TCE CONTAMINATION OF GROUNDWATER AND SOIL: PRODUCTION OF MIXED METHANOTROPHIC CULTURE

BY

ZHENG LU

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Civil and Geological Engineering
University of Manitoba
Winnipeg, Manitoba

ISBN 0-315-92317-2

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- General 0578
- Ancient 0579
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- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
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History of Science 0585
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Political Science
- General 0615
- International Law and Relations 0616
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Recreation 0814
Social Work 0452
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- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
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- Neuroscience 0317
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- Public Health 0573
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- Speech Pathology 0460
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Home Economics 0386

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- Organic 0490
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- Polymer 0495
- Radiation 0754

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Physics
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- Acoustics 0986
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- Molecular 0609
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- Materials Science 0794
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- Metallurgy 0743
- Mining 0551
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- Packaging 0549
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- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
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Enseignement secondaire 0533
Enseignement spécial 0529
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Finances 0277
Formation des enseignants 0530
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- Germanique 0311
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- Archéologie 0324
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- Commerce-Affaires 0505
- Économie agricole 0503
- Économie du travail 0510
- Finances 0508
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- Histoire générale 0578
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- Africaine 0331
- Canadienne 0334
- États-Unis 0337
- Européenne 0335
- Moyen-orientale 0333
- Latino-américaine 0336
- Asie, Australie et Océanie 0332

Histoire des sciences 0585
Loisirs 0814
Planification urbaine et régionale 0999
Science politique
- Généralités 0615
- Administration publique 0617
- Droit et relations internationales 0616

Sociologie
- Généralités 0626
- Aide et bien-être social 0630
- Criminologie et établissements pénitentiaires 0627
- Démographie 0938
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- Structure et développement social 0700
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Transports 0709
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- Agronomie. 0285
- Alimentation et technologie alimentaire 0359
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- Élevage et alimentation 0475
- Exploitation des pâturages 0777
- Pathologie animale 0476
- Pathologie végétale 0480
- Physiologie végétale 0817
- Sylviculture et faune 0478
- Technologie du bois 0746

Biologie
- Généralités 0306
- Anatomie 0287
- Biologie (Statistiques) 0308
- Biologie moléculaire 0307
- Botanique 0309
- Cellule 0379
- Écologie 0329
- Entomologie 0353
- Génétique 0369
- Limnologie 0793
- Microbiologie 0410
- Neurologie 0317
- Océanographie 0416
- Physiologie 0433
- Radiation 0821
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- Généralités 0786
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- Alimentation et nutrition 0570
- Audiologie 0300
- Chimiothérapie 0992
- Dentisterie 0567
- Développement humain 0758
- Enseignement 0350
- Immunologie 0982
- Loisirs 0575
- Médecine du travail et thérapie 0354
- Médecine et chirurgie 0564
- Obstétrique et gynécologie 0380
- Ophtalmologie 0381
- Orthophonie 0460
- Pathologie 0571
- Pharmacie 0572
- Pharmacologie 0419
- Physiothérapie 0382
- Radiologie 0574
- Santé mentale 0347
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- Soins infirmiers 0569
- Toxicologie 0383

SCIENCES PHYSIQUES
Sciences Pures
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- Généralités 0485
- Biochimie 487
- Chimie agricole 0749
- Chimie analytique 0486
- Chimie minérale 0488
- Chimie nucléaire 0738
- Chimie organique 0490
- Chimie pharmaceutique 0491
- Physique 0494
- PolymÈres 0495
- Radiation 0754

Mathématiques 0405
Physique
- Généralités 0605
- Acoustique 0986
- Astronomie et astrophysique 0606
- Électronique et électricité 0607
- Fluides et plasma 0759
- Météorologie 0608
- Optique 0752
- Particules (Physique nucléaire) 0798
- Physique atomique 0748
- Physique de l'état solide 0611
- Physique moléculaire 0609
- Physique nucléaire 0610
- Radiation 0756

Statistiques 0463

Sciences Appliqués Et Technologie
Informatique 0984
Ingénierie
- Généralités 0537
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- Conditionnement (Emballage) 0549
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- Génie nucléaire 0552
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TCE CONTAMINATION OF GROUNDWATER AND SOIL

PRODUCTION OF MIXED METHANOTROPHIC CULTURE

BY

ZHENG LU

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

Mixed cultures of methanotrophic microorganisms were successfully incubated from three different seed materials: activated sludge, anaerobically digesting municipal sludge and river sediment, in three open laboratory fill and draw breeder-reactors fed with natural gas and air (15:85, vol:vol). The average specific growth rate of methanotrophs was found to be 0.018 d^{-1}. All three cultures thus developed, were able to degrade TCE at initial concentration of 30 $mg \cdot L^{-1}$ in experiments without addition of methane into the water phase. The maximum TCE degradation rate (23°C) was 14.6 $mg(TCE) \cdot [g(VS)]^{-1} \cdot d^{-1}$. Effects of temperature and pH on the degradability of TCE by methanotrophs were studied. The rate of TCE removal decreased from approximately 8.6 $mg(TCE) \cdot [g(VS)]^{-1} \cdot d^{-1}$ at 23°C down to 4.2 $mg(TCE) \cdot [g(VS)]^{-1} \cdot d^{-1}$ at 4°C. Optimum removal was found at pH = 7 to 7.4. Both the temperature and pH effects were fully reversible.

Addition of thus prepared mixed methanotrophic cultures to soil slurry reactors resulted in TCE degradation in all reactors with rates inversely proportional to the size of soil fraction and amount of organic matter in soil. Biodegradation was superior in the water phase; sorption hindered degradation. Bio-degradation of TCE by addition of the methanotrophic culture sieved through glass fibre filters was also evaluated using laboratory column packed with loamy sand, compared with a parallel soil column receiving fresh water at the same flow

rate. The culture in soil showed limited ability to remove TCE with best results obtained for filter size 0.7 to 1 μm. Unscreened biomass addition was also applied to a column. The biomass did not move through the soil column blocking the pores. Size selection of mixed methanotrophic culture was conducted using simple glass fibre filter screening.

Study of the storage temperature for the produced methanotrophic culture revealed that the lower the temperature, the more active the biomass remained. Simple freezing of the culture at -20°C retained 92% of the TCE degradation ability during the 73 days of storage.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The completion of this thesis was assisted and encouraged by following people. My special thanks are extended to:

Dr. Jan A. Oleszkiewicz for his attention to transferring my study from McGill University to University of Manitoba and choosing the topic, as well as his guidance, knowledge, enthusiasm and financial aid; Dr. Maria Elektorowicz of Concordia University (formerly McGill University) for her unselfish advice and consultation; Professor Richard R. Sparling of Dept. of Microbiology, the University of Manitoba for his scientific input and expert advice & consultation; Ms. Judy Tingley for her setting up of GC and methodology for analyses. Mr. Wacek Trebacz for his long-term technical assistance; Mr. Saibal Basu for providing phosphate pH buffer for experiment.

All staff and students in Environmental Engineering Division: for their friendship and help in various aspect.

Staff in Northernwest Lab., for providing used analyzed soil sample.

My family; my wife, Ms. Xiao Guang Li, for her understanding, year-long waiting to unite, and for fully support; My mom, dad, who gave their sincere hope and support.

DEDICATION

To the People's Republic of China, my motherland, who has raised me for more than thirty years.

LIST OF TABLES

* App. = Appendix

LIST OF FIGURES

LIST OF ABBREVIATIONS

APHA	American Public Health Association
AWWA	American Water Works Association
DCE	Dichloroethylene
DNAPL	Dense Non-Aqueous Phase Liquid
GC	Gas Chromatography
MMO	Methane Monooxygenase
NAD	Nicotinamide Adenine Dinucleotide
$NADH_2$	Nicotinamide Adenine Dinucleotide Hydrogen
NEWPCC	North End Water Pollution Control Centre
pMMO	Particulate (membrane-bound) Methane Monooxygenase
SITE	Superfund Innovative Technology Evaluation
sMMO	Soluble Methane Monooxygenase
TCE	Trichloroethylene
USEPA	United States Environmental Protection Agency
USA	United States America
WEF	Water Environment Federation

I. INTRODUCTION

Trichloroethylene (TCE) is a significant environmental pollutant in contaminated soil and groundwater in developed countries due to its common use in industry for degreasing, dry cleaning and as solvents (Westrich et al., 1984). The U. S. Environmental Protection Agency has listed TCE as a priority pollutant, and remediating contaminated aquifers will be a formidable task (Fliermans et al., 1988). Efforts are currently underway to develop new methods for remediation of soil and groundwater. Possible remediation strategies (Lu et al., 1993) include some processes, such as, soil stripping (venting); soil washing; excavation and landfill; trench recovery; UV light, alkaline polymer surfactant washing soil; and biological treatment by using pump-and-treat method and bio-stimulation methods.

In some remediation technologies mentioned above, TCE is not destroyed but simply evaporates or is immobilized with solid matrix. Biological treatment of contaminated soil and groundwater, based on the ability of microorganisms to degrade these contaminants, is a promising method, which is a viable treatment alternative and which can safely, effectively and permanently reembodied TCE.

TCE can be degraded under both aerobic and anaerobic conditions. Under methanogenic conditions, TCE is degraded by a reductive de-chlorination to dichloroethylene (DCE) and then vinyl chloride (VC) (Vogel & McCarty, 1985).

Unfortunately vinyl chloride is more mutagenic and carcinogenic in humans than TCE. Under aerobic conditions TCE can be degraded cometabolically by special groups of bacteria, e.g. *Alcaligenes eutrophus JMP134* (Harker et al., 1990). *Pseudomonas putida F1* (Wackett et al., 1989), and *Pseudomonas cepacia* (Nelson et al., 1988); phenol utilizing microorganisms (Hopkins & McCarty, 1993); ammonia-oxidizing bacteria *Nitrosomonas europaea* (Arciero et al., 1989); toluene-oxidizing bacteria (Nelson et al., 1986; Parsons et al., 1984) also have the potential to degrade trichloroethylene.

Methanotrophs are a group of microorganisms which grow on methane as sole source of energy and as a major source of carbon. The methane monooxygenase (MMO) system present in certain methanotrophic bacteria has been found to be capable of oxidizing a wide range of carbon substrate including TCE when copper becomes limiting (Burrows et al., 1984; Scott et al., 1981). A lot of efforts on degradation of TCE from soil and groundwater have been done in last ten years using methanotrophs.

The primary objective of this work was to investigate the possibility of incubation of mixed methanotrophic cultures using different easily available sources; to determine the rates of biomass growth, methane and oxygen consumption in a closed incubator-reactor; to determine if the mixed methanotrophs were cultures capable of TCE biodegradation from contaminated

water phase or slurry; to find out how environmental factors, eg. temperature and pH affect TCE degradation; to test a simple methods for storing methanotrophic biomass for mid-to-long term usage; and finally to conduct soil column study on degradation of TCE using screened and unscreened culture.

II. LITERATURE REVIEW

2.1 Trichloroethylene (TCE) and Usage

Trichloroethylene (TCE) is a chlorinated organic solvent widely used by various industries such as metal processing, electronics, dry cleaning, paint, pulp and paper, and textile manufacturing. TCE has been used ubiquitously for the last 50 years because of its low flammability, low flash point and cleansing ability far superior to the alkaline cleansers. The production of TCE was reported at 234,000 tons in the United States in 1980 (USEPA, 1980), 70,000 tons in Japan in 1992 (Inaba & Hirata, 1992) and 16,200 t/a were used in Canada in 1983 (Environment Canada, 1992). Some important characteristics of TCE is listed in Table 2.1.

2.2 Scope of the TCE Contamination

Sloppy handling and storage procedures have often led to spills and subsequent seepage of TCE to groundwater where, depending on the prevailing environmental conditions it remained at concentrations in excess of the established drinking water standards which are 5 μg/L in USA (Mahaffey et al., 1992), 50 μg/L in Canada (Environment Canada, 1992) and 30 μg/L in Japan (Hirata et al., 1991). The TCE concentrations in groundwater depend on the magnitude of contamination, the characteristics of soil, the mobility of groundwater and physico-

Table 2.1 Some Important Characteristics of Trichloroethylene

Molecular Structure:	Cl Cl C = C Cl H
Molecular Weight:	131.40
Density:	1.46 $g \cdot cm^{-3}$ > 1.00 $g \cdot cm^{-3}$ (H_2O)
Boiling Point:	86.7 °C < 100 °C @ 760 mm Hg
Vapour Pressure:	20 mm Hg @ 0°C 60 mm Hg @ 20°C
Water Solubility:	1100 $mg \cdot L^{-1}$ @ 25°C
Evaporation from H_2O:	90% for 63-Minutes
Nonflammable	
Miscible with Common Organic Solvent	
Biological Effects:	
* Toxicity Threshold (Cell Multiplication Inhibition Test):	
- bacteria (*Pseudomonas putida*):	65 $mg \cdot L^{-1}$
- algae (*Microcystis aeruginosa*):	63 $mg \cdot L^{-1}$
- protozoa (*Entosiphon sulcatum*):	1200 $mg \cdot L^{-1}$
* Severe Toxic Effects to Man:	2000 $\mu g \cdot L^{-1}$ @ 60 min.
- unsatisfactory	>400 $\mu g \cdot L^{-1}$
- symptoms of illness	800 $\mu g \cdot L^{-1}$

* adapted from Handbook of Environmental Data on Organic Chemicals (2nd Edition, Verschueren Karel)

chemical as well as microbiological characteristics of the site. The concentrations varied from traces to 20 $mg \cdot L^{-1}$ (UMA, 1992).

Trichloroethylene is a dense non-aqueous phase liquid (DNAPL) of density higher than water and of low viscosity. It may be transported vertically through aquifers due to high gravity and the action of capillary forces (Fig.2.1). In the initial stages of the spill, in the unsaturated zone, TCE can exist dissolved in the water phase; in air phase as vapour; adsorbed on to solid phase; and in the immiscible phase as DNAPL. In the saturated zone TCE will be present in all phases except in the air phase. Relatively small releases of TCE can produce substantial and long-lasting sources of groundwater contamination due to TCE's high density, low viscosity and low solubility which allows sinking into aquifers. Once in the saturated zone the slow solubility will cause significant contamination and natural or even man-induced remediation processes may take decades and centuries before completion (Tavis et al., 1990). The issue of TCE tailing (i.e. persistence above the theoretically calculated time for complete removal of TCE from the pore volume) and recurrence of contaminant after the cessation of remedial action has been documented (Hirata et al., 1991).

TCE was found in 3.3% of 11.000 wells in Japan (Muraoka and Hirata, 1990). The level of TCE in the U.S. in St. Joseph, Savannah, and Moffett sites were 600-7000 $\mu g \cdot L^{-1}$, 5000 $\mu g \cdot L^{-1}$, and 100 $\mu g \cdot L^{-1}$, respectively (McCarty et al.,

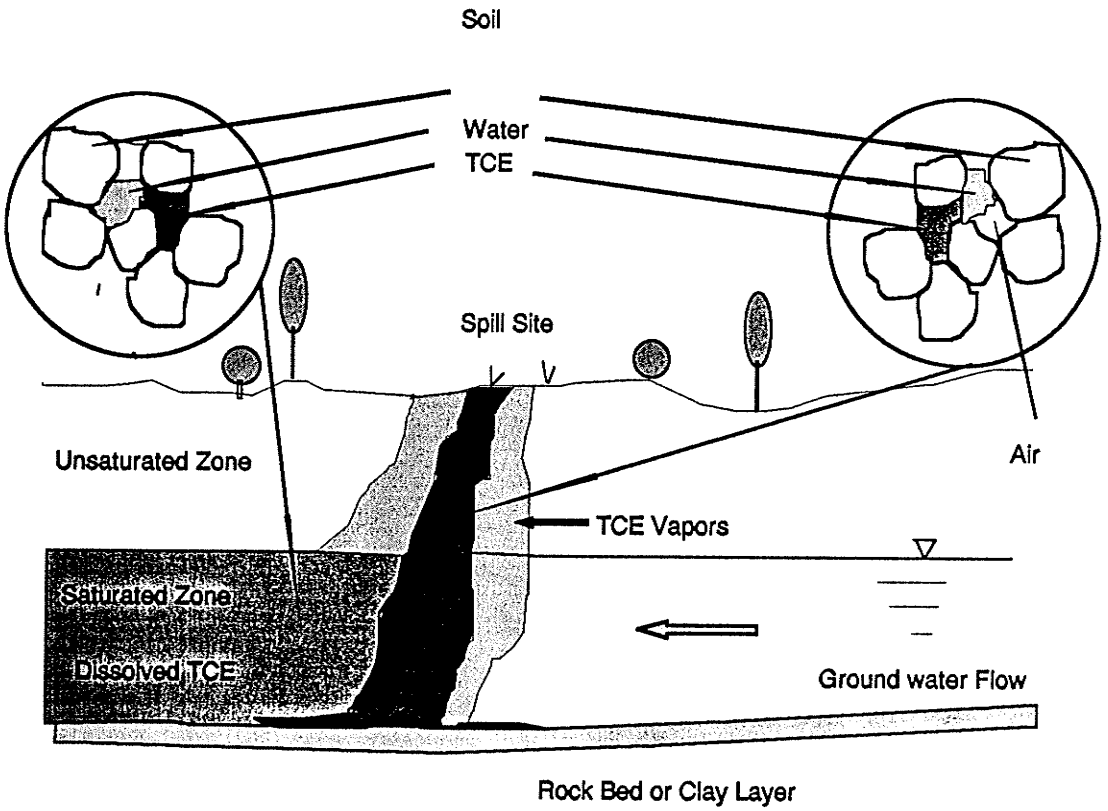

Figure 2.1 Migration of TCE in Soil and Groundwater

1991; Nichols et al., 1992; GASRep, 1990). Many aquifers underlying military facilities in the U.S. and Canada (e.g., Tyndall Air Force Base, FL, and Borden Canadian Forces Base, ON) are contaminated with TCE to higher levels (McFarland et al., 1992; UMA, 1992). The site at Stony mountain, Manitoba, had TCE concentrations in the "hot spots" as high as 13.2 $mg \cdot L^{-1}$, respectively (UMA, 1992). However in most cases at a distance from "hot spots", the observed concentration of TCE in pumped groundwater was very low, normally in 1 $\mu g \cdot L^{-1}$ to 1 $mg \cdot L^{-1}$ range (American Chemical Society, 1986; UMA Engineering 1992). Anderson et al. (1992) concluded that the main potential reason for low aqueous-phase concentrations was the tendency of majority of the dense solvent fluid to accumulate on the top of bedding planes in the saturated zone and form one or more flat source zones that present very low cross-sectional areas to the on-coming groundwater flow, thus preventing TCE from fully dissolving into groundwater (Fig.2.2) .

Municipal landfill leachate plumes often contain TCE at lower levels, perhaps because of high sorption onto the organic matrix of the waste. Barker (1987) found TCE levels of 0 to 21 $\mu g \cdot L^{-1}$ in Ontario landfill leachate plumes. The Ottawa Gloucester landfill leachate had TCE as high as 90 $\mu g \cdot L^{-1}$ (Simovic and Jones, 1987).

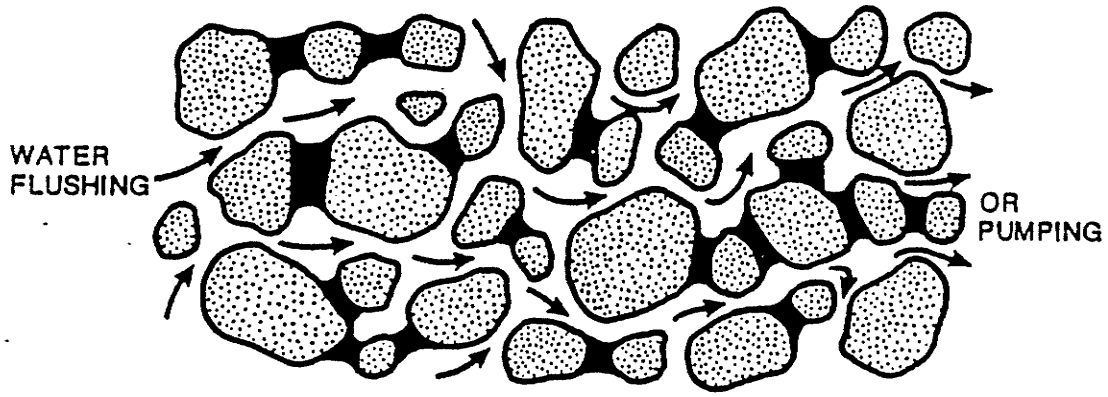

Figure 2.2 Trapped TCE at Residual Saturated Soil

2.3 Some Removal Methods

A number of engineering solutions are in the early stages of development. Table 2.2 summarizes features of some technologies used to remove TCE from both unsaturated and saturated zone (Lu et al., 1993)

Table 2.2 Some of the Feasible Remediation Technologies

	Technology	Ex/On/In Situ	Cost	Soil Zone
1	**Stripping**	In-situ	H*	Both
	+ Hot air or Steam	in-situ		
	+Carbon absorbtion	in-situ		
	+ Distillation	in-situ		
2	**Soil Washing**	In-situ	M & H	Both
3	**Excavation**	Ex-situ	M & H	unsaturated
	+ Chemical extraction	on, off site		
	+ Stripping	on, off site		
	+ Land application	off site		
4	**Trench Recovery System**	In-situ	L & M	Both
	+ Pure TCE collection	on site		
	+Physical treatment	on site		
	+Bio-treatment, etc.	on site		
5	**Pump and Treat Method**	In-situ	H	Saturated
	+ Physical treat	on site		
	+ Chemical treat	on site		
	+ Bio-treatment	on site		
6	**In-Situ Bioremediation**	In-situ	M**	Saturated
	+ Bio-stimulation (CH_4 & O_2)	in -situ		
7	**Passive Remediation**	In-situ	L***	Both

Note: * High
** Median
*** Low

Stripping

In-Situ stream/hot air stripping is an in situ stripping process that utilizes steam and hot air to treat soils contaminated with TCE without the need for

excavation. Developed along the lines of soil vacuum extraction of vapour form of DNAPL, the most recent full scale work was successfully conducted by Toxic Treatment Inc. (Anon, 1991) There are two major elements: the process tower and the process train. The process tower includes hollow augers that drill into the soil to be treated. The process train consists of two systems which operate in conjunction during the treatment of the soil: the process gas treatment system provides the conditioning of stream/hot air and volatile TCE so that the air can be reintroduced into the process. The liquid treatment system separates the disposal or recycling and the condensed steam is recycled. The level of contaminant removal can be as high as 85% and is dependent on the contaminants present, treatment time, soil conditions and chemistry of TCE soil interaction.

The advantages of this system are addresses both vadose and saturated zones to target levels with removal rate approximately 85%; provides permanent treatment; closed vapour-collect systems reduce air pollution and reduce exposure; downward migration of TCE is mitigated. The process limitations are: the subsurface of the working site must be free of major obstructions such as large boulders etc., increased treatment times are needed in high clay soils; higher capital cost. In summary, the in-situ steam/hot air system appears applicable to less extensive pollution sites, very soon after the spill, in fairly permeable soil characteristics and to sites with maximum depth below 9 meter.

Soil Washing

In this technology an alkaline polymer surfactant (APS) is used to flush the contaminated site and the mixture of surfactant and TCE is pumped out, and separated, so that surfactant can be reused. The aquifer rehabilitation is completed by circulating water, using a conventional pump-and-treat operation. Surfactant soil flushing can proceed on two distinctly different mechanistic levels: enhanced dissolution of adsorbed and dissolved phase contaminants, and displacement of free-phase non-aqueous contaminants. These two mechanisms may occur simultaneously during soil flushing (Huling et al., 1991). The surfactant and alkali reduce the surface tension between the TCE and water which increases the mobility. Polymer is added to increase the viscosity of the flushing fluid to minimize the fingering effects and to maintain hydraulic control and improve flushing efficiency.

Generally better removal efficiencies, greater than 89%, were obtained in more heavily contaminated soils (Mahaffey, et al., 1991). In-situ soil washing may effect a quick removal of TCE both in saturated and unsaturated zones. The effects of the presence of groundwater in the saturated zone and the effect of changing of the indigenous microbial population caused by APS residual are not very well determined, as yet.

Excavation

This ex-situ technology consists of removal of the contaminated soil in the unsaturated zone for off-site treatment which could include chemical extraction; stripping; landfilling and other methods.

This technique is best applicable to small recent spill sites or very impermeable soil (such as clay and silt) at sites where dense TCE is located in unsaturated zone. Polluted sites where TCE had already penetrated into the saturated zone, but still very high TCE content remains confined in the unsaturated zone are also likely candidates.

Trench Recovery (Dual Drain-lines) System

Trench systems could be used to recover TCE when the TCE reservoir is located near the ground surface. Two drain-lines are horizontally placed on the top of the impermeable layer in different vertical levels. for collecting dense TCE. The condensed TCE can be collected from the bottom line by pumping. Groundwater with dissolved TCE is withdrawn from the upper screen which results in an upwelling of the TCE which is collected in the lower line. Both streams are treated for TCE recovery. An advantage of the dual drain system is that the TCE/water separation requirements on site are reduced (Sale & Kuhn, 1988).

Pump-and-Treat Method

The pump and treat Figure 2.3 is the most widely used technique to clean up TCE from the aquifer. In a typical pump and treat system operating at a spill site TCE contaminated groundwater withdrawn from extraction wells is treated above the ground and re-injected or discharged to surface waters. This technology can also be used as a hydraulic barrier to prevent off-site migration of contaminant plumes from spill site. This is accomplished by lowering the groundwater levels near a line of wells, thus diverting groundwater flow toward the pumping wells (Mercer et al., 1990).

The remediation by using this method is seldom permanent. The concentration of DNAPL often rises again once pumps are turned off (Travis et al., 1990, Hirata et al., 1991), pointing to the main limitations of the process: dependence on the predominant hydrogeological conditions of the contaminated soil. Low permeability zones not only restrict the flow of TCE toward extraction wells but also cause increased TCE adsorption on the soil. If the hydraulic conductivity (K) is below 10^{-7} cm/s, pump and treat solution is not feasible (Mercer et al., 1990). The second limitation is due to TCE being a highly absorbable compound on the soils thus being held back (retarded) by its adherence to the soil particles during pumping. The un-desolveded TCE, because of its lower solubility,

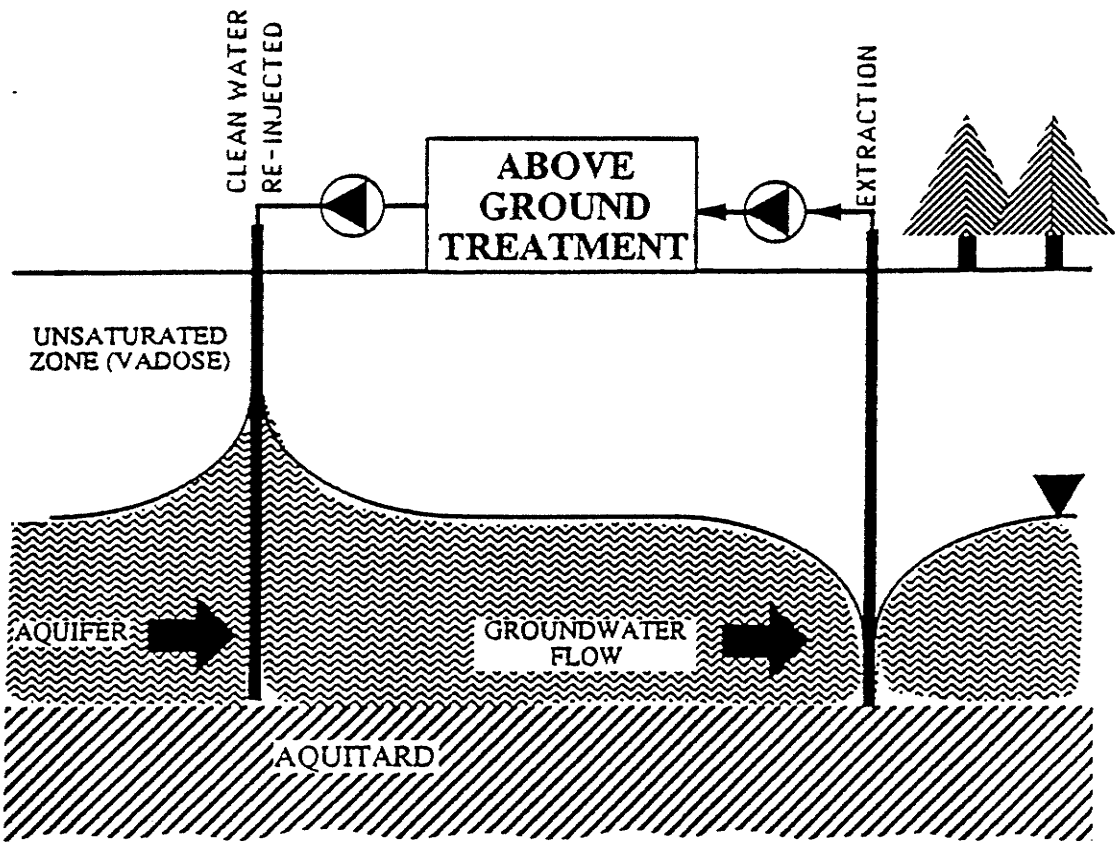

Figure 2.3 Typical Schematic for Pump-and-Treat Method

(adapted from Oleszkiewicz, J. A. et al., 1993)

will slowly to be added or released to the groundwater. Switching from continuous pumping to pulsed pumping is one modification that may improve the efficiency of TCE removal.

