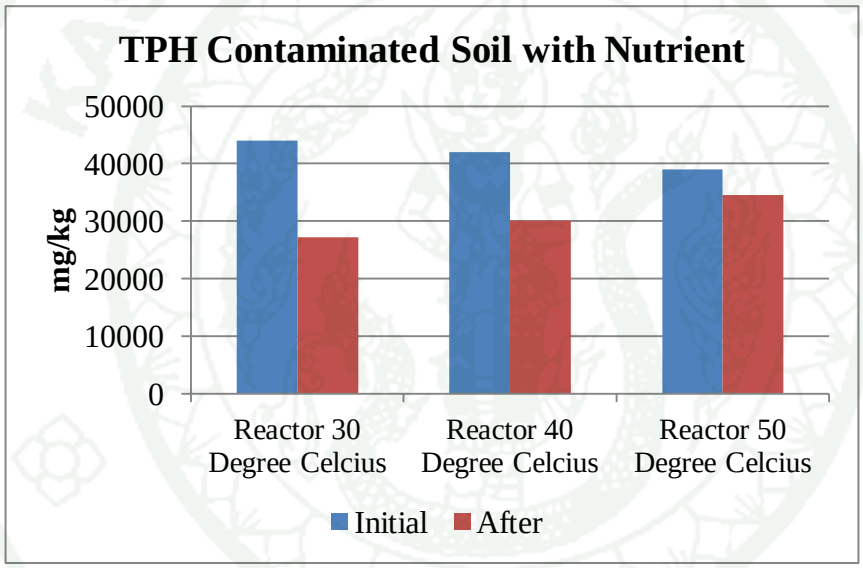


Figure 27 TPH Contaminated Soil with Nutrient

In the figure 27, TPH concentration of contaminated soil with nutrient was reduced around 25 mg/kg in 30°C, 30 mg/kg in 40°C, and 35 mg/kg in 50°C. In the table 12, the reduction efficiency of TPH contaminated soil with nutrient was 38.22% in 30°C, 28.27% in 40°C, and 11.37% in 50°C respectively. Thus, the optimum temperature conditions for nutrients were 30°C and 40°C to degrade the TPH concentration in soil by feeding nutrients C/N/P molar ratios: 100:2:0.2 (Hutchinson, 2001).

3.6 Moisture Content, Volatile Solids, Total Organic Carbon, COD, pH, Nitrogen, and Phosphorous

In table 13, the remaining percent values of volatile solids and total organic carbons of TPH contaminated soil without sterilization, with sterilization, and with nutrients in 30°C, 40°C, and 50°C were almost around 7% and 5%. The two values of VS and TOC were not much different from each other because the measurement of the TOC-V_{CSH} (SSM-5000A) used the very small amount of the TPH contaminated soil samples (60 mg for Total Carbons and 150 mg for Inorganic Carbons), and so it could have some deviation in the results of VS and TOC.

The moisture contents of TPH contaminated soil with sterilization were 51.35% in 30°C, 50.82% in 40°C, and 52.4% in 50°C, see table 13. The moisture contents of the TPH contaminated soil without sterilization were 51.77% in 30°C, 50.96% in 40°C, and 59.39% in 50°C, and TPH contaminated soil with nutrients were also 53.21% in 30°C, 56.28% in 40°C, and 53.34% in 50°C, see table 13. The moisture contents were to degrade the TPH in soils for all temperatures of 30°C, 40°C, and 50°C. The pH values of all three reactors in all temperature were around 7 in neutral.

The COD remaining in 40°C of all TPH contaminated soil without sterilization, with sterilization, and with nutrients was reduced to demanding than the hydrocarbons degrading temperature in 30°C and 50°C, see table 13. During the higher temperature, COD remaining was decreased in 30°C, 40°C, and 50°C. Remaining COD in 30°C was more than 40°C and 50°C. Therefore, the optimal temperatures for degradation of hydrocarbons in this research were between 30°C and 40°C, and also when temperature was increased the VS, TOC, and COD were decreased, see table 13.

Table 13 MC, VS, TOC, COD, and pH values

	Remaining Values				
	Moisture Content	Volatile Solids	Total Organic Carbons	COD	pH
	TPH contaminated soil without Sterilization				
Reactor 30°C	51.77%	6.70%	4.572%	169,385 mg/kg	6.59
Reactor 40°C	50.96%	6.26%	4.574%	130,982 mg/kg	7.08
Reactor 50°C	59.39%	6.70%	4.704%	147,744 mg/kg	7.27
	TPH Contaminated Soil With Sterilization				
Reactor 30°C	51.35%	6.69%	4.357%	113,720 mg/kg	6.64
Reactor 40°C	50.82%	6.25%	4.375%	134,320 mg/kg	7.11
Reactor 50°C	52.40%	6.78%	5.070%	134,340 mg/kg	7.10
	TPH Contaminated Soil With Nutrient				
Reactor 30°C	53.21%	7.04%	4.798%	182,319 mg/kg	6.29
Reactor 40°C	56.28%	7.18%	4.268%	114,189 mg/kg	6.61
Reactor 50°C	53.34%	7.26%	5.468%	158,765 mg/kg	6.78

