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The Influence of Soil Organic Matter on the Fate of Trichloroethylene in Soil

by

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November, 1997

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfilment of the requirements for the degree of
Doctor of Philosophy

ABSTRACT

Trichloroethylene (TCE) contamination of soil and groundwater is extensive in Canada and the U.S. Under saturated soil conditions, TCE partitions with the organic fraction of soil and under anaerobic conditions is subject to biodegradation to dichloroethylene (DCE) isomers and vinyl chloride (VC). However, biodegradation is slow, and TCE often persists with accumulation of the DCE isomers and VC. The objective of the present study was to determine the effect of soil organic matter (SOM) on the fate of TCE in a saturated soil environment under anaerobic conditions. In natural soil, the presence of inorganic minerals as well as indigenous microorganisms greatly complicates the study of the concurrent processes of sorption and anaerobic biodegradation. To overcome such difficulties, a surrogate soil organic matter (SSOM) was used in conjunction with the pure strain *Desulfomonile tiedjei* to determine the effects of sorption and desorption on the anaerobic biodegradation of TCE. Composted sphagnum moss, sterilized so as to eliminate indigenous microbial activity, was implemented as a SSOM since it is representative of natural SOM. *Desulfomonile tiedjei* was utilized as a model of anaerobic consortia that exist in soil since it is a well studied microorganism. Results of the present study indicate that as the initial contact time (*i.e.* aging) between the TCE and SSOM increases, TCE becomes increasingly resistant to biodegradation. The extent of biodegradation declined by 75 % when the SSOM was aged with TCE for 30 d. This decline was attributed to TCE partitioning with SSOM and possibly due to complexation with dissolved organic matter (DOM). Results of this study indicate that soils contaminated with TCE for prolonged time periods may be less amenable to bioremediation efforts. Resistance of TCE to biodegradation was not parallelled by resistance to desorption for the 30 d aging that was studied. Results of longer term biodegradation tests (*i.e.* incubation times of up to 24 d) indicate that *Desulfomonile tiedjei* is capable of sustaining itself on the SSOM. It would appear that the bacteria degraded the SSOM itself to produce a compound with the following molecular formula: $C_3H_2ON_4$. In the longer term biodegradation tests, dechlorination of TCE was approximately 20 times greater in the presence of the SSOM compared to the case where the SSOM was absent. Since VC was never detected following biodegradation, it would appear that *Desulfomonile tiedjei* is a potential candidate for bioaugmentation for the purposes of remediation of contaminated soil. However, it should be noted that the major product of reductive dechlorination by *Desulfomonile tiedjei* was *cis*-1,2-DCE, and although it is considered to be less toxic than TCE or VC, it was also found to be less amenable to sorption by the SSOM compared to TCE. Hence, depending on the concentration of *cis*-1,2-DCE, a subsequent aerobic treatment may be necessary for complete conversion of this intermediate compound to CO_2.

RÉSUMÉ

La contamination des sols et eaux souterraines au trichloroéthylène (TCE) est importante au Canada et aux États-Unis. Sous des conditions de sols saturés, le TCE se partitionne avec la fraction organique du sol et est aussi sujet à la biodégradation en anaérobie qui forme des isomères de dichloroéthylène (DCE) et du chlorure de vinyle (CV). Par contre, cette dégradation est lente et souvent le TCE persiste avec l'accumulation des isomères de DCE et du CV. L'objectif de cette étude est de déterminer l'effet de la matière organique du sol (MOS) sur le sort du TCE dans un sol saturé, donc en milieu d'anaérobie. Dans les sols naturels, la présence de minéraux inorganiques ainsi que de micro-organismes indigènes complique grandement l'étude simultanée des processus d'adsorption et de biodégradation en anaérobie. Pour surmonter ces difficultés, nous avons utilisé un surrogat de matière organique du sol (SMOS) conjointement avec une lignée pure de *Desulfomonile tiedjei* pour déterminer les effets de l'adsorption et de la désorption sur la biodégradation en anaérobie du TCE. La mousse de sphagne compostée et stérilisée pour éliminer l'activité microbienne indigène, a été utilisée comme SMOS parce qu'elle est représentative de la MOS naturelle. Le *Desulfomonile tiedjei* a été utilisé comme modèle pour les consortium en anaérobie existant dans les sols parce qu'il est le sujet d'intenses recherches à travers le monde. Les résultats de cette étude indiquent que lorsque le temps de contact initial (i.e. vieillissement) entre le TCE et le SMOS augmente, le TCE devient de plus en plus résistant à la biodégradation. L'ampleur de la biodégradation décroît de 75% quand le SMOS est vieilli en présence de TCE pendant 30 jours. Nous attribuons cette diminution au fait que le TCE s'est réparti dans le SMOS et possiblement due à une complexation avec de la MOS. Les résultats de cette étude indiquent que les sols contaminés avec du TCE pendant des périodes de temps prolongées peuvent être moins susceptibles à la biodégradation. Dans cette étude avec un vieillissement de 30 jours, la résistance du TCE à la biodégradation n'a pas été accompagnée par la résistance à la désorption. Les résultats pour les tests de biodégradation de plus longue durée (i.e. temps d'incubation jusqu'à 24 jours) indiquent que le *Desulfomonile tiedjei* est capable de se maintenir sur le SMOS. Il apparaît que la bactérie dégrade le SMOS lui-même pour produire un composé dont la formule moléculaire est $C_8H_2ON_4$. Lors d'études de biodégradation de longue durées, la déchlorination du TCE est approximativement 20 fois plus grande en présence du SMOS comparativement au cas ou le SMOS est absent. Puisque le CV n'a jamais été détecté après les études de biodégradation, il apparaît que le *Desulfomonile tiedjiei* est un candidat potentiel de bio-augmentation pour la remédiation des sols contaminés. Par contre, on note que le produit majeur de la déchlorination réductrice du *Desulfomonile tiedjei* est le cis 1,2-DCE; et bien qu'il soit considéré comme étant moins toxique que le TCE ou le CV, il est tout de même plus mobile que le TCE. Ainsi, selon la concentration de cis 1,2-DCE, un traitement en aérobie peut être nécessaire pour la conversion de ce produit intermédiaire en CO_2.

Acknowledgements

In writing an acknowledgement at the end of ones doctoral studies, there are three words that are frequently used in recognizing others : 'thanks', 'support', and 'guidance'. These words appear constantly throughout this acknowledgement because there were many people who contributed directly and otherwise to the completion of this research.

I would like to thank Professor R.N. Yong for his support and guidance. In addition, this work was funded by the Natural Sciences and Engineering Research Council of Canada through a grant to Professor R.N. Yong.

Professor Jim Nicell provided invaluable support and guidance in the completion of this work. Thank you Jim. I will always be grateful for your support. I would also like to thank Professor Subhasis Ghoshal for his guidance and expert advice in the completion of this work. I have greatly benefited from our discussions and your suggestions.

I especially owe thanks to the Biotechnology Research Institute of the National Research Council of Canada for support and assistance that was critical to the completion of this work. In particular, I would like to thank Dr. Serge Guiot, Leader of the Environmental Bioengineering Group for his assistance and support. Your expertise and experience in the anaerobic biodegradation of chlorinated solvents were invaluable to this research. Dr. Jalal Hawari, Leader of the Environmental Analytical Chemistry Laboratory, also provided much needed assistance. I would also like to thank Professor Stephen Brown of Queen's University whose advice was extremely helpful in the preparation of this thesis.

A very special thanks goes to Professor Jim Tiedje and Jim Cole of Michigan State University for providing the gift of the *Desulfomonile tiedjei* culture (more than once), and for their invaluable and insightful discussions throughout the course of this research.

Thanks also goes to Sylvie Rocheleau for her assistance in establishing the necessary laboratory protocols required in working with strict anaerobes. The technical assistance of Stephane Deschamps, Ed Zhou, Alan Corriveau, and Louise Paquet in regards to analytical chemistry are most gratefully acknowledged.

Thanks also goes to the former Geotechnical Research Centre of McGill University for use of facilities and expertise of technical staff during the initial stages of this research. In particular, Dr. A.M.O. Mohamed and Sangeeta Khanna provided much needed technical support.

I would like to express my deepest gratitude to my family for their constant support and encouragement throughout this adventure. To my husband Diarmid and my daughter Islay Campbell, I absolutely could not have done it without your support and humour. To my mother Margaret Sheremata for helping me with my computer and other woes at the end. To my father William Sheremata for his support and encouragement in realizing my goals. To my sister (fellow Ph.D. candidate) Willow, who was a great friend and source of support. To my parents in-law Marion and Ian Campbell for their support.

I want to thank Kim Hibbeln for her friendship and technical support through the past four years at McGill and BRI.

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ABBREVIATIONS

ABE	: Adsorption Bond Energy
AC	: Activated Carbon
AEC	: Anion Exchange Capacity, meq/100 g
ArOH	: Aromatic hydroxyl or phenolic functional group
ARV	: Aqueous Reactor Volume
BCA	: Bicinchoninic acid
CAA	: Cold Anaerobic Assay
$Ca(OH)_2$	: Calcium hydroxide
$Ca(NO_3)_2$	: Calcium nitrate
CEC	: Cation Exchange Capacity, meq/100 g
CMC	: Critical Micelle Concentration
2,4-D	: 2,4-Dichlorophenoxy-acetic acid
DCE	: Dichloroethylene
DOM	: Dissolved Organic Matter
DNAPL	: Dense Nonaqueous Phase Liquid
EDB	: 1,2-Dibromoethane
EGME	: Ethylene Glycol Monoethyl Ether
FID	: Flame Ionization Detector
GAC	: Granular Activated Carbon
GC	: Gas Chromatography
GCMS	: Gas Chromatography-Mass Spectroscopy
HCl	: Hydrochloric acid
HEPES	: N-2[hydroxyethyl]-N'-[2-ethanesulfonic acid], hemisodium salt
IOMD	: Intra-Organic Matter Diffusion
IR	: Infrared
IRS	: Infrared Spectroscopy/Spectrophotometry
K	: Sorption partitioning coefficient, L/kg or mL/g
K_a	: Acidity constant
K_b	: Basicity constant
K_d	: Desorption partitioning coefficient, L/Kg or mL/g
K_{oc}	: Chemically specific sorption coefficient normalized for organic carbon, L/kg or mL/g
KBr	: Potassium bromide
KCl	: Potassium chloride
kGy	: Kilo Grey
MCL	: Maximum Concentration Limit, mg/L
MCLG	: Maximum Concentration Limit Goal, mg/L
MF	: Molecular Formula
MPN	: Most Probable Number
MW	: Molecular Weight
NaCl	: Sodium chloride

NaOH	: Sodium hydroxide
NOC	: Nonionic Organic Contaminant
NMR	: Nuclear Magnetic Resonance
% OC	: % Organic Carbon
PAH	: Polyaromatic Hydrocarbon
PCE	: Perchloroethylene
PCDD	: Penta- to heptachlorinated dibenzo-p-dioxin
PCDF	: Dibenzofuran
PCP	: Pentachlorophenol
PD	: Pore Diffusion
ROH	: Aliphatic hydroxyl or alcohol group
SOM	: Soil Organic Matter
SRPD	: Sorption Retarded Pore Diffusion
SSA	: Specific Surface Area, m^2/g
SSOM	: Surrogate Soil Organic Matter
TCE	: Trichloroethylene
TNT	: Trinitrotoluene
TOC	: Total Organic Carbon
UV	: Ultra Violet
U.S. EPA	: United States Environmental Protection Agency
VC	: Vinyl Chloride

TERMINOLOGY

Abiotic losses: Refers to contaminant losses that do not result from biological activity.