On Site Water Treatment Facilities

The contaminated water removed by the trench or well-based pump-and-treat techniques can be treated by a number of new and established technologies introduced under the USEPA's Superfund Innovative Technology Evaluation (SITE) (Anon, 1992), such as air stripping with aqueous-phase carbon adsorption; thermal incineration; membrane process; heating with distillation (Naft, 1992); ultra-violet light catalyzed oxidation using ozone and hydrogen peroxide (Anon, 1989); oxidation and stripping (Simovic et al., 1987). Often these methods are either too expensive or result in transfer of TCE to other locations. Biological treatment on the other hand can destroy TCE and several processes were demonstrated in laboratory and pilot scale, which will be described in section 2.4 for Aerobic and Anaerobic process and section 2.6 for Methanotrophic Process.

Passive Remediation

The principle of passive remediation is to leave the site to naturally occurring processes of dispersion, sorption, and biotransformation. The rate of passive remediation will be controlled by indigenous bacteria and soil environment, i. e. dissolved oxygen, temperature, salinity, pH, etc..

Low capital cost, (site investigation and installation of the monitoring wells) is the principal advantage of passive remediation. The most obvious disadvantage is unpredictability of length of time required for completion (Preslo, 1988). This method would not be a suitable remediation alternative in most cases. But it may fit a small scale spill, where the distance which the TCE plume could travel during the time required for passive remediation must be less than the distance to the nearest potential drinking water receptors; or a spill site with a very impermeable soil which will not influence the downstream groundwater user.

Selection Process

The choice of method will depend on the scale of TCE spill (unsaturated zone only or both vadose zone and saturated zone); geological conditions (permeability) of the soil; biotreatability in soil; local regulation and public opinion, and characteristics of various technologies themselves. One or several technologies may be used at the same time.

Basically, recovery of pure TCE should be the focus initially to minimize further migration. The action must be conducted as early as possible after the spill, because even small delays can exacerbate the problem. In-situ bioremediation will not be usually used at this stage; it would be introduced later to reduce the dissolved TCE in the saturated zone. TCE recovery preceding bioremediation will improve bioremediation by reducing the toxicity time, resources and labour (Huling

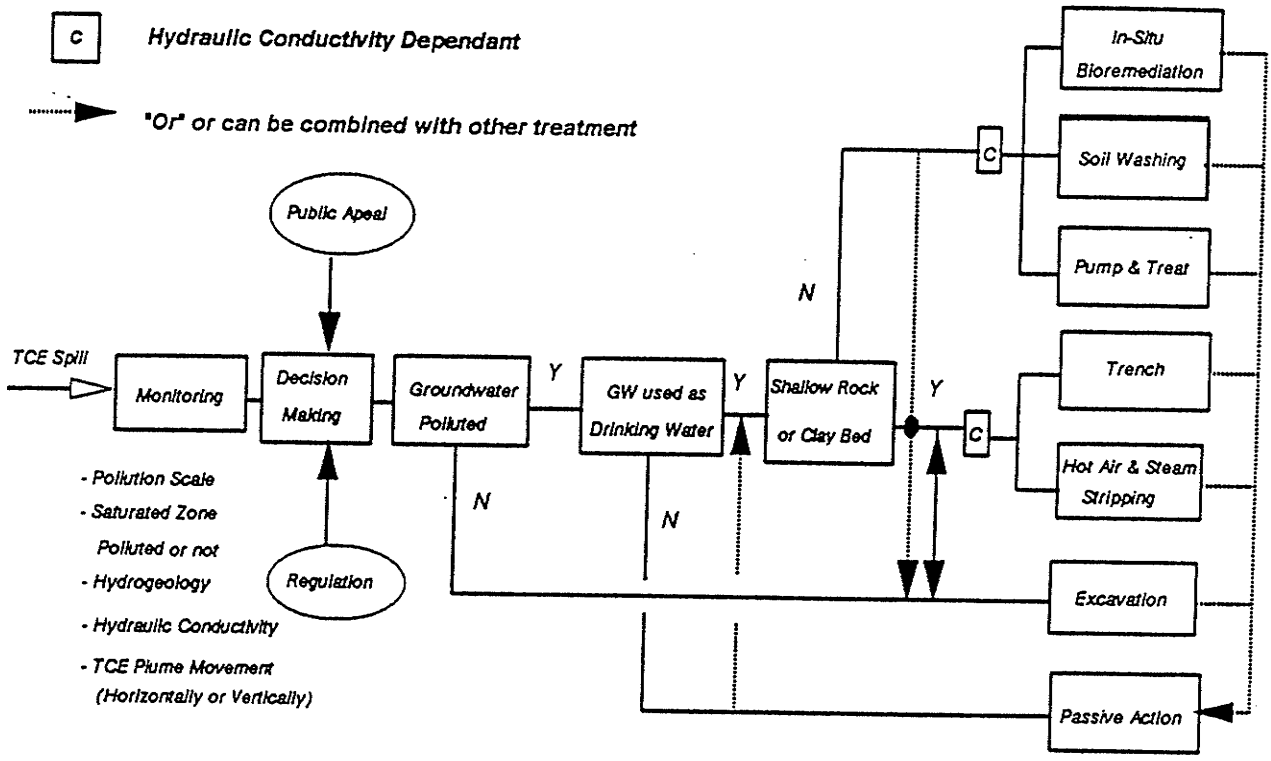

Figure 2.4 Groundwater Remediation Selection

(reprinted from Lu & Oleszkiewicz, 1993)

et al., 1991). Although pump-and-treat is ineffective for aquifer restoration, it has great value for plume containment and contaminant mass reduction. Figure 2.4 shows one possible decision tree for the seven technologies discussed here. Often a combination of more than two methods will have to be used.

2.4 Bioremediation

Biodegradation of TCE in water and soil phases has been widely studied in recent years. In anaerobic conditions, TCE can be converted by reductive dehalogenation to dichloroethylene and ultimately to a more toxic intermediate vinyl chloride in human (Vogel & McCarty, 1985, Freedman & Gossett, 1989). These reports showed that biodegradation of TCE under anaerobic conditions, often naturally dominant in groundwater, occurs very slowly, if at all. This is unfortunate since anaerobic conditions prevail in groundwaters.

It was also found that many aerobic bacteria, listed in Table 2.3 are also able to degrade trichloroethylene.

Additionally, it is found from recent studies that methanotrophic bioremediation may be the most promising technology for removing soluble TCE from contaminated soil and groundwater. The great potential for use of these

organisms for TCE bioremediation requires one describes in great detail of the physiology and diversity of these organisms (hence section 2.5).

Table 2.3 Bacteria Shown to Cometabolize TCE

Stain	Substrate	Reference
Alcaligenes eutrophus JMP134		Harker et al., (1990)
Mycobacterium vaccae JOB5	Propane	COGNIS, Inc.(1991)
Nitrosomonas europaea	Ammonia	Arciero et al., (1989)
phenol utilizing microorganisms	Phenol	Hopkins & McCarty, (1993)
Pseudomonas putida F1	Toluene	Wackett et al., (1989)
Pseudomonas cepacia	Toluene	Nelson et al., (1988)
Pseudomonas mendocina KR-1	Toluene	COGNIS, Inc.(1991)
Rhodococcus erythropolis	Isoprene	COGNIS, Inc.(1991)
toluene-oxidizing bacteria	Toluene	Nelson et al., 1986; Parsons et al.,1984
Methanotrophic Bacteria	Methane	in section 2.5

2.5 Methanotrophic Bacteria

According to Hanson's review (Hanson et al., 1992), Kaser and Shongen first reported the existence of methane-oxidizing bacteria in 1906. The ubiquity of these organisms and their importance to the conservation of organic material was generally recognized by Aiyer in 1920. In 1949, Hutton and Zobell succeeded in

isolating pure cultures of methane-oxidizing bacteria from several sources including gas field soils, beach sand, and mud from marine and freshwater samples (Hanson et al., 1992). Whittenbury (1984) reported the isolation of over 100 strains of methane-utilizing bacteria and classified them into five genera. The five genera fall into three major groups described in Table 2.4 with typical stains:

Table 2.4 Major Groups of Methanotrophs

Group I	**Group II**	**Group X**
Methylococcus, Methylomonas, Methylobacter	*Methylosinus Methylocystis*	*Methylococcus capsulatus*
Example:		
-Methylomonas methanica 68-1	*-Methylosinus trichosporium OB3b* *-Methylosinus sporium* *-Methylocystis parvus*	*Methylococcus capsulatus*
Characteristics:		
stacks of disc-shaped membrane structures	paired intrayto-plasmic membranes	the same as group I
mol % G+C = 50-59	mol % G+C = 62-67	DNA has higher G+C
ribulose mono-phosphate pathway	serine pathway	contains ribulose bisphosphate carboxylase hybrid properties of I & II

The pathway for degradation of methane (Figure 2.5) is described below (Broholm et al., 1992):

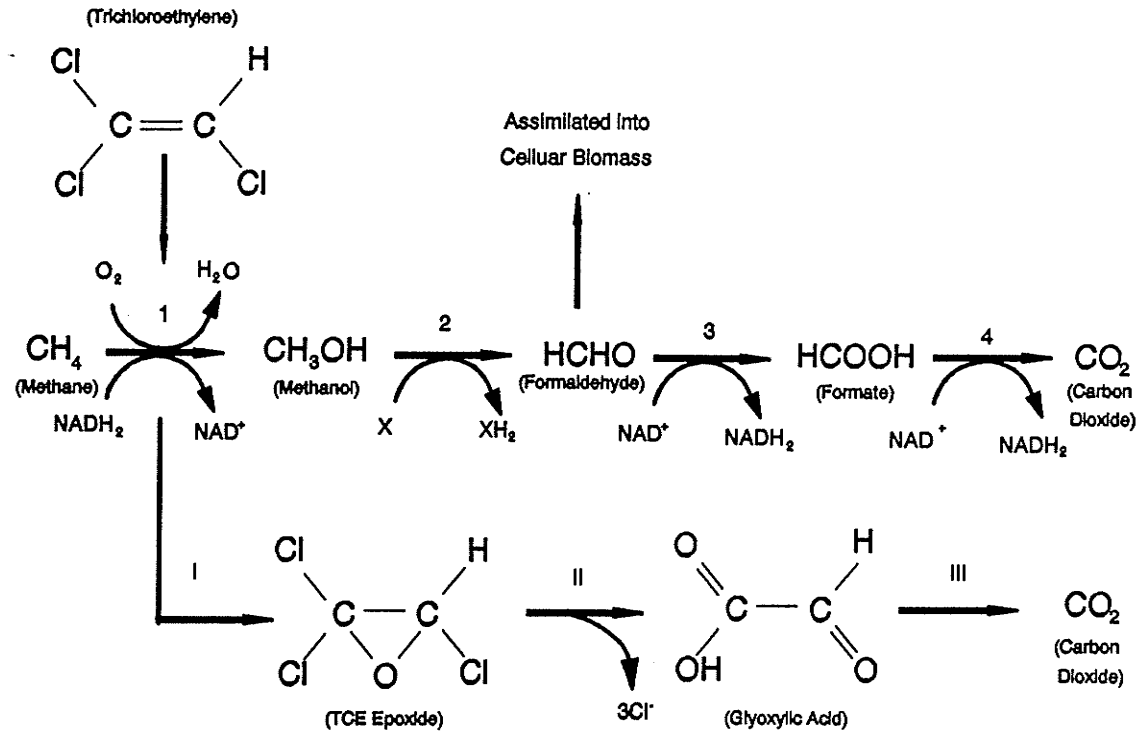

Figure 2.5 The Pathway for Methane and TCE Degradation

(1-4 Methane Degradation Pathway, I-III TCE Degradation Pathway,

Adapted from McFarland M. J. et al., 1992)

In the first step, methanotrophs employ a monooxygenase to catalyze the first reaction in the oxidation of methane to methanol. This methane monooxygenase (MMO) has received growing interest as a potentially valuable tool for industrial scale biotransformation and ecologically important biodegradation reactions. Both a particulate (membrane-bound) methane monooxygenase (pMMO) (Dalton and Higgins 1987; DiSpirito et al., 1992) and a soluble monooxygenase (sMMO) (Dalton and Higgins 1987; Tsien et al., 1989) have been identified. The first reaction also requires an electron donor, such as $NADH_2$, which is generated in the later oxidation steps. Methanol is oxidized to formaldehyde, which is either assimilated into bacterial biomass or is further oxidized to formate and carbon dioxide generating $NADH_2$ required for the initial oxidation of methane and as electrons source for respiration. MMO seems to be responsible for the initial oxidation of the chlorinated aliphatics (Forgel et al., 1986). From the pathway shown in Figure 2.5, it can be seen that methanol may be an alternative growth substrate (electron donor and carbon source) for the methanotrophs, whereas formate only may serve as an alternative electron donor.

It is believed that sMMO is found only in group II and group X methanotrophs in copper-limiting conditions (Murrell 1992), and relatively high TCE degradation rates are only found in some species of type II and X methanotrophs, which were recently studied (Brusseau et al., 1990, Oldenhuis et al., 1989; Tsien et al., 1989; Tsien et al., 1992). A recent study reported that pMMO can degrade

TCE but degradation rates are several orders of magnitude lower that those with sMMO (DiSpirito et al., 1992). The sMMO has a wider substrate specificity than the pMMO (Burrows et al., 1984). The soluble enzyme can catalyze the incorporation of oxygen into a broad range of substrate including alkanes, alkenes, aromatic hydrocabons, alicyclic hydrocarbons, halogenated alkanes, and chlorinated aromatic hydrocarbons (Hanson et al., 1992). This enzyme may be useful on an industrial scale both for the production of chemicals and the breakdown of industrial pollutants. For example, sMMO might aid in the production of alcohols from alkanes and in the formation of specific expoxides for subsequent construction of polymers (Large and Bamforth, 1988).

In addition, sMMO can catalyze the breakdown of trichloroethylene (TCE), a major industrial pollutant (Oldenhuis et al., 1989; Tsien et al., 1989). When TCE is the substrate for MMO, the first chemical intermediate is TCE epoxide (Fig. 2.4). This compound is unstable and is rapidly transformed to glyoxylic acid with the removal of chloride. Glyoxylic acid may then be chemically or biologically oxidized to carbon dioxide (McFarland et al., 1992).

2.6 Methanotrophic Remediation of TCE

Current Studies in Laboratory

In recent years, the methane-oxidizing bacteria have been studied

extensively for degradation of TCE. Several chemical factors influence the degradation of chlorinated aliphatics. The copper concentration seems to control whether methane-oxidizing bacteria express soluble or particulate MMO (Stanley et al., 1983). During copper limitation, when the soluble form is expressed, higher degradation rates for the chlorinated aliphatics have been observed compared to substitutions with copper in excess (Oldenhuis et al., 1989, Tsien et al., 1989). Studies on the interactions between TCE and methane degradation have revealed that the methane concentration has a negative effect on the degradation of TCE because of competition between TCE and methane for MMO (Lanzarone and McCarty, 1990; Broholm et al., 1992, Oldenhuis et al., 1991; Semprini et al., 1991). The highest TCE degradation rates have been observed by resting cells in the absence of additional methane. Resting cells have a finite TCE degradation capacity which may be due to toxicity of some of TCE degradation products (Alvarez-Cohen and McCarty, 1991; Oldenhuis et al., 1991). The use of alternative electron donors such as methanol and formate has been proposed to avoid the competition between TCE and methane. Methanol and formate are both miscible with water in contrast to methane which only has a limited solubility in water of 24 $mg \cdot L^{-1}$ at 20°C (Windholz, 1983). Formate has been used successfully as an electron donor to enhance the TCE degradation by resting cells for shorter periods of time from a few hours to a few days (Oldenhuis et al., 1989; Alvarez-Cohen and McCarty, 1991; Semprini et al., 1991; McFarland et al., 1992), whereas the use of methanol so far has resulted in differing results (Janssen et al., 1988; Oldenhuis

et al., 1989). Henry and Grbic-Galic (1990) have shown that TCE degradation rates for different mixed cultures of methane-oxidizing bacteria grown in slightly different mineral media varied.

Batch Scale Experiments

Studies of pure and mixed methane oxidizing (or methanotrophic) cultures revealed that they were able to degrade TCE. Efforts in constructing various methanotrophic reactors for the purpose of removing TCE from groundwater have also been reported, such as fixed biofilm reactor (Strand et al., 1991; Arvin, 1991); Continuous-Recycle Expanded-Bed Bio-reactors (Phelps et al., 1990; Phelps et al., 1991). Some researchers tried to build two-stage bio-reactor to avoid the competition between TCE and methane (Alvarez-Cohen L. et al., 1991; McFarland et al., 1992).

Column microcosm studies of biological degradation of TCE (Wilson et al., 1985; Lanzarone & McCarty, 1990) on stimulation of indigenous methanotrophic culture using natural gas and air were followed by successful field demonstration of biodegradation TCE and DCE and to some extent vinyl chloride using methane and air at Moffett Naval Air Station (Robert et al., 1990; Semprini et al., 1990).

In-Situ Bioremediation (Bio-stimulation)

A method gaining more widespread attention recently is in-situ

bioremediation , the treatment of subsurface pollutants by stimulating the native microbial population (Charboneau et al., 1990). In-situ bioremediation involves addition of nutrients, additional oxygen or other electron acceptors (Fig.2.6) combined with hydraulic control to minimize migration of the plume during the in-situ treatment (Thomas, 1987). In treating TCE contaminated sites the method allows for: treatment of TCE adsorbed to aquifer material or trapped in pore spaces; removal of TCE held in the unsaturated and capillary zones by using an infiltration gallery. The efficiency of TCE removal using in-situ bioremediation can be much higher than that of pump-and-treat (McCarty et al., 1991). The area of treatment using bioremediation can be larger than with other remedial technologies because the treatment moves with the plume and can reach areas that should otherwise be inaccessible. In-situ bioremediation (stimulation) often costs less than other methods of remediation because it utilizes the indigenous bacteria.

The disadvantages of TCE in-situ bioremediation include clogging of the injection wells by the profuse microbial growth and difficulty of implementation in low-permeability aquifers that do not permit the transport of adequate supplies of nutrients or oxygen to active microbial populations. Aquifer with K values of 10^{-4} $cm \cdot s^{-1}$ or greater, are usually considered good candidates for in-situ bioremediation (Thomas & Ward, 1989).

Application of methanotrophic bioremediation in-situ has been demonstrated

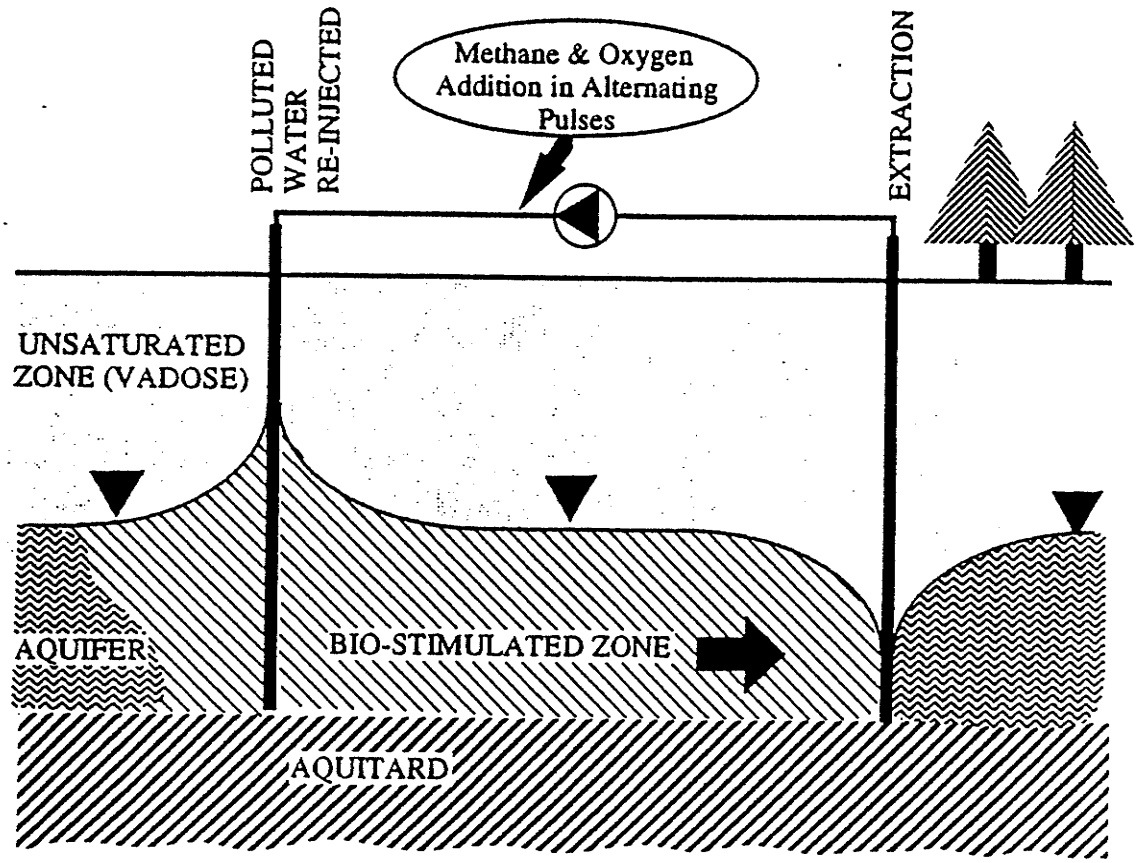

Figure 2.6 In-Situ Biological Stimulation of Indigenous Methanotrophic Biomass in the Saturated Zone (reprinted from Oleszkiewicz, J. A. et al. 1993)

(McCarty et al., 1991; Semprini & McCarty 1990). The resulting transformations were: TCE 20-30%; cis-DCE 45-55%; trans-DCE 80-90%; and VC 90-95%, and indicated that the rate and extent of biotransformation decreased with the increase of the degree of chlorination of the organic compounds. (Semprini & McCarty, 1990). Research should continue on the competition between methane and TCE for the MMO reaction site; sorption of TCE onto the aquifer solids and increasing the efficiency of bioremediation.

III. OBJECTIVES

1). to asses the feasibility of enrichment and incubation of mixed methanotrophic bacteria from easily available inoculum sources such as activated sludge, anaerobically digested sludge, and river sediment, in open systems.

2). to determine the effectiveness of incubating a mixed methanotrophic culture in an incubator with a closed head space with natural gas and air recycling. Methane and oxygen consumption and carbon dioxide production will be determined to establish the kinetics of biomass production.

3). to study the effects of cessation of methane addition and the effects of pH and temperature on TCE degradation in groundwater and soil.

4) to test the potential for TCE removal from excavated contaminated soil using separately prepared and stored cultures.

5) to evaluate the potential for in-situ bioremediation of TCE by using sieved biomass added to laboratory soil columns and to compare this with washing (pump and treat) the contaminated column.

6) to determine if simple freezing could be used as an inexpensive method of

storing the methanotrophic biomass produced in an off-site incubator-reactor.

4. MATERIALS & METHODS

4.1 Open Methanotrophic Breeder-Reactor

Three 2.5 litre dark glass reactors (Fig.4.1 & Fig.4.2) were filled with 1.5 litre of nutrient medium (Table 4.1.). Reactor I was inoculated with activated sludge collected from the pure oxygen system at the North End Water Pollution Control Centre (NEWPCC) in Winnipeg; Reactor II was inoculated with anaerobic digester sludge from NEWPCC; Reactor III was inoculated with about 100 mL of river sediment from the Red River in Winnipeg. A mixture of approximately 10 - 15% methane from a natural gas tap in the laboratory and 85 - 90% air was continuously fed from a diffuser at the bottom of each reactor at rate of 12.5 -17.5 $mL \cdot min.^{-1}$ The composition of natural gas in the laboratory tap was 95.5% methane, 2% ethane, 1.93% nitrogen with some trace gases including CO_2. A magnetic stirrer was employed to mix the culture. The reactor operated at room temperature approximately 23°C and in a fill and draw mode. Once a week 1 litre of the culture supernatant was replaced with fresh nutrient medium.