CONCLUSION AND RECOMMENDATION

Conclusion

The reduction efficiency of TPH contaminated soils was much more in no sterilization TPH contaminated soils in 40°C. The TPH reduction efficiency of TPH contaminated soil with nutrient was less than the TPH contaminated soil without sterilization in all temperature conditions of 30°C, 40°C, and 50°C. If temperature was more increased from 30°C to 50°C in TPH contaminated soil with nutrients, the reduction efficiency was also higher in 30°C and 40°C than in 50°C. The reduction efficiency of TPH contaminated soil with Sterilization was not apparently changed to decrease total petroleum hydrocarbons because of killing most anaerobic microorganism.

CO_2 productions of nutrient added TPH contaminated soil in 40°C was higher than in 30°C and 50°C. Few amount of CO_2 production in sterilized TPH contaminated soils occurred in the bottle reactor because initial remained VS in the head space of reactor bottle already existed to produce CO_2. CH_4 production of non-sterilized TPH contaminated soils were increased more than nutrients added TPH contaminated soils while temperature is increased. The results of the CO_2 and CH_4 productions in non sterilized TPH contaminated soils and nutrients added TPH contaminated soils highlighted to show that the optimum condition temperatures were around 30°C and 40°C. Thus, if temperature was increased, CO_2 and CH_4 production were produced more, and it increased the TPH degradation rate.

Recommendation

Therefore, the rates of biodegradation of hydrocarbons are highly dependent on the temperature conditions for anaerobic bioremediation. The microbial degradation of hydrocarbon pollutants is a complex process, and it is also depending on localized environmental condition such as moisture contents during anaerobic process for further biodegradation. Therefore, the increasing temperature by double for anaerobic bioremediation increases the TPH reduction in soil. From this research, the optimum temperatures for degrading total petroleum hydrocarbons (TPH) are between 30^{o}C and 40^{o}C for each of (30) days period. Some of the strong and persistent of hydrocarbon contaminants such as tar are unknown and will be required for future research.

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APPENDICES

Appendix A

TPH, CO_2, CH_4, TOC, COD, VS, MC, and pH Results

Appendix Table A1 First Phase Experiment for Soil Contaminated with Total Petroleum Hydrocarbons (Non-Sterilization: 30°C, 40°C, and 50°C)

Day	Non-sterilization				Remark
	Area	$\%CH_4$	Area	$\% CO_2$	
1	0	0	342.777	3.0857	
2	0	0	1638.47	14.749	
3	0	0	2413.48	21.726	
4	0	0	2463.27	22.174	
5	0	0	2732.93	24.602	
6	0	0	2959.43	26.641	
8	0	0	2825.99	25.439	
9	0	0	3088.18	27.8	30°C operation
10	0	0	3088.85	27.806	
11	9.615	0.0235	2880.02	25.926	
12	14.948	0.0365	2977.03	26.799	
13	21.168	0.0517	3272.55	29.46	
14	27.087	0.0985	3248.1	29.239	
15	40.317	0.1272	3322.72	29.911	
16	31.5099	0.231	3237	29.14	
17	46.6046	0.3417	3504.99	31.552	
18	75.0976	0.5506	3690.94	33.226	
19	87.9291	0.6446	3592.51	32.34	
20	116.211	0.852	3655.76	32.909	
21	164.596	1.2067	3890.42	35.022	
22	193.202	1.4165	3933.12	35.406	40°C operation
23	223.204	1.6364	3754.44	33.798	
24	238.923	1.7517	3744.54	33.708	
25	291.002	2.1336	4031.39	36.291	
26	302.877	2.2206	3903.82	35.142	
27	332.923	2.4409	3836.56	34.537	
28	351.925	2.5802	3893.49	35.049	
29	376.097	2.7574	3772.03	33.956	
30	334.123	2.4497	3893.39	35.048	

Appendix Table A1 (Continued)

Day	Non-sterilization				Remark
	Area	%CH_4	Area	% CO_2	
31	448.744	3.2901	3788.07	34.1	
32	527.85	3.8701	3845.86	34.621	
33	597.556	4.3811	3679.65	33.124	
34	823.791	6.0399	4084.26	36.767	
35	928.659	6.8087	3745.87	33.72	
36	1305.81	9.5739	4748.36	42.745	
37	1118.46	8.2003	5301.04	47.72	50^oC operation
38	1169.8	8.5767	6088.04	54.805	
39	1134.12	8.3151	5986.23	53.888	
40	1180.89	8.6581	6217.72	55.972	
41	1129.33	8.2801	5979.83	53.831	
42	1124.65	8.2457	5986.07	53.887	
43	1123.49	8.2372	5953.59	53.595	
44	1075.44	7.8849	5702.59	51.335	
45	1150.54	8.4356	6125.33	55.141	