Aging: The time that a contaminant is resident in soil prior to the introduction of microorganisms for the purpose of biodegradation or prior to the introduction of conditions conducive to desorption.

Bioconversion/Biodegradation/Biotransformation: Are used interchangeably to refer to the partial microbial breakdown of organic contaminants.

Biotic losses: Contaminant losses that are due to microbial breakdown or complete mineralization.

Mineralization: The complete microbial conversion of an organic contaminant to CO_2.

Enrichment culture: A culture of microorganisms capable of degrading a contaminant(s) due to prior contact.

Chapter 1

Introduction and Problem

1.1 Introduction

Since its discovery in 1864, trichloroethylene (TCE) has become a widely used industrial and commercial solvent (Aviado *et al*, 1976). It has been used as a medical analgesic, a degreaser of machinery, an adhesive, a solvent in hair spray, a component in spot removers, cleaning solvents, and as a low temperature heat transfer fluid (Lloyd *et al*, 1975). Although some of its uses have been curtailed, until recently annual production was 234,000 tonnes worldwide (Kleopfer *et al*, 1985). In 1976, it was estimated that 540×10^6 kg were emitted to the atmosphere and 9.1×10^6 kg to the ocean in the U.S. on an annual basis (Fuller, 1976).

Until 1985, there were two production plants in Shawinigan, Que. that produced TCE by chlorination of ethylene (Environment Canada, 1993). Presently, 1.4 kt of TCE are imported annually into Canada. It is estimated that much of the TCE imported into Canada enters the environment due to its dispersive usage. Major uses are in the automotive industry for the cleaning of parts. Minor uses include adhesive and copolymer production, dry cleaning, and textile manufacturing.

Entry into the environment is by atmospheric emissions, effluent discharges, landfill leachate, and by accidental spills (Environment Canada, 1993). Since TCE is a dense non-aqueous phase liquid (DNAPL), it tends to migrate downward to groundwater supplies in the subsurface where it forms pools (Kueper and McWhorter, 1992). Chlorinated solvents, such as TCE, tend to solubilize in water and contaminate groundwater (Anderson *et al*, 1992). Subsequently, soil becomes contaminated with TCE by two means: the downward migration of the solvent and by movement of groundwater containing dissolved TCE.

Groundwater contamination by TCE in Canada is widespread (Table 1.1). Data for soils and sediments are less available; however, the limited data that are available indicates that this is also a problem (Table 1.2). Furthermore, although TCE has not been used for food preparation or in imports since 1977, it has been detected in food following limited sampling programs (*i.e.* 51-410 μg/kg in cheese, butter, and fruit pies) (Environment Canada, 1993). The presence of TCE within the food chain is a concern since it is on the Priority Substances List[1].

Table 1.1. Reported concentrations of TCE in groundwater (Environment Canada, 1993).

Site	Concentration range, μg/L
Ville Mercier landfill, Mercier Ville Quebec	102-12,950
Municipal landfill, Glouster Ontario	<d.l. (1 μg/L)-2480
Industrial site, Vancouver B.C.	59.5-21,900
Chemical transfer station, Toronto Ontario	425,000
Industrial site, Manitoba	1165 below site, 470 several km away

d.l. detection limit

[1] Priority Substances List is compiled by the Ministers of Health and the Environment. This list identifies substances that may be harmful to the environment or human health.

Table 1.2. Reported concentrations of TCE in soil and sediment (Environment Canada, 1993).

Site	Concentration range
Sediments, St. Clair river	<0.01-110,000 μg/kg
Soil, chemical warehouse & distribution facility, Vancouver	<d.l.- 4500 μg/kg
Soil, industrial site, Manitoba	<d.l.- 1000 mg/L

d.l. detection limit

In the hydrologic cycle, water in soil is subject to transpiration from groundwater and infiltration from the surface (Domenico and Schwartz, 1990). These inputs of groundwater and surface water to a saturated soil may contain 8 to 12 mg/L of dissolved oxygen. However, the dissolved oxygen is quickly depleted by microbial respiration and chemical oxidation (Howler and Bouldin, 1971). Therefore, if the rate of incoming dissolved oxygen (*i.e.* through transpiration and infiltration) is less than the rate of oxygen depletion, a saturated soil will be anaerobic. Under such conditions, TCE is biodegraded to 1,1-dichloroethylene (1,1-DCE), *cis*-1,2-DCE, *trans*-1,2-DCE, and vinyl chloride (VC) by reductive dechlorination under passive conditions by indigenous bacteria (Mohn and Tiedje, 1992; Vogel and McCarty, 1985). The *cis*-1,2-DCE isomer predominates over *trans*-1,2-DCE, and 1,1-DCE is the least significant intermediate (Bouwer, 1993). Within the family of TCE and its lesser chlorinated ethenes (*i.e.* TCE, DCE isomers, and VC), TCE is the most amenable to reductive dechlorination because it is the most favourable electron acceptor and has the most negative value in the change of Gibbs free energy (Adriaens and Vogel, 1995). Hence, the intermediate products (*i.e.* DCE isomers and VC) often persist for long periods (Fatherpure and Tiedje, 1994).

The intermediate products are also toxic and have been found to accumulate where TCE

contamination has occurred (Gregi & Université Laval, 1993; Kleopfer *et al*, 1985; Pavlostathis and Mathavan, 1992). The relative toxicity of TCE and its intermediate products are reflected by the U.S. Environmental Protection Agency's drinking water standards. Particularly, the maximum contaminant level (MCL) of TCE, *cis*-1,2-DCE, and VC are 5, 70, and 2 μg/L, respectively (Pontius, 1996). Hence, VC is considered to be more toxic than TCE and *cis*-1,2-DCE, as indicated by the MCLs just cited. Therefore, partial degradation of TCE by reductive dechlorination does not eliminate the toxicity associated with TCE. Consequently, remediation of contaminated sites is imperative.

Pump-and-treat is inefficient and slow for subsurface remediation of chlorinated solvents due to their sorption by soils (McCarty and Semprini, 1993). However, pump-and-treat technologies are most useful in preventing further migration of contaminant plumes (Mackay and Harmon, 1993). More importantly, the treatment aspect of said technology usually involves air stripping that merely transfers the contaminant from one medium to another. Hence, biological processes are preferable to physical ones since they are capable of converting the contaminant to innocuous products. *In situ* anaerobic bioremediation is a desirable option for saturated soils. Under such conditions, regardless of permeability, aerobic bioremediation is not feasible due to limited gas flow (Hinchee, 1993). Currently, the use of electron acceptors other than oxygen is under investigation so as to stimulate indigenous or introduced microorganisms for anaerobic degradation of chlorinated solvents (Reinhard, 1993). However, biodegradation studies have been traditionally carried out in the absence of soil thereby eliminating the effects of sorption and desorption on the availability of the contaminant for biodegradation. For *in situ* anaerobic bioremediation to be implemented more successfully in the field, the influence of sorption on contaminant availability must be better understood. To achieve this, further studies involving the concurrent processes of sorption, desorption, and anaerobic biodegradation of TCE are required.

1.2 Problem Development

From the literature, it is known that TCE sorption is associated with the organic fraction of soil (Carter and Weber, 1994; Zytner, 1992). In many cases it has been observed that organic contaminants sorbed by soil are unavailable to bacteria for degradation (Alvarez-Cohen *et al*, 1993; Apajalahti and Salkinoja-Salonen, 1984; Ogram *et al*, 1985). Hence, the rate of desorption and the strength of contaminant association with soil organic matter (SOM) are critical to the biodegradation process. It has also been found that the longer a contaminant is resident in soil (*i.e.* aging) the more resistant it is to desorption (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992) and to biodegradation (Alexander, 1995; Guerin and Boyd, 1993; Hatzinger and Alexander, 1995). An understanding of the effect of aging on the interactive processes of sorption, desorption, and biodegradation of TCE in the presence of SOM is crucial in order to predict the fate of this contaminant in soil and to subsequently implement more effective bioremediation strategies.

It is desirable to study such interactions using only the organic soil fraction since clay minerals in soil may oxidize organic contaminants such as TCE (Yong *et al*, 1997). Hence, isolation of the SOM, that predominantly interacts with nonionic subsurface contaminants such as TCE, must be achieved in order to carry out such a study. However, SOM in natural soils is intricately associated with other minerals and is impossible to extract (Rice and MacCarthy, 1989). To overcome this limitation, others have implemented such surrogate materials as lignin, collagen, and cellulose to represent SOM in sorption studies (Xing *et al*, 1994). However, such surrogate materials do not adequately represent SOM since they have not undergone the biologically mediated humification process that occurs in natural soils. Composted waste materials, including wood shavings, sawdust, peat moss, grape marc, and sewage sludge, have been proposed as amendments for soil depleted of its natural SOM by poor farming practices. However, to the best knowledge of the author, such composted materials have not been utilized to study soil-contaminant interactions.

Anaerobic consortia readily carry out reductive dehalogenation of TCE in soils, sediment, and groundwater (Mohn and Tiedje, 1992). However, such microbial systems are not well understood. Hence, it is thought that the use of pure strains in defined media may prove useful in the study of microbially mediated reductive dehalogenation in the laboratory (Bedard and Quensen, 1995). There have been a number of pure strains capable of carrying out reductive dehalogenation of TCE (Neuman *et al*, 1994; Scholz-Muramatsu *et al*, 1995; Sharma and McCarty, 1996; Shelton and Tiedje, 1984). However, the most extensively studied organism has been *Desulfomonile tiedjei* (Bedard and Quensen, 1995); hence, it can serve as a useful model in the study of microbial dehalogenation in the laboratory. Although *Desulfomonile tiedjei* was originally isolated from an anaerobic sewage sludge (Shelton and Tiedje, 1984), only very recently has it been demonstrated to be capable of dechlorination activity in soil (Fantroussi *et al*, 1997). In the latter study, dechlorination activity was demonstrated for 3-chlorobenzoate, the inducer of the dehalogenase enzyme, and not TCE itself. Hence, not only can this organism serve as a useful model for the study of microbially mediated reductive dehalogenation in the laboratory, it has the potential to be used for bioaugmentation in the bioremediation of soils contaminated with TCE.