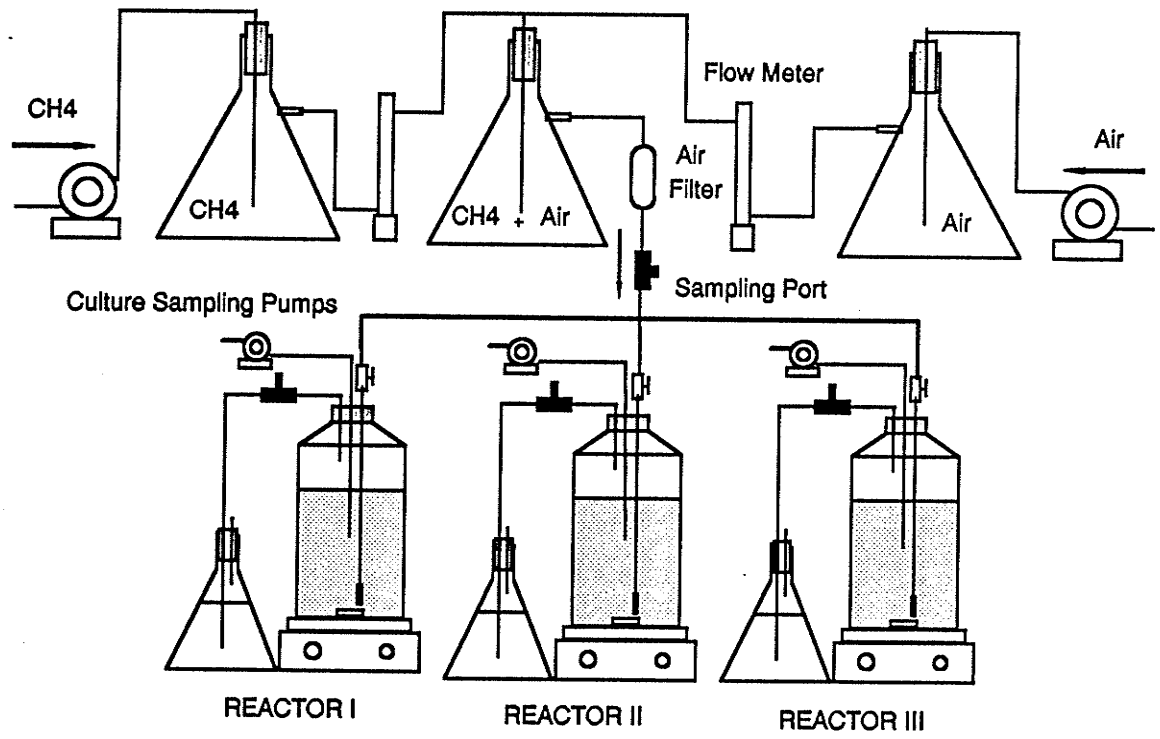

Figure 4.1 Schematic Diagram of the Open Methanotrophic Breeder-Reactor (REACTOR I -- Activated Sludge; REACTOR II -- Anaerobic Digester Sludge; REACTOR III -- River Sediment)

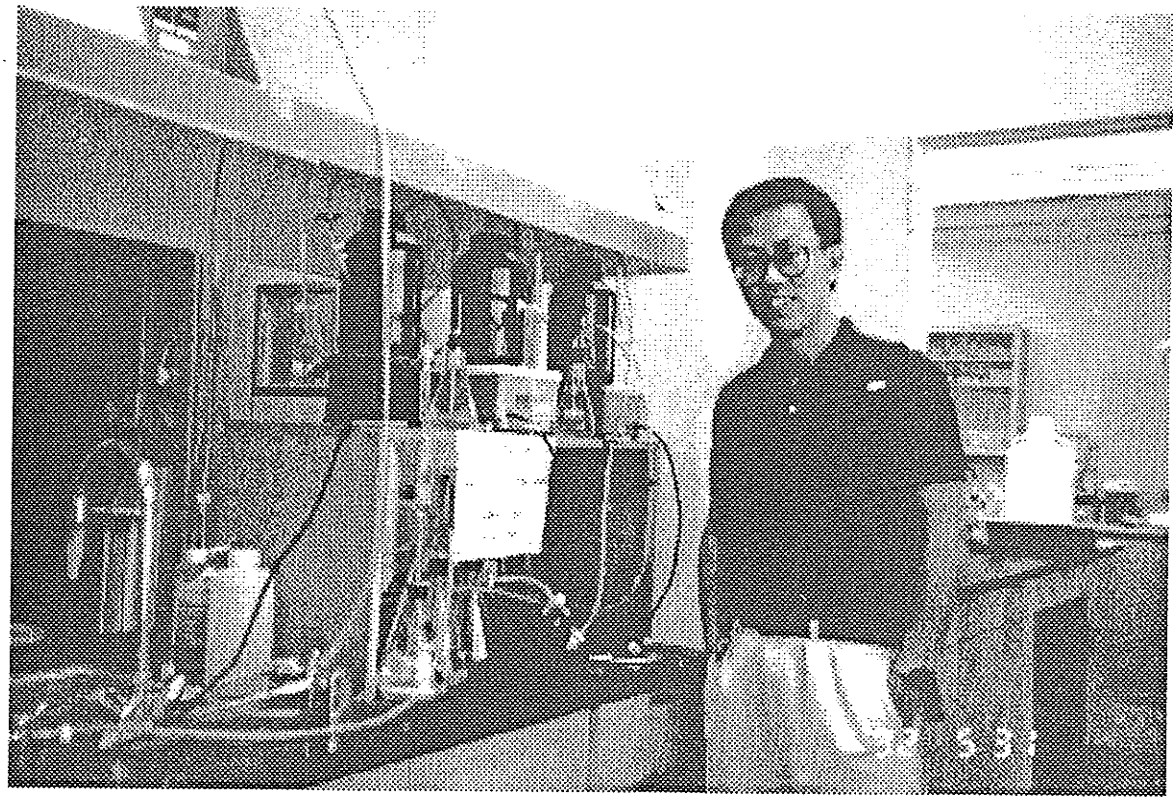

Figure 4.2 Operating Open Methanotrophic Breeder-Reactor

Table 4.1 Composition of Nutrient Medium*

	Name of Chemicals	Concentration ($g \cdot L^{-1}$)
Basic:		
	$MgSO_4.7H_2O$	1.0
	KNO_3	1.0
	$CaCl_3.2H_2O$	0.20
	KH_2PO_4	0.26
	$Na_2HPO_4.7H_2O$	0.70
Mineral Salts:		
	$FeCl_3.4H_2O$	0.0018
	$CoCl_2.6H_2O$	0.0020
	$MnSO_2.H_2O$	0.0009
	$ZnCl_2$	0.0010
	$CaCl_2.2H_2O$	0.0005
	$Na_2MoO_4.2H_2O$	0.0005
	$NiCl_2.6H_2O$	0.0012

* modified from Wittenburg, et al. 1970

4.2 Closed Methanotrophic Incubator-Reactor

A 2.5 L closed dark glass reactor (Fig.4.3 & Fig.4.4) was filled with 1.5 L medium (Table 4.1) and inoculated with 75 mg (as volatile solids) of mixed methanotrophic culture from the open methanotrophic reactor (Fig.4.1). The gas with a known composition of methane (approximately 20% and air (80%) from a 53.6 L tank was continuously cycled between the reactor and the gas tank at rate of 12.5 - 17.5 $mL \cdot min.^{-1}$. A magnetic stirrer was also employed to mix the culture. Gas sampling ports were positioned on the gas collection tank and on the gas lines coming in and out of the reactor. Once the pressure inside the tank

decreased due to consumption by the culture, a prepared gas mixture, containing 20% of CH_4 and 80% of air was fed, using 100 mL syringe through one of the gas sampling ports to keep the air pressure the same inside and outside of the system. A cycling air pump on the gas collection tank was turned on periodically (every 10 minutes for 10 minutes) to mix gases with different density. When methane content fell below 15%, all aeration was stopped, the culture was allowed to settle and 500 mL of supernatant was withdrawn and replaced with the fresh nutrient medium. The gas in the gas tank was also changed to the initial proportion of 20% of CH_4, 80% of air. The experiment was conducted at room temperature.

4.3 Facilities used for TCE Degradation in Water Phase

28 mL culture tubes were filled with 18 mL of culture from open reactors without addition of CH_4. The tubes were capped using teflon faced butyl rubber stoppers and aluminum crimp seals and were covered tightly with aluminum foil to avoid photo-degradation. A control fed with deionized water was incubated alongside. TCE (Fisher Scientific, 99.99%) saturated water of exactly specified quantity was injected below water level to reach the desired concentration. The culture with TCE was mixed by rotating test tubes at about 20 rpm mounted on a rotating shaft stirrer (Fig.4.5). The whole test tube was then centrifuged at 4000 rpm in IEC HN-S centrifuge for 10 minutes before collecting the sample from the supernatant.

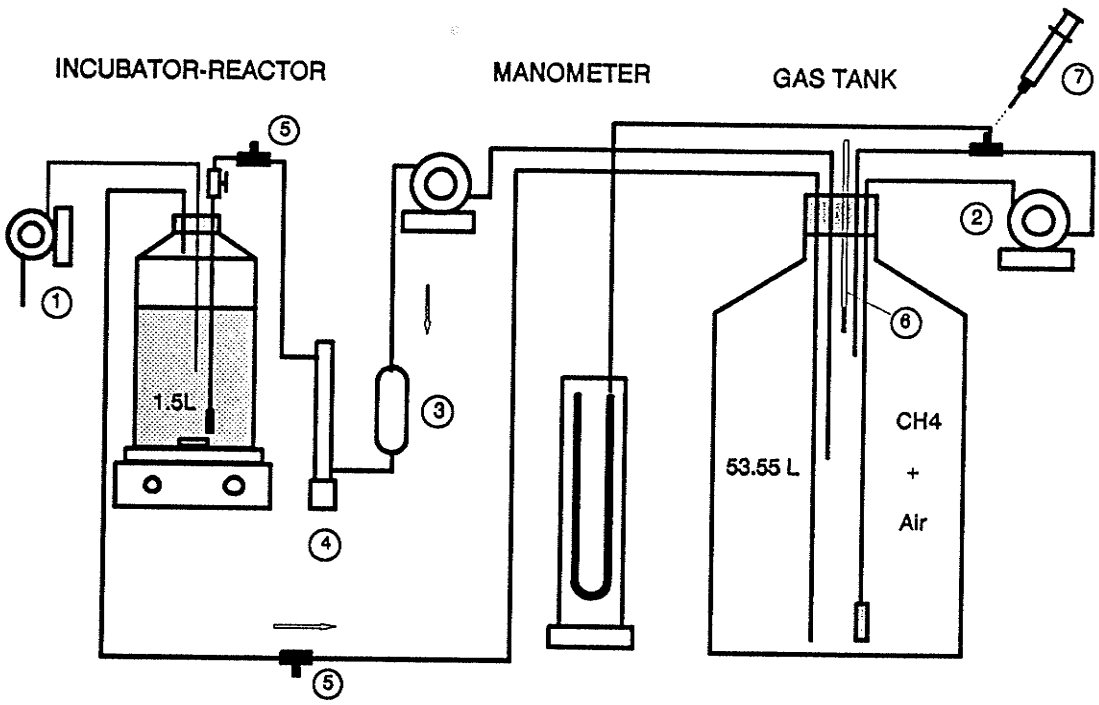

Figure 4.3 Schematic Diagram of Closed Methanotrophic Incubator-Reactor (1) Culture Sampling; (2) Gas Recycling Pump; (3) Air Filter; (4) Flow Meter; (5) Air Sampling; (6) Thermometer; (7) 100 mL Syringe.

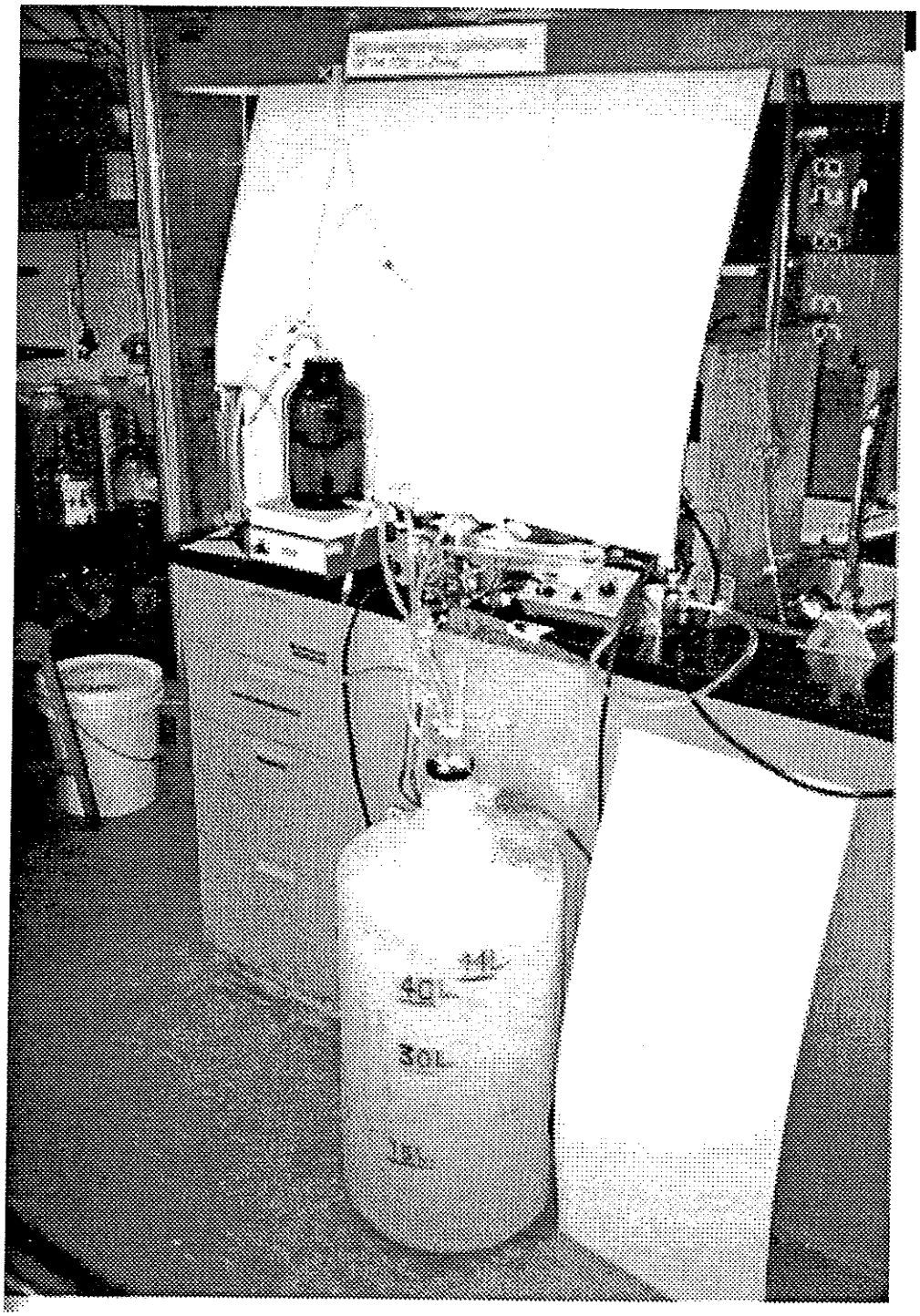

Figure 4.4 Operating Closed Methanotrophic Incubator-Reactor

4.4 Facilities used for TCE Degradation in Slurry Reactors

Degradation of TCE in soil and water slurry was conducted in 28 mL test tubes filled with 10 g samples of dried soil and 14 mL phosphate- buffered (pH = 7) water to prevent pH change. The tubes, in effect slurry reactors, were capped using Teflon-faced butyl rubber stoppers and crimp seals and were covered tightly with aluminium foil to avoid photo-degradation. A control fed with 14 mL deionized water was incubated alongside. TCE (Fisher Scientific, 99.99%) saturated water of exactly specified quantity was injected with below water level to reach the desired concentration. The tubes were then mixed by rotating them at 20 rpm on a rotating shaft stirrer (Fig.4.5) for three days. After TCE level in the water phase stabilized following sorption, 2 mL of thickened methanotrophic culture was injected into each test tubes. The thickening was obtained by centrifuging the original culture from the methanotrophic incubator-reactor at 4500 rpm in IEC HN-S centrifuge for 30 minutes and decanting the supernatant. The procedure yielded approximately 45 mg (dried weight volatile solids -- VS) of methanotrophic culture in each test tube. The whole test tube was then rotated again at about 20 rpm and then centrifuged at 4500 rpm in IEC HN-S centrifuge for 10 minutes before collecting the sample of the supernatant. A 1 μL syringe was used for sampling. To avoid pH change during the experiment, 0.1 M phosphate (pH = 7) buffer at 25°C was used to neutralize soil with various pHs, when conducting the test of TCE degradation from the soils in the test tube experiment.

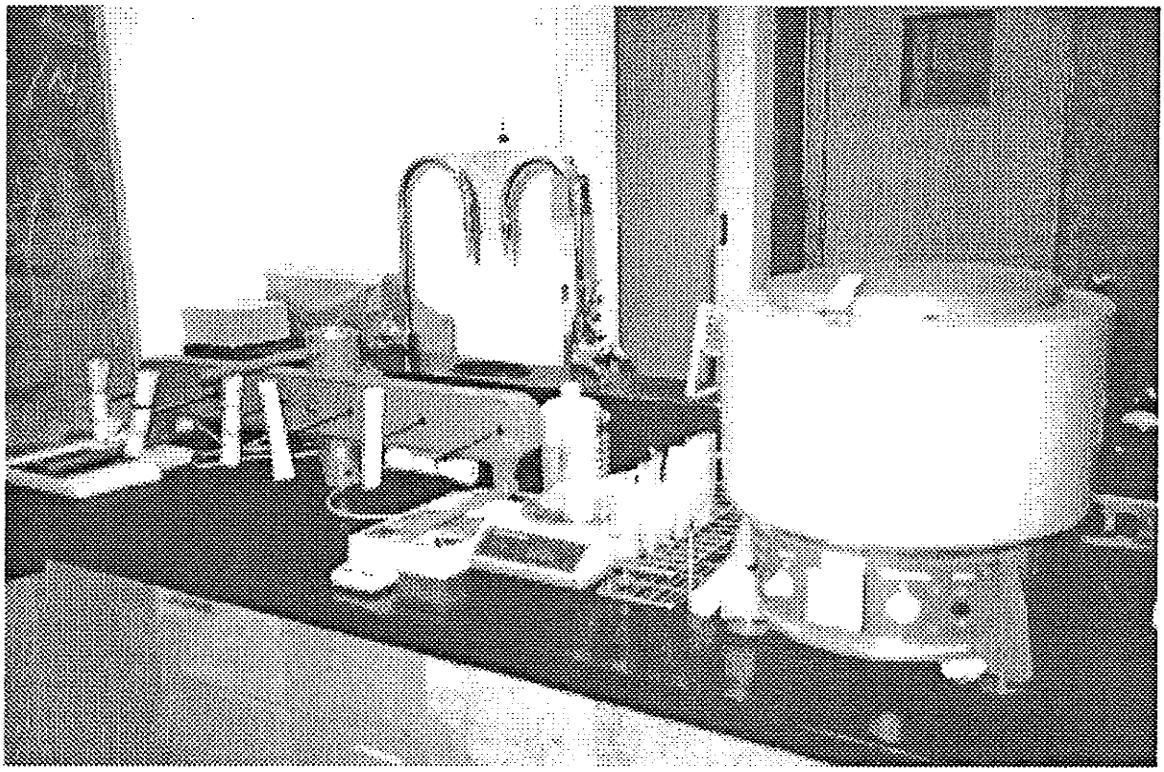

Figure 4.5 Mixing and Centrifuging Equipments

4.5 Equipment of TCE Degradation in Soil Column

Some 440 mL (1200 g) of analyzed dry sandy soil (described in Table 4.2 and Table 4.3) was packed into two dispensing burette (3.75 inner diameter), covered tightly with aluminium foil to avoid photodegradation, to a depth of 40 cm (Fig.4.6 and 4.7). At beginning, tap water was run at speed of average 158 - 162 $mm \cdot d^{-1}$, which resulted in 14.3 - 14.7 $cm \cdot d^{-1}$ of moving rate of water through the column vertically. The flow rates for both column were controlled by a parallel micro tubing pump (model 371, Sage Instruments) drawn the liquid from the bottom. Then water was replaced by water containing a known concentration of TCE. Samples from sampling ports located in influent and effluent were analyzed periodically using direct injection method. After TCE concentration in the effluent was stabilized for few days, feeding for column I was changed with mixed methanothrophic culture screened through 1.2 μL glass fibre filter and column II was fed tap water again. When TCE level for both columns in effluent dropped and stabilized in very low level, known concentrated TCE solution was injected again into two columns. This procedure was repeated several times, while we observed the difference of TCE degradation in the two columns.

4.6 Soil

The soil samples used in the study, originated from an agricultural project

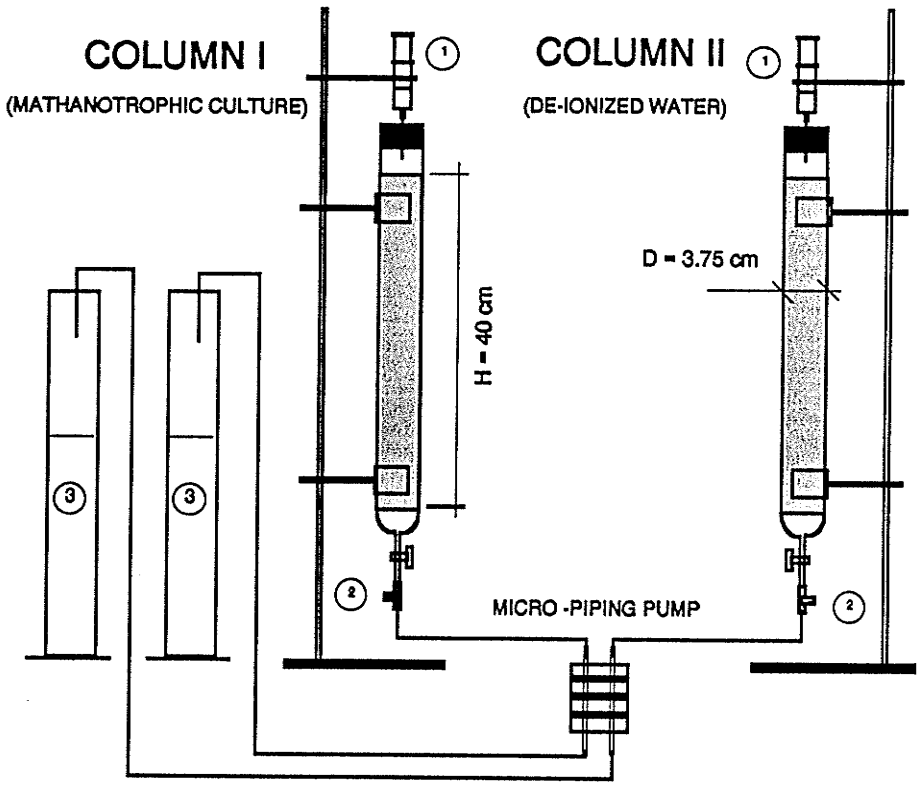

Figure 4.6 Set up of the Column Study (1) Syringes (100 mL). (2) Effluent Sampling Ports. (3) Graduated Cylinders (1000 mL)

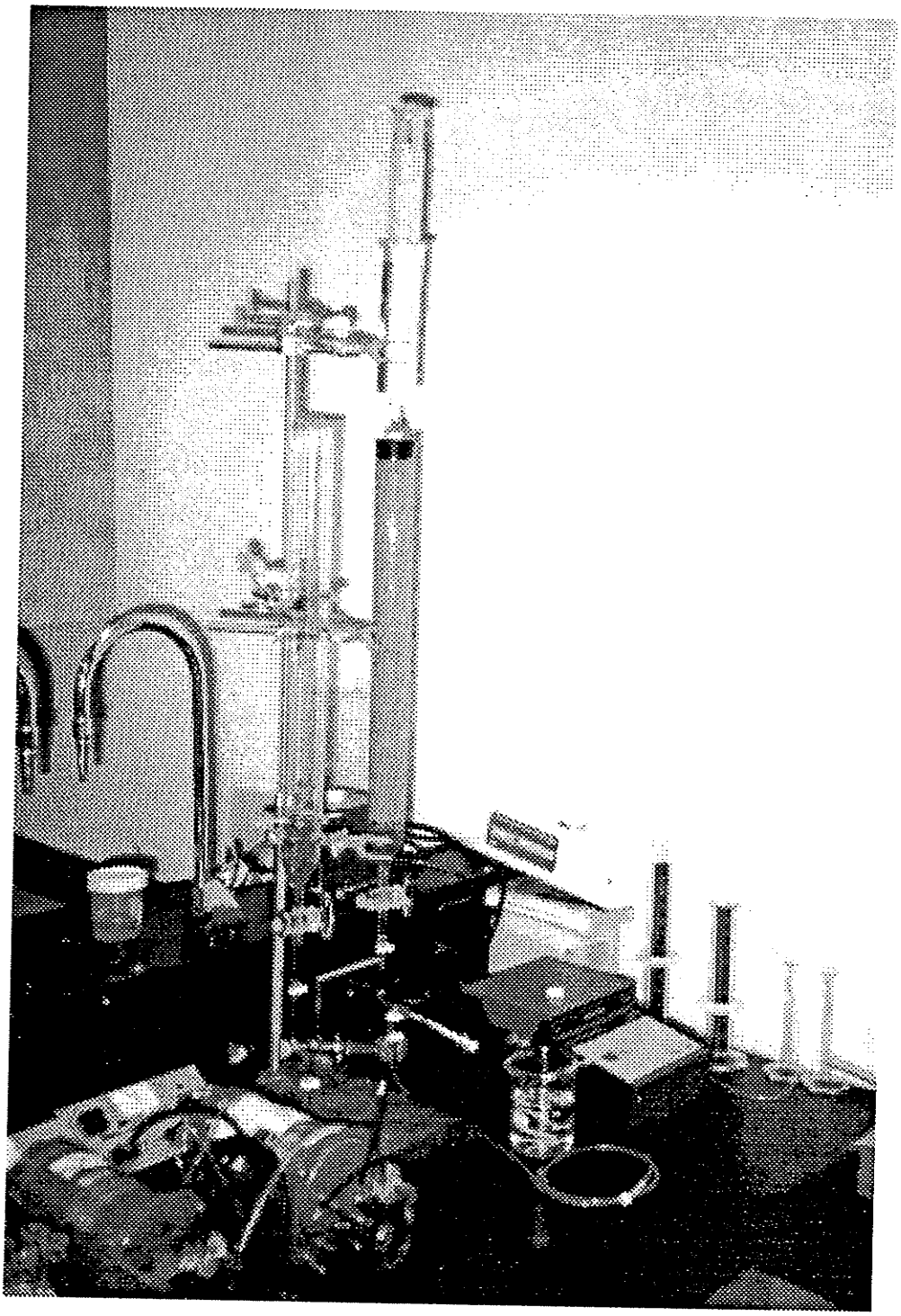

Figure 4.7 Operating Soil Column

in western Ontario, were fully analyzed by Northernwest Labs - an Agricultural Services Complex at Winnipeg, Manitoba. The results are presented in Table 4.2 and Table 4.3.

Table 4.2. Soil Analysis Results: Composition of the Three Soils used in the Slurry Reactors and Column Studies

Experiment	Sample No.	Clay	Silt	Sand					Textural
				Very Coarse	Coarse	Media	Fine	Very Fine	
		%	%	%	%	%	%	%	
I.									
	12	2	8	4	10	26	31	19	Sand
	48	56	37	0	0	4	2	1	Clay
	67	3	48	0	0	1	3	53	Very Fine Sandy Loam
II.									
	4	5	13	5	8	15	23	31	Loamy Sand
	6	2	21	3	8	19	26	21	Loamy Sand
	36	6	12	7	16	19	18	22	Loamy Sand
	21	5	15	3	10	21	25	21	Loamy Sand
III.									
	1	12	24	5	7	14	19	20	Loamy Sand

Table 4.3. Chemical Analysis of Soil used in the Slurry Reactors and Column Studies

Experiment	Sample No.	Organic Carbon	Organic Matter	pH	CEC	Exchangeable Ions (ppm)					Total N (ppm)
						Ca	Mg	Na	K	P	
I.											
	12	0.6	0.35	4.9	3.2	329	56	57	27	2.9	0.05
	48	0.6	0.35	6.3	26.7	3900	997	84	215	2	0.06
	67	0.6	0.35	5.6	3.7	100	23	25	18	2	0.04
II.											
	4	0.8	0.46	5.5	3.8	158	43	32	30	2.2	0.06
	6	1.3	0.75	5.2	5.3	128	31	44	30	2	0.06
	36	2.0	1.16	4.6	7.0	185	54	43	40	5.5	0.06
	21	5.6	3.25	4.5	21.7	103	34	53	45	3	0.19
III.											
	1	0.5	0.29	5.8	7.4	605	408	56	42	3	0.05

*Analyzed by Northernwest Laboratory.

4.7 Conditions for Study of the pH Effects

The pH experiment was conducted by adjusting the pH using 0.2 M of sodium phosphate buffer at 25°C. The pH range tested was from 5.8 to 8.0. The 44 mg (as volatile solids) of centrifuged methanotrophic culture was transferred into each test tube already set at the predetermined pH value.

4.8 pH Buffer for Slurry Reactors

To avoid pH change during the experiment, 0.1 M phosphate (pH = 7) buffer at 25°C was used to neutralize soil with various pHs, when conducting the test of TCE degradation from the soils in the test tube experiment.

4.9 Analyses

A thermal conductivity gas chromatography Gow MAC Model 550 was used to measure the concentration of CH_4, CO_2 and O_2 in the gas. The carrier gas, helium was allowed to flow through the stainless steel 1.8 × 6 mm columns, packed with PoropakQ, 80/100 mesh at flow rate 60 $mL \cdot min^{-1}$, which was regulated at 30 psi. The temperature of the column, detector and injector were 55°C, 110°C and 95°C, respectively. A one mL pressure loc gas-tight sampling

syringe was used for sampling.

TCE concentration was determined from direct injection of 1 μL of centrifuged supernatant into a Hewlett Packard 5890A gas chromatography equipped with an F.I.D. detector and connected to a Waters integrator. The separation was achieved at 45°C using a DB-5 column, 15 m × 0.53 mm I.D.× 1.5 μm film thickness. Injector and detector temperatures were both 200°C. Concentrations were measured with a Waters 740 Data Module, calibrated with appropriate standards.

Total and volatile solids were determined using Standard Methods (APHA, AWWA, WEF 1992).