Appendix Table A2 First Phase Experiment for Soil Contaminated with Total Petroleum Hydrocarbons (Sterilization: 30°C, 40°C, and 50°C)

Day	Sterilization				Remark
	Area	$\%CH_4$	Area	$\% CO_2$	
1	0	0	155.568	0.34707	
2	0	0	607.117	0.5358	
3	0	0	940.255	0.43663	
4	0	0	944.5	0.55487	
5	0	0	1047.62	0.5933	
6	0	0	1194.77	2.0183	
8	0	0	1492.45	5.7015	30°C operation
9	0	0	1594.77	7.9281	
10	0	0	2065.12	13.483	
11	0	0	2360.98	17.7431	
12	0	0	2775.45	22.6988	
13	0	0	2423.86	18.4439	
14	0	0	2458.98	19.1418	
15	0	0	2443.44	19.053	
16	0	0	2193.1	19.7426	
17	0	0	2213.6	19.9272	
18	0	0	2252.42	20.2766	
19	0	0	2276.76	20.4958	
20	0	0	2330.2	20.9768	40°C operatrion
21	0	0	2400.63	21.6109	
22	0	0	2405.57	21.6553	
23	0	0	2295.9	20.6681	
24	0	0	2393.08	21.5429	
25	0	0	2502.28	22.526	
26	0	0	2506.65	22.5653	
27	0	0	2171.38	19.5471	
28	0	0	1904.59	17.1454	
29	0	0	1887.3	16.9898	
30	0	0	2423.16	21.8137	

Appendix Table A2 (Continued)

Day	Sterilization				Remark
	Area	%CH_4	Area	% CO_2	
31	0	0	2567.48	23.1128	
32	0	0	2709.52	24.3915	
33	0	0	2640.67	23.7717	
34	0	0	2322.08	20.9037	
35	0	0	2693.66	24.2487	
36	0	0	2951.84	26.5729	50^oC operation
37	0	0	3056.11	27.5116	
38	0	0	3175.78	28.5888	
39	0	0	3237.69	29.1462	
40	0	0	2855.38	25.7046	
41	0	0	3320.1	29.8881	
42	0	0	3346.01	30.1213	
43	0	0	3487.33	31.3935	
44	0	0	3465.04	31.1928	
45	0	0	3583.47	32.2589	

Appendix Table A3 TPH Contaminated Soil (Non-Sterilization) in 30°C for First Phase experiment

	Non-Sterilized soil Reactor 1 (30°C)		Non-Sterilized soil Reactor 2 (30°C)		Non-Sterilized soil Reactor 3 (30°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
1	0	3.6284	0.0000	4.5622	0	3.6284
2	0	11.526	0.0000	15.725	0	14.645
3	0	19.789	0.0000	18.071	0	21.920
4	0	20.568	0.0000	19.327	0	22.598
5	0	21.661	0.0000	21.223	0	24.114
6	0	22.878	0.0000	24.173	0	24.518
7	N/A	N/A	0.0000	24.072	0	25.160
8	0	5.8341	0.0000	18.720	0	25.863
9	0	20.504	0.0000	3.0199	0	25.205
10	0	23.518	0.1167	20.946	0	26.028
11	0	25.221	0.1756	22.469	0	25.249
12	0	26.383	0.2390	24.296	0	26.526
13	0	26.939	0.3151	24.467	0	26.462
14	0	27.251	0.4765	26.770	0	26.415
15	0	26.962	0.6061	25.390	0	26.042

Appendix Table A4 TPH Contaminated Soil (Non-Sterilization) in 40°C for First Phase experiment

	Non-Sterilized soil Reactor 1 (40°C)		Non-Sterilized soil Reactor 2 (40°C)		Non-Sterilized soil Reactor 3 (40°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
16	0.1244	25.643	0.9972	26.122	0.0000	27.621
17	0.1980	28.423	1.4338	25.953	0.0000	28.491
18	0.3168	28.809	2.1366	27.984	0.0671	29.542
19	0.4893	28.502	2.4495	24.463	0.0889	30.013
20	0.8139	28.665	2.9758	24.866	0.1331	31.401
21	1.3136	28.696	3.9676	29.604	0.2079	32.159
22	1.8264	28.743	4.3817	29.505	0.3042	32.526
23	2.3030	28.601	4.6415	28.580	0.4366	30.799
24	2.7504	28.511	4.7843	26.140	0.6280	33.207
25	3.1568	28.614	5.5961	29.887	0.8635	33.808
26	3.3702	26.688	5.6730	28.521	1.1088	34.308
27	3.7208	27.045	6.3891	30.616	1.3944	34.157
28	3.8859	28.086	6.4842	29.240	1.7354	34.245
29	4.1230	28.481	7.4547	31.696	2.2229	34.849
30	1.5217	26.331	7.1423	28.646	2.7025	34.405