1.3 Objective

The objective of the present research was to determine the effect of SOM on the fate of TCE in soil under saturated and anaerobic conditions. As described in the previous section, the fate of TCE is governed by chemical interactions of TCE with soil that are predominantly defined by sorption to and desorption from SOM, as well as by microbially mediated reductive dehalogenation of TCE to lesser chlorinated species such as DCE isomers and VC. Furthermore, the availability of TCE for biodegradation is highly dependent on the processes of sorption, desorption, and aging. Therefore, to study the fate of TCE in soil, the processes of sorption, desorption, and anaerobic biodegradation (*i.e.* reductive dechlorination) will be examined in the presence of SOM as a function of aging.

1.4 Tasks

In order to achieve the objective of this research, the following tasks must be performed:

1. Simulate a surrogate soil organic matter (SSOM) in a manner similar to that which occurs in a natural soil environment. In addition, it was necessary to select a method of sterilizing the SOM that minimizes the alteration of surface properties that may affect sorption. This study represents the first attempt to simulate a SSOM that is truly representative of natural SOM. Sterilization of the SSOM will eliminate potential biological interactions with TCE that could be mistakenly interpreted as irreversible sorption in the sorption-desorption study (described in *task # 2*). Sterilization will also eliminate potential competition between *Desulfomonile tiedjei* and the indigenous microorganisms present in the SSOM.

2. Determine the effect of aging up to 30 d on the following:

- The anaerobic biodegradation of TCE contaminated SSOM by the pure strain bacterium *Desulfomonile tiedjei.*

- The sorption and desorption of TCE and *cis*-1,2-DCE (*i.e.* a major product of biodegradation) by and from SSOM.

3. Determine if *Desulfomonile tiedjei* has the capability of degrading the SSOM itself (*i.e.* utilizing it as a carbon source). If so, this could contribute to the release of soil-bound TCE to the more mobile aqueous phase.

In determining the effects of aging on TCE biodegradation and on the reversibility of sorption (Task # 2), insights that will benefit *in situ* bioremediation technology will be realized. Although the effects of aging on desorption of TCE from soil have been studied (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992), to the knowledge of the author, such effects on TCE biodegradation have not been studied until now. As well, no studies were found in the literature that pertain to the sorptive/desorptive behaviour of *cis*-1,2-DCE in soil. This is an important consideration since *cis*-1,2-DCE is a major product of TCE dechlorination, and further biodegradation of *cis*-1,2-DCE will likely depend on its sorptive/desorptive behaviour.

Identification of possible release of soil-bound TCE by microbial processes (task # 3) has not been identified as such in the literature. The implication of such a finding would be that although TCE bound to soil for long periods may be somewhat resistant to desorption, release to the mobile aqueous phase is feasible by other mechanisms. Identification of microbial release of soil-bound TCE under passive field conditions would render remediation of contaminated sites a more pressing issue.

1.5 Organization of Thesis

This thesis consists of eight chapters and two appendices. An overview of their organization is as follows:

Chapter1: Provides an introduction and development of the problem at hand, the objective and associated tasks of the research, and the organization of the thesis.

Chapter2: Review of the literature with emphasis on the sorptive/desorptive behaviour of TCE and other nonionic organic contaminants, possible mechanisms for sorption and desorption, anaerobic biodegradation of TCE, a portrait of the pure strain dechlorinator

Desulfomonile tiedjei, a summary of the known effects of sorption and desorption on biodegradation, a description of the nature and formation of soil organic matter and sterilization techniques, as well as a review of existing and emerging technologies for *in situ* bioremediation.

Chapter 3: The experimental methodology employed to achieve the objectives and associated tasks outlined in Chapter 1 is described.

Chapter 4: Results and discussion related to the simulation and sterilization of the SSOM that was utilized for this research. This part of the research has been published in the journal Soil Science & Plant Analysis. The candidate is first author for this publication.

Chapter 5: Results and discussion pertaining to the sorption of TCE as a function of aging by the sterile SSOM and its effect on TCE biodegradation by *Desulfomonile tiedjei*. In addition, results pertaining to experiments that were carried out to determine whether the bacterium could survive with SSOM over long periods while maintaining dehalogenation activity are also presented in this chapter.

Chapter 6: Results and discussion for the sequential desorption of TCE and *cis*-1,2-DCE from sterile SSOM as a function of aging. Also included in this chapter are the results from analysis of the SSOM-TCE complex using infrared spectroscopy (IRS).

Chapter 7: An overall summary, conclusions, and recommendations for further study are provided in this chapter.

Chapter 8: This chapter presents statements of originality of this research.

Appendix A: Description of methods for preparation of two media tested for determination

of optimal growth of *Desulfomonile tiedjei*.

Appendix B: Detailed calculations to determine the extent of the dissociation of the oxygen-containing functional groups of the SSOM.

Chapter 2

Literature Review

TCE contamination of the subsurface is widespread and persistent; furthermore, there is concern that TCE has made its way into the food chain (Environment Canada, 1993). In providing remedial measures for such vast subsurface contamination, anaerobic *in situ* bioremediation offers the advantages of cost effectiveness and technical suitability for saturated soils since they are less amenable to oxygen delivery required for aerobic treatment (Hinchee, 1993). However, due to the processes of sorption and desorption, the availability of TCE for biodegradation may be limited. This chapter describes the chemical and physical interactions between TCE and the organic component of soil, and the effect that such interactions may have on the availability of TCE for biodegradation. Furthermore, the process of anaerobic biodegradation of TCE by reductive dechlorination is described and various microorganisms that are capable of carrying out the process are discussed. However, emphasis is placed on *Desulfomonile tiedjei* since this organism was used in the present study.

Subsequently, the nature and formation of soil organic matter, the principal soil component that reacts with nonionic contaminants such as TCE, are described. The role of compost as a potential surrogate soil organic matter is discussed. Also included in this section are methods of soil sterilization, since inclusion of such a procedure was necessary in the present study.

Finally, existing and emerging technologies in the area of *in situ* bioremediation of soil and groundwater contaminated with TCE are discussed so as to provide a framework for the present study.

2.1 TCE Behaviour in the Subsurface

The factors that are known to influence the fate of TCE within the soil system are reviewed in this section. The emphasis of this review is on saturated soil systems. With no net influx of dissolved oxygen, such systems are anaerobic by virtue of the fact that soil gas has been replaced by pore water and any residual oxygen has been consumed by anaerobic or facultative microorganisms (Liss and Baker, 1994).

2.1.1 Sorption of TCE

The term *sorption* is a general one, and is used to describe a variety of mechanisms involved in associations of organic chemicals such as TCE with the solid phase of soil. There is much debate as to the defining mechanism of the sorption of nonionic organic contaminants (NOC), such as TCE, by soil (Pignatello, 1993). The various models of sorption that have been proposed are the subject of a subsequent section in this chapter. There are several chemical and physical properties of TCE and soil that affect the partitioning process.

TCE is a low molecular weight halocarbon that is slightly soluble in water (1100 mg/L at 20 °C; Anderson *et al*, 1992) and its sorption is controlled by the organic carbon content of soil. The transfer of NOCs from water to soil has been described in terms of a solute partitioning process with SOM (Chiou *et al*, 1979). The following ratio has been used to define the sorption parameter K_{OC}:

$$K_{OC} = \frac{(\mu g\ adsorbed/g\ organic\ carbon)}{(\mu g/mL\ solution)} \qquad (1)$$

The chemically specific sorption parameter, K_{OC}, has been useful in assessing the fate and

transport of contaminants in soil (Lyman *et al*, 1990). The K_{OC} is in turn determined from the partition coefficient, K, as follows:

$$K_{OC} = \frac{K}{\%OC} \times 100 \qquad (2)$$

Where % OC is the percentage of organic carbon in the soil. For TCE sorption by soil and model sorbents, the partition coefficient, K, has been defined by the empirical Freundlich sorption isotherm (Carter and Weber, 1994; Grathwohl, 1990; Xing *et al*, 1996, Zytner, 1992). The Freundlich isotherm relates the mass of solute that partitions with the sorbent to the equilibrium concentration of the solute in the aqueous phase, and is defined as follows (Benefield *et al*, 1982):

$$x/m = KC^{1/n} \qquad (3)$$

Where x/m (mg/kg) is the mass of sorbate sorbed per mass of sorbent, K is the sorption capacity constant, or partitioning coefficient, used to define K_{OC} (L/kg), C is the aqueous solute concentration at equilibrium (mg/L), and n is a constant that relates to the intensity of sorption and the heterogeneity of sites. The Freundlich model is valid for the concentration range, solute, and temperature that were implemented in determining the constants K and n (Freundlich, 1926). Hence, K and K_{OC} may be different for varying solute concentration ranges. Furthermore, in the development of the empirical Freundlich sorption isotherm, it was assumed that the sorbent was heterogeneous and was composed of different classes of sorption sites (Freundlich, 1926).

Earlier studies involving the sorption of hydrophobic compounds by soil found that the following simplified partition equation was applicable (Karickhoff *et al*, 1979; Means *et al*, 1980; Schwarzenbach and Westall, 1981):

$$x/m = KC \qquad (4)$$

Where 1/n = 1. However, as noted above, this simplified partition equation is not valid for TCE (Carter and Weber, 1994; Grathwohl, 1990; Xing *et al*, 1996; Zytner, 1992).

In one study, the Langmuir sorption isotherm was found to be applicable in describing TCE uptake by silicalite, a synthetic zeolite (Alvarez-Cohen *et al*, 1993). The Langmuir isotherm may be expressed as follows (Benefield *et al*, 1982):

$$x/m = \frac{abC}{(1 + aC)} \tag{5}$$

Where x/m and C are the same as in equations (3) and (4), and a and b are constants.

The Langmuir isotherm assumes that the solute has equal affinity for all sorption sites and that monolayer sorption behaviour will occur (Langmuir, 1918). Alvarez-Cohen *et al* (1993) found that equilibrium sorption of TCE by a synthetic zeolite fit the Langmuir isotherm. However, it is unlikely that the inherent assumptions of the Langmuir isotherm are applicable to soil, as is evidenced by the number of reports that have found that the Freundlich isotherm is valid for TCE sorption by soil and model sorbents (Carter and Weber, 1994; Grathwohl, 1990; Xing *et al*, 1996; Zytner, 1992). This likely stems from the fact that the Freundlich isotherm assumes a heterogenous surface containing different classes of sorption sites (Freundlich, 1926). Furthermore, each class of sorption sites follows the Langmuir isotherm behaviour.