5. RESULTS AND DISCUSSIONS

5.1 Development of Methanotrophic Culture in Open Breeder- Incubators from Three Inocula

Viable mixed methanotrophic cultures were developed from all three inocula, i.e. activated sludge, anaerobic digesting sludge and river sediment by feeding 10-15% natural gas and 85-90% air and changing the nutrient medium once per week. The total solids and volatile solids content of the three cultures stabilized for approximately a month in day 118 are shown in Table 5.1 (refer to Appendix A). The colour of cultures incubated in dark glass containers turned to orange/pink. After completion of over three months of incubation, all the cultures from different inocula were characterized by comparing their capacity to degrade TCE (Fig.5.1, refer to Appendix B). The culture incubated from the anaerobic digester sludge with the least dry weight of biomass expressed the best specific degradation activity at 14.6 $mg(TCE)\cdot g(VS)^{-1}\cdot d^{-1}$. The 32.74 $mg\cdot L^{-1}$ TCE introduced in 20 mL test tube were totally degraded within 24 hours. The culture developed from the Red River sediment exhibited the lowest rate and biodegradation activity at 0.82 $mg(TCE)\cdot g(VS)^{-1}\cdot d^{-1}$.

Figure 5.2 (refer to Appendix B) shows the difference of TCE degradation capability of fresh un-acclimated activated sludge, mixed methanotrophic biomass

developed from activated sludge and methanotrophic biomass inhibited by sodium azide (added at a dose of 2 $g \cdot L^{-1}$). Un-acclimated activated sludge and inhibited methanotrophs did not degrade TCE. It is interesting to note that there is virtually no sorption of TCE on the surface of the activated sludge and inhibited biomass floc. This indicates that there is no abiotic removal of TCE.

Table 5.1 Change in the Course of Incubation

	Total Solids [$g \cdot L^{-1}$]	Volatile Solids [$g \cdot L^{-1}$]	TCE Degradability [$mg(TCE) \cdot g(VS)^{-1} \cdot d^{-1}$]
Activated Sludge			
Day 0	4.70	3.66	
Day118	3.16	2.80	11.2
Digested Sludge			
Day 0	10.47	2.80	
Day 118	3.84	2.24	14.6
River Sediment			
Day 0	88.56*	4.44	
Day 118	4.16	3.34	8.2

Notes:

1. * Original Sample contained sand and clay
2. The initial TCE concentration in test tubes was 32.7 $mg \cdot L^{-1}$.

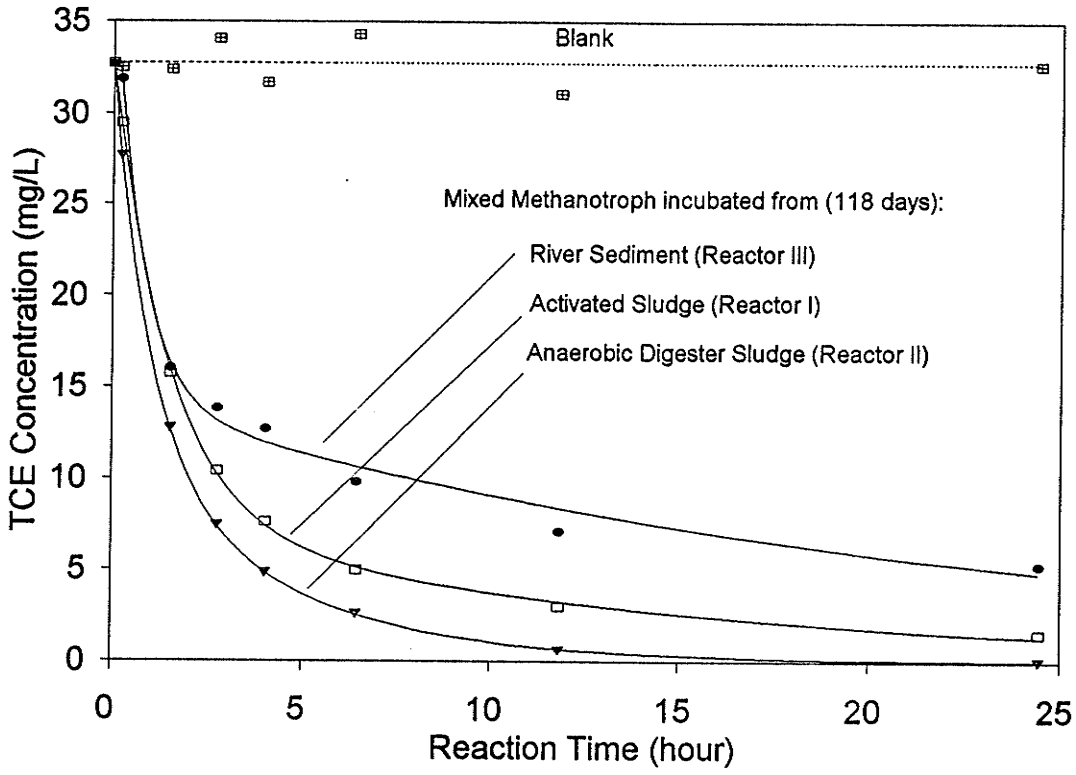

Figure 5.1 Degradation of TCE by Methanotrophs Developed from Different Sources

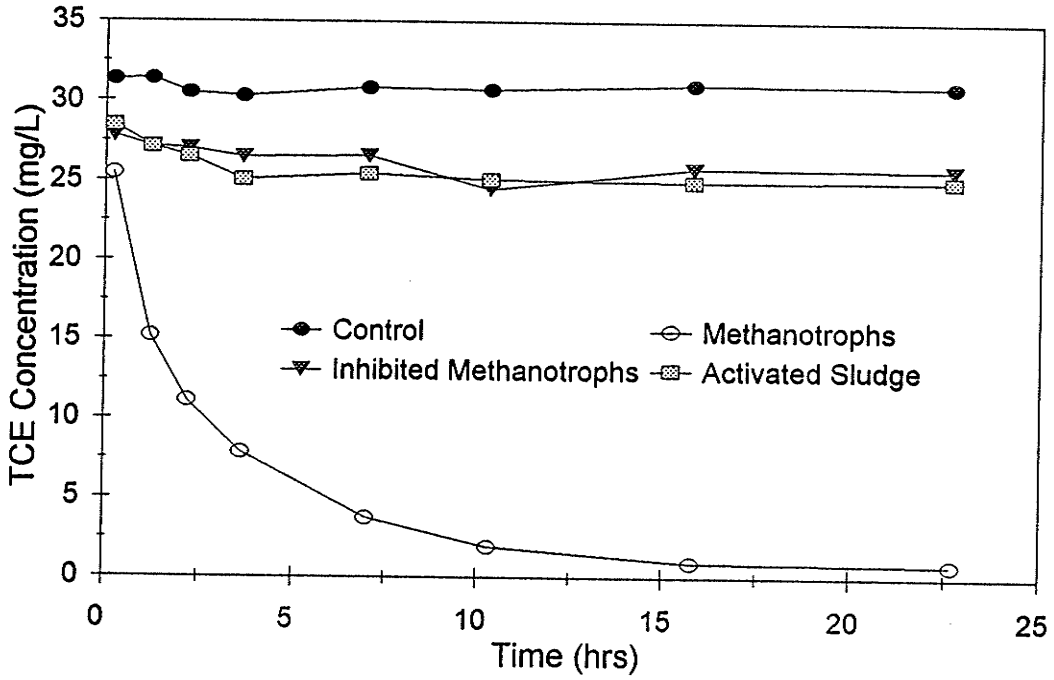

Figure 5.2 TCE Degradation by Methanotrophs, Inhibited Methanotrophs and Activated Sludge

5.2 Mixed Methanotrophic Culture Grown in Closed Incubator Reactor

So far only few studies have dealt with TCE present at high concentrations (Broholm et al 1990, Alvarez-Cohen & McCarty 1991). TCE removal efficiency attained in this study was comparatively high, the probable reasons may include: 1) high aeration rate of the methanotrophic culture with methane and air provided better contact of bacteria with methane - the sole carbon and energy source; 2) there was no competition between TCE and methane, because no methane was fed during TCE degradation in the test tubes. Former studies (Broholm et al. 1990) showed that increasing methane feed rate and concentration in the culture resulted in a decreased TCE degradation. In order to avoid the competition, some researchers (Alvarez-Cohen et al, 1991, McFarland et al 1992) designed a two-reactors system, consisting of a methanotrophic growth unit and a TCE degradation unit. Biomass, which was used for TCE degradation without addition of methane, was reactivated in the methanotrophic growth unit.

This study was aimed at finding an economical method of incubating mixed methanotrophic culture at a bioremediation plant site. TCE degradation would be affected by adding the biomass to groundwater at the polluted site by any feasible method, determined by soil column studies or by addition to the excavated polluted soil treatment system, either in a solid phase mode of a disposal cell (analogous to compost cells) or a slurry reactor (analogous to the soil wash system).

To investigate the feasibility of efficient biomass production, a closed methanotrophic reactor was constructed (Fig.4.3 & Fig.4.4). Mixed methanotrophic biomass (75 mg as volatile solids) from one of the open methanotrophic breeder-reactors was introduced to the medium aerated with methane and air. Since the reactor headspace was closed, methane and oxygen consumption and carbon dioxide production were quantified when growing the methanotrophic population. The experiment, extended for 97 days, was divided into four phases. When methane content fell below 15%, all aeration was stopped, the culture was allowed to settle and 500 mL of supernatant was withdrawn and replaced with the fresh nutrient medium. Figure 5.3 & Figure 5.4 illustrate determination the methane consumption by the methanotrophic culture (oxygen consumption and carbon dioxide production shown in Fig. 5.5, Fig. 5.6, Fig.5.7 and Fig. 5.8. All results please refer to Appendix C). The difference between the methane fed and concentration of methane measured in the gas tank indicate the consumption of methane by mixed methanotrophic population in the reactor. Increase of methane and oxygen consumption, and of carbon dioxide production was observed once biomass growth was initiated (Fig. 5.9). The ratios of methane consumption, oxygen consumption, and carbon dioxide production versus biomass production expressed in grams dry-weight of volatile solids were found to be 42.8 $g(O_2)\cdot[g(biomass)]^{-1}$, 21.5 $g(CH_4)\cdot[g(biomass)]^{-1}$ and 6.5 $g(CO_2)\cdot[g(biomass)]^{-1}$, respectively. Mixed methanotrophic bacteria formed a slow-growing culture in comparison to other aerobic bacterial masses, such as, mixed population of

activated sludge, which may exhibit rates in the order of $0.1d^{-1}$ and higher. The growth rate of the methanotrophs in this system was approximately 18.2 $mg \cdot g^{-1} \cdot d^{-1}$ at 23°C, or 0.018 d^{-1}.

One can speculate that a methanotrophic incubator-reactor built at a wastewater treatment plant site using anaerobic sludge digestion would be the most economical because of the free methane source and the source inoculum (digesting sludge).

It was also observed that the pH in the incubator varied from about 7.5 to 6.7 during each batch incubation period after and before each exchange of the gases in the head space (Fig. 5.10). This was caused by increased concentration of carbonic acid resulting from carbon dioxide dissolution in liquid. A carbon dioxide stripper or a trap (e.g. KOH solution) can be inserted in the gas line between incubator and gas storage tank to offset the potential negative effect of the pH drop.

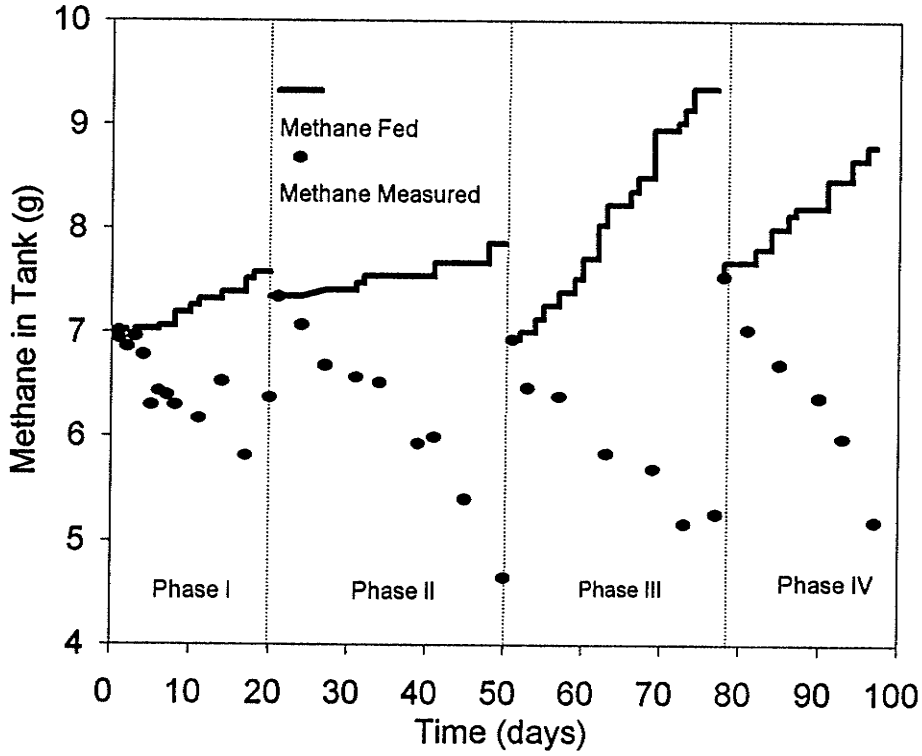

Figure 5.3 Methane Consumption in Closed Incubator-Reactor

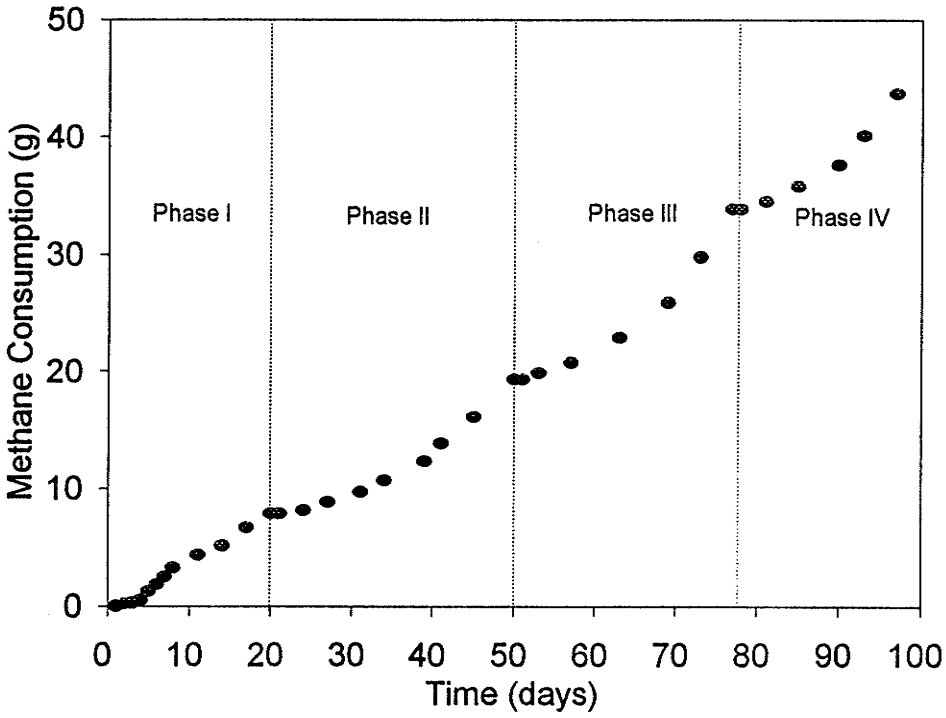

Figure 5.4 Methane Consumption Accumulation in Closed Incubator-Reactor

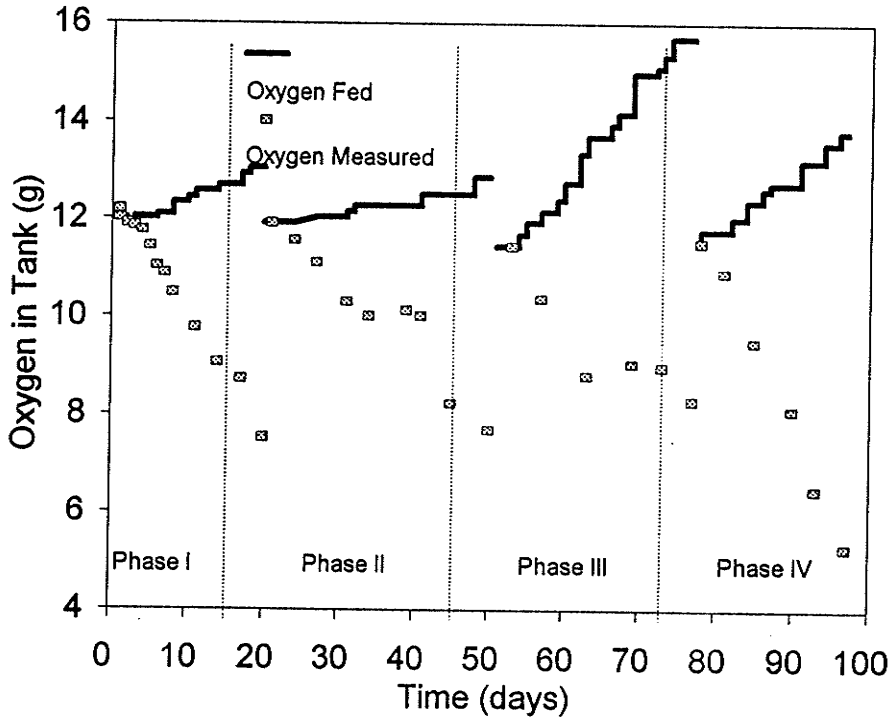

Figure 5.5 Oxygen Consumption in Closed Incubator Reactor

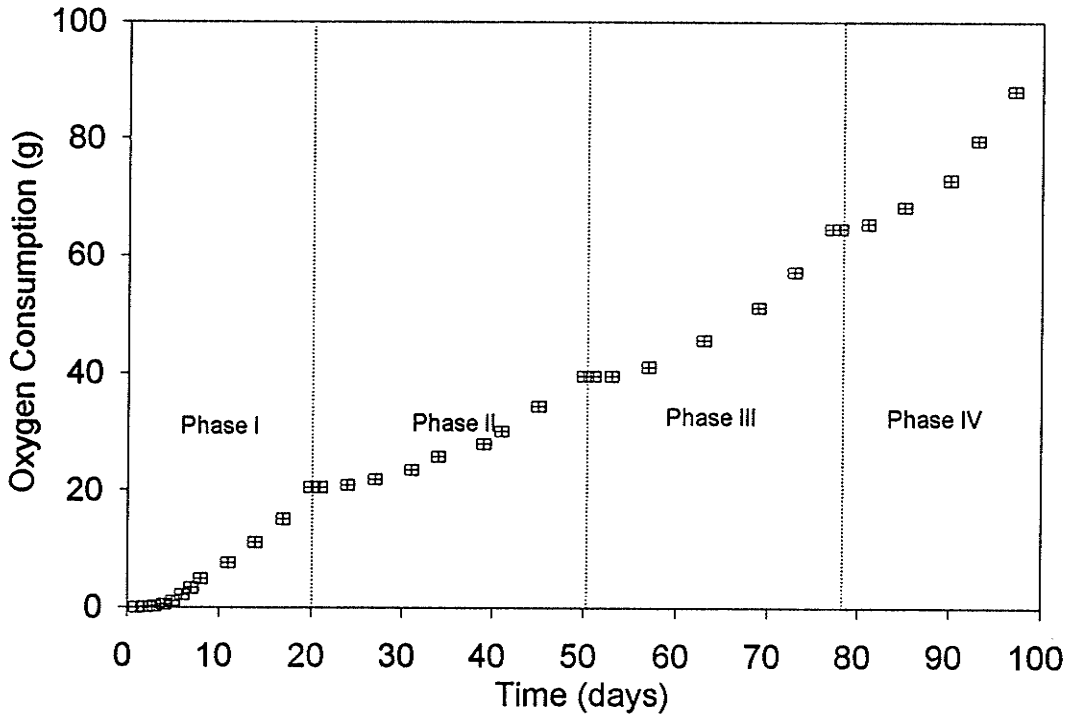

Figure 5.6 Oxygen Consumption Accumalation in Closed Incubator-Reactor

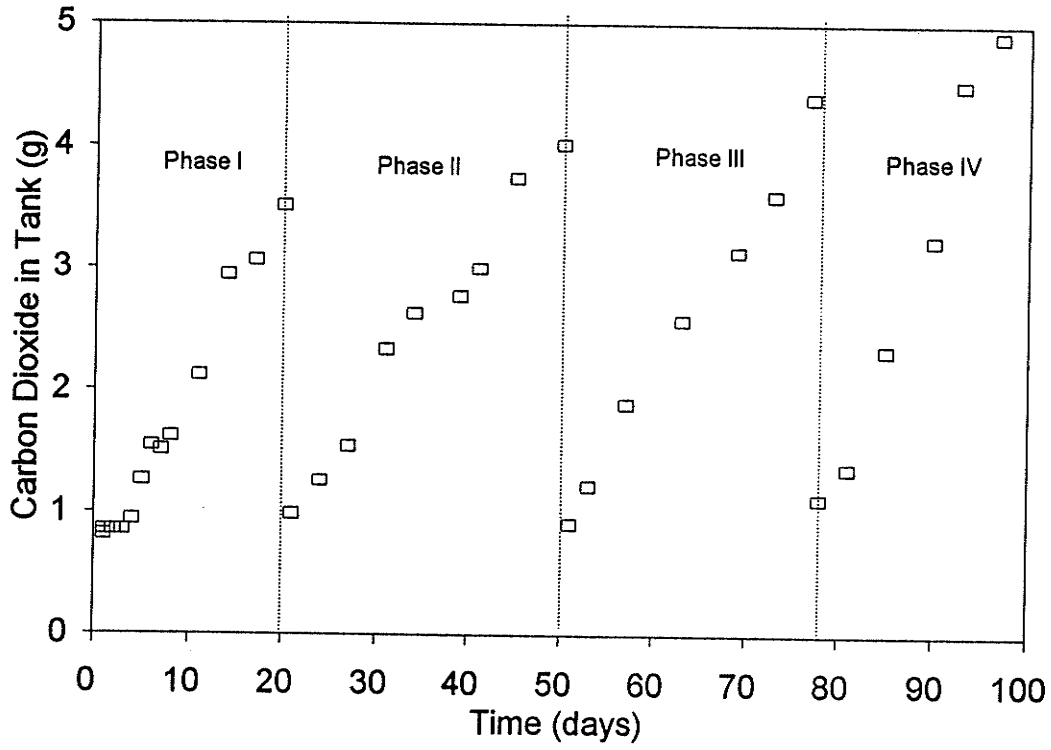

Figure 5.7 Carbon Dioxide Production in Closed Incubator-Reactor

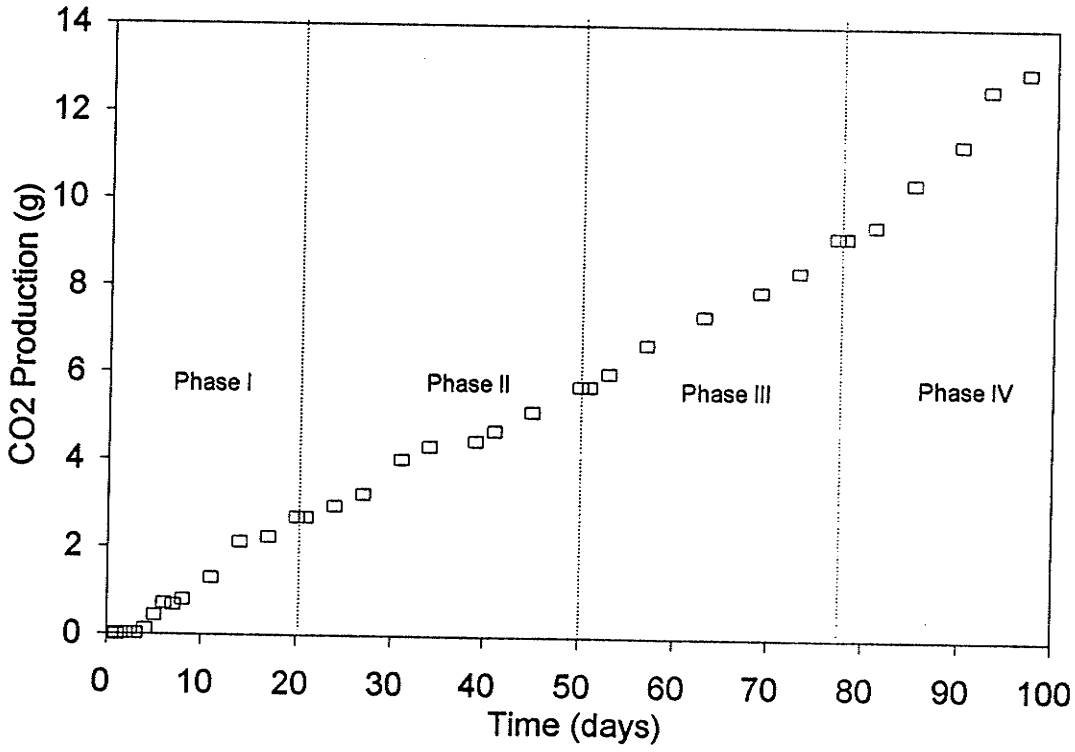

Figure 5.8 Carbon Dioxide Production Accumulation in

Closed Incubator-Reacotor

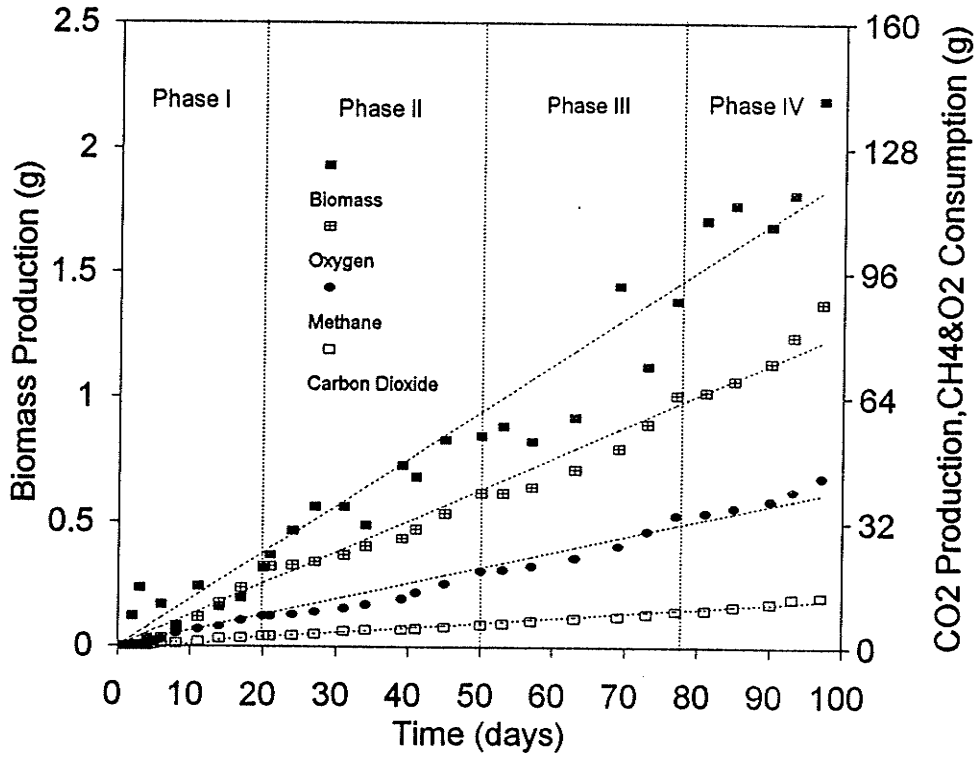

Figure 5.9 Biomass and Gases Observation in Closed Incubator-Reactor

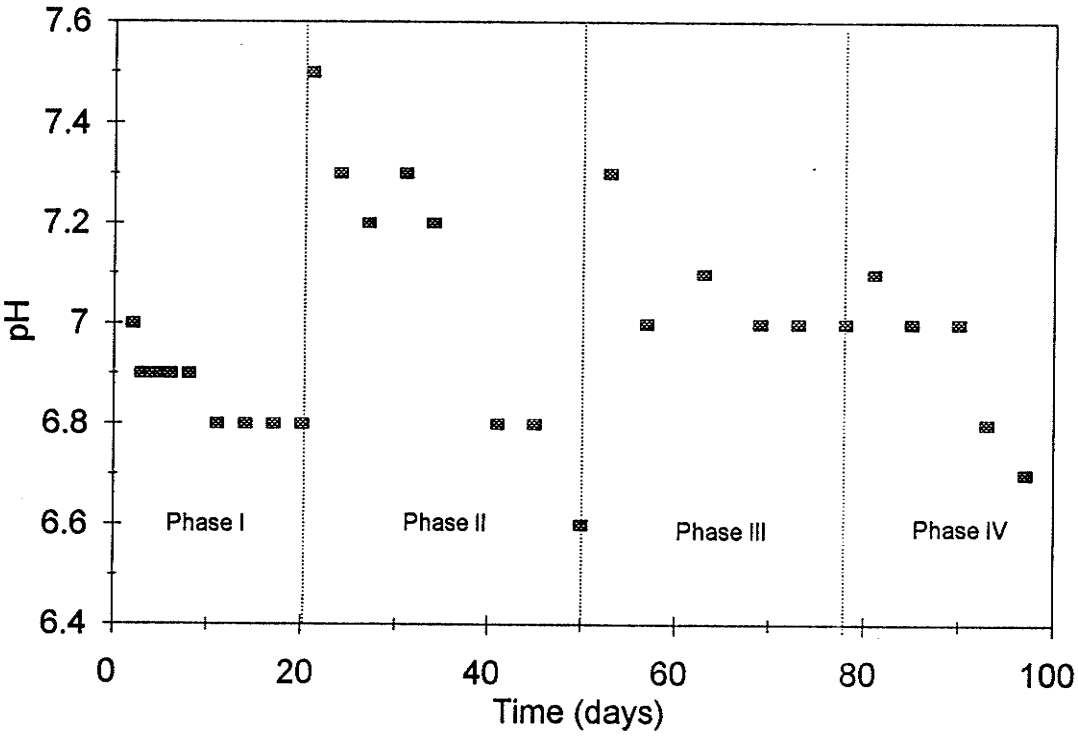

Figure 5.10 pH Change in Clsoed Incubator-Reactor

5.3 Factors Affecting Biodegradation of TCE

A known amount of 66 mg (as volatile solids) of methanotrophic culture was taken from the closed-headspace reactor and distributed into test tubes belonging to three different groups (two test tubes for each group): the first group was placed in a 23°C low temperature incubator (model 307, Fisher Scientific), the second was placed in a refrigerator set at 8°C, and the third group was placed in a walk-in cold chamber set at 4°C. The culture with TCE was mixed by rotating test tubes at about 20 rpm. The efficiency of TCE degradation decreased with the decrease in temperature (Fig.5.11, refer to Appendix D). The differences in rates of TCE degradation at the three temperatures became obvious within the 24 hours. As shown in Table 5-2, the removal rates of 8.6, 6.0 and 4.2 $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$, the thresholds of TCE degradation of 1.2 $mg\cdot L^{-1}$, 11 $mg\cdot L^{-1}$ and 15 $mg\cdot L^{-1}$, based on 24 hours data, were obtained for the cultures placed in 23°C, 8°C and 4°C, respectively. Increasing temperature is one general way in which the rate of chemical reaction can be increased, because it increases the thermal motion of the molecules and thus increases the fraction having sufficient internal energy to enter the transition state. Usually the rate of reaction is about doubled by a 10°C rise in temperature (Lehninger, 1987).