Appendix Table A5 TPH Contaminated Soil (Non-Sterilization) in 50^{o}C for First Phase experiment

	Non-Sterilized soil Reactor 1 (50^{o}C)		Non-Sterilized soil Reactor 2 (50^{o}C)		Non-Sterilized soil Reactor 3 (50^{o}C)	
time (days)	%CH4	%CO2	7.9634	29.848	%CH4	%CO2
31	3.8019	25.185	9.7217	31.912	3.5012	34.179
32	3.2901	25.895	10.779	33.991	5.0588	33.531
33	4.1489	27.706	12.770	34.807	5.7412	28.256
34	5.4737	27.486	17.143	39.561	7.9315	28.121
35	7.5246	28.736	17.412	36.992	12.724	36.341
36	9.9569	31.740	14.454	39.902	14.09	34.854
37	7.1895	29.422	14.343	42.954	13.684	46.652
38	8.5166	39.879	13.526	40.736	12.933	45.860
39	8.3576	40.305	13.508	42.500	13.204	47.083
40	8.7437	39.778	12.700	40.460	13.221	46.945
41	8.8206	39.623	12.595	40.968	13.394	47.255
42	9.138	40.494	12.597	42.485	13.579	47.644
43	9.1499	39.266	13.080	44.609	13.577	47.530
44	6.5547	30.534	12.462	42.796	13.818	47.840
45	9.4366	40.885	7.9634	29.848	14.016	48.030

Appendix Table A6 The Carbon Dioxide production by Non-Sterilization of Soil contaminated with Total Petroleum Hydrocarbons (% in $3O^{O}C$, $4O^{O}C$, and $5O^{O}C$ for reactor 1, 2, and 3)

Temperature ($30^{o}C$)		Temperature ($40^{o}C$)		Temperature ($50^{o}C$)	
Time (Days)	Average (%) CO_2	Time (Days)	Average (%) CO_2	Time (Days)	Average (%) CO_2
1	3.0857	16	29.14	31	34.1007
2	14.749	17	31.5524	32	34.6210
3	21.726	18	33.22646	33	33.1248
4	22.174	19	32.34033	34	36.7671
5	24.602	20	32.90973	35	33.7209
6	26.641	21	35.02216	36	42.7455
7	25.439	22	35.40653	37	47.7207
8	27.800	23	33.79806	38	54.8055
9	27.806	24	33.7089	39	53.8889
10	25.926	25	36.2912	40	55.9729
11	26.799	26	35.1427	41	53.8313
12	29.460	27	34.5373	42	53.8875
13	29.239	28	35.0498	43	53.5951
14	29.911	29	33.9564	44	51.3355
15	29.158	30	35.0489	45	55.1411
				Total Average	34.8201
				Standard Deviation	6.06586

Appendix Table A7 The Methane production by Non-Sterilization of Contaminated Soil with Total Petroleum Hydrocarbons (% in $3O^{O}C$, $4O^{O}C$, and $5O^{O}C$ for reactor 1, 2, and 3)

	Temperature ($30^{o}C$)		Temperature ($40^{o}C$)		Temperature ($50^{o}C$)
Time (Days)	Average (%) CH_4	Time (Days)	Average (%) CH_4	Time (Days)	Average (%) CH_4
1	0	16	0.2310	31	3.2901
2	0	17	0.3417	32	3.8701
3	0	18	0.5506	33	4.38116
4	0	19	0.6446	34	6.0399
5	0	20	0.8520	35	6.80876
6	0	21	1.2067	36	9.5739
7	0	22	1.4165	37	8.2003
8	0	23	1.6364	38	8.5767
9	0	24	1.7517	39	8.3151
10	0.0235	25	2.1336	40	8.6581
11	0.0365	26	2.2206	41	8.2801
12	0.0517	27	2.4409	42	8.2457
13	0.0662	28	2.5802	43	8.2372
14	0.0985	29	2.7574	44	7.8849
15	0.1272	30	2.4497	45	8.4356
				Total Average	2.942533
				Standard Deviation	0.95487

Appendix Table A8 TPH Contaminated Soil (Sterilization) in 30^{o}C for First Phase experiment