The sorption studies conducted to date have utilized concentrations that are less than the solubility limit (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992; Zytner, 1992). This reflects the fact that TCE contamination exists primarily in the dissolved form. As mentioned previously, DNAPLs such as TCE form pools in the subsurface that act as continuous sources for dissolution into groundwater (Kueper and McWhorter, 1992). Hence,

groundwater contamination by TCE, and other DNAPL contaminants, in the soluble form is extensive (Anderson *et al*, 1992).

Zytner (1992) studied the characteristics of TCE sorption by a sandy loam soil, an organic top soil, peat moss, and granular activated carbon (GAC). These experiments were conducted in batch mode with soil slurries containing dissolved TCE (175-875 mg/L). The time required to reach apparent equilibrium for sorption was found to be 2 d, on average. Furthermore, the average K_{OC} (118 L/kg) determined for these soils indicates that TCE is moderately mobile in soil. Compounds with K_{OC}'s that are less than 100 L/kg are considered to be highly mobile; whereas, compounds with K_{OC}'s in the range of 1000 L/kg are considered as immobile in soil (Zytner, 1992). This study concluded that soils high in organic carbon are effective in sorbing and retaining dissolved TCE.

In another study conducted by Pavlostathis and Jaglal (1991) batch sorption tests were conducted for an initial TCE concentration that ranged from 7.14-50.52 μg/L. It was observed that up to 88 % of TCE was removed by a natural soil that contained 0.13 % organic carbon. The time required to reach apparent equilibrium with respect to sorption was found to be 3 d. Xing *et al* (1996) found 48 h as sufficient time to reach apparent equilibrium for TCE sorption by natural soil, peat, humic acid, cellulose, chitin, and polyethylene. In addition, Grathwohl (1990) determined that after 1 d, additional sorption of TCE by a variety of natural soils did not occur in the subsequent 2 d.

As mentioned earlier, sorption of TCE by soil from water is governed by the organic fraction of soil since it is an NOC (Chiou *et al*, 1983). In saturated soil, metal ions that are adsorbed onto mineral surfaces are hydrated. This renders the mineral surface hydrophilic, and hydrophobic nonionic organic compounds such as TCE are unable to approach. However, Estes *et al* (1988) concluded that a sodium saturated montmorillonite clay was capable of sorbing up to 75 % of dissolved TCE at 50 μg/L over a 28 d period. In these experiments,

the clay mineral was treated with 20 % H_2O_2 to remove the organic material. Despite this pretreatment, the clay still contained 0.29 % organic carbon. This was more than double the amount present in the natural soil studied by Pavlostathis and Jaglal (1991). As noted above, this natural soil was quite capable of TCE sorption at these low levels of organic matter. Therefore, in both cases it is possible that the trace quantity of organic carbon was responsible for the observed uptake of TCE at such low concentrations as opposed to the montmorillonite clay, as concluded by Estes *et al* (1988).

The time frame for sorption in soils is several years as compared with several days that is often implemented in the laboratory (Pignatello, 1993). As mentioned above, in the laboratory, sorption of TCE over 1-2 d has been described by the Freundlich sorption isotherm (Grathwohl, 1990; Xing *et al*, 1996; Zytner, 1992). However, the apparent partition coefficient, K_{APP}, has been observed to increase over prolonged periods (Pignatello and Xing, 1996). In this last study, the apparent Freundlich partition coefficients for long (*i.e.* > 10 d) contact times ($K_{APP,LONG}$) were compared to those obtained for short (*i.e.* < 3 d) contact times ($K_{APP,SHORT}$). This study reported ratios of apparent partitioning coefficients obtained over long periods to those obtained over shorter periods (*i.e.* $K_{APP,LONG}$ / $K_{APP,SHORT}$). These ratios were found to vary from 3 (for PCE) to 10 (for 1,2,4,5-tetrachlorobenzene). Karickhoff (1980) proposed a two compartment model for NOC sorption kinetics by soil that may explain this observed bi-phasic sorption over long periods. According to this model that is illustrated in Figure 2.1, the distribution of sorbed NOCs between the labile and non-labile phases is dependent on the percentage of organic carbon in the soil, the solubility of the NOC, and the residence time. As the residence time for contamination and the percentage of organic carbon increases and as the solubility of the NOC decreases, the fraction of non-labile sorbed NOC (*i.e.* S_2) increases and the labile (*i.e.* S_1) and dissolved (*i.e.* C) fractions decrease. According to this two-compartment model, the kinetics of mass transfer between the labile phase and the aqueous phase are much faster than the kinetics of mass transfer between the non-labile and labile phases. Furthermore, in the initial rapid uptake of the

contaminant, the apparent partition coefficient (*i.e.* $K_{APP,SHORT}$) can be orders of magnitude less than the apparent partition coefficient of the second slower (*i.e.* $K_{APP,LONG}$) phase of contaminant uptake (Pignatello and Xing, 1996). The division between these two phases can be hours or days.

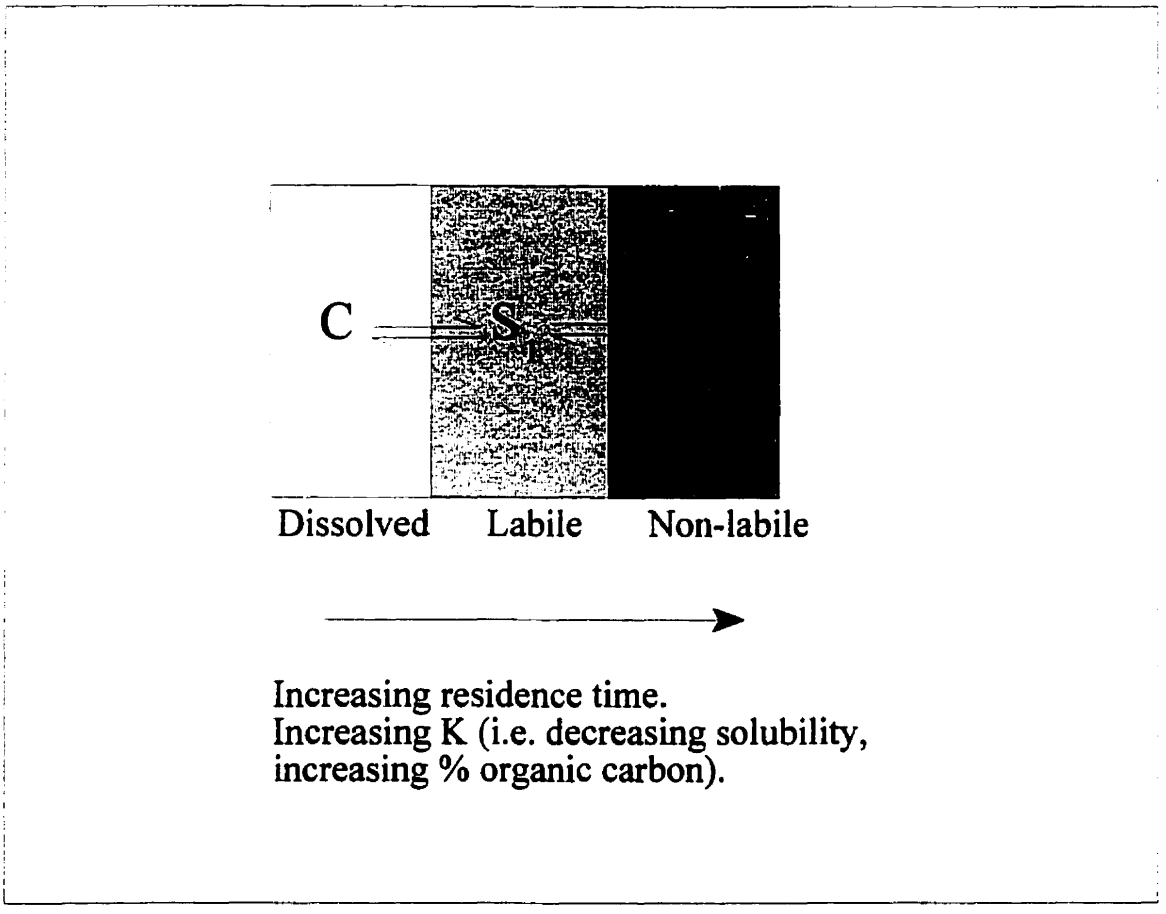

Figure 2.1. Conceptual representation of the two-compartment model of sorption kinetics for NOC (adapted from Karickhoff, 1980).

It is thought that short term laboratory studies merely represent labile sorption kinetics, and that the longer term non-labile, or non-equilibrium, sorption kinetics are not represented in the "tens of thousands of experiments....carried out with equilibration times of 72 h or less..." (Pignatello, 1993).

2.1.2 Desorption of TCE

An understanding of the desorptive behaviour of soil bound NOCs, such as TCE, is critical in determining their fate and transport. However, it is difficult to adequately study desorption in the laboratory for the purposes of determining the reversibility of sorption since the residence times often employed (*i.e.* 24-74 h) do not adequately represent field conditions (Pavlostathis and Mathavan, 1992).

As well as studying sorption, Zytner (1992) studied the desorption of TCE from the four granular materials mentioned previously (*i.e.* sandy loam soil, organic top soil, peat moss, and GAC). Following sorption of TCE by the granular materials for a period of 48 h, all of the supernatant was removed and replaced by de-ionized water, and the slurries were equilibrated for time periods in the range of 100 h. The time required to reach equilibrium for desorption was considerably longer than that required for sorption (107 h, on average). The materials with a higher proportion of organic carbon were found to contain a higher proportion of TCE that resisted desorption. Desorption was expressed by the following Freundlich desorption isotherm:

$$x_d/m = K_d C^{1/n_d} \qquad (6)$$

Where x_d was defined as the mass of sorbate released (mg), K_d as the equilibrium desorption constant, n_d as a constant, and C and m were unchanged from the previously defined Freundlich sorption isotherm (equation 3). Of course, this equation is only valid for equilibrium desorption conditions and is subject to error as a result of the solid-liquid separation required in removing the supernatant and replacing it with de-ionized water. Entrained solids containing sorbed TCE in the supernatant removed for analysis is a potential

source of error inherent in this desorption procedure.

Other studies have indicated that the resistance of TCE to desorption increases with the residence time (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992). In one set of tests, the effect of residence time on desorption was studied. The residence time was varied (2.5, 5.5, and 15.5 months), and the TCE remaining in the soil following 12 d of desorption with de-ionized water was found to be proportional to the residence time (10, 32, and 45 % TCE remaining, respectively) (Pavlostathis and Mathavan, 1992). Furthermore, most of the TCE was desorbed within the first 24 h.