After 24 hours, the two cultures maintained at the lower temperatures were transferred to the 23°C chamber. After raising the temperature to room

Table 5-2. Temperature Affecting Biodegradation

Temperature	23°C	8°C	4°C
Removal Rate (24 hrs) $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$	8.6	6.0	4.2
Threshold of TCE Degradation ($mg\cdot L^{-1}$)	1.2	11	15

temperature, the efficiency of TCE degradation increased to the level comparable to the 23°C chamber within 24 hours. The threshold of TCE degradation at each temperature were obtained and estimated, based on the data in the experiment. It was observed that higher thresholds of TCE degradation occurred the cultures placed in lower temperature. It can be speculated that the enzyme (MMO) can not recognize substrate at lower temperature.

This study illustrates that temperature is an important factor, when using off-site developed methanotrophic culture removing TCE. Raising the temperature before contacting culture with TCE may be economically justified if the rate of TCE oxidation is critical. The effects of temperature decrease were fully reversible. It would be interesting to explore in future research wether biomass incubated at e.g. 9°C and biomass brought down to 9°C from 23°C, have the same viability.

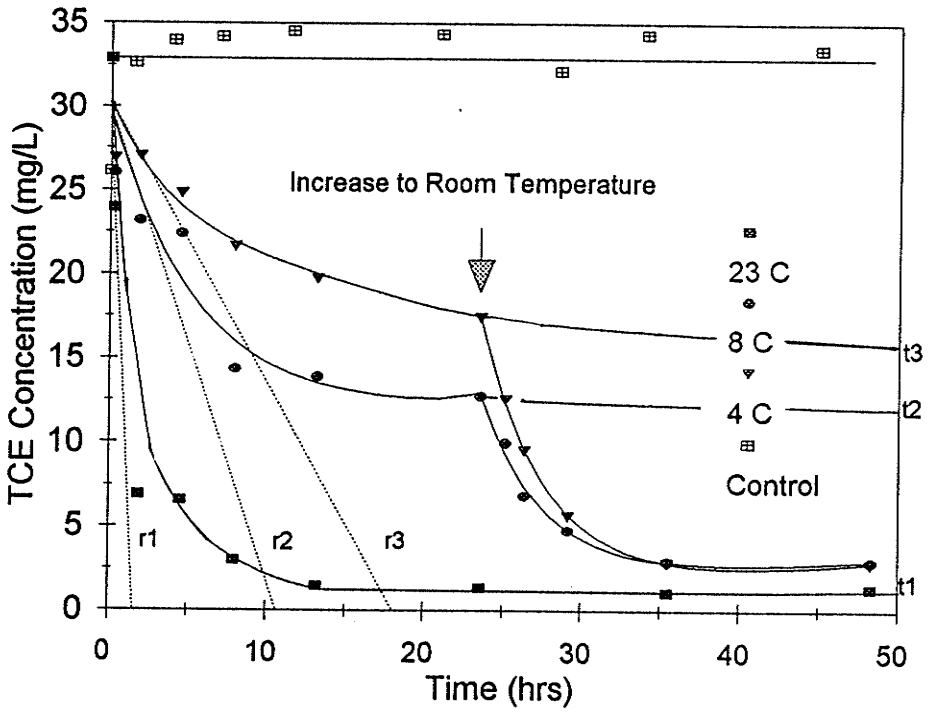

Figure 5.11 Effect of Temperature on the Degradation of TCE

(r_1, r_2 and r_3 represent initial rates of TCE degradation, t_1, t_2 and t_3 represent thresholds of TCE degradation)

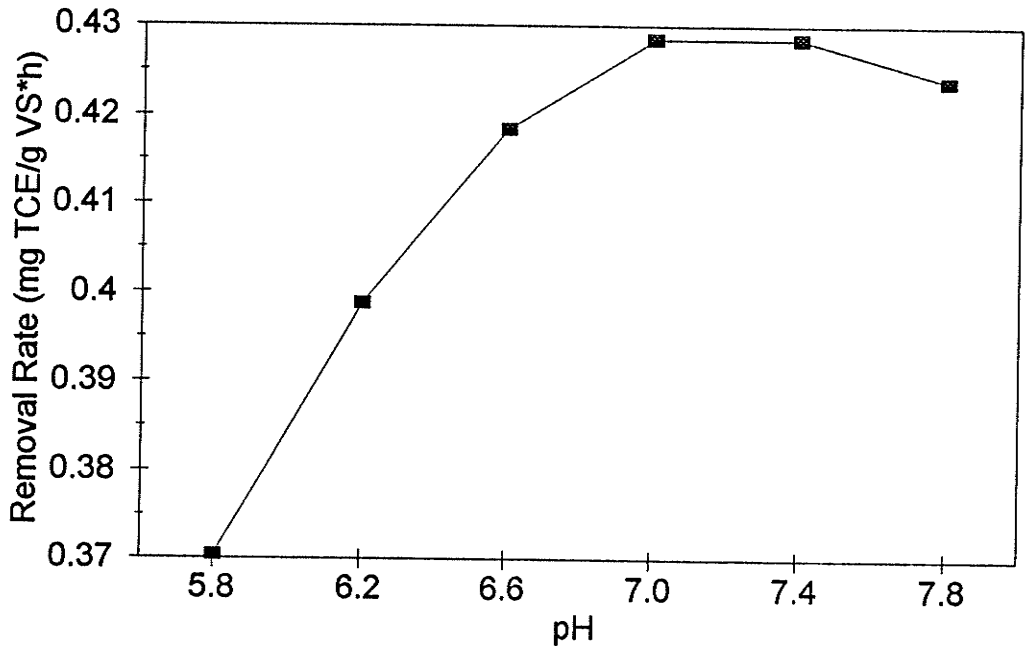

Figure 5.12 Effect of pH on the Degradation of TCE

The effect of various pH values on the TCE degradation potential is shown in Fig.5.12 (refer to Appendix D). The methanotrophs have shown optimum performance at pH range from 7.0 to 7.4 resulting in 100% removal of 24.5 $mg \cdot L^{-1}$ of TCE. Lower pH (below 7.0) and higher pH (above 8) inhibited TCE degradation.

5.4 Degradation of TCE in Soil Slurry Reactors

Excavated soil containing high TCE concentrations can be treated in soil slurry reactors. The test tube slurry reactor experiment was conducted by mixing pre-analyzed soils with the ready-made methanotrophic culture. Three types of soil were chosen with similar carbon contents but different fraction size, eg. clay, silt and very fine sand (Exp.I, described in Table 4.2). 14 mL of phosphate buffer was mixed with soil in each test tube to maintain the pH at 7. The tubes with a known amount of TCE were equilibrated by rotating them at about 20 rpm on a rotating shaft stirrer for three days. A pellet of centrifuged mixed methanotrophic cultures (45 mg as volatile solids) was injected into each test tube which were then rotated continuously except for periods of sample collection.

It was found that the biomass injected into tubes with different soils had retained its capability to degrade TCE, however the extent of degradation was not comparable. TCE removal rates were lower in the soil slurry and water environment when compared to removal in water only phase (Fig.5.13, refer to

Appendix E). Higher TCE removal rate was observed in the test tube containing clay rather than those containing sand and very fine sandy loam. The reason might be that clay provided large contact surface to allow adsorbed TCE to get in touch with the suspended biomass. It was not possible to conclude here that rough size-fractionated soil had lower reaction rate of TCE degradation, because there were many other factors involved in the case.

In the next experiment, three soils contained different quantity of organic carbon but had similar size fraction (Exp.II, described in Table 4.2). The soil containing higher concentration of organic matter (Fig.5.14, refer to Appendix E) sorbed more TCE than the ones with lower organic carbon, resulting in lower TCE concentration in the supernatant. As in the preceding experiment, the added mixed methanotrophic culture degraded TCE in the soil slurry system to a lesser extent than in tubes without soil, in water phase only. Both test tube experiments illustrated that mixed methanotrophic bacteria incubated in an off-site bio-reactor can be applied to remove TCE when added to excavated polluted soil in slurry-type reactors. The effects of TCE removal from different soils will depend on soil fraction size; organic carbon contents and pH. Conditions in slurry reactors treating excavated soil off-site, could be engineered to optimize the degradation by mixed ready-made methanotrophic cultures incubated independently.

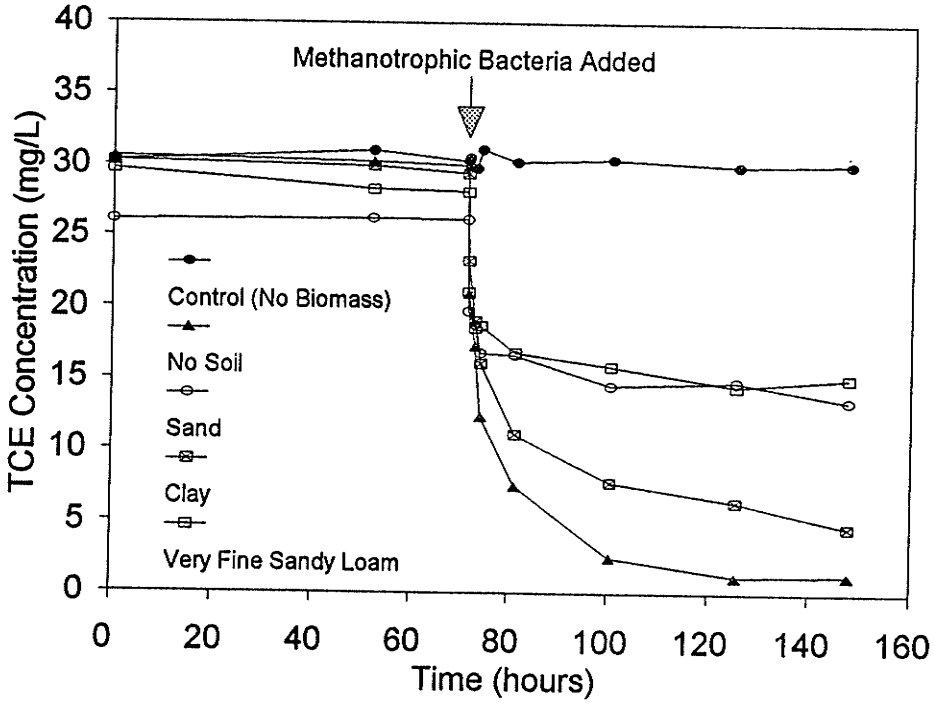

Figure 5.13 Degradation of TCE from Soils (I)

(TCE removal rates were lower in the soil slurry reactor than in water reactor. Higher TCE removal were observed in the test containing clay rather than those containing sand and very fine sandy loam)

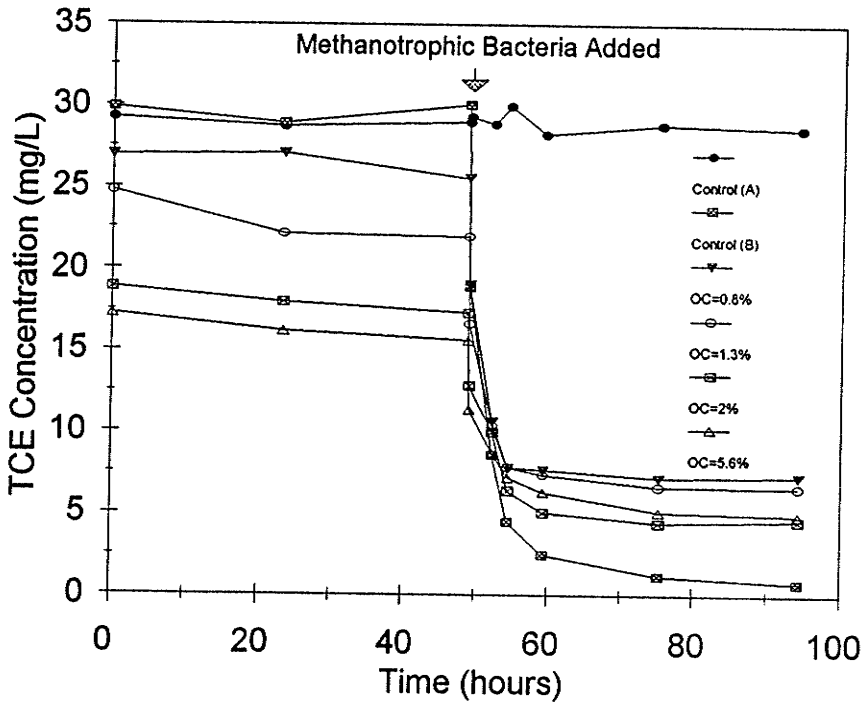

Figure 5.14 Degradation of TCE from Soils (II)

(The soil containing higher concentrations organic matter sorbed more TCE than ones with lower organic carbon. Mixed methanotrophic culture degraded TCE in the soil slurry system to a lesser extent than in water phase only)

5.5 Effects of Size of Biomass on TCE Removal: The Effect of Filtration

Introduction of ready-made bacteria to a polluted soil site is one method of site bioremediation. It was found that the magnitude of sorption on the soil particles and bacterial cell size determined the transport of bacteria through the soil (Alexander et al., 1991). In order to effectively be able to transfer the developed microbial mass to a polluted microcosm, one must find the smallest size of the active biomass cell in the mixed culture that will still remove TCE. Glass fibre filters with the same material and different pore size (Table 5.2) were selected, according to Logan (1993), to screen the biomass from the incubator-reactor, starting with some 4.05 $g \cdot L^{-1}$ of volatile solids.

It was found that the rate of degradation of TCE decreased with the decrease in the size of biomass passed through the filter. Naturally, the amount of biomass filtered through progressively smaller diameter filters decreased. The ratio of VS/TS decreased at the same time. Based on data in Fig. 5.15 (refer to

Table 5.3 Results of Biomass Screening using Glass Fibre Filters

Parameter	Biomass	Diameter of Glass Fibre Filter 2.7μm	1.6μm	1.0μm	0.7μm
Total Solids ($g \cdot L^{-1}$)	5.52	4.74	4.56	4.08	2.46
Volatile Solids ($g \cdot L^{-1}$)	4.05	3.37	3.20	2.73	1.16
VS/TS (%)	73.4	71.1	70.2	66.9	47.15

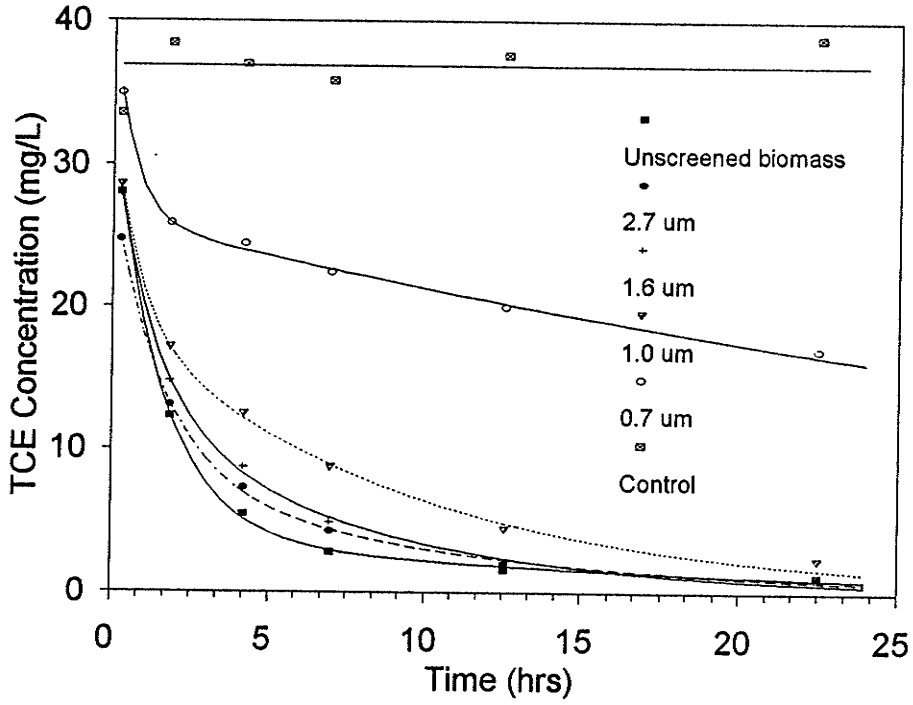

Figure 5.15 Biomass Size Selection

Appendix F), one can conclude that the most effective size of TCE -degrading biomass ranged from 0.75μm to 1.0μm.

5.6 Effects of Biomass Storage Conditions on TCE Biodegradation Ability

The use of ready-made methanotrophic mixed culture produced for addition to a contaminated site or for slurry reactor treatment may require middle-to-long term biomass storage. In order to find the simplest and most economical storage method, storage in aquatic conditions at 20°C, 4°C and -20°C was tested.

The amount of 5.16 mg (as volatile solids) of methanotrophic culture collected from the incubator-reactor was put into each test tube in three different bundles (10 test tubes for each bundle, sub-divided into 5 batches): the first bundle was placed in a 20°C temperature incubator (model 307, Fisher Scientific), the second and third were placed in a walk-in cold chamber set at 4°C and deep freezer (Zenith) at -20°C. TCE degradation experiments were conducted at 1st, 7th, 13th, 20th, 41th and 73th day of storage using the procedure described earlier for liquid samples with soil slurry reactor after all cultures were brought back to the room temperature at an average of 23°C.

The activity of the biomass (Fig.5.16, refer to Appendix G) was defined by measuring the specific rate of TCE removal in $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$. At 20°C storage

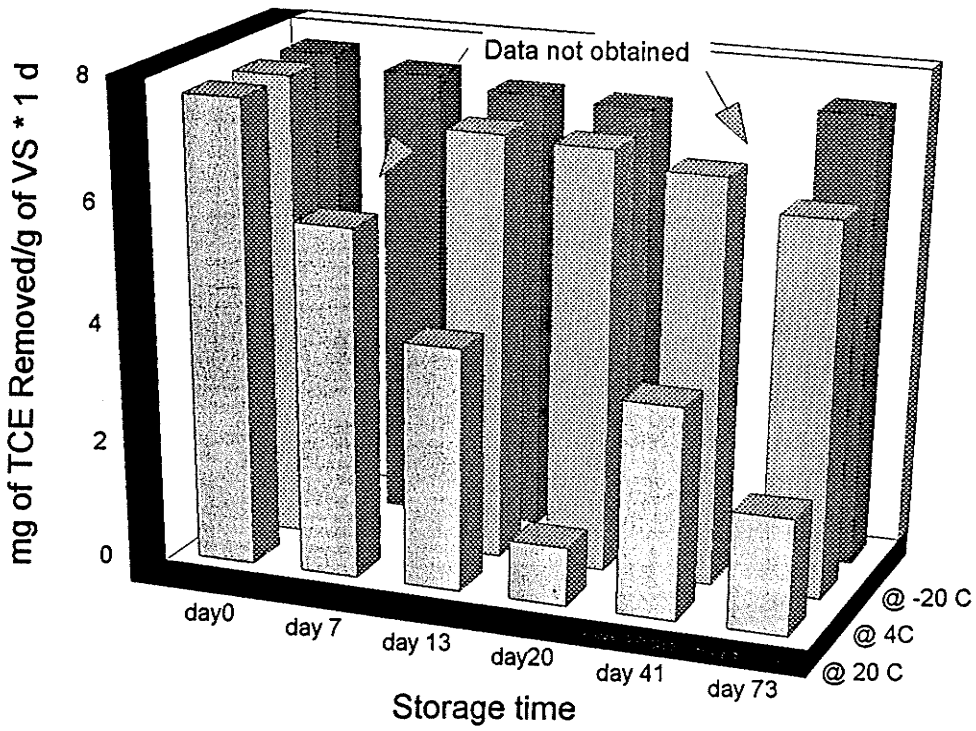

Figure 5.16 Biomass Freezing Storage

, the specific activity of the biomass has dropped very quickly, down to 50% of the original activity in less than two weeks. The decline in the specific activity was much less severe even after prolonged storage at 4°C. Loss in activity was minimal after a prolonged storage at -20°C.

The rapid loss of activity after storage in liquid form (temperature of 4°C and 20°C) could be attributed to lack of substrate available and endogenous respiration processes. One conclusion relevant to bioremediation in situ is that lower groundwater temperature usually (5°C to 10°C) will prolong the survival of the introduced ready-made bacteria.

Unexpectedly, the freezing to -20°C in water phase, without dehydration, allowed the mixed culture to retain virtually undiminished activity of the key degradative enzyme -- methane monooxygenase (MMO). This method of biomass storage is generally available at the lowest cost, using simple inexpensive freezing equipment.

5.7 Column Studies with Screened Culture

Two parallel soil columns (Exp. III, described in Table 4.2) were used to evaluate the effects of TCE removal by screened methanotrophic culture added to the influent water fed to Column I and compared to the reference column II fed

water only. Figure 4.6 and Figure 4.7 illustrates the column setup, operation of column and Figure 5.17 (refer to Appendix H) illustrates both the influent and effluent TCE concentrations before and after feeding a known concentrations of TCE to both columns (TCE feeding period is marked in Fig.5.17 as "Feeding"). Methanotrophs screened through a 1.2 μm glass fibre filter were mixed and continuously fed to column I. Tap water was fed to column II in the remediation period. The influent TCE concentration for two columns was in the range 23 - 33 $mg \cdot L^{-1}$. After several days of feeding TCE, the effluent TCE concentration more or less stabilized at 12 - 18 $mg \cdot L^{-1}$. A probable explanation for this apparent removal of TCE was adsorption to the surface of the soil.

The identical increasing trend of TCE concentrations in the effluent of both columns in the feeding period in phase I illustrated that conditions for both columns were roughly the same. When the TCE effluent concentration stabilized, the feeding to column I was supplemented with a methanotrophic culture, which screened through a D = 1.2 μm glass fibre filter, suspended in tap water; the feeding solution to column II was unadulterated tap water. It was observed that TCE contents in both columns dropped very quickly during the remediation period primarily as a result of simple washout, but when the effluent TCE concentration reached about 5 $mg \cdot L^{-1}$, removal (washout) rate for Column II, fed with tap water, became slower than that in column I. This situation was also observed true in phase II and phase III of the experiment. The trend of TCE increase in column I

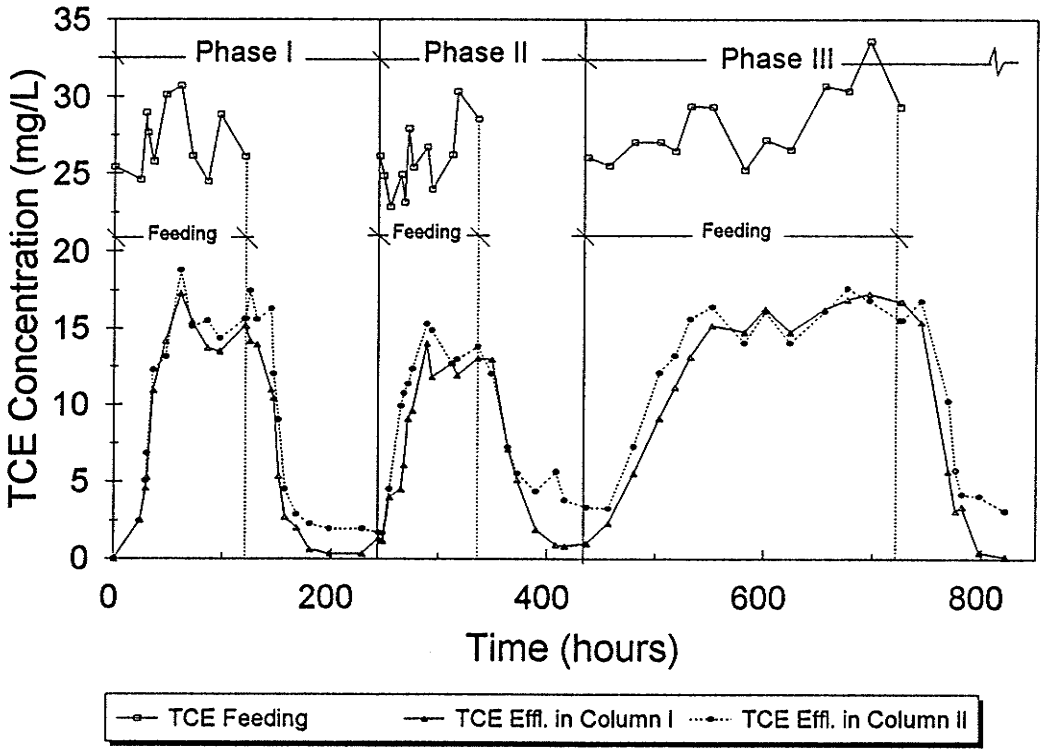

Figure 5.17 TCE Removal from Soil Column

during the TCE feed period was retarded in comparison to that in column II in the subsequent experimental phases II and III.

Higher removal rates and complete removal at the end of the remediation period and retardation of the TCE increase during the active TCE feeding period for column I have confirmed the activities of methanotrophic culture during the operation. The leachate (effluent) in column I became turbid a few days later after changing TCE feeding to screened methanotrophic culture. It was also observed that some of the methanotrophic cultures accumulated at the top of the column, demonstrating that the soil hindered the passage of the culture through the soil.