	Sterilized soil Reactor 4 (30^{o}C)		Sterilized soil Reactor 5 (30^{o}C)		Sterilized soil Reactor 6 (30^{o}C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
1	0	0.3597	0	0.3361	0	0.3858
2	0	0.5324	0	0.5522	0	0.5448
3	0	0.5985	0	0.6133	0	0.1541
4	0	0.7369	0	0.7333	0	0.1782
5	0	1.329	0	0.1291	0	0.3045
6	0	3.0897	0	2.4033	0	0.399
7	0	6.7437	0	5.093	0	5.1637
8	0	10.305	0	8.4800	0	9.2051
9	0	15.026	0	11.965	0	12.395
10	0	15.423	0	16.545	0	17.705
11	0	26.135	0	17.046	0	18.455
12	0	15.935	0	17.148	0	18.768
13	0	16.166	0	17.648	0	18.793
14	0	16.550	0	17.792	0	18.915
15	0	16.224	0	17.414	0	18.802

Appendix Table A9 TPH Contaminated Soil (Sterilization) in 40^{o}C for First Phase experiment

	Sterilized soil Reactor 4 (40^{o}C)		Sterilized soil Reactor 5 (40^{o}C)		Sterilized soil Reactor 6 (40^{o}C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
16	0	0.3597	0	18.239	0	19.493
17	0	0.5324	0	18.436	0	19.755
18	0	0.5985	0	18.477	0	19.769
19	0	0.7369	0	18.475	0	19.772
20	0	1.329	0	19.011	0	20.166
21	0	3.0897	0	19.558	0	20.457
22	0	6.7437	0	19.737	0	20.720
23	0	10.305	0	19.789	0	21.040
24	0	15.026	0	19.887	0	21.387
25	0	15.423	0	20.287	0	21.594
26	0	26.135	0	20.725	0	21.742
27	0	15.935	0	17.531	0	21.673
28	0	16.166	0	14.058	0	21.742
29	0	16.550	0	11.865	0	22.142
30	0	16.224	0	21.343	0	21.764

Appendix Table A10 TPH Contaminated Soil (Sterilization) in 50^{o}C for First Phase experiment

	Sterilized soil Reactor 4 (50^{o}C)		Sterilized soil Reactor 5 (50^{o}C)		Sterilized soil Reactor 6 (50^{o}C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
31	0	19.314	0	22.009	0	22.231
32	0	18.389	0	24.847	0	23.759
33	0	21.785	0	23.377	0	24.726
34	0	20.488	0	24.711	0	23.562
35	0	22.315	0	25.693	0	24.949
36	0	22.803	0	26.419	0	24.602
37	0	21.478	0	25.519	0	25.722
38	0	23.441	0	27.446	0	25.657
39	0	25.884	0	27.433	0	26.501
40	0	24.158	0	28.672	0	27.281
41	0	28.781	0	27.646	0	27.597
42	0	27.095	0	29.196	0	27.864
43	0	27.183	0	29.464	0	27.527
44	0	27.432	0	29.402	0	28.067
45	0	28.758	0	29.514	0	28.477

Appendix Table A11 The Methane production by Sterilization of Soil contaminated with Total Petroleum Hydrocarbons (% in 3O^{O}C, 4O^{O}C, and 5O^{O}C for reactor 4, 5, and 6)

	Temperature (30^{o}C)		Temperature (40^{o}C)		Temperature (50^{o}C)
Time (Days)	Average (%)	Time (Days)	Average (%)	Time (Days)	Average (%)
	CH_4		CH_4		CH_4
1	0	16	0	31	0
2	0	17	0	32	0
3	0	18	0	33	0
4	0	19	0	34	0
5	0	20	0	35	0
6	0	21	0	36	0
7	0	22	0	37	0
8	0	23	0	38	0
9	0	24	0	39	0
10	0	25	0	40	0
11	0	26	0	41	0
12	0	27	0	42	0
13	0	28	0	43	0
14	0	29	0	44	0
15	0	30	0	45	0
				Total Average	0
				Standard Deviation	0

Appendix Table A12 The Carbon Dioxide production by Sterilization of Soil contaminated with Total Petroleum Hydrocarbons (% in $3O^{O}C$, $4O^{O}C$, and $5O^{O}C$ for reactor 4, 5, and 6)

Temperature ($30^{o}C$)		Temperature ($40^{o}C$)		Temperature ($50^{o}C$)	
Time (Days)	Average (%) CO_2	Time (Days)	Average (%) CO_2	Time (Days)	Average (%) CO_2
1	0.347067	16	19.74257	31	23.1128
2	0.5358	17	19.92717	32	24.3915
3	0.436633	18	20.27663	33	23.7717
4	0.554867	19	20.49577	34	20.9037
5	0.5933	20	20.97683	35	24.2487
6	2.0183	21	21.61087	36	26.5729
7	5.7015	22	21.6553	37	27.5116
8	7.9281	23	20.66813	38	28.5888
9	13.48303	24	21.54287	39	29.1461
10	17.7431	25	22.52597	40	25.7046
11	22.6988	26	22.5653	41	29.8880
12	18.4439	27	19.5471	42	30.1213
13	19.14177	28	17.14543	43	31.3935
14	19.05303	29	16.98977	44	31.1927
15	19.14283	30	21.8137	45	32.2589
				Total Average	19.2025
				Standard Deviation	4.65781