In another study, a soil that had been contaminated with TCE for at least 18 years was found to be resistant to desorption regardless of variations in pH (Pavlostathis and Jaglal, 1991). Results of batch tests indicate that about 25 % of TCE was desorbed with de-ionized water after 22 h, and that little more TCE was desorbed for the remaining 6 d of the test. In the same study, continuous desorption was employed in a column. After 24,000 pore volumes of organic-free distilled water were passed through the column, 72 % of the TCE was desorbed and further quantities of TCE in the effluent were non-detectable. The soil in this study consisted of 0.13 % organic carbon, 12 % clay (unidentified), 32 % silt, and 56 % sand.

In the last two studies cited, biphasic desorption was evident. In the first phase, a majority of TCE was desorbed fairly rapidly. This was followed by a second phase where desorption occurred more slowly. In both studies, it was concluded that TCE desorption in the second phase was a function of the extended time periods that the soil had been contaminated. This was consistent with the results of Zytner (1992) for freshly contaminated soils where monophasic desorption of TCE was observed. As mentioned previously, desorption of TCE from freshly contaminated soil fit the Freundlich desorption isotherm outlined by equation (6). Such biphasic desorption is consistent with the two-compartment sorption model proposed by Karickhoff (1980) that is outlined in Figure 2.1. The first phase of rapid

desorption observed in soil that had been contaminated for long periods (Paviostathis and Jaglal, 1991; Paviostathis and Mathavan, 1992) may be likened to the labile fraction (*i.e.* S_1) and the second phase of slower desorption to the non-labile fraction (*i.e.* S_2).

In a recent study by Deitsch and Smith (1995), TCE desorption from two soils with varying levels of organic carbon (*i.e.* 24 % soil A and 1.36 % soil B) was studied for varying aging times (*i.e.* 1 and 4 wks). Desorption was carried out in a continuous-flow stirred tank reactor following aging of the two soils with an equivalent of 250 mg/L TCE for the two time periods indicated above. For soil A that contained 24 % organic carbon, 48 % of the TCE was removed in 20 aqueous reactor volumes (ARVs) following 1 wk aging, and 38 % TCE was removed in the same number of ARVs for 4 wk aging. For soil B, all of the TCE was removed in 20 ARVs following 1 wk aging. Hence, as illustrated in Figure 2.1, increased residence time (*i.e.* aging) and organic carbon content both increase the amount of the non-labile fraction (*i.e.* S_2).

In the study of desorption of three NOCs (*i.e.* naphthalene, phenanthrene, and p-dichlorobenzene) from a natural sediment containing 0.27 % organic carbon, Kan *et al* (1994) implemented an innovative technique of sequential desorption. Data from sorption tests conducted over 1-4 d fit a Freundlich isotherm model, and the calculated K_{OC}'s compared well with those reported in the literature. Desorption was carried out with successive dilutions by removing 60 to 80 % of the supernatant and replacing it with fresh electrolyte solution over a time period of 137 d. The time required for desorption was significantly longer (*i.e.* 137 d) compared with the time implemented for sorption (*i.e.* 1-4 d). Another advantage of conducting successive desorption, although it was not pointed out in this study, is that errors resulting from entrained solids in the supernatant are eliminated. When sorption is calculated as the difference between the total chemical concentration measured and that measured in the aqueous phase, entrained solids in the aqueous/supernatant will result in under estimation of sorption. Although this may not be a

problem for soils containing low levels of SOM that are amenable to centrifugation, as the SOM content of a soil increases, solid-liquid separation by centrifugation becomes more difficult. Hence, the desorption technique identified by Kan *et al* (1994) appears to be an alternative method for soils with high levels of SOM.

2.1.3 Possible Mechanisms of Sorption and Desorption

As mentioned previously in this chapter, the defining mechanism for sorption, and hence desorption, is the subject of considerable debate (Brusseau *et al*, 1991; Pignatello, 1993; Pignatello and Xing, 1996; Wu and Gschwend, 1986). Although some of the overall patterns of sorption and desorption were discussed, this section outlines some mechanisms that have been proposed in the literature that may help to explain these rate-limited processes.

As outlined earlier, sorption time scales in the field are lengthier than what is normally implemented in the laboratory (*i.e.* years versus days). Consequently, the sorption-desorption process may be rate limiting to other dissipation processes, namely biodegradation (Pignatello, 1993). Four possible mechanisms responsible for retarded sorption-desorption are discussed in this section and are depicted in the conceptual diagram of Figure 2.2.

2.1.3.1 Pore Diffusion (PD)

According to this concept, sorption-desorption is retarded as a result of molecular diffusion through pore water where advection is absent. Retarded pore diffusion in fixed-pore systems of catalyst beads and zeolites has been described by the following equation (Brusseau *et al*, 1991, where the ratio D_p/D_o was incorrectly transposed in the paper just cited):

$$\log(D_p/D_o) = -0.5 - 1.98\,\lambda \qquad (7)$$

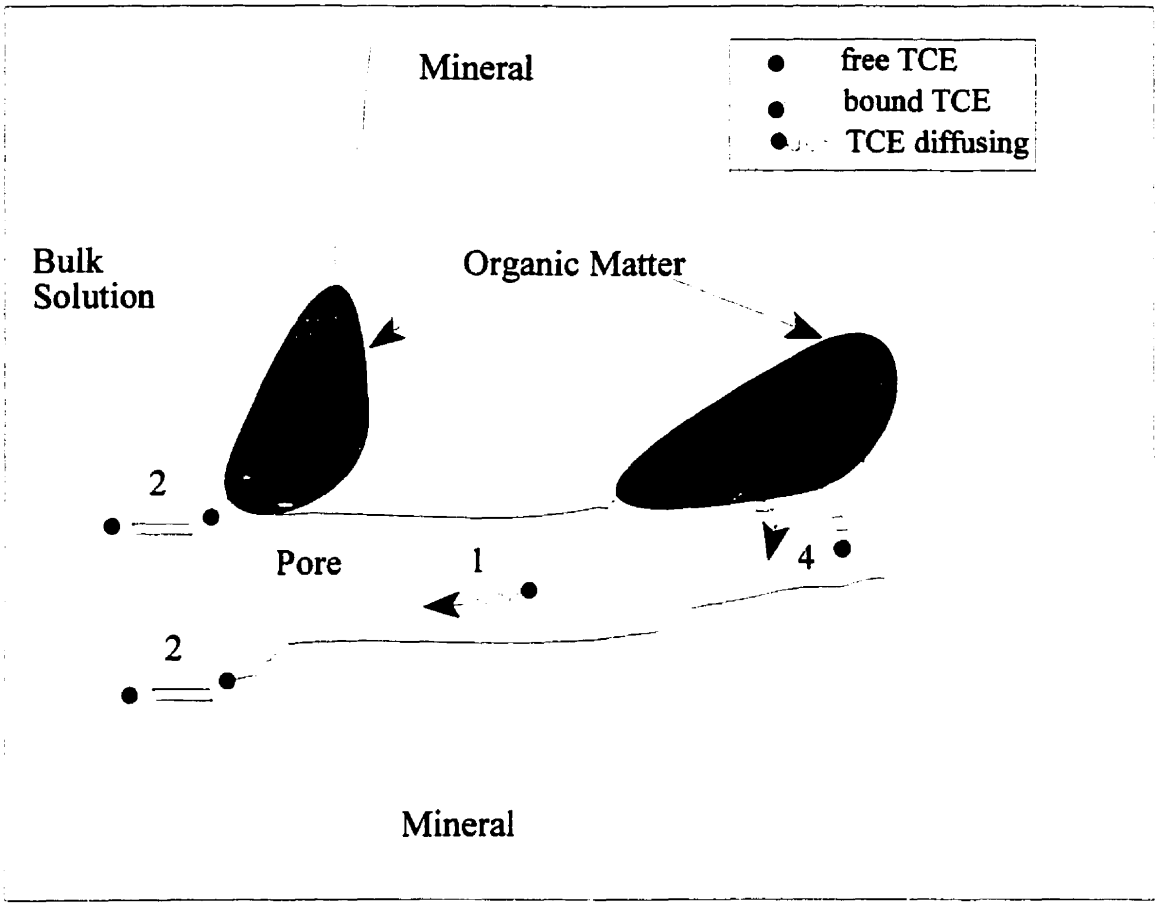

Figure 2.2. Four possible mechanisms for rate-limited sorption of organic contaminants in natural soil: (1) pore diffusion in pore water (PD); (2) adsorption bond energy (ABE); (3) intra-organic matter diffusion (IOMD); and (4) sorption-retarded pore diffusion (SRPD) (adapted from Pignatello, 1993).

Where D_o is the aqueous diffusion coefficient (L^2/T), D_p is the pore diffusion coefficient, and λ is the ratio of the solute molecular diameter to the pore diameter. The regression coefficients are averages of those reported in a number of studies. For a molecular diameter of < 1nm, the pore diameter would have to be approximately 25 nm for there to be retarded diffusion. The pore size distribution of a sandy aquifer was reported to be as follows: 80 % of the internal pore volume was composed of pores with diameters that were greater than 25 nm and 90 % of the internal pore volume had pore sizes greater than 10 nm (Brusseau *et al*, 1991). Based on these results, it was not expected that retarded pore diffusion would be a

factor in nonequilibrium, or retarded, sorption or desorption of solutes in a sandy aquifer material. However, in soils containing clay sized particles and organic matter, aggregates may contain an appreciable proportion of pore diameters that are significantly less than 25 nm. As an example of PD, Ball and Roberts (1991b) found that pore diffusion coefficients (D_p) for PCE and 1,2,4,5-tetrachlorobenzene (TeCB) in a sandy aquifer material were 2-3 orders of magnitude lower than the bulk aqueous diffusion coefficients (D_o).

2.1.3.2 Adsorption Bond Energy (ABE)

NOCs can form van der Waals interactions, dipole-dipole interactions, ion-dipole interactions, and hydrogen bonds with organic matter in soil (Pignatello, 1993). As mentioned previously, such interactions do not occur between NOCs and the mineral components of soil under saturated conditions, but rather with the organic fraction. The energy required to form these individual bonds is just a few kcal/mol, and they are formed and broken in short time periods.

Dipole-dipole interactions and van der Waals forces are weak, but in large molecules (*i.e.* parathion, alachlor, cycloate, benzonitrile, and DDT) their cumulative effect is considered significant in associations with humic substances (Senesi, 1993). However, for smaller sized molecules such as TCE, the cumulative effect is not considered to be significant.