Table 5.3 shows results of the TCE mass balance calculation for the two columns which revealed that out of approximately 100 mg TCE fed to each column in 120 day, in column I only 53.45 mg TCE, while column II approximately 60.8 mg TCE were detected in the effluent respectively. The amount of TCE actually removed by biodegradation cannot be established. The amount retained in column II due to sorption was almost 40 mg. There will continue to be a significant long-term leaching from that column of TCE in concentration well exceeding the typical "accepted" level of TCE of 20 -50 $\mu g \cdot L^{-1}$. The concentration of TCE in column I dropped to zero at the end of the third phase prolonged remediation period, indicating that the bacteria retained in the soil matrix were actively removing the sorbed TCE. What is more important - there is legitimate expectation that there

will not be any TCE leaching out after some time.

Table 5.4 Mass Balance of TCE for Column I and Column II

	Column I	Column II	Position
TCE Feeding (mg)	99.13	100.60	Influent
TCE Recovery (mg)	53.45	60.77	Effluent

TCE concentrations fed to each column were very close, because the flow rate were controlled by a parallel micro-pump. The difference in TCE recovery (60.77 - 53.45 = 7.32 mg) confirmed biodegradation by the methanotrophic culture added.

Whole unscreened biomass addition to column I was tried after the completion of the screened biomass test described. It was found that the biomass rapidly accumulated on the top of the column packed with loamy sand which hindered and finally stopped leachate from passing through the soil column. The experiment had to be stopped at that moment because of non-equal flow rates in the two columns. It was concluded that the addition of the whole unfiltered culture could be unsuccessful in full scale.

6. CONCLUSIONS

(1). Mixed methanotrophic cultures incubated in open breeder-reactors from different seed materials were all able to degrade high concentrations of trichloroethylene. Maximum degradation rates of 14.6 $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$, 11.2 $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$ and 8.2 $mg(TCE)\cdot[g(VS)]^{-1}\cdot d^{-1}$ were obtained for cultures developed from digester sludge, activated sludge and river sediment, respectively, after three months.

(2). Closed headspace reactor was demonstrated to be a technically and economically feasible method for growing mixed cultures of methanotrophs. The specific oxygen consumption, specific methane consumption ratio and specific carbon dioxide production expressed per unit of biomass production were 42.8 $g(O_2)\cdot[g(biomass)]^{-1}$, 21.5 $g(CH_4)\cdot[g(biomass)]^{-1}$, and 6.5 $g(CO_2)\cdot[g(biomass)]^{-1}$, respectively. The growth rate of the methanotrophs in this system was approximately 18.2 $mg\cdot g^{-1}\cdot d^{-1}$ ($0.018 d^{-1}$) at room temperature. Carbon dioxide produced in the system would have to be removed in order to maintain the pH of the culture near a neutral level which is the optimum for the culture.

(3). The optimum pH for methanotrophs degrading TCE was in range 7.0 to 7.4. Temperature was also found to be a very important factor, regulating the activities of methanotrophic cultures. The lower the temperature, the less active were the

methanotrophs. The rate of TCE removal decreased from 8.6 mg(TCE)·[g(VS)]$^{-1}$·d^{-1} at 23°C to 4.2 mg(TCE)·[g(VS)]$^{-1}$·d^{-1} at 4°C. These two factors should be taken into account when designing and applying the proposed technology of producing TCE-degrading biomass.

(4). Soil slurry reactors with added biomass removed TCE at a rate much slower and to a lesser extent than when TCE contaminated water was contacted with biomass only, without soil added.

(5). Removal of TCE from the liquid phase in soil slurry reactors was higher for soils with higher organic content and for clay soil - the material with the smallest grain size.

(6). Mixed culture of methanotrophs screened on a glass fibre filter exhibited TCE degradation capacity decreasing with the decrease of pore size. Some 50 % loss of this capacity was demonstrated for 0.7 μm size. Relatively small losses were experienced for biomass filtered on pore sizes larger than 1 μm.

(7). Straight freezing (without dehydration) was found to be the simplest long-term storage technique for mixed methanotrophic biomass. Freezing the culture at -20°C preserved at least 92 % of TCE degradation ability for as long as 73 days.

(8). Lower temperature storage (+4°C)preserved more of the degradation ability of the biomass, than storage at +20°C.

(9). Biomass screened on 1.2 μm filter easily penetrated a sandy loam laboratory soil column. Unfiltered biomass clogged the same column and completely blocked its leaching ability.

(10). The reference column through which only tap water (without added biomass) was passed produced a lasting TCE residue in excess of 3 $mg \cdot L^{-1}$ (3000 ppb). No TCE residue was found at the end of experiment in the leachate from the column with added screened biomass.

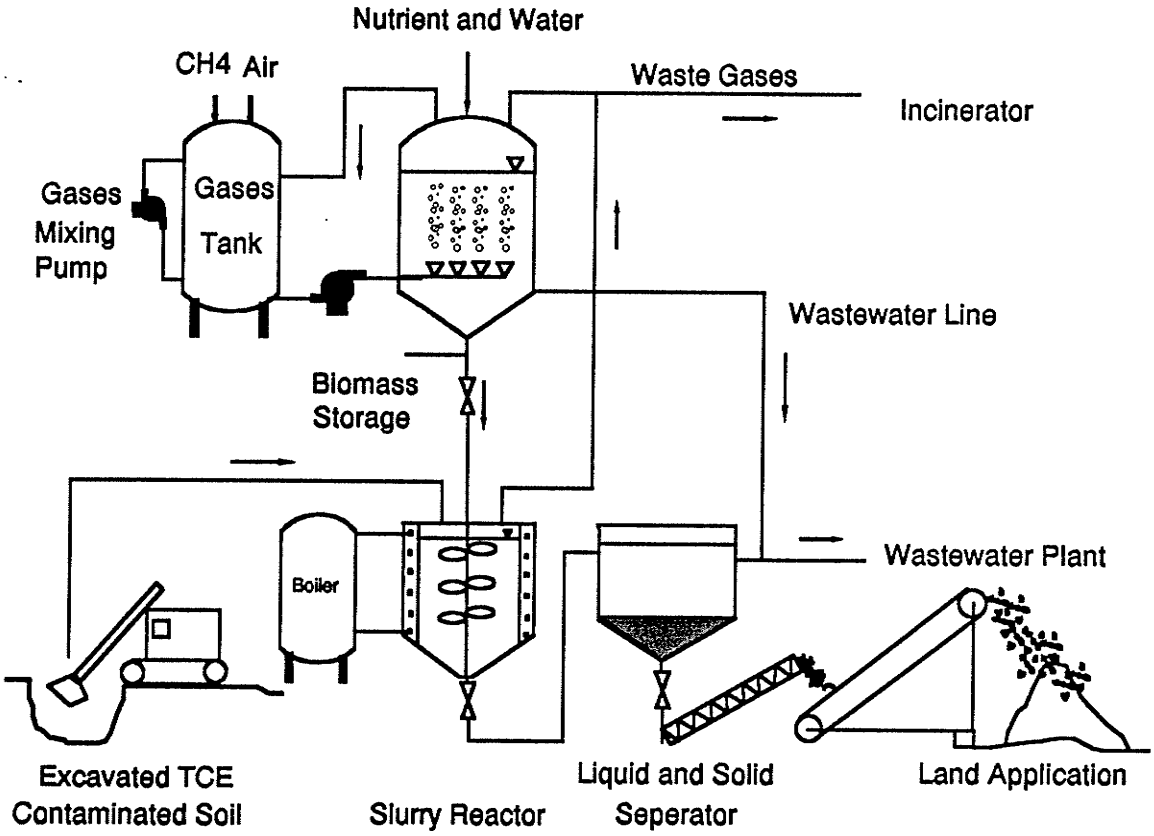

Figure 5.18 Schematric of Application of Bioremediation System

7. FUTURE RESEARCH

For completion of slurry methanotrophic bio-reactor process based on current studies, following suggestions are made for future studies:

(1) to use mixed gases from anaerobic digester as methane source to incubate mixed methanotrophs. At the same time, to investigate the effects of gases from anaerobic digester, such as H_2S, CO and CO_2 on biomass growth and on TCE degradation ability by using the biomass. If so, find methodology to get rid of unhealthy and toxic gases, e.g. using alkaline (KOH) trapper to remove CO_2 to keep pH in closed incubator-reactor constant.

(2) to find if biomass incubated at e.g. 5-10°C and the biomass brought down to 5-10°C from 23°C, have the same ability to degrade TCE. This is particularly important for sludge-addition process -- injection of ready-made methanotrophic bacteria in higher temperature to polluted site with lower ambient temperature.

(3) to decide minimum (reducing) water ratio in slurry reactor which allows proper mechanical mixing and optimal TCE degradation.

(4) to do pilot-scale experiments, which is particularly important before applying this technology and conduct economical evaluation to compare with other

techniques.

(5) to investigate migration behavior of methanotrophs in different kind of soil.

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APPENDIX A

Experimental Results Methanotrophic Cultures in Breeder-Incubator

Table A01. Experimental Results for Methanotrophic Cultures in Incubator-Reactor O (Activated Sludge) 1992

DATE	PH	MLSS (g/l)	MLVSS (g/l)	D.O2 (mg/l)	CH4:O2 (%)	MIXED AIR (ml/min)	SVI (ml/g)	TEMP. (C)	MICRO.CHECK
July 27	7.2	0.38	0.34	4	(10-20)	75	-	23	-
July 31	7.5	0.46	0.33	-	(10-15)	75	-	23	-
August 8	8.3	0.71	0.41	8.4	(10-15)	75	35.21	24	-
August 14	7.7	1.34	0.87	6.8	(10-15)	75	26.12	23	protozoa, rotifer
August 21	7.5	2.66	1.79	6	(10-15)	75	24.44	23	-
September 4	7.4	2.51	1.73	-	(10-15)	75	39.84	25	rotifer,aspidisca costate (p)(more)
September 10	7.6	2.45	1.72	2.8	(10-15)	75	46.94	-	aspidisca costate
September 17	7.6	2.68	-	5.9	(10-15)	75	46.64	25	rotifer(m), aspidisca costate(l)
September 27	7.6	0.75	0.61	2	(10-15)	75	300.00	24	rotifer(less)
October 10	7	-	-	2000	(10-15)	75	-	-	-
October 17	7.7	3.17	2.25	1	(10-15)	75	70.98	23	rotifer(m), aspidisca costate(l)
October 26	7.5	3.62	2.56	1.2	(10-15)	75	89.78	21.5	rotifer(m), aspidisca costate(l)
November 6	7.5	2.72	1.9	1	(10-15)	75	82.72	-	figerprint-like bacteria
November 15	7.5	4.86	3.46	1.3	(10-15)	75	63.79	23	figerprint-like bacteria
November 22	7.6	3.58	2.56		(10-15)	75	111.73	23	figerprint-like bacteria
November 30	7.6	4.06	2.84	1.4	(10-15)	75	110.84	23	-
December 9	7.5	3.94	2.82	0.9	(10-15)	75	101.52	23	-

Table A02. Experimental Records for Incubator-Rector 0 1992

DATE	MLSS AND MLVSS						SETTLEBILITY				pH of medium	Action
	No.	W.CRUCIB (g)	W.DR.+CR (g)	W.IR.+CR. (g)	MLSS (g/l)	MLVSS (g/l)	V.INCUBATOR (ml)	V.SLUDGE (ml)	SV (%)	SVI (ml/g)		
July 27	C 24	16.8635	16.8673	16.8639	0.38	0.34	2000	-	0	0.00		
July 31	C 24	16.8638	16.8684	16.8651	0.46	0.33	2000	-	0	0.00		
August 8	C 24	16.8629	16.8700	16.8659	0.71	0.41	2000	50	2.5	35.21		
August 14	C 24	16.8637	16.8771	16.8684	1.34	0.87	2000	70	3.5	26.12		
August 21	C 24	16.8637	16.8903	16.8724	2.66	1.79	2000	130	6.5	24.44	solution PH=6.8	
September 4	T 21	15.1897	15.2148	15.1975	2.51	1.73	2000	200	10	39.84	solution PH=6.2	
September 10	P 3	18.2273	18.2518	18.2346	2.45	1.72	2000	230	11.5	46.94	solution PH=6.4	
September 17	T 21	15.1893	15.2161		2.68	-	2000	250	12.5	46.64	solution PH=6.4	volve for methane supply changed
September 27	C 12	15.8540	15.8615	15.8554	0.75	0.61	2000	450	22.5	300.00		maybe not mixing well when taking sample
October 10	-	-	-	-	-	-	2000	300	15	-		
October 17	T 51	15.8356	15.8673	15.8448	3.17	2.25	2000	450	22.5	70.98		Air and Methane Supply system change,
October 26	59	18.3087	18.3268	18.3140	3.62	2.56	2000	650	32.5	89.78		
November 6	C 31	15.7640	15.7776	15.7681	2.72	1.9	2000	450	22.5	82.72		
November 15	C 8	16.9244	16.9487	16.9314	4.86	3.46	2000	620	31	63.79	solution PH=6.8	
November 22	C 2	17.2277	17.2456	17.2328	3.58	2.56	2000	800	40	111.73	solution PH=6.8	
November 30	T14	14.8458	14.8661	14.8519	4.06	2.84	2000	900	45	110.84		
December 9	S3	15.5389	15.5586	15.5445	3.94	2.82	2000	800	40	101.52		

Table A11. Experimental Results for Methanotrophic Cultures in Incubator-Reactor I (Activated Sludge) 1992

DATE	PH	MLSS (g/l)	MLVSS (g/l)	D.O2 (mg/l)	CH4:O2 (%)	MIXED AIR (ml/min)	SVI (ml/g)	TEMP. (C)	MICRO.CHECK
July 27	7.65	4.7	2.68	2.05	(10-20)	75	-	23	-
July 31	7.5	5.65	3.33	-	(10-15)	75	75.22	23	-
August 7	7.55	6.55	3.95	0.6	(10-15)	75	72.52	24	-
August 14	7.4	6.05	3.62	0.5	(10-15)	75	82.64	24.5	Protozoa, Rotifer
August 21	7.4	5.92	3.82	0.4	(10-15)	75	101.35	25	-
August 26	7.5	5.86	3.88	1	(10-15)	75	85.32	26	rotifer(l),aspidisca costate (p)(more)
September 4	7.6	4.76	3.16	-	(10-15)	75	113.45	25	rotifer(l),aspidisca costate (p)(more)
September 10	7.5	6.06	4.38	0.2	(10-15)	75	115.51	-	Rotifer(m),protozoa(l)
September 17	7.5	6.24	-	1.3	(10-15)	75	120.19	27	rotifer(m),protozoa(l)
September 27	7.7	4.32	3.26	2.7	(10-15)	75	138.89	24.5	rotifer(m),protozoa(l)
October 10	-	-	-	-	(10-15)	75	-	-	-
October 17	7.9	6.19	4.81	4.6	(10-15)	75	92.89	24	rotifer(m),protozoa(l),Aspidisca costate(l)
October 26	7.7	5.36	4.08	0.9	(10-15)	75	139.93	21	rotifer(m),protozoa(l),Aspidisca costate(l)
November 6	7.6	4.02	3.24	0.9	(10-15)	75	199.00	-	Aspidisca costate(l)
November 15	7.5	3.82	3.08	0.9	(10-15)	75	157.07	23	protozoa(l),Aspidisca costate(normal)
November 22	7.6	2.8	2.32	-	(10-15)	75	196.43	23	figer print bacteria, Aspidasca costate
November 30	7.55	1.98	1.96	1.1	(10-15)	75	277.78	23	-
December 9	7.5	2.66	2.32	0.8	(10-15)	75	0.00	23	-

Table A1.2. Experimental Records for Incubator-Rector I 1992

DATE	MLSS AND MLVSS						SETTLEBILITY				pH of medium	Action
	No.	W.CRUCIB (g)	W.DR.+CR (g)	W.IR.+CR. (g)	MLSS (g/l)	MLVSS (g/l)	V.INCUBATOR (ml)	V.SLUDGE (ml)	SV (%)	SVI (ml/g)		
July 27	C 26	15.9675	16.0145	15.9877	4.7	2.68	2000	-	0	0.00		
July 31	C 26	15.9682	16.0247	15.9914	5.65	3.33	2000	850	42.5	75.22		
August 7	C 26	15.9689	16.0344	15.9949	6.55	3.95	2000	950	47.5	72.52		
August 14	C 26	15.9698	16.0303	15.9941	6.05	3.62	2000	1000	50	82.64		
August 21	C 26	15.9701	15.9997	15.9806	5.92	3.82	2000	1200	60	101.35	solution PH=6.8	throw sludge
August 26	C 26	15.9682	15.9975	15.9781	5.86	3.88	2000	1000	50	85.32	solution PH=6.2	
September 4	C 26	15.9704	15.9942	15.9784	4.76	3.16	2000	1080	54	113.45	solution PH=6.4	throw some sludge
September 10	T 58	17.6334	17.6637	17.6418	6.06	4.38	2000	1400	70	115.51	solution PH=6.4	throw some sludge
September 17	C 31	15.766	15.7972	-	6.24	-	2000	1500	75	120.19		
September 27	C 36	16.8922	16.9138	16.8975	4.32	3.26	2000	1200	60	138.89		
October 10	-	-	-	-	-	-	2000	1200	60	-		
October 17	white	17.7162	17.7781	17.73	6.19	4.81	2000	1150	57.5	92.89		
October 26	white	17.2659	17.2927	17.2723	5.36	4.08	2000	1500	75	139.93		
November 6	P 10	18.6265	18.6466	18.6304	4.02	3.24	2000	1600	80	199.00	solution PH=6.8	
November 15	P 5	18.6945	18.7136	18.6982	3.82	3.08	2000	1200	60	157.07	solution PH=6.8	
November 22	white	17.7165	17.7305	17.7189	2.8	2.32	2000	1100	55	196.43		
November 30	P9	18.0219	18.0318	18.022	1.98	1.96	2000	1100	55	277.78		
December 9	KT7	17.286	17.2993	17.2877	2.66	2.32	2000	800	40	0.00		

Table A21. Experimental Results for Methanotrophic Cultures in Incubator-Reactor II (River Sediment) 1992

DATE	PH	MLSS (g/l)	MLVSS (g/l)	D.O2 (mg/l)	CH4:O2 (%)	MIXED AIR (ml/min)	SVI (ml/g)	TEMP. (C)	MICRO.CHECK
July 27	7.3	88.56	6.3	0.5	(10-20)	75	-	23	-
July 31	7.5	23.4	2.24	-	(10-15)	75	-	23	-
August 7	7.5	20.42	2.38	0.8	(10-15)	75	-	24	-
August 14	7.5	14.92	2.6	0.3	(10-15)	75	-	26	Aspidisca costata
August 21	7.4	7.32	1.8	0.2	(10-15)	75	-	26.5	-
August 26	7.5	3.52	1.16	0.8	(10-15)	75	120.74	28	asidisca costata (p) (less)
September 4	7.5	3.85	1.64	-	(10-15)	75	116.88	27	asidisca costata (p) (less)
September 10	7.4	5.52	2.68	0.2	(10-15)	75	117.75	-	asidisca costata (p) (less)
September 17	8.3	3.32	-	7.5	(10-15)	75	90.36	26	non-moving bacteria observed
September 27	7.9	3.58	1.6	0.3	(10-15)	75	75.42	24.5	non-moving bacteria observed
October 10	-	-	-	-	(10-15)	75	-	-	-
October 17	7.8	5.98	3.04	0.7	(10-15)	75	41.81	24	asidisca costata (p) (less)
October 26	7.4	3.9	2.38	0.9	(10-15)	75	38.46	21.5	asidisca costata (p) (less)
November 6	7.6	1.68	1.32	0.75	(10-15)	75	104.17	-	asidisca costata,rotifer,protozoa
November 15	7.5	3.76	2.72	0.7	(10-15)	75	66.49	23	asidisca costata (more)
November 22	7.5	4.16	3.08	-	(10-15)	75	72.12	24	asidisca costata (m) figerprint bacteria
November 30	7.5	3.76	2.86	0.9	(10-15)	75	93.09	22	-
December 7	7.5	3.82	2.54	0.7	(10-15)	75	65.45	23	-

Table A2.2. Experimental Records for Incubator-Reactor II 1992

DATE	MLSS AND MLVSS						SETTLEBILITY				pH of medium	Action
	No.	W.CRUCIB (g)	W.DR.+CR (g)	W.IR.+CR. (g)	MLSS (g/l)	MLVSS (g/l)	V.INCUBATOR (ml)	V.SLUDGE (ml)	SV (%)	SVI (ml/g)		
July 27	T 21	15.1875	16.0731	16.0101	88.56	6.3	2000	-	0	0.00		can not see sludge settling
July 31	T 21	15.1898	15.4238	15.4014	23.4	2.24	2000	-	0	0.00		can not see sludge settling
August 7	T 21	15.1897	15.2918	15.2799	20.42	2.38	2000	-	0	0.00		throw soil, cann't see sludge settling
August 14	T 21	15.189	15.2636	15.2506	14.92	2.6	2000	-	0	0.00		throw soil, cann't see sludge settling
August 21	T 21	15.1894	15.226	15.217	7.32	1.8	2000	-	0	0.00	solution PH=6.8	throw soil, cann't see sludge settling
August 26	T 21	15.1871	15.2047	15.1989	3.52	1.16	2000	850	42.5	120.74	solution PH=6.2	throw soil
September 4	C 24	16.8642	16.9027	16.8863	3.85	1.64	2000	900	45	116.88	solution PH=6.4	
September 10	F 10	18.031	18.0586	18.0452	5.52	2.68	2000	1300	65	117.75	solution PH=6.4	
September 17	white	17.7161	17.7327	-	3.32	-	2000	600	30	90.36		stirring stop & throw sludge
September 27	white	17.5464	17.5643	17.5563	3.58	1.6	2000	540	27	75.42		CH4 line blocked, sludge and supernatant seperation
October 10	-	-	-	-	-	-	2000	500	25	-		
October 17	F 5	17.8502	17.8801	17.8649	5.98	3.04	2000	500	25	41.81		
October 26	P14	18.8327	18.8522	18.8403	3.9	2.38	2000	300	15	38.46		
November 6	T 58	17.6337	17.6421	17.6355	1.68	1.32	2000	350	17.5	104.17	solution PH=6.8	Some sludge leaking by accident
November 15	T 52	14.6523	14.6711	14.6575	3.76	2.72	2000	500	25	66.49	solution PH=6.8	
November 22	C 12	15.8546	15.8754	15.8600	4.16	3.08	2000	600	30	72.12		
November 30	43	18.4508	18.4696	18.4553	3.76	2.86	2000	700	35	93.09		
December 7	white	17.7194	17.7385	17.7258	3.82	2.54	2000	500	25	65.45		

Table A31. Experimental Results for Methanotrophic Cultures in Incubator-Reactor III (Anaerobic Digester Sludge) 1992

DATE	PH	MLSS (g/l)	MLVSS (g/l)	D.O2 (mg/l)	CH4:O2 (%)	MIXED AIR (ml/min)	SVI (ml/g)	TEMP. (C)	MICRO.CHECK
July 27	8	10.47	4.96	3.8	(10-20)	75	-	23	-
July 31	8.3	8.9	3.47	-	(10-15)	75	-	23	-
August 7	7.9	8.14	3.58	0.5	(10-15)	75	-	24	-
August 14	7.4	5.1	2.34	0.4	(10-15)	75	-	24	Aspidisca costata, protozoa
August 21	7.4	15.32	5.96	0.3	(10-15)	75	19.58	24.5	-
August 26	7.5	10.04	4.4	0.6	(10-15)	75	37.35	26	aspidisca costata (p) (more)
September 4	7.5	10	4.46	-	(10-15)	75	40.50	26	aspidisca costata (p) (less)
September 10	7.2	8.7	3.84	1.2	(10-15)	75	57.47	-	rotifer(l),aspidisca costata(m)
September 17	7.5	11.64		1	(10-15)	75	64.43	27	rotifer(l),aspidisca costata(l)
September 27	7.6	10.9	5.54	0.3	(10-15)	75	-	24.5	rotifer(l),aspidisca costata(l)
October 10	-	-	-	-	(10-15)	75	-	-	-
October 17	8	22.32	13.52	4.2	(10-15)	75	38.08	24.5	rotifer(l),aspidisca costata(m) warm
October 26	7.6	27.96	24.68	0.7	(10-15)	75	26.82	20	rotifer(l),aspidisca costata(m)
Novermer 6	7.6	5.62	3.62	0.65	(10-15)	75	124.56	-	fingerprint like bacteria
Novermer 15	7.6	6.02	4.02	0.6	(10-15)	75	99.67	23	fingerprint like bacteria
November 22	7.7	5.8	3.98	-	(10-15)	75	103.45	23	fingerprint like bacteria, others
November 30	7.7	5.98	4.4	0.7	(10-15)	75	150.50	22	-
December 7	7.6	3.46	2.78	0.6	(10-15)	75	79.48	23	-

Table A3.2. Experimental Records for Incubator-Reactor III 1992

DATE	MLSS AND MLVSS						SETTLEBILITY				pH of medium	Action
	No.	W.CRUCIB (g)	W.DR.+CR (g)	W.IR.+CR. (g)	MLSS (g/l)	MLVSS (g/l)	V.INCUBATOR (ml)	V.SLUDGE (ml)	SV (%)	SVI (ml/g)		
July 27	C 22	17.0627	17.1674	17.1178	10.47	4.96	2000	-	0	0.00		can't see sludge settling
July 31	C 22	17.0644	17.1534	17.1187	8.9	3.47	2000	-	0	0.00		can't see sludge settling
August 7	C 22	17.0643	17.1457	17.1099	8.14	3.58	2000	-	0	0.00		can't see sludge settling
August 14	C 22	17.0671	17.0926	17.0809	5.1	2.34	2000	-	0	0.00		can't see sludge settling
August 21	C 22	17.0652	17.1418	17.112	15.32	5.96	2000	600	30	19.58	solution PH=6.8	
August 26	C 22	17.0646	17.1148	17.0928	10.04	4.4	2000	750	37.5	37.35	solution PH=6.2	
September 4	C 22	17.0664	17.1164	17.0941	10	4.46	2000	810	40.5	40.50	solution PH=6.4	
September 10	P 10	18.625	18.6685	18.6493	8.7	3.84	2000	1000	50	57.47	solution PH=6.4	
September 17	P 10	18.627	18.6852		11.64		2000	1500	75	64.43		
September 27	5	18.8015	18.856	18.8283	10.9	5.54	2000	-	0	-		can't see sludge settling
October 10	-	-	-	-	-	-	2000	-	0	-		
October 17	P 5	18.6933	18.8049	18.7373	22.32	13.52	2000	1700	85	38.08		
October 26	T 10	14.7931	14.9329	14.8095	27.96	24.68	2000	1500	75	26.82		
Novermer 6	S 4	15.1912	15.2193	15.2012	5.62	3.62	2000	1400	70	124.56	solution PH=6.8	
Novermer 15	P 7	18.3575	18.3876	18.3675	6.02	4.02	2000	1200	60	99.67	solution PH=6.8	
November 22	Lu	17.4509	17.4799	17.46	5.8	3.98	2000	1200	60	103.45		
November 30	C24	16.864	16.8939	16.8719	5.98	4.4	2000	1800	90	150.50		
December 7	C36	16.8924	16.9097	16.8958	3.46	2.78	2000	550	27.5	79.48		stirer does not work

APPENDIX B

TCE Degradation by Methanotrophs Develped from Different Seeds

Table B01. TCE Degradation by Methanotrophs Developed from Different Seeds

Date: December 23, 1992

Condition:
Temperature: 22 C
Air Presure: 75.58 cm Hg
Volume of TCE
Saturated Solut.: 500 ul
Volume of Cuture: 18 mL
V. of Test Tube: 28 mL

(Comparision for 4 Reactors)