Appendix Table A13 First Phase Experiment of TPH concentration for before and after experiment

TPH Reduction			
TPH Contaminated Soil without Sterilization			
	Initial (Concentration)	After (Concentration)	% Reduction
Reactor 1	50,000 mg/kg	27,888.45 mg/kg	44.22 %
Reactor 2	50,000 mg/kg	14,000 mg/kg	72 %
Reactor 3	50,000 mg/kg	33,864 mg/kg	32.27 %
TPH Contaminated Soil with Sterilization			
	Initial (Concentration)	After (Concentration)	% Reduction
Reactor 4	50,000 mg/kg	45,854.355 mg/kg	8.29 %
Reactor 5 (leaked)	50,000 mg/kg	36,328.87 mg/kg	27.34 %
Reactor 6	50,000 mg/kg	47,524.75 mg/kg	4.95%

Initial Conditions for TPH concentrations = (66,000 + 34,000 + 50,000)/3 = 50,000 mg/kg

Appendix Table A14 MS, VS, TOC, COD and pH values after First Phase Experiment

	% Remaining				
	Moisture Content	Volatile Solids	Total Organic Carbons	COD	pH
	TPH contaminated soil without sterilization				
Reactor 1	52.73%	3.152%	3.417%	12.89 %	6.98
Reactor 2	59.65%	3.185%	3.436%	12.31%	6.92
Reactor 3	52.03%	3.596%	3.108%	10.92%	6.97
	TPH Contaminated Soil With Sterilization				
Reactor 4	51.092%	3.928%	3.293%	10.05%	6.89
Reactor 5	leaked	Leaked	leaked	Leaked	Leaked
Reactor 6	45.899%	3.809%	3.227%	12.66%	6.81

Appendix Table A15 Second Phase Experiment of TPH Contaminated Soil in 30°C

	Sterilized soil Reactor 1 (30°C)		Non-Sterilized soil Reactor 2 (30°C)		Nutrient soil Reactor 3 (30°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
1	0	0.0760	0.0000	0.9329	0.0000	0.3659
2	0	0.1104	0.0000	9.8353	0.0000	5.0201
3	0	0.1260	0.0000	18.516	0.0000	9.5181
4	0	0.1350	0.0000	19.453	0.0000	12.698
5	0	0.1513	0.0000	21.000	0.0000	15.384
6	0	0.1612	0.0000	22.712	0.0000	17.896
7	0	0.1762	0.1010	24.715	0.0000	20.182
8	0	0.1892	0.2771	25.101	0.0000	22.066
9	0	0.2009	0.6482	26.751	0.0000	24.220
10	0	0.2036	1.3531	28.314	0.0000	24.285
11	0	0.2129	2.5810	29.783	0.0000	24.226
12	0	0.2270	4.2556	30.352	0.0000	24.389
13	0	0.2436	6.4865	31.346	0.0561	25.072
14	0	0.2381	7.8831	31.252	0.0656	25.353
15	0	0.2462	8.5791	31.527	0.0805	25.696

Appendix Table A15 (Continued)

	Sterilized soil Reactor 1 (30°C)		Non-Sterilized soil Reactor 2 (30°C)		Nutrient soil Reactor 3 (30°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
16	0	0.2556	8.8723	31.822	0.1074	26.244
17	0	0.2683	9.0236	32.020	0.1244	26.454
18	0	0.2897	9.2480	32.710	0.1778	27.191
19	0	0.2994	9.1739	31.939	0.2454	27.060
20	0	0.3094	9.4539	32.418	0.3201	28.307
21	0	0.3204	9.4455	31.892	0.3998	28.499
22	0	0.3317	9.9958	33.112	0.4825	29.745
23	0	0.3469	10.210	33.489	0.6074	30.156
24	0	0.3636	10.298	32.441	0.6912	30.394
25	0	0.3753	11.067	33.457	0.7668	30.735
26	0	0.3816	11.479	32.773	0.8036	31.011
27	0	0.3887	11.840	32.303	0.8368	30.410
28	0	0.3985	12.305	32.406	0.9192	30.996
29	0	0.4061	12.707	32.652	0.9758	31.218
30	0	0.4061	12.933	32.291	1.0200	31.324