Ion-dipole interactions and hydrogen bonding can occur between the oxygen containing functional groups of SOM (that are ionized and hence negatively charged) and partial charges of slightly polar NOCs. Ion-dipole interactions can occur between ionized carboxyl and phenolic functional groups of the SOM and the partial dipole moment of the H atom of TCE. Hydrogen bonding between the chlorine atoms of TCE and the hydrogen atoms of the oxygen-containing functional groups of humic substances stem from the following factors (Smith, 1973):

1. The chlorine atom is basic (*i.e.* has the ability to donate electrons).

2. The oxygen-containing functional groups (*i.e.* phenolic and carboxylic acid) are acidic.

3. Absence of steric effects. The small size and lack of branching in TCE and DCE isomers enable them to approach more freely than some larger sized molecules.

Some consider ABE as unlikely to be responsible for the retarded long-term desorption behaviour that has been observed for small molecules such as TCE (Pignatello, 1993).

2.1.3.3 Intra-Organic Matter Diffusion (IOMD)

In IOMD, the contaminant (*i.e.* sorbate) diffuses by diffusive mass transfer within the organic matter matrix. It has been proposed that this process is the rate-limiting mechanism in sorption of NOCs by soil (Brusseau *et al*, 1991). The primary assumption in this model is that SOM is a polymeric-type substance within which sorbates can diffuse. Conceptually, IOMD is similar to the partitioning process of NOCs with SOM (Chiou, 1989).

2.1.3.4 Sorption-Retarded Pore Diffusion (SRPD)

SRPD assumes intraparticle radial diffusion kinetics in which an effective diffusion coefficient, D_{eff}, is of the form (Pignatello, 1993):

$$D_{eff}=\frac{D_w k_r}{\tau(1+\beta K_p/\theta)} \qquad (8)$$

Where D_w is the bulk aqueous diffusivity, $k_r (\leq 1)$ is a term to account for pore constriction and molecular steric hindrance, and τ (≥ 1) is a term that accounts for non-linear diffusion paths, dead end paths, and pores with variable diameters. Furthermore, K_p is a partition

coefficient for equilibrium sorption on pore walls, β is the particle density, and θ is the particle porosity. By inspection of the above equation, it is evident that diffusion of contaminant solutes in pore water can be retarded by sorption $(1 + \beta K_p/\theta)$, tortuosity (τ), and constriction (k_r). An important assumption of the SRPD model is that it assumes that most sorption occurs inside particles.

In comparing IOMD with SRPD, two basic physical differences between diffusion in organic matter and micropores must be emphasized (Brusseau *et al*, 1991). The first difference is related to the pore size. In the case of organic matter, the pores are comparable in size with that of the diffusing solute; whereas, in the case of particles, the pores in which the contaminant solutes diffuse are much larger than the solutes themselves. The second difference relates to the rigidity of the porous network. The porous network of organic matter is dynamic in that it is undergoing constant change, and the porous structure of soil particles is considered relatively fixed. Due to these physical differences, IOMD should not be considered in the same way as diffusion in fixed porous structures.

2.1.4 Migration Potentials of Chlorinated Ethenes

Specific migration potentials of compounds have been compared on the basis of K_{OC} values (Nyer, 1993). As was described in the previous section, K_{OC} is determined from the partition coefficient K. Where K has been found to be represented by the Freundlich sorption isotherm for TCE (Carter and Weber, 1994; Grathwohl, 1990; Xing *et al*, 1996; Zytner, 1992). In determining K_{OC} from the partition coefficient K, sorption studies are typically carried out with soil as described by Lyman *et al* (1990). In general, K_{OC} values obtained for specific compounds are relatively constant and reasonably independent of soil or sediment; however, variations have been observed. In particular, K_{OC} values obtained from a variety of soils and sediments can have coefficients of variation of 10 to 140 % for the same compound (Lyman *et al*, 1990). Hence, it would appear preferable to determine the relative K_{OC} of TCE and *cis*-1,2-DCE, its major intermediate, for the particular soil of interest in bioremediation studies. The K_{OC}'s for TCE, utilizing a variety of sorbent materials, have been reported by Zytner (1992), and are listed in Table 2.1.

The variation in K_{OC} described by Lyman *et al* (1990) is well illustrated by the results in Table 2.1 for TCE. Values of K_{OC} obtained experimentally for *cis*-1,2-DCE using soil were not found in the literature at the time of this study.

Table 2.1. K_{OC} of TCE for a variety of sorbent materials (Zytner, 1992).

Material	K_{OC} (mL/g, or L/Kg)
Sandy loam	50
Organic top soil	115
Peat moss	189

To overcome the difficulty posed by determining the K_{OC} from the partition coefficient K using soil, the octanol-water partition coefficient, K_{OW}, is commonly used to estimate K_{OC}. The advantage to this approach is that the costs associated with experimentally determined sorption isotherms using soil are avoided. Values of K_{OW} for a number of contaminants can be found in the literature (Nyer, 1993). The K_{OW} of a compound is defined by the following ratio (Lyman *et al*, 1990):

$$K_{OW} = \frac{\text{Concentration in octanol phase}}{\text{Concentration in aqueous phase}} \quad (9)$$

The K_{OC} has been related to the K_{OW} and the solubility (S) of a chemical compound according to the following equations (Lyman *et al*, 1990):

$$\log K_{OC} = a \log K_{OW} + b \quad (10)$$

$$\log K_{OC} = c \log S + d \quad (11)$$

Where a, b, c, and d are constants. Estimations of K_{OC} using equation (10) are preferable to those obtained using equation (11) (Lyman *et al*, 1990). No reason for this was provided by

the authors, but octanol-water partitioning is probably a better prediction of sorption behaviour in soil as compared to solubility. Values of K_{ow} for perchloroethylene (PCE)[1], TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, and VC are summarized in Table 2.2. On the basis of the K_{ow}'s, one would expect *cis*-1,2-DCE to be more mobile in soil than TCE. Furthermore, one would also expect that PCE would be the least mobile of the chlorinated ethenes listed in Table 2.2.

In estimating K_{oc} from K_{ow} according to equation (10), some studies have demonstrated this relationship to be valid for NOCs (Means *et al*, 1980; Schwarzenbach and Westall, 1981). On the other hand, there are some reported limitations to the validity of this relationship (Xing *et al*, 1994). In this last study, it was found that the quality of the sorbent significantly influenced the partitioning of α-naphthol (an NOC) in aqueous systems that utilized various organic sorbents. The authors concluded that if this observation was true for all NOCs, then K_{oc} cannot be accurately predicted from K_{ow}. In another study, it was found that sorption of the pesticide napromide (another NOC) by sediments was greater than what was observed for soil on an organic matter basis (Gerstl and Kliger, 1990). Equation (10) assumes uniformity of the sorption capacity of organic matter, regardless of its origin. According to Xing *et al* (1994), this assumption may not be valid. In addition, according to the results of Zytner (1992) that are summarized in Table 2.1, it is evident that the quality of the organic matter also affects TCE partitioning.

[1]PCE is the precursor to TCE in the reaction pathway outlined in Figure 2.3 that follows.

Table 2.2. Octanol-water partition coefficients, K_{ow}, for some chlorinated ethenes (Nyer, 1993).

Compound	K_{ow}
PCE	390
TCE	240
cis-1,2-DCE	5.0
trans-1,2-DCE	3.0
VC	24

To illustrate the effect of organic matter quality on sorption, consider that Xing *et al* (1994) found that the K_{OC} for α-naphthol increased from 83 to 436 mL/g as the aromaticity of the organic matter increased from 23 to 35 % (as calculated from NMR spectra). In another study, TCE vapour sorption by a variety of wet soils indicated that the measured K_{OC}'s were very much dependent on the nature and type of SOM (Grathwohl, 1990). For example, the measured K_{OC} was 363 mL/g for a weathered shale and 2692 mL/g for an unweathered shale. The difference between the K_{OC}'s was attributed to the increase in the oxygen-containing functional groups. Particularly, the weathered shale contained more oxygen-containing functional groups that rendered it less hydrophobic, hence the reduced K_{OC}. Two estimates of K_{OC} from the K_{OW} (using a form of equation (10)) were reported as 93 and 141 mL/g by Grathwohl (1990) in the same study. Hence, K_{OC}'s estimated from the K_{OW} (*i.e.* 93 and 141 mL/g) were far below the values measured using soil (*i.e.* 363 and 2692 mL/g).

Hence, based on the work of Gerstl and Kliger (1990), Grathwohl (1990), Xing *et al* (1994), and Zytner (1992), it would appear appropriate to conduct sorption/desorption experiments under the same conditions as those carried out for biodegradation in order to study the possible effects of sorption on biodegradation. Furthermore, utilizing the K_{OW} to estimate contaminant mobility in soil is not accurate. The chemical characteristics of the SOM must

also be considered.

2.1.5 Biodegradation of TCE

TCE undergoes biodegradation in both aerobic and anaerobic environments. In an aerobic environment, microorganisms that oxidize compounds such as propane, methane, and phenol produce an enzyme called monooxygenase (Fan and Scow, 1993; Fliermans *et al*, 1988; Fogel *et al*, 1986; Little *et al*, 1988; Wilson and Wilson, 1985). Monooxygenase is non-specific in that it is able to oxidize a variety of environmental contaminants such as TCE. This is a co-metabolic process since it only occurs in the presence of other organic compounds that serve as the primary energy source (McCarty, 1988).

In anaerobic environments, TCE is dechlorinated by reductive dehalogenation (Mohn and Tiedje, 1992). The halogen substituent is removed from the molecule with concurrent addition of electrons. Such dehalogenation is illustrated for TCE in Figure 2.3. Depending on which chlorine atom is removed first, either 1,1-DCE, *cis*-1,2-DCE, or *trans*-1,2-DCE are formed. However, *cis*-1,2-DCE is the predominant product of reductive dechlorination of TCE following the removal of the first chlorine atom, and 1,1-DCE is the least significant intermediate (Freedman and Gossett, 1989). Removal of the second chlorine atom results in the formation of VC. VC is eventually reductively dechlorinated to ethylene (Freedman and Gossett, 1989) and is finally converted to CO_2. The mechanism by which ethylene is converted to CO_2 is unknown (Mohn and Tiedje, 1992). The transformation of TCE is slow and intermediate products (1,1-DCE, *cis/trans*-1,2-DCE, and VC) can accumulate (Kleopfer *et al*, 1985). Furthermore, the chemically mediated breakdown of TCE and its intermediates under saturated soil conditions is negligible. The fate of the DCE isomers and VC in soil has not been studied and is not well understood (Fogel *et al*, 1986). However, their accumulation has been related to the slow rate of dechlorination of the parent compound. In particular, as chlorine atoms are removed, the rate of dechlorination becomes slower (Chu and Jewell,

1994). As described in Chapter 1, accumulation of these intermediate compounds is of concern since they are on the Priority Substances List. Hence, the partial degradation of TCE does not eliminate the potential toxicity associated with it.