Time	Blank Water	R 0 Activatd Sludge	R I Activated Sludge	R II River Sedimentation	R III Digestor Sludge
MLSS (g/L)		3.56	3.16	4.16	3.84
MLVSS (g/L)		2.62	2.8	3.34	2.24
MLSS/MLVSS		0.74	0.89	0.58	0.80
TCE Degradation					
(hr)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
0.22	32.51	28.06	29.49	31.86	27.66
1.53	32.38	13.04	15.74	16.04	12.74
2.77	34.09	7.47	10.44	13.82	7.44
4.03	31.70	4.74	7.67	12.72	4.81
6.43	34.33	1.99	4.99	9.82	2.60
11.83	31.11	0.31	3.01	7.13	0.61
24.43	32.67	0.00	1.52	5.28	0.00
Average	32.74				
mg of TCE removed by 1 g of MLVSS (after 24.43 hours)					
		12.49	11.15	8.22	14.61

Table B 02. TCE Degradation by Methanotrophs, Inhibited Methanotrophs and Activated Sludge

Date: June 24, 1993

Condition:
Temperature: 24.5 C
Air Presure: 75.65 cm Hg
Volume of TCE Saturated Solution: 500 uL
Volume of Culture: 18 mL
V.of Test Tube: 28 mL

	TCE Concentration			
(hour)	Control (mg/L)	Methanotrophs Biomass (mg/L)	Inhibited M. Biomass (mg/L)	Activated Sludge (mg/L)
0.22	31.37	25.47	27.81	28.47
1.22	31.40	15.24	27.13	27.14
2.22	30.54	11.10	27.00	26.54
3.63	30.32	7.87	26.48	25.05
7.00	30.82	3.77	26.56	25.45
10.3	30.71	1.95	24.48	25.07
15.77	31.04	0.94	25.79	24.94
22.67	30.96	0.80	25.70	25.00
pH (1)	7.8	7.1	7.0	8.2
pH (2)	6.8	6.9	6.9	7.3
WC		26.5914	26.5914	28.5330
WC + TS		26.6357	26.6357	28.5794
WC+NVS		26.6038	26.6038	28.5462
TS		4.43	4.43	4.64
VS		3.19	3.19	3.32
VS/TS(%)		72.0	72.0	71.6

APPENDIX C

The Experimental Analysis for Closed Incubator

Table C01. The experimental analysis for closed incubator 1993

	General			Gas in Tank												Gas in Outlet			Total and Volitile Solid				Ratio		
			Presure		CH4			CO2				O2							Total	Volatile	VS	Biomas	VS	VS	CH4
Time	PH	Temp	In Tank	Percent	Accum.	Measure	Consum	Perce	Accum.	Measure	Consum	Percent	Accum.	Measure	Consum	CH4	CO2	O2	Solid	Solid	TS	Growth	CH4	O4	O2
(hrs)		(C)	(M H2O	(%)	(g)	(g)	(g)	(%)	(g)	(g)	(g)	(%)	(g)	(g)	(g)	(%)	(%)	(%)	(g/L)	(g/L)	(%)	(g)	(g/g)	(g/g)	(g/g)
0	0.0	23.5	10.187	20.2	7.01	7.01	0	0.9	0.82	0.82	0.00	17.3	12.01	12.01	0	15.3	0.9	18.3	2.20	0.29	13.18	0.00	0.00	0.00	0.00
					7.01								12.01						2.19	0.39	17.81				
22		25.4	10.194	20.1	7.01	6.94	0.07	0.9	0.86	0.86	0.04	17.7	12.01	12.19		20.9	0.9	17.3							
35	7.0	25.4	10.192	19.9	7.01	6.86	0.23	0.9	0.86	0.86	0.04	17.3	12.01	11.90	0.11	20.7	0.9	17.3	2.18	0.35	16.06	0.12	0.77	1.08	1.40
					7.01								12.01						2.21	0.49	22.17				
58	6.9	25.4	10.188	20.2	7.03	6.96	0.30	0.9	0.86	0.86	0.04	17.2	12.03	11.85	0.29	20.7	1.0	16.7	2.27	0.55	24.23	0.23	3.40	1.30	0.38
					7.03								12.01						2.25	0.44	19.56				
82	6.9	25.0	10.190	19.6	7.03	6.78	0.55	1.0	0.95	0.95	0.13	17.0	12.03	11.76	0.56	19.8	1.6	15.5	2.15	0.36	16.74	0.03	0.12	0.11	0.93
					7.03								12.01						2.15	0.36	16.74				
111	6.9	26.5	10.181	18.4	7.03	6.30	1.27	1.3	1.26	1.26	0.44	16.7	12.03	11.43	1.15	18.9	2.1	13.8	2.23	0.37	16.59	0.01	0.02	0.03	1.22
					7.03								12.01						2.25	0.33	14.67				
136	6.9	26.5	10.168	18.8	7.03	6.43	1.86	1.6	1.54	1.54	0.72	16.1	12.03	11.03	2.15	19.0	2.2	14.2	2.15	0.48	22.33	0.17	0.28	0.17	0.59
					7.03								12.01						2.11	0.42	19.91				
137		26.5	10.188		7.06								12.09												
159		27.0	10.196	18.6	7.06	6.39	2.53	1.6	1.51	1.51	0.69	15.9	12.09	10.89	3.35	19.0	2.2	14.0							
182	6.9	25.6	10.139	18.4	7.06	6.30	3.29	1.7	1.62	1.62	0.80	15.3	12.09	10.49	4.95	18.8	2.4	13.1	2.13	0.40	18.78	0.08	0.11	0.05	0.48
					7.06								12.09						2.12	0.39	18.40				
184		25.6	10.203		7.19								12.33												
237		26.5	10.153		7.19								12.33												
237		26.5	10.197		7.25								12.44												
250	6.8	25.1	10.157	17.9	7.25	6.17	4.37	2.2	2.12	2.12	1.30	14.2	12.44	9.77	7.63	17.6	3.0	11.8	2.24	0.49	21.87	0.24	0.22	0.09	0.40
					7.25								12.44						2.26	0.51	22.57				
251		25.4	10.198		7.32								12.56												
322	6.8	24.9	10.151	19.0	7.32	6.53	5.16	3.1	2.95	2.95	2.13	13.2	12.56	9.06	11.13	17.8	3.5	12.0	2.09	0.44	21.05	0.16	0.20	0.04	0.22
					7.32								12.56						2.10	0.45	21.43				
326	0.0	24.9	10.195		7.38								12.68												
398	6.8	24.3	10.137	16.9	7.38	5.82	6.72	3.2	3.06	3.06	2.24	12.7	12.68	8.72	15.09	16.6	3.7	11.0	2.12	0.45	21.23	0.20	0.12	0.05	0.40
					7.38								12.68						2.13	0.49	23.00				
400		24.3	10.204		7.51								12.92												
424		24.0	10.164		7.51								12.92												
424		24.0	10.200		7.57								13.03												
467	6.8	22.0	10.186	18.3	7.57	6.37	7.92	3.7	3.51	3.51	2.69	10.8	13.03	7.53	20.59	16.8	4.1	9.5	2.12	0.56	26.42	0.32	0.26	0.06	0.22
474		24.5	10.178		7.34								11.91						2.10	0.54	25.71				
490	7.5	24.6	10.183	21.3	7.34	7.34	7.92	1.0	0.99	0.99	2.69	17.2	11.91	11.91	20.59	20.8	1.3	16.4	2.82	0.59	20.92	0.37	0.31	0.07	ERR
					7.34								11.91						2.83	0.58	20.49				
562	7.3	24.5	10.186	20.5	7.34	7.07	8.19	1.3	1.26	1.26	2.96	16.7	11.91	11.57	20.93	20.2	1.7	15.8	2.32	0.65	28.02	0.47	0.32	0.08	0.79
					7.34								11.91						2.32	0.65	28.02				
633		24.5	10.151		7.40								12.03												
634	7.2	24.5	10.175	19.4	7.40	6.68	8.91	1.6	1.54	1.54	3.24	16.1	12.03	11.11	21.85	18.7	2.2	14.7	2.32	0.73	31.47	0.56	0.29	0.09	0.78
					7.40								12.03						2.30	0.70	30.43				
730	7.3	22.9	10.156	19.0	7.40	6.57	9.75	2.5	2.33	2.33	4.03	14.9	12.03	10.31	23.57	18.3	2.8	13.8	2.30	0.67	29.13	0.56	0.28	0.08	0.49
					7.40								12.03						2.29	0.76	33.19				
734		22.9	10.193		7.47								12.14												
754		23.1	10.147		7.47								12.14												
754		23.1	10.182		7.53								12.26												

Table C01. The experimental analysis for closed incubator 1993

	General			Gas in Tank												Gas in Outlet			Total and Volitile Solid				Ratio		
					CH4			CO2				O2													
Time (hrs)	PH	Temp (C)	Presure In Tank (M H2O	Percent (%)	Accum. (g)	Measure (g)	Consum (g)	Perce (%)	Accum. (g)	Measure (g)	Consum (g)	Percent (%)	Accum. (g)	Measure (g)	Consum (g)	CH4 (%)	CO2 (%)	O2 (%)	Total Solid (g/L)	Volatile Solid (g/L)	VS TS (%)	Biomass Growth (g)	VS CH4 (g/g)	VS O4 (g/g)	CH4 O2 (g/g)
802	7.2	22.4	10.230	18.7	7.53	6.52	10.76	2.7	2.63	2.63	4.33	14.3	12.26	10.02	25.81	19.3	3.1	13.1	2.32	0.65	28.02	0.49	0.22	0.06	0.45
					7.53								12.26						2.36	0.68	28.81				
922		24.0	10.171	17.2	7.53	5.93	12.36	2.9	2.77	2.77	4.47	14.7	12.26	10.13	27.94	14.9	3.3	12.8	2.73	1.01	37.00	0.73	0.26	0.10	0.75
					7.53								12.26						2.62	0.64	24.43				
974	6.8	24.0	10.161	17.4	7.53	5.99	13.90	3.2	3.00	3.00	4.70	14.5	12.26	10.01	30.19	16.8	3.6	12.3	2.53	0.77	30.43	0.68	0.25	0.09	0.69
					7.53								12.26						2.56	0.82	32.03				
976		24.0	10.211		7.66								12.50												
1068	0.0	24.0	10.162	15.6	7.66	5.40	16.17	3.6	3.75	3.44	5.14	11.9	12.50	8.23	34.46	15.7	4.4	10.3	2.69	0.89	33.09	0.83	0.24	0.08	0.53
1143		23.5	10.140		7.66								12.50												
1143		23.5	10.210		7.79								12.73												
1145		23.0	10.148		7.79								12.73												
1145		24.5	10.203		7.86								12.85												
1189	6.6	23.5	10.140	13.5	7.86	4.65	19.37	4.2	4.02	4.02	5.72	11.1	12.85	7.69	39.62	13.0	5.0	8.0	2.80	0.93	33.21	0.85	0.19	0.08	0.62
1211		24.0	10.190	20.0	6.93	6.93	19.37	1.0	0.91	0.91	5.72		11.44		39.62				2.89	0.88	30.45				
1238		23.5	10.138		6.93								11.44												
1238		23.5	10.188		7.00								11.44												
1264	7.3	24.0	10.170	18.7	7.00	6.47	19.90	1.3	1.22	1.22	6.03	16.6	11.44	11.44	39.62	18.4	1.9	-2.2	2.71	0.95	35.06	0.89	0.51	0.16	ERR
					7.00								11.44						2.69	0.91	33.83				
1287					7.00								11.44												
1287		24.5	10.250		7.13								11.44												
1309		24.5	10.118		7.13								11.68												
1309		24.5	10.239		7.26								11.68												
1355	7.0	23.5	10.163	18.5	7.26	6.39	20.77	2.0	1.89	1.89	6.70	15.0	11.92	10.37	41.16	17.1	2.6	13.3	2.63	0.90	34.22	0.82	0.40	0.12	0.56
					7.26								11.92						2.63	0.88	33.46				
1367		24.5	10.118		7.26								11.92												
1367		24.5	10.245		7.39								11.92												
1403		24.5	10.113		7.39								12.15												
1403		24.5	10.250		7.52								12.15												
1426		23.8	10.058		7.52								12.39												
1426		23.8	10.238		7.71								12.39												
1476		22.5	10.123		7.71								12.74												
1476		22.5	10.258		7.84								12.74												
1488		21.0	10.068		7.84								12.98												
1488		21.0	10.248		8.03								12.98												
1498	7.1	22.0	10.129	16.8	8.03	5.83	22.97	2.7	2.58	2.58	7.39	12.7	13.33	8.79	45.70	16.6	3.4	10.9	2.54	0.93	36.61	0.92	0.27	0.09	0.48
					8.03								13.33						2.59	0.98	37.84				
1510		21.5	10.069		8.03								13.33												
1510		21.5	10.249		8.23								13.33												
1583		21.0	10.154		8.23								13.69												
1583		21.0	10.279		8.36								13.69												
1608		20.0	10.137		8.36								13.92												
1608		20.0	10.267		8.49								13.92												
1641		22.5	10.076		8.49								14.16												
1641		22.5	10.246		8.69								14.16												
1644	7.0	22.5	10.201	16.3	8.69	5.69	25.98	3.3	3.14	3.14	7.95	13.0	14.52	9.04	51.18	16.7	3.8	11.1	2.75	1.34	48.73	1.45	0.34	0.13	0.55
					8.69								14.52						2.71	1.27	46.86				

Table C01. The experimental analysis for closed incubator 1993

	General			Gas in Tank											Gas in Outlet			Total and Volitile Solid				Ratio		
			Presure		CH4				CO2				O2												
Time	PH	Temp	In Tank	Percent	Accum.	Measure	Consum	Perce	Accum.	Measure	Consum	Percent	Accum.	Measure	Consum	CH4	CO2	O2	Total Solid	Volatile Solid	VS TS	Biomas Growth	VS CH4	VS O4	CH4 O2
(hrs)		(C)	(M H2O	(%)	(g)	(g)	(g)	(%)	(g)	(g)	(g)	(%)	(g)	(g)	(g)	(%)	(%)	(%)	(g/L)	(g/L)	(%)	(g)	(g/g)	(g/g)	(g/g)
1656		20.0	10.068		8.69								14.52												
1656		20.0	10.328		8.95								14.99												
1714		20.2	10.180		8.95								14.99												
1714		0.0	10.254		9.02								15.11												
1738	7.0	20.2	10.183	14.7	9.02	5.17	29.83	3.7	3.60	3.60	8.41	12.8	15.11	8.97	57.31	14.1	4.4	11.1	2.77	1.08	38.99	1.13	0.22	0.10	0.63
					9.02								15.11						2.75	1.10	40.00				
1743		20.2	10.176		9.02								15.11												
1743		20.2	10.313		9.15								15.34												
1762		21.5	10.133		9.15								15.34												
1762		21.5	10.353		9.34								15.70												
1835		21.0	10.216	15.0	9.34	5.26	33.91	4.6	4.41	4.41	9.21	11.8	15.70	8.29	64.72	13.9	5.0	9.6	3.00	1.23	41.00	1.39	0.26	0.11	0.55
1860	7.0	21.0	10.213	21.5	7.54	7.54	33.91	1.2	1.12	1.12	9.21	16.4	11.52	11.52	64.72	15.3	1.6	16.4	3.05	1.30	42.62				
1871		21.0	10.151		7.54								11.52												
1871		21.0	10.273		7.67								11.76												
1930	7.1	21.0	10.258	20.0	7.67	7.03	34.55	1.4	1.37	1.37	9.47	15.5	11.76	10.92	65.56	19.3	2.1	13.9	3.25	1.51	46.46	1.71	0.93	0.27	0.76
					7.67								11.76						3.25	1.45	44.62				
1958		21.5	10.168		7.67								11.76												
1959		21.5	10.310		7.80								12.00												
2015		18.5	10.117		7.80								12.00												
2015		18.5	10.323		8.00								12.35												
2036	7.0	20.0	10.358	18.8	8.00	6.70	35.85	2.4	2.34	2.34	10.44	13.3	12.35	9.48	68.43	17.8	3.1	11.8	3.27	1.52	46.48	1.77	0.71	0.21	0.45
					8.00								12.35						3.29	1.52	46.20				
2055		21.5	10.182		8.00								12.35												
2055		21.5	10.257		8.00								12.35												
2060		20.0	10.157		8.00								12.35												
2060		20.0	10.291		8.13								12.59												
2084		20.0	10.271		8.13								12.59												
2084		20.0	10.341		8.20								12.71												
2151	7.0	22.5	10.307	18.1	8.20	6.38	37.68	3.3	3.24	3.24	11.33	11.5	12.71	8.10	73.04	17.5	4.2	9.8	3.42	1.49	43.57	1.69	0.56	0.17	0.40
					8.20								12.71						3.35	1.44	42.99				
2178		20.5	10.207		8.20								12.71												
2178		20.5	10.344		8.33								12.95												
2184		17.0	10.194		8.33								12.95												
2184		17.0	10.329		8.46								13.18												
2224	6.8	21.5	10.343	16.9	8.46	5.98	40.16	4.6	4.51	4.51	12.60	9.1	13.18	6.47	79.75	16.2	5.7	6.8	3.38	1.55	45.86	1.82	0.49	0.15	0.37
					8.46								13.18						3.37	1.55	45.99				
2243		20.0	10.205		8.46								13.18												
2243		20.0	10.340		8.66								13.54												
2294		19.5	10.221		8.66								13.54												
2294		19.5	10.323		8.79								13.78												
2314	6.7	20.5	10.255	14.7	8.79	5.18	43.77	5.1	4.91	4.91	13.01	7.5	13.78	5.29	88.24	16.3	5.7	4.9	3.44	1.83	53.20	2.20	0.46	0.16	0.43
2304					8.79								13.78						3.55	1.78	50.14				

Table C 02. The experimental records for closed incubator 1993

* - Change gas and air; ** - Change Nutrient; and *** - Adding 20 mL culture from open incubator

Date	Day	O'Clock	General				Total and volitile Soild				Gases in Tank			Gases in Outlet			Gases Standard									Std.Gas Added (L)	Act.
			PH	Temp (C)	P (in) (cm H2O)	P(out) (cm Hg)	SC	WC (g)	WC+TS (g)	WC+NVS (g)	CH4 Read	CO2 Read	O2 Read	CH4 Read	CO2 Read	O2 Read	CH4 10 (%)	CH4 15 (%)	CH4 20 (%)	CO2 1 (%)	CO2 5 (%)	CO2 10 (%)	O2 5 (%)	O2 15 (%)	O2 20 (%)		
Aug.28	1	14 : 27		23.5	0.9	75.00	2	26.5920	26.6140	26.6111	62.0	0.2	29.2	47.2	0.2	30.8		46.3	61.3	0.5	9.1			25.3	33.7		
	1	:					n	28.5760	28.5979	28.5940																	
	1	21 : 30		25.4	1.8	74.99					61.7	0.3	29.8	64.1	0.2	29.2		46.3	61.3	0.5	9.1			25.3	33.7		
Aug.29	2	10 : 30	7	25.4	1.6	74.99	N	28.6696	28.6914	28.6879	61.0	0.3	29.1	63.3	0.3	29.1		46.3	61.3	0.5	9.1			25.3	33.7		
	2						18	26.5163	26.5384	26.5335																	
Aug.30	3	9 : 50	6.9	25.4	1.0	75.00	G	26.9353	26.9580	26.9525	61.9	0.3	29.0	63.5	0.6	28.1		46.3	61.3	0.5	9.1			25.3	33.7		***
	3	:					13	27.7990	27.8215	27.8171																	
Aug.31	4	9 : 35	6.9	25.0	1.0	75.02	20	28.3933	28.4148	28.4112	61.0	0.5	28.8	61.5	1.9	26.3		46.5	62.1	0.5	9.5			25.4	33.7	0.1	
	4						D3	26.3517	26.3732	26.3696																	
Sep.1	5	15 : 20	6.9	26.5	0.3	75.00	M	29.2939	29.3162	29.3125	58.1	1.3	28.7	59.6	3.2	24.1		48.6	62.7	0.5	9.9			26.0	34.1		
	5	:					7	28.7204	28.7429	28.7396																	
Sep.2	6	15 : 30	6.9	26.5	-1.0	75.00	I	29.2645	29.2860	29.2812	59.5	2.0	28.4	60.2	3.3	25.5		48.6	63.0	0.5	9.9			26.7	34.4		
	6						5	28.5207	28.5418	28.5376																	
	6	16 : 30		26.5	1.0	75.00																				0.3	
Sep.3	7	14 : 35		27.0	2.0	74.99					58.7	2.0	28.2	59.6	3.3	25.2		48.2	62.6	0.6	9.9			26.8	34.8		
Sep.4	8	14 : 0	6.9	25.6	-3.9	75.00	10	28.3316	28.3529	28.3489	56.7	2.2	27.5	57.7	3.9	24.0		47.5	61.1	0.5	9.9			27.0	35.1		
	8						M	29.2945	29.3157	29.3118																	
	8	16 : 0		25.6	2.5	75.00																					
Sep.6	10	21 : 0		26.5	-2.5	75.00																				1.0	
	10	21 : 10		26.5	1.9	75.00																					
Sep.7	11	10 : 10	6.8	25.1	-2.1	75.00	20	28.3942	28.4166	28.4117	57.0	3.4	25.5	56.1	5.2	21.6		48.8	62.7	0.5	9.8			26.8	35.0	0.5	
	11						T	29.4939	29.5165	29.5114																	
	11	11 : 10		25.4	2.0	75.00																					
Sep.10	14	9 : 50	6.8	24.9	-2.9	75.02	n	28.5779	28.5988	28.5944	58.6	5.0	23.2	55.1	5.9	21.3		47.1	61.5	0.4	9.1			26.2	34.4	0.5	
	14						13	27.8004	27.8214	27.8169																	
	14	14 : 0		24.9	1.5	75.02																					
Sep.13	17	13 : 45	6.8	24.3	-3.9	74.99	17	27.5656	27.5868	27.5823	52.5	5.3	22.0	51.6	6.2	19.2		47.4	60.8	0.6	9.0			25.9	34.3	0.5	
	17						2	26.5939	26.6152	26.6103																	
	17	15 : 35		24.3	2.8	74.99																					
Sep.14	18	16 : 0		24.0	-1.2	74.99																				1.0	
	18	16 : 5		24.0	2.4	74.99																					
Sep.16	20	11 : 0	6.8	22.0	0.8	75.00	10	28.3338	28.3550	28.3494	55.2	6.2	20.0	51.3	7.1	17.9		46.7	59.6	0.6	9.0			26.7	34.7	0.5	
	20						P	28.1847	28.2057	28.2003																	
	20	18 : 0		24.5	0.0	75.00					68.3	0.5															
Sep.17	21	9 : 50	7.5	24.6	0.4	75.01	5	28.5163	28.5445	28.5386	65.6	0.6	29.5	64.2	1.2	28.0		48.2	62.1	0.5	8.9			25.5	34.4		* & **
	21						I	29.2599	29.2882	29.2824																	
Sep.20	24	10 : 0	7.3	24.5	1.1	74.98	N	28.6701	28.6933	28.6868	62.8	1.2	28.6	62.1	2.0	27.0		47.6	61.5	0.5	9.0			25.5	34.4		
	24						13	27.8010	27.8242	27.8177																	
Sep.23	27	8 : 30		24.5	-2.4	74.98																					
	27	10 : 0	7.2	24.5	0.0	74.98	19	28.8834	28.9066	28.8993	63.8	2.1	28.9	61.6	3.4	26.5		50.0	65.8	0.6	10.2			27.0	35.7	0.5	
	27						S	30.1969	30.2199	30.2129																	
Sep.27	31	9 : 45	7.3	22.9	-2.0	74.99	n	28.5787	28.6017	28.5950	61.9	3.8	26.0	59.9	4.6	24.1		50.0	65.0	0.5	9.6			26.2	35.0		
	31						P	31.5657	31.5886	31.5810																	
	31	14 : 15		22.9	1.8	74.98																					
Sep.28	32	9 : 45		23.1	-3.1	75.00																				0.5	
	32	9 : 46		23.1	0.4	75.00																				0.5	

Table C 02. The experimental records for closed incubator 1993

* - Change gas and air; ** - Change Nutrient; and *** - Adding 20 mL culture from open incubator

Date	Day	O'Clock	General				Total and volitile Soild				Gases in Tank			Gases in Outlet			Gases Standard									Std.Gas Added (L)	Act.
			PH	Temp (C)	P (in) (cm H2O)	P(out) (cm Hg	SC	WC (g)	WC+TS (g)	WC+NVS (g)	CH4 Read	CO2 Read	O2 Read	CH4 Read	CO2 Read	O2 Read	CH4 10 (%)	CH4 15 (%)	CH4 20 (%)	CO2 1 (%)	CO2 5 (%)	CO2 10 (%)	O2 5 (%)	O2 15 (%)	O2 20 (%)		
Sep.30	34	9 : 40	7.2	22.4	5.5	74.98	5	28.5208	28.5440	28.5375	61.5	4.5	25.5	63.5	5.4	23.2		50.1	65.7	0.5	9.7			26.7	35.7		
	34						P	28.1866	28.2102	28.2034																	
Oct. 5	39	10 : 5		24.0	-0.9	75.02	17	27.5673	27.5946	27.5845	59.5	5.0	26.5	52.8	5.9	23.2		53.0	68.0	0.5	9.9			27.1	36.0		**
	39	:					7	28.7247	28.7509	28.7445																	
Oct. 7	41	14 : 20	6.8	24.0	-2.2	75.04	H	27.2017	27.2270	27.2193	58.7	6.0	26.1	56.8	7.2	22.1		51.0	67.3	0.6	10.6			27.0	36.1		
	41						N	28.6703	28.6959	28.6877																	
	41	16 : 0		24.0	2.8	75.04																				1.0	
Oct.11	45	11 : 30	6.8	24.0	-1.6	75.00	K	29.1242	29.1511	29.1422	54.2	7.0	22.3	54.5	8.8	19.3		52.1	68.5	0.5	10.4			27.9	37.0		
Oct.12	48	15 : 0		23.5	-4.0	75.02																					
	48	15 : 5		23.5	3.0	75.02																					
Oct.14	48	17 : 0		23.0	-3.0	75.00																				1.0	
	48	17 : 5		24.5	2.5	75.00																					
Oct.16	50	12 : 50	6.6	23.5	-4.0	75.02	10	28.3336	28.3616	28.3523	49.3	8.7	21.5	47.8	10.5	16.3		54.0	69.4	0.6	10.6			27.9	36.2	0.5	
	50						S	30.1984	30.2273	30.2185																	
Oct.17	51	11 : 0		24.0	1.0	75.02					69.5	0.5						54.0	69.4	0.6	10.6			27.9	36.2		* & **
Oct.18	52	14 : 10		23.5	-4.0	75.00																					
	52	14 : 15		23.5	1.0	75.00																					
Oct.19	53	16 : 0	7.3	24.0	-1.0	75.02	3	28.2498	28.2769	28.2674	66.1	1.2	30.0	65.0	2.8			54.7	70.0	0.5	10.2			27.5	35.5	0.5	
	53						P	28.1859	28.2128	28.2037																	
Oct.20	54	14 : 35		24.5	-7.0	75.02																					
	54	14 : 40		24.5	7.0	75.02																					
Oct.21	55	12 : 50		24.5	-6.5	75.04																				1.0	
	55	12 : 55		24.5	5.6	75.04																					
Oct.23	57	11 : 0	7	23.5	-1.5	75.00	18	26.5201	26.5464	26.5374	64.1	3.0	26.2	59.7	4.5	23.2		53.0	69.0	0.7	10.0			26.2	34.9	1.0	
	57						W	26.3545	26.3808	26.3720																	
	57	23 : 0		24.5	-6.0	75.00																					
	57	23 : 5		24.5	7.0	74.98																					
Oct.25	59	11 : 15		24.5	-6.5	75.00																				1.0	
	59	11 : 20		24.5	7.2	75.00																					
Oct.26	60	9 : 30		23.8	-12.0	75.00																				1.0	
	60	9 : 35		23.8	6.0	75.00																					
Oct.28	62	11 : 30		22.5	-5.5	75.00																				1.5	
	62	11 : 35		22.5	8.0	75.00																					
	62	23 : 30		21.0	-11.0	75.00																				1.0	
	62	23 : 35		21.0	7.0	75.00																					
Oct.29	63	10 : 0	7.1	22.0	-5.0	75.01	S	30.2002	30.2256	30.2163	45.9	3.6	18.0	45.2	4.9	15.7		41.3	53.8	0.4	7.9			21.0	27.5	1.5	
	63						10	28.3350	28.3609	28.3511																	
	63	22 : 0		21.5	-11.0	75.01																					
	63	22 : 5		21.5	7.0	75.01																					
Nov.1	66	22 : 30		21.0	-6.0	75.27																				1.5	
	66	22 : 35		21.0	6.5	75.27																					
Nov.2	67	23 : 30		20.0	-7.5	75.25																				1.0	
	67	23 : 35		20.0	5.5	75.25																					
Nov.4	69	9 : 15		22.5	-14.0	75.28																				1.0	
	69	9 : 20		22.5	3.0	75.28																					
	69	12 : 0	7	22.5	-1.5	75.28	7	28.7255	28.7530	28.7396	52.6	6.5	22.5	53.6	7.8	19.2		48.7	63.4	0.4	11.1			26.1	35.0	1.5	
	69						2	26.5926	26.6197	26.6070																	