Appendix Table A16 Second Phase Experiment of TPH Contaminated Soil in 40°C

	Sterilized soil Reactor 1 (40°C)		Non-Sterilized soil Reactor 2 (40°C)		Nutrient soil Reactor 3 (40°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
1	0	0.1479	0.0000	1.6828	0.0000	2.9444
2	0	0.5357	0.0000	21.197	0.0000	26.721
3	0	0.5250	0.0000	24.334	0.0000	27.920
4	0	0.5941	0.0000	26.501	0.0000	26.137
5	0	1.1939	0.0000	27.773	0.0000	30.566
6	0	0.6065	0.0000	28.009	0.0000	28.655
7	0	1.3021	0.0000	19.976	2.4180	45.783
8	0	1.4131	0.0000	28.391	2.4891	45.760
9	0	1.4865	0.0000	28.145	2.5086	46.327
10	0	1.3797	0.0000	27.259	2.7557	46.377
11	0	1.3631	0.0000	27.526	3.1622	46.375
12	0	1.4102	0.0000	25.824	3.5010	46.121
13	0	1.2562	0.0000	25.517	3.9223	46.612
14	0	1.3211	0.0000	25.666	4.4273	43.335
15	0	1.4727	3.6191	24.449	4.7728	46.710

Appendix Table A16 (Continued)

	Sterilized soil Reactor 1 (40°C)		Non-Sterilized soil Reactor 2 (40°C)		Nutrient soil Reactor 3 (40°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
16	0	1.4604	3.5012	34.180	4.8143	46.864
17	0	1.3949	5.0588	33.532	5.2843	46.164
18	0	1.5103	5.7412	28.257	5.4236	45.312
19	0	1.4139	7.9315	28.122	6.1429	49.187
20	0	1.8188	12.725	36.342	6.3309	46.922
21	0	1.5548	14.090	34.855	6.3275	46.249
22	0	1.5891	13.685	46.652	6.4066	46.027
23	0	1.4197	12.933	45.861	6.8500	46.265
24	0	1.4514	13.205	47.083	6.2677	43.591
25	0	1.5575	13.221	46.946	7.4442	46.008
26	0	1.5396	13.395	47.256	7.5563	47.101
27	0	1.4739	13.579	47.644	7.9779	46.591
28	0	1.4439	13.578	47.530	8.5071	46.578
29	0	1.3373	13.818	47.840	8.6045	45.716
30	0	1.6851	14.017	48.031	9.0734	44.791

Appendix Table A17 Second Phase Experiment of TPH Contaminated Soil in 50°C

	Sterilized soil Reactor 1 (50°C)		Non-Sterilized soil Reactor 2 (50°C)		Nutrient soil Reactor 3 (50°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
1	0	0.3203	0.0000	0.9169	0.0000	0.3659
2	0	1.6881	0.0000	27.844	0.0000	5.0201
3	0	1.2493	0.0000	26.717	0.0000	9.5181
4	0	1.4117	0.0000	29.884	0.0000	12.698
5	0	1.3179	0.0000	33.440	0.0000	15.385
6	0	1.2719	0.0000	32.490	0.0000	17.897
7	0	1.3150	0.0000	34.351	0.0000	20.183
8	0	1.4173	0.0000	33.304	0.0000	22.066
9	0	1.9652	0.0000	35.311	0.0000	24.220
10	0	1.9716	0.0000	35.712	0.0000	24.286
11	0	2.3287	0.0000	35.068	0.0000	24.227
12	0	2.7785	0.0000	34.615	0.0000	24.390
13	0	3.6344	0.0000	35.276	0.0000	25.087
14	0	5.1461	0.0000	32.206	0.0000	25.370
15	0	6.9964	7.9634	29.848	3.8217	24.734

Appendix Table A17 (Continued)

	Sterilized soil Reactor 1 (50°C)		Non-Sterilized soil Reactor 2 (50°C)		Nutrient soil Reactor 3 (50°C)	
time (days)	%CH4	%CO2	%CH4	%CO2	%CH4	%CO2
16	0	8.9010	9.7217	31.912	4.9486	24.973
17	0	7.7057	10.780	33.991	5.3850	25.061
18	0	8.1824	12.770	34.807	7.8261	25.108
19	0	8.6060	17.143	39.562	8.8863	24.716
20	0	7.8293	17.412	36.992	8.8104	25.896
21	0	7.5761	14.454	39.902	9.1134	26.006
22	0	6.5478	14.343	42.955	9.3408	27.098
23	0	6.5828	13.526	40.737	9.6415	27.415
24	0	6.4554	13.509	42.501	9.5190	27.693
25	0	7.5601	12.701	40.461	10.972	27.575
26	0	7.7314	12.595	40.968	10.568	27.958
27	0	11.311	12.597	42.485	10.556	27.429
28	0	11.320	13.080	44.609	11.684	27.629
29	0	12.621	12.462	42.797	11.373	27.941
30	0	13.290	13.974	44.151	11.826	27.905