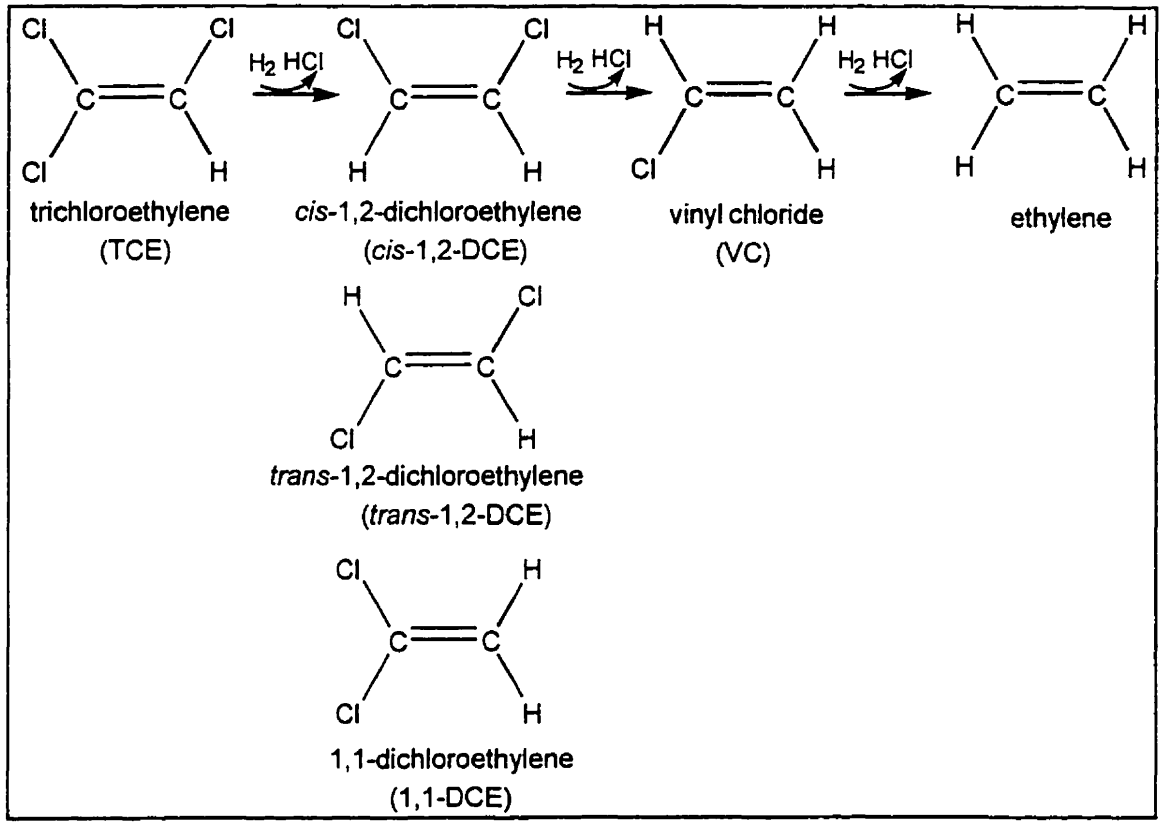

Figure 2.3. Reductive dehalogenation of TCE (Freedman and Gossett, 1989; Mohn and Tiedje, 1992).

Table 2.3 lists some strains of bacteria that have been reported to be capable of carrying out TCE degradation. Although it has been widely reported that reductive dehalogenation of PCE and TCE to DCE and VC is initiated by anaerobic bacteria, there is evidence that these transformations can also be carried out by a facultative aerobic bacterium (*i.e.* strain MS-1) in the absence of oxygen (Sharma and McCarty, 1996).

Table 2.3. Pure strain bacteria reported to degrade PCE and TCE.

Bacterium	Physiological group based on gaseous atmosphere	Biodegradation mechanism/pathway	Reference
strain MS-1, with characteristics matching *Enterobacteriaceae* family members.	facultative aerobe	PCE→TCE→*cis*-1,2-DCE (*i.e.* reductive dehalogenation)	Sharma and McCarty (1996)
Pseudomonas putida F1; *Pseudomonas* sp. strain JS150; *Pseudomonas fluorescons* CFS215; *Pseudomonas* sp. strain W31	aerobe	toluene dioxygenases and monoxygenases	Leahy *et al* (1996)
Pseudomonas cepacia G4	aerobe	monoxygenase	Krumme *et al* (1993)
Methylosinus trichosporium OB3b PP358	aerobe	soluble methane monoxygenase (sMMO)	Fitch *et al* (1996)
Dehalospirillum multivorans	strict anaerobe	PCE→TCE→*cis*-1,2-DCE(*i.e.* reductive dehalogenation)	Neuman *et al* (1994); Scholz-Muramatsu *et al* (1995)
Desulfomonile tiedjei	strict anaerobe	PCE→TCE→*cis*-1,2-DCE(*i.e.* reductive dehalogenation)	Cole *et al* (1995); Shelton and Tiedje (1984)

2.1.5.1 *Desulfomonile tiedjei*

Of the pure strains described in Table 2.3, *Desulfomonile tiedjei*[2] has been the most extensively studied since first reports of its isolation (Shelton and Tiedje, 1984). Dechlorination activity in *D. tiedjei* is induced by 3-chlorobenzoate (Cole and Tiedje, 1990). However, more recently it has been reported that dechlorination is more efficiently induced by 3-fluorobenzoate (Cole *et al*, 1995). In particular, 3-fluorobenzoate itself is not dehalogenated as is 3-chlorobenzoate since the fluorine atom is more electronegative than the chlorine atom; hence, more energy is available for dechlorination of TCE.

Since its isolation from an anaerobic sewage sludge in the early eighties, *D. tiedjei* has been characterized as an obligate anaerobe that requires a reduced oxygen free environment created by other organisms (Mohn and Tiedje, 1992; Shelton and Tiedje, 1984). This organism is thought to be syntrophic[3] within an anaerobic system since some of its products, such as sulphide, are toxic at high concentrations to the organism itself but are removed by other organisms within an anaerobic consortium. Furthermore, *D. tiedjei* is a sulphate reducing bacterium that utilizes pyruvate as a source of carbon. It also possesses a unique morphology in that it is a rod shaped bacillus with a "collar" where it is thought that cell division may occur (Shelton and Tiedje, 1984).

In undefined cultures, or consortia, reductive dechlorination occurs quite readily (Mohn and Tiedje, 1992). Furthermore, sterilization of undefined cultures has resulted in inhibition of reductive dehalogenation. However, such consortia are not well understood (Bedard and Quensen, 1995). Hence, the use of pure strains in defined media may, in some cases, prove

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[2]Herein referred to as *D. tiedjei*.

[3]A type of symbiosis where two or more organisms living together benefit each other by exchanging nutrients with one another.

to be more useful in studying microbial reductive dechlorination in the laboratory. In the study of reductive dechlorination, *D. tiedjei* offers the distinct advantage over other pure strains, some of which are listed in Table 2.3, and consortia in that it has been the most extensively studied (Bedard and Quensen, 1995). Hence, it is for this reason that *D. tiedjei* was selected as a model organism for the present study.

2.1.6 Effect of Sorption and Desorption on Biodegradation

From the previous discussion, it is evident that TCE sorption onto and desorption from soil are governed by the presence of organic matter. Furthermore, the longer a soil is contaminated, the more resistant TCE is to desorption. Hence, the behaviour of contaminants in the subsurface and the success of *in situ* bioremediation are dependent upon the concurrent processes of sorption and biotransformation (Alvarez-Cohen *et al*, 1993). A number of studies have been conducted in the area of TCE sorption and desorption (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992: Zytner, 1992) as well as TCE biodegradation (Fan and Scow, 1993; Fogel *et al*, 1986; Folsom *et al*, 1990; Kleopfer *et al*, 1985; Little *et al*, 1988). However, little research has been conducted concerning the interactions between sorption, desorption, and biodegradation.

A simple model that depicts the bioavailability of contaminants in a saturated soil is described by McCarty (1988) and Pavlostathis and Jaglal (1991). According to this model, the contaminant in the solution phase is biodegraded as opposed to that in the sorbed phase. This model is illustrated for TCE in Figure 2.4. It has been postulated that sorption of contaminants will result in a reduction in the overall rate and degree of biodegradation. When the contaminant in the solution phase is degraded, a concentration gradient between the sorbed and solution phases exist. According to Pavlostathis and Jaglal (1991), this concentration gradient will result in desorption of the contaminant into solution. Furthermore, according to this model, the overall rate of contaminant removal is a function

of the rates of desorption and bacterial utilization. Some research results are consistent with this model (Alvarez-Cohen *et al*, 1993; Ogram *et al*, 1985; Hatzinger and Alexander, 1995; Scow and Alexander, 1992) while others are not (Apajalahti and Salkinoja-Salonen, 1984; Oldenhuis *et al*, 1991). Both sets of differing results are discussed in this section.

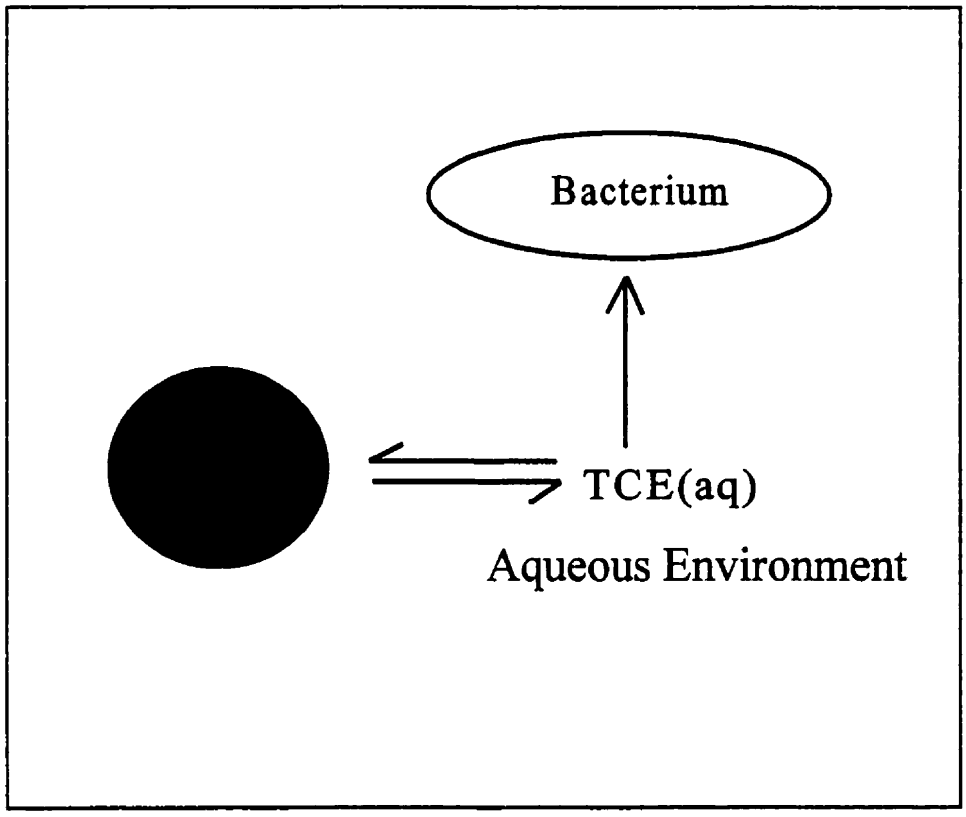


Figure 2.4. Conceptual diagram of the interaction of sorption and biodegradation (adapted from McCarty, 1988).