Table C 02. The experimental records for closed incubator 1993

* - Change gas and air; ** - Change Nutrient; and *** - Adding 20 mL culture from open incubator

Date	Day	O'Clock	General				Total and volitile Soild				Gases in Tank			Gases in Outlet			Gases Standard									Std.Gas Added (L)	Act.
																	CH4			CO2			O2				
			PH	Temp (C)	P (in) (cm H2O)	P(out) (cm Hg)	SC	WC (g)	WC+TS (g)	WC+NVS (g)	CH4 Read	CO2 Read	O2 Read	CH4 Read	CO2 Read	O2 Read	10 (%)	15 (%)	20 (%)	1 (%)	5 (%)	10 (%)	5 (%)	15 (%)	20 (%)		
	69	23 : 45		20.0	-14.5	75.26																					
	69	23 : 50		20.0	11.5	75.26																					
Nov.7	72	10 : 0		20.2	-4.0	75.31																				2.0	
	72	10 : 5		20.0	3.4	75.31																					
Nov.8	73	9 : 35	7	20.2	-3.0	75.26	n	28.5770	28.6047	28.5939	56.6	8.2	22.6	54.3	9.9	19.1		57.5	75.0	0.6	11.7			27.0	37.0	0.5	
	73						A	28.1475	28.1750	28.1640																	
	73	15 : 10		20.2	-3.7	75.26																					
	73	15 : 15		20.2	10.0	75.26																					
Nov.9	74	9 : 30		21.5	-8.0	75.26																				1.0	
	74	9 : 35		21.5	14.0	75.26																					
Nov.12	77	10 : 30		21.0	0.0	75.28	19	28.8834	28.9134	28.9011	54.0	9.9	20.3	50.3	11.1	16.2		54.0	70.7	0.8	11.0			26.2	35.5	1.5	
	77						18	26.5188	26.5493	26.5363																	
Nov.13	78	12 : 0	7	21.0	0.0	75.26					77.0	1.0	30.2	58.2	2.2	30.2		57.2	72.4	0.6	10.5			27.7	36.4		* & **
	78	22 : 30		21.0	-6.2	75.26																					
	78	22 : 35		21.0	6.0	75.26																					
Nov.16	81	10 : 10	7.1	21.0	4.5	75.26	2	26.5918	26.6243	26.6092	68.0	1.8	28.6	65.9	3.5	25.8		53.1	68.1	0.8	10.4			27.7	36.6	1.0	
	81						7	28.7243	28.7568	28.7423																	
Nov.17	82	14 : 25		21.5	-5.0	75.30																					
	82	14 : 30		21.5	9.2	75.30																					
Nov.19	84	23 : 0		18.5	-10.0	75.29																				1.0	
	84	23 : 5		18.5	10.6	75.29																					
Nov.20	85	19 : 40	7	20.0	14.0	75.30	17	27.5677	27.6004	27.5852	66.2	4.2	24.8	63.3	5.9	22.2		54.5	70.0	0.7	10.8			27.7	36.2	1.5	
	85						8	26.4875	26.5204	26.5052																	
Nov.21	86	15 : 0		21.5	-3.5	75.29																					
	86	15 : 5		21.5	4.0	75.29																					
	86	19 : 50		20.0	-6.0	75.29																					
	86	19 : 55		20.0	7.4	75.29																					
Nov.22	87	20 : 10		20.0	0.0	75.69																				1.0	
	87	20 : 15		20.0	7.0	75.69																					
Nov.25	90	15 : 15	7	22.5	3.7	75.68	S	30.1990	30.2332	30.2183	65.6	7.4	21.2	63.3	9.8	18.0		54.0	72.6	0.9	12.0			27.7	37.0	0.5	
	90						n	28.5779	28.6114	28.5970																	
Nov.28	91	17 : 45		20.5	-7.2	75.75																					
	91	17 : 50		20.5	6.5	75.75																					
	91	23 : 30		17.0	-8.5	75.75																				1.0	
	91	23 : 35		17.0	5.0	75.75																					
Nov.30	93	15 : 30	6.8	21.5	7.0	75.70	2	26.5929	26.6267	26.6112	70.2	11.7	19.0	67.5	14.9	13.9		62.6	82.9	1.0	12.8			31.9	42.9	1.0	
	93						8	26.4885	26.5222	26.5067																	
Dec.1	94	10 : 30		20.0	-6.5	75.68																					
	94	10 : 35		20.0	7.0	75.68																					
Dec.3	96	13 : 40		19.5	-5.2	75.70																				1.5	
	96	13 : 45		19.5	5.0	75.70																					
Dec.4	97	9 : 50	6.7	20.5	-2.0	75.72	W	30.2002	30.2346	30.2163	62.0	13.1	18.0	68.0	15.0	13.2		63.1	81.8	1.0	12.9			32.1	41.5	1.0	
	97						19	28.8834	28.9189	28.9011																	

Table C 03. Regresson Results (For Figure 5-9)

Regression Output:(CH4)		
Constant		0.0000
Std Err of Y Est		1.9154
R Squared		0.9800
No. of Observations		31.0000
Degrees of Freedom		30.0000
X Coefficient(s)	0.4057	
Std Err of Coef.	0.0068	

Regression Output:(CO2)		
Constant		0.0000
Std Err of Y Est		0.4172
R Squared		0.9887
No. of Observations		31.0000
Degrees of Freedom		30.0000
X Coefficient(s)	0.1228	
Std Err of Coef.	0.0015	

Regression Output:(O2)		
Constant		0.0000
Std Err of Y Est		3.2225
R Squared		0.9849
No. of Observations		31.0000
Degrees of Freedom		30.0000
X Coefficient(s)	0.8085	
Std Err of Coef.	0.0115	

Regression Output:(Biomass)		
Constant		0.0000
Std Err of Y Est		0.1375
R Squared		0.9516
No. of Observations		31.0000
Degrees of Freedom		30.0000
X Coefficient(s)	0.0189	
Std Err of Coef.	0.0005	

APPENDIX D

Effect of Temperature and pH

Table D01. Effect of Temperature

Date: August 26, 1993

Condition:
Temperature: 23.5 C
Air Presure: 74.99 cm Hg
Volume of TCE
Saturated Solution: 500 uL
Volume of Culture: 18 mL
V.of Test Tube: 28 mL

Time	TCE Concentration									
(hour)	23.5 C No.1 (mg/L)	23.5 C No.2 (mg/L)	23.5 C Average (mg/L)	8 C No.1 (mg/L)	8 C No2 (mg/L)	8 C Average (mg/L)	4 C No.1 (mg/L)	4 C No.2 (mg/L)	4 C Average ()	Blank (mg/L)
0.27	22.01	25.81	23.91	26.14	25.9	26.02	27.29	26.54	26.92	26.15
1.87	13.83	14.32	14.08	23.32	23.01	23.17	28.04	26.04	27.04	32.68
4.53	6.21	6.97	6.59	22.49	22.32	22.41	25.69	23.95	24.82	33.98
7.92	2.79	3.23	3.01	14.85	13.91	14.38	19.89	23.35	21.62	34.22
13.15	1.45	1.51	1.48	13.73	14.06	13.90	18.67	20.82	19.75	34.58
23.58	1.38	1.34	1.36	14.43	11.06	12.75	16.5	18.37	17.44	34.39
25.15				9.58	10.43	10.01	11.85	13.3	12.58	
26.38				7.16	6.54	6.85	9.84	9.28	9.56	
29.15				4.98	4.63	4.81	5.75	5.57	5.66	32.22
35.50	1.04	1.15	1.10	3.23	2.68	2.96	3.33	2.42	2.88	34.39
48.30	1.35	1.36	1.36	3.31	2.57	2.94	3.41	2.25	2.83	33.49
pH (1)	7.4	7.4	7.40	7.4	7.4	7.40	7.4	7.4	7.40	Ave.
pH (2)	6.8	6.8	6.80	6.8	6.8	6.80	6.8	6.8	6.80	32.90

Note:

pH (1) - measued pH before putting TCE.
pH (2) - pH after TCE degradation experiment.

Total and Volitile Solids

Crucible#	A	8	Average
WC	28.1494	26.4864	
WC + TS	28.2016	26.539	
WC + NVS	28.1650	26.5023	
TS (g/L)	5.22	5.26	5.24
VS (g/L)	3.66	3.67	3.66
VS/TS (%)	70.11	69.77	69.94

Table D02. Effect of pH

Date: June 3, 1993

Condition:
Temperature: 23.2 C
Air Presure: 75.75 cm Hg
Volume of TCE
Saturated Solution: 500 uL
Volume of Culture: 18 mL
V.of Test Tube: 28 mL

	TCE Concentration						
pH (c)	5.8	6.2	6.6	7.0	7.4	7.8	Blank
(hour)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
0.27	19.00	18.59	19.81	18.37	18.26	19.05	27.13
2.67	11.21	9.44	8.03	7.03	5.80	5.89	23.76
6.00	8.60	6.27					
6.20			4.14	3.42	2.66	3.08	23.87
11.63	6.00	3.41	2.16	1.42	0.91	1.17	24.00
23.15	3.32	1.70	0.58	0.00	0.00	0.27	23.75
Remov. Rate (%)	86.45	93.06	97.63	100.00	100.00	98.90	
Remov. Rate (mg TCE/g VS*h)	20.58	22.15	23.24	23.81	23.81	23.54	
pH (1)	5.7	6.0	6.5	6.8	7.3	7.8	Ave.
pH (2)	5.6	6.0	6.5	6.8	7.3	7.6	24.502
(a) (mL)	18.4	16.3	12.5	7.8	3.5	1.7	
(b) (mL)	1.6	3.7	7.5	12.2	16.5	18.3	

Note:

1. 0.2 M Sodium Phosphate Buffer is prepared.
   * (a) NaH2PO4 MW = 138.01 13.8 g/500mL
   * (b) Na2HPO4 MW = 268.00 26.8 g/500mL
2. pH (c) - Calculated pH.
   pH (1) - measued pH before putting TCE.
   pH (2) - pH after TCE degradation experiment.

Total and Volatile Solids

Crucible#	A	8	Average
WC	26.9341	29.3209	
WC + TS	26.9746	29.3619	
WC + NVS	26.9507	29.3363	
TS	4.05	4.10	4.07
VS	2.39	2.56	2.47
VS/TS (%)	59.0	62.4	60.73

APPENDIX E

TCE Degradation from Soil

Table E01. TCE Degradation from Soil (I)

Date: December 15, 1993
Condition:
Temperature: 22 C
Air Presure: 75.45 cm Hg
Volume of TCE
Saturated Solution: 500 uL
Volume of Culture: 18 mL
V.of Test Tube: 28 mL

Time				TCE Concentration (mg/L)										
						Sand			Clay			Very Fine Sandy Loam		
Date	Days	O'clock	(hour)	Ctrl (A)	Ctrl (B)	12-1	12-2	12-Ave.	48-1	48-2	48-Ave.	67-1	67-2	67-Ave.
Dec.15	1	11 : 0	0.00	30.27	30.56	26.27	25.94	26.11	31.40	29.12	30.26	28.93	30.42	29.68
Dec.17	3	15 : 0	52.00	31.02	30.27	26.17	26.31	26.24	29.88	30.02	29.95	27.16	29.47	28.32
Dec.18	4	10 : 25	71.42	30.31	29.99	26.03	26.31	26.17	29.52	29.37	29.45	27.30	29.00	28.15
Dec.18	4	10 : 35	71.58	30.57	20.97	19.84	19.60	19.72	24.07	22.50	23.29	20.09	22.08	21.09
Dec.18	4	12 : 5	73.08	29.80	17.29	18.49	19.09	18.79	19.77	18.33	19.05	17.71	19.40	18.56
Dec.18	4	13 : 15	74.25	31.10	12.34	16.44	17.16	16.80	15.44	16.80	16.12	18.01	19.50	18.76
Dec.18	4	20 : 15	81.25	30.26	7.54	16.73	16.62	16.68	10.62	11.59	11.11	16.32	17.36	16.84
Dec.19	5	15 : 30	100.50	30.45	2.52	14.60	14.49	14.55	7.34	8.23	7.79	15.17	16.63	15.90
Dec.20	6	16 : 35	125.58	29.98	1.21	15.64	14.01	14.83	6.64	6.25	6.45	14.59	14.43	14.51
Dec.21	7	14 : 50	147.83	30.12	1.30	13.94	12.98	13.46	4.63	4.74	4.69	15.25	14.95	15.10
pH Initial				7.1	7.1	6.9	6.9	6.9	6.4	6.4	6.4	7	6.9	6.95
pH end				7.1	6.9	6.7	6.7	6.7	6.5	6.3	6.4	6.9	6.9	6.9

Note:
- put 10 g of dried soil sample into each test tube.
- mix 14 ml deioniaed water with soil.
- inject 400 uL of saturated TCE solution to each test tube.
- take 300 ml of Methanotroph culture from Reactor I.
- centrifuge the culture @ 4500 rpm for 30 miuntes.
- extract 20 mL of condensed culture from 300 mL of the culture.
- inject 2 ml of condensed culture to each test tube.
- centrifuge soil containing culture @ 4500 rpm for 10 min. before taking sample.

Crucible#	N
WC	28.6695
WC + TS	28.8178
WC + NVS	28.6839
TS (g/L)	24.72
VS (g/L)	22.32
VS/TS (%)	90.29

Table E02. TCE Degradation from Soil (II)

Date: December 28, 1993
Condition:
Temperature: 19 C
Air Presure: 75.5
Volume of TCE
Saturated Solutio 500 uL
Volume of Cultur 18 mL
V.of Test Tube: 28 mL

Date	Conditions T (C)	Air pres. (mm Hg)	Time Days	O'clock			(hour)	TCE Concentration (mg/L) Cont. (A)	Cont.(B)	OC=0.8% 4-1	4-2	4-Ave.	OC=1.3% 6-1	6-2	6-Ave.	OC=2.0% 36-1	36-2	36-Ave.	OC=5.6% 21-1	21-2	21-Ave.
Dec.29	19	75.5	1	11	:	10	0.00	29.22	29.88	27.11	26.78	26.95	25.06	24.49	24.78	18.49	19.22	18.86	17.51	17.03	17.27
Dec.30	19	75.46	2	10	:	30	23.33	28.74	28.94	27.09	broken	27.09	21.90	22.40	22.15	18.01	17.97	17.99	15.85	16.51	16.18
Dec.31	19	75.46	3	12	:	10	49.00	29.01	30.06	25.57	-	25.57	21.67	22.31	21.99	17.25	17.39	17.32	15.45	15.86	15.66
Dec.31	19	75.46	3	12	:	20	49.17	29.30	18.93	19.08	-	19.08	16.55	16.78	16.67	13.47	12.2	12.84	11.04	11.69	11.37
Dec.31	19	75.46	3	15	:	30	52.33	28.89	8.57	10.61	-	10.61	10.67	10.08	10.38	9.53	10.46	10.00	8.47	8.96	8.72
Dec.31	19	75.46	3	17	:	45	54.58	30.01	4.50	7.79	-	7.79	8.00	7.69	7.85	6.48	6.31	6.40	7.56	6.86	7.21
Dec.31	20	74.45	3	22	:	30	59.33	28.26	2.47	7.68	-	7.68	7.45	7.32	7.39	5.20	4.98	5.09	6.41	6.24	6.33
Jan.1	20	75.5	4	14	:	25	75.25	28.87	1.21	7.20	-	7.20	6.99	6.35	6.67	4.63	4.31	4.47	5.12	5.12	5.12
Jan.2			5	9	:	30	94.33	28.61	0.75	7.27	-	7.27	6.87	6.36	6.62	4.57	4.63	4.60	4.85	5.01	4.93
pH Ini.								7.20	7.1	7.1	7	7.05	7.1	7	7.05	6.5	6.6	6.55	6.5	6.5	6.50
pH End								7	7	7	-	7	7	7	7	6.5	6.4	6.45	6.5	6.5	6.5

Note:

- put 10 g of dried soil sample into each test tube.
- mix 14 ml deioniaed water with soil.
- inject 400 uL of saturated TCE solution to each test tube.
- take 300 ml of Methanotroph culture from Reactor I.
- centrifuge the culture @ 4500 rpm for 30 miuntes.
- extract 20 mL of condensed culture from 300 mL of the culture.
- inject 2 ml of condensed culture to each test tube.
- centrifuge soil containing culture @ 4500 rpm for 10 min. before taking sample.

Crucible#	N
WC	28.6695
WC	26.5195
WC + TS	26.8645
WC + NVS	26.5648
TS (g/L)	34.50
VS (g/L)	29.97
VS/TS (%)	86.87

APPENDIX F

Size Selection of Methanotrophic Biomass

Table F01. TCE Degradation using Screened Methanotrophic Cultur

Date: August 23, 1993

Air Presure: 74.98 cm Hg
Volume of TCE
Saturated Solution: 500 uL
Volume of Culture: 18 mL
V.of Test Tube: 28 mL

	TCE Concentration (mg/L)						
			Glass fibre filter with different pore size				
Time (hr)	Control	Sludge	2.7 um	1.6 um	1.2 um	1.0 um	0.7 um
0.27	33.61	28.03	24.71	27.91	28.25	28.57	34.97
1.85	38.49	12.32	13.13	14.79	12.31	17.11	25.87
0.00	37.02	5.53	7.33	8.74	7.06	12.49	24.46
6.95	35.86	2.85	4.34	4.97	3.70	8.76	22.41
12.57	37.65	1.63	2.06	2.23	1.94	4.49	19.99
22.50	38.86	1.20	1.20	1.23	1.19	2.37	17.03
46.97	37.36	1.43	1.16	1.11	1.07	1.48	13.98
PH (I)	7.4	7.4	7.4	7.4	7.4	7.4	7.4
PH (E)	7.4	6.8	6.8	6.8	6.8	6.9	7.2
	Total Solid and Volitile Soild						
Crucible#	3	5	E	T	I	M	no
WC	28.2442	28.5204	28.9536	29.494	29.2642	29.2931	31.5655
WC + TS	28.2999	28.5756	29.001	29.5396	29.3138	29.3339	31.5901
WC + NVS	28.2590	28.5351	28.9673	29.5076	29.2781	29.3066	31.5785
TS (g/L)	5.57	5.52	4.74	4.56	4.96	4.08	2.46
VS (g/L)	4.09	4.05	3.37	3.20	3.57	2.73	1.16
VS/TS (%)	73.43	73.37	71.10	70.18	71.98	66.91	47.15

APPENDIX G

Methanotrophic Biomass Freezing Storage

Table G01. Methanotrophic Biomass Freezing Storage

Time (hour)	Blank (mg/L)	June 15 (d 23 C (mg/L)	June 22 (Day 7) 23 C (mg/L)	4 C (mg/L)	-20 C (mg/L)	June 28 (Day 13) 23 C (mg/L)	4 C (mg/L)	-20 C (mg/L)	July 5 (Day 20) 23 C (mg/L)	4 C (mg/L)	-20 C (mg/L)	July 26 (Day 41) 23 C (mg/L)	4 C (mg/L)	-20 C (mg/L)	August 27 (Day 73) 23 C (mg/L)	4 C (mg/L)	-20 C (mg/L)
0.27	30.97	23.66	20.75	21.50	26.03	23.38	27.73	27.96	24.71	24.51	26.03	25.64	23.49	26.88	25.90	21.35	26.05
1.57	30.50	9.19															
1.70																	
1.88												26.30	13.01	broken			
1.95			14.83	8.02	7.48	21.96	11.45	11.87									
3.50									20.19	10.54	9.17						
4.08	31.30	3.61										19.55	9.89				
5.22			8.82	Broken	2.03												
5.50						14.64	6.26	4.11									
6.30									26.45	4.74	3.30						
8.12												19.48	4.92				
8.93	25.28	0.55															
11.22						13.73	2.95	2.02									
14.00									23.91	3.20	2.33						
18.72			7.40		1.25												
20.83																	
21.25															21.36	5.78	1.20
21.63												15.53	3.97				
25.03						13.65	2.76	1.84									
25.45	24.16	0.44							24.96	2.97	2.03						
27.20			7.22		1.15												
41.00																	
42.25															21.36	5.21	1.67
												15.77	4.07				
Average	28.442																
@24 hrs		0.45	7.25		1.19	13.63	2.72	1.8	24.86	2.99	2.06	15.56	3.98		21.36	5.77	1.27
% of removal		98.4	74.5	100.0	95.8	52.1	90.4	93.7	12.6	89.5	92.8	45.3	86.0	100.0	24.9	79.7	95.5
S/X (mgTCE/gVS*d)		7.7	5.9		7.5	4.1	7.1	7.4	1.0	7.0	7.3	3.6	6.8		2.0	6.3	7.5

Total & Volitile Solids

No.of C	18	19	
WC	26.5151	28.8775	
WC + TS	26.5646	28.9281	
WC + NVS	26.5288	28.8915	
TS (g/L)	4.95	5.06	5.00
VS (g/L)	3.58	3.66	3.62
VS/TS (%)	72.32	72.33	72.33

APPENDIX H

Methanotrophic Degradation of TCE from Soil in Column

Table H01. Methanotrophic Degradation of TCE from Soil in Columns

Date	Day	O'Clock	Time (hr)	Conditions T (C)	Conditions Air P (cm Hg)	Feeding TCE (mg/L)	Feeding PH	Column I TCE (mg/L)	Column I Volume (mL)	Column I PH	Column II TCE (mg/L)	Column II Volume (mL)	Column II PH	Notes
Dec. 17	1	9 : 15	0	22.0	75.43	25.46	7.2	0.00	0		0.00	0		Experiment Started
Dec. 18	2	9 : 30	24	22.0	75.41	24.64		2.52	150	7.6	2.49	150	7.6	Add TCE
	2	14 : 50	30	22.0	75.41	28.97		4.61	48	7.6	5.08	51	7.6	
	2	16 : 0	31	22.0	75.41	27.68		5.29			6.84			
	2	21 : 45	37	22.0	75.41	25.82		10.95	115		12.29	90		
Dec. 19	3	8 : 40	47	21.7	75.41	30.15		14.20	190	7.5	13.16	190	7.5	
	3	22 : 45	62	21.5	75.42	30.74		17.31	105		18.80	110		
Dec. 20	4	9 : 15	72	20.5	75.42	26.17		15.49	180	7.6	15.16	182	7.6	
	4	23 : 30	86	22.0	75.42	24.53		13.73	105	7.6	15.52	105	7.6	
Dec. 21	5	10 : 45	98	20.2	75.42	28.86		13.51	180	7.4	14.38	180	7.4	
Dec. 22	6	9 : 50	121	21.0	75.41	26.13		15.17	170	7.5	15.63	170	7.5	
	6	10 : 45	122					15.17	0		15.63	0		Add Culture
	6	14 : 35	125	21.0	75.41			14.21	25		17.48	25		
	6	21 : 0	132					13.96	87		15.60	85		
Dec. 23	7	10 : 15	145	20.5	75.41			10.99	177	7.2	16.30	174	7.5	
	7	12 : 0	147					10.46	15		12.01	15		
	7	17 : 0	152					5.39	50		9.03	50		
	7	22 : 45	158					2.72	90		4.55	90		
Dec. 24	8	9 : 30	168	20.4	75.41			2.05	162	7.8	2.90	165	7.6	
	8	21 : 45	181					0.63	90		2.28	90		
Dec. 25	9	15 : 30	198	20.0	75.41			0.34	220	7.8	1.97	200	7.7	
Dec. 26	10	22 : 0	229					0.34	0		1.97	0		
Dec. 27	11	13 : 10	244	20.0	75.48	26.15		1.35	120	7.9	1.72	75	7.7	Add TCE
	11	17 : 0	248			24.89		1.20	30		1.67	32		
	11	23 : 30	254			22.88		4.02	75		4.56	75		
Dec. 28	12	9 : 50	265	19.5	75.48	24.96		4.51	147	8.1	9.93	135	7.6	
	12	13 : 0	268			23.17		6.12	50		10.76	17		
	12	16 : 40	271			27.96		9.09	52		11.37	50		
	12	20 : 45	276			25.41		9.61	75		12.36	73		
Dec. 29	13	10 : 10	289	19.5	75.50	26.76		14.00	180		15.28	165		
	13	15 : 0	294			24		11.82			14.89			
Dec. 30	14	9 : 30	312	17.0	75.46	26.22		12.71	175	7.9	12.70	185	7.8	
	14	14 : 0	317			30.34		11.96	35		13.00	40		
Dec. 31	15	9 : 30	336	20.0	74.50	28.55		13.05	176	7.9	13.82	191	8	
	15	22 : 0	349	20.0	74.50			12.97	95		12.02	105		Add Culture
Jan. 1	16	13 : 15	364	20.0	75.60			7.13	204	7.6	7.26	223	7.7	
	16	22 : 10	373	22.0	75.41			5.16	60		5.57	52		
Jan. 2	17	15 : 20	390	20.0	75.48			1.93	204	7.8	4.41	180	8.3	
Jan. 3	18	10 : 10	409	20.0	75.50			0.94	75		5.67	25		
	18	18 : 0	417					0.84	125		3.82	75		Add TCE
Jan. 4	19	13 : 50	437	20.0	75.48	26.1		1.01	285		3.37	220		
Jan. 5	20	9 : 40	456	20.0	75.48	25.55		2.34	150	7.8	3.30	135	8.1	
Jan.6	21	9 : 30	480	21.0	75.51	27.06		5.54	185	7.6	7.33	180	7.8	
Jan. 7	22	9 : 30	504	21.0	75.51	27.06		9.13	175	7.5	12.1	160	7.7	
	22	23 : 30	518	21.5	75.46	26.49		11.16	80		13.21	80		
Jan. 8	23	13 : 30	532	21.8	75.50	29.4		13.16	190	7.5	15.62	185	7.6	
Jan. 9	24	9 : 30	552	21.0	75.49	29.36		15.21	160	7.5	16.43	160	7.6	
Jan. 10	25	15 : 45	582	22.0	75.47	25.24		14.78	150	7.4	14.07	225	7.5	
Jan. 11	26	10 : 30	601	22.0	75.48	27.2		16.29	135	7.7	16.14	135	7.8	
Jan. 12	27	9 : 30	624	22.0	75.46	26.58		14.78	170	7.6	14.07	170	7.7	
Jan. 13	28	17 : 0	656	22.0	75.49	30.73		16.29	230	7.4	16.14	245	7.6	
Jan.14	29	14 : 10	677	22.0	75.46	30.41		16.90	170	7.5	17.62	162	7.6	
Jan. 15	30	10 : 30	697	20.1	75.26	33.62		17.30	135	7.6	16.87	110	7.7	
Jan.16	31	14 : 0	725	21.0	75.50	29.33		16.74	182	7.2	15.55	200	7.5	
	31	16 : 0	727	21.0	75.50			16.74	0		15.55	0		Add culture
Jan. 17	32	9 : 20	744	20.0	75.48			15.44	135	7.5	16.79	112	7.5	
Jan. 18	33	10 : 30	769	21.0	75.49			5.70	175	7.3	10.24	172	7.8	
	33	17 : 20	776	21.5	75.49			3.13	51		5.76	49.5		
	33	23 : 10	782					3.39			4.17			
Jan. 19	34	15 : 0	798	20.0	75.48			0.42	235	7.7	4.07	175	7.7	
Jan. 20	35	15 : 0	822	20.5	75.49			0.16	100		3.12	100		