Appendix B

The 1st International Conference on Environmental Science, Engineering and Management, Phowadol Resort & Spa Chiang Rai, Thailand, March 21, 2012

Biodata

Title	Ex-Situ Anaerobic Bioremediation for Soil Contaminated with Total Petroleum Hydrocarbon
Description	The ex-situ anaerobic bioremediation for soil contaminated with total petroleum hydrocarbon (TPH) was operated under the temperature of 30°C, 40°C, and 50°C. These temperatures were orderly maintained for 45 days in each batch-scale reactor. The methane (CH_4) and carbon dioxide (CO_2) production as well as the biodegradation of TPH were determined from sterilization and non-sterilization contaminated soils. The results showed that for sterilization contaminated soil there was no CH_4 production occurred while the CO_2 production was slightly found and for non-sterilization contaminated soil the CH_4 production was an average of 0.06%, 1.55% and 7.26% while the CO_2 production was an average of 9.48%, 13.59% and 19.68% at 30°C, 40°C and 50°C, respectively. This was an important evident to show that there was the microbial activity occurred in the reactors although there was no inoculated in the reactor. Also, the volatilization of TPH components was found about 6.62% due to high temperature operation. In addition, the TPH reduction about 31.63% might be occurred due to anaerobic microbial degradation. This should be confirmed by determining the microbial population in the contaminated soil before and after operation. Further study would be required for the effects of temperatures on the anaerobic bioremediation of TPH reduction in the contaminated soil.
Name of Presenter	Ye Hlaing Win
Designation	Master of Engineering (Environmental Engineering)
Gender	Male
Nationality	Myanmar
Email Address	yehlaingwin@hotmail.com and yehlaingwin2007@gmail.com
Telephone	0870375526 and 0820518410
Academic Background	Mechanical Engineer
Brief Work Experience	HVAC Air Duct Design, Heat Exchanger Design, M& E AutoCAD, and current electronic manufacturing experience (Cal Comp Electronics Thailand)

CERTIFICATE

This is to certify that

Ye Hlaing Win

Presented the paper entitled

Ex-Situ Anaerobic Bioremediation for Soil Contaminated with Total Petroleum Hydrocarbon

at

The 1st International Conference on Environmental Science, Engineering and Management

Phowadol Resort & Spa Chiang Rai, Thailand

March 21, 2012

Ksemsan Suwarnarat

President of Environmental Engineering Association of Thailand

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Ex-Situ Anaerobic Bioremediation for Soil Contaminated with Total Petroleum Hydrocarbon

Ye Hlaing Win 1 and Suchat Leungprasert 2*

1Graduate student ; Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok 10900, Thailand

2 Lecturer, Department of Environmental Engineering, Faculty of Engineering, Kasetsart University, Bangkok*

10900, Thailand; Tel : 02942855 ext 1027, Fax : 025790730 E-mail : fengscl@ku.ac.th*

ABSTRACT

The ex-situ anaerobic bioremediation for soil contaminated with total petroleum hydrocarbon (TPH) was operated under the temperature of 30oC, 40oC, and 50oC. These temperatures were orderly maintained for 45days in each batch-scale reactor. The methane (CH4) and carbon dioxide (CO2) production as well as the biodegradation of TPH were determined from sterilization and non-sterilization contaminated soils. The results showed that for sterilization contaminated soil there was no CH4 production occurred while the CO2 production was slightly found and for non-sterilization contaminated soil the CH4 production was an average of 0.06%, 1.55% and 7.26% while the CO2 production was an average of 9.48%, 13.59% and 19.68% at 30oC, 40oC and 50oC, respectively. This was an important evident to show that there was the microbial activity occurred in the reactors although there was no inoculated in the reactor. Also, the volatilization of TPH components was found about 6.62% due to high temperature operation. In addition, the TPH reduction about 31.63% might be occurred due to anaerobic microbial degradation. This should be confirmed by determining the microbial population in the contaminated soil before and after operation. Further study would be required for the effects of temperatures on the anaerobic bioremediation of TPH reduction in the contaminated soil.

Keywords: ex-situ anaerobic bioremediation; soil contaminated; total petroleum hydrocarbon; methane production

CURRICULUM VITAE

NAME : Mr. Ye Hlaing Win

DATE OF BIRTH : July 17, 1974

BIRTH PLACE : Mandalay, Myanmar

EDUCATION:

YEAR	INSTITUTE	DEGREE/DIPLOMA
2008	Thammasat Univ.	B.Eng (Mech)

POSITION/TITLE : Mechanical Engineer

WORK PLACE : Cal Comp Electronics, Thailand (CCET)
Phetchaburi Plant
http://www.calcomp.co.th/

SCHOLARSHIP : B.K.Kee Foundation (USA), Norwegian Burma Committee (NBC, Norway), and Prospect Burma (UK)