In one study, the effect of TCE sorption by a synthetic zeolite on its cometabolic transformation by methanotrophic bacteria was investigated (Alvarez-Cohen *et al*, 1993). The availability of sorbed TCE to microorganisms for mineralization was examined by using ^{14}C-labeled TCE within a mixed methanotrophic culture. TCE was able to sorb onto the zeolite by virtue of its hydrophobic properties (Chen, 1976). Results indicate that transformation of solution-phase TCE induced the desorption of TCE from the zeolite.

Furthermore, the rate of TCE transformation by microorganisms was found to be reduced in the presence of the zeolite. It was proposed that TCE transformation by the methanotrophic bacteria is proportional to the concentration of TCE in the aqueous phase and is independent of the mass in the sorbed phase.

Estrella *et al* (1993) examined the effects of biodegradation and sorption of 2,4-dichlorophenoxyacetic acid (2,4-D) on its transport through soil columns. Biodegradation, and to a lesser extent sorption, were found to retard 2,4-D transport in soil. In another study, Ogram *et al* (1985) found that sorbed 2,4-D was unavailable for biodegradation by a pure strain bacterium. It was proposed that the sorbed 2,4-D was either protected from degradation or that it was located sufficiently deep within the soil organic matter matrix that the bacteria were unable to attack it. In another study, the kinetics of biodegradation of benzylamine (0.95-25 mg/L initial concentrations) by a bacterial isolate in the presence and absence of montmorillonite (in a suspension of 10 g/L with an inorganic salts solution) was studied by Miller and Alexander (1991). It was found that the rate of biodegradation was less in the clay suspension than in the inorganic salt solution.

Gordon and Millero (1985) found that the presence of soil may hinder biodegradation by limiting the availability of low molecular weight organic acids and sugars. Sorption of glucose, acetic acid, succinic acid, glutamic acid, and citric acid by hydroxyapatite was negatively correlated with biodegradation rates by the bacterium *Vibrio alginolyticus*. This study suggests that adsorption of organic compounds in the nutrient medium by soil may retard the biodegradation of contaminants.

Mihelcic and Luthy (1991) reported that the process of naphthalene sorption-desorption were reversible and rapid compared with the rate of microbial degradation under denitrifying conditions. Furthermore, the maximum rate of degradation was proportional to the soil:water ratio. Guerin and Boyd (1993) studied naphthalene mineralization in four sandy loam soils

(organic carbon contents that ranged from 0.76 to 5.36 %) with two bacteria strains. It was found that the availability of naphthalene was strain dependent. In the case of mineralization by *Pseudomonas putida*, results indicate that the bacterium had access to labile-sorbed naphthalene, as evidenced by the fact that the rate of mineralization was greater than what would be expected on the basis of aqueous phase naphthalene. Consistent with this hypothesis, it was observed that aging up to one year resulted in a decrease in the rate of mineralization, as labile-sorbed naphthalene entered into the non-labile phase. In contrast, sorbed phase naphthalene was unavailable for mineralization by NP-Alk (an isolate from petroleum contaminated soil). Results indicate that when aqueous phase naphthalene was degraded, desorption and subsequent degradation occurred.

Hatzinger and Alexander (1995) examined the effect of aging on biodegradation and extractability. It was found that aging resulted in an increased resistance to biodegradation for phenanthrene and also for 4-nitrophenol. In addition, it was found that with aging both compounds were more resistant to a butanol extraction.

Adriaens *et al* (1995) recently published some results regarding long-term removal patterns of sediment sorbed penta- to heptachlorinated dibenzo-p-dioxin (PCDD) and dibenzofuran (PCDF) congeners. It was found that removal patterns were consistent with the labile and non-labile desorption model. Furthermore, the rate of anaerobic biotransformation by indigenous microorganisms was found to be limited by the rate of desorption.

Scow *et al* (1986) also found the two compartment model (Figure 2.1) fit the mineralization rate of phenol at concentrations of 0.32-100 ng/g of soil and also for aniline at concentrations of 0.30-500 μg/g soil. This was apparent from the biphasic CO_2 evolution from monitoring the mineralization kinetics of ^{14}C-labelled phenol and aniline. It was postulated that the first compartment could represent the substrate initially available to cells and thereby subject to rapid mineralization, and the second compartment could be sequestered deep within the

micropores thereby limiting the rate of mineralization by diffusion. Hence, uptake of contaminants in the micropores of aggregated soil appears to limit its availability for biodegradation. In a subsequent study by Scow and Alexander (1992), phenol mineralization from synthetic kaolinite aggregates was found to be retarded compared to mineralization of unaggregated clay. Steinberg *et al* (1987) attributed the resistance of 1,2-dibromomethane (EDB), that had been resident in soil for 19 yrs, to desorption and biodegradation to the entrapment of EDB in soil micropores. Upon pulverization, the EDB was no longer resistant, and freshly contaminated soil exhibited no resistance.

In contrast to the studies cited thus far, in some cases sorption has been found to enhance biodegradation. For example, sorption of pentachlorophenol (PCP) by bark chips was found to be essential for the biodegradation of PCP for concentration levels greater than 10 μM (Apajalahti and Salkinoja-Salonen, 1984). Sorption of PCP was thought to detoxify the medium, thus allowing degradation to occur at PCP concentrations of up to 200 μM. As degradation proceeded, PCP was desorbed and subsequently degraded. In another study, TCE degradation by *Methylosinus trichosporium* OB3b, by a cometabolic process, was found to be enhanced by the addition of activated carbon (Oldenhuis *et al*, 1991). Apparently, sorption of TCE by the activated carbon reduced the toxicity of the aqueous environment. Furthermore, slow desorption of TCE from the activated carbon resulted in partial degradation.

From the studies cited above, it is evident that two scenarios can occur in the process of biodegradation when solid sorptive materials are present. In the first scenario, biodegradation is limited by desorption, and the overall rate of biodegradation is reduced by sorption. Most of the studies cited seem to support this first scenario. Furthermore, it was also suggested that aging results in increased resistance to desorption and biodegradation. In the second scenario, sorption apparently detoxified the medium for the working microorganism, and the rate and degree of biodegradation increased.

2.2 Soil Organic Matter (SOM)

This section describes the nature of SOM and some of its distinctive chemical characteristics. This is followed by a description of composting and how this technique has been applied for reconditioning of soil that has lost its natural organic matter and how this could be implemented as a surrogate soil organic matter (SSOM) to study the interactions of TCE with soil that determine its fate.

The use of infrared spectroscopy (IRS) to characterize surface functionality of SOM, and the use of IRS in the study of contaminant interactions in soil is also discussed.

2.2.1 Nature of SOM

The organic fraction of mineral soils ranges from 0.5-5 % (Yong *et al*, 1992). This fraction consists of a humic and a non-humic fraction (Schnitzer, 1978). The non-humic fraction is readily degraded by soil microbes and has distinctive recognizable chemical components, such as carbohydrates, proteins, peptides, amino acids, and waxes to name a few. The humic fraction arises from the biodegradation of non-humic materials; hence, it does not contain the distinctive chemical components described above. The humic fraction is composed of the following constituents:

a) Humic acid Humic acid is extracted from soil by alkali solution, but precipitates upon acidification. The mechanism by which humic substances are formed from non-humic (*i.e.* dead plants and animals) is not known. However, it has been suggested that the more complex high molecular weight humic substances are degraded by oxidation processes to humic acids that are subsequently converted to fulvic acid (Schnitzer, 1978).

b) Fulvic acid Fulvic acid is extracted from soil by alkali solution and does not precipitate upon acidification.

c) Humin Cannot be extracted from soil.

Although the organic fraction of soil is small, it has a significant effect on the soils' engineering, agricultural, and transport properties (Yong and Warkentin, 1975). Aggregate formation occurs when organic matter attaches to the clay surface by hydrogen bonds and electrostatic attraction. The water holding capacity of soil is enhanced by the small intra-aggregate pores of the aggregates and also by the functional groups associated with the organic matter (Yong *et al*, 1992). Soil strength has also been shown to increase with the presence of polysaccharides that enhance aggregate formation (Mourato, 1990). In this last study, polysaccharides were examined since they are the major constituent of carbohydrates that in turn make up 10 % of the organic matter in soil (Lowe, 1978).

Functional groups are defined as chemically reactive sites, and they include hydroxyls, carboxyls, phenols, and amines. Specifically, they have the ability to protonate or deprotonate depending on the pH of the aqueous environment. The oxygen containing functional groups in organic matter have been identified as the most reactive, and these include the carboxylic (CO_2H), phenolic OH, alcoholic OH, and the carbonyl (C=O) groups (Schnitzer, 1982). The exchange capacity of humic substances arises from the acidity of the aromatic and aliphatic CO_2H and phenolic OH functional groups. These functional groups enable humic materials to bind to minerals, metals, and organic compounds. The exact molecular structures of humic acid and fulvic acid are unknown; however, the carboxylic, phenolic, and alcoholic groups render them acidic. It has been reported that nearly complete dissociation occurs at pH 5-7, and so the net charge of humic acid and fulvic acid is negative (Sposito, 1989).

Furthermore, there are four features that render humic acid and fulvic acid reactive:

1. **Polyfunctional.** The acids possess a wide variety of functional groups.

2. **Macromolecular.** The negative charge exhibited by the ionization of the functional groups is exhibited all over the molecule.

3. **Hydrophilicity.** The macromolecule is capable of forming strong hydrogen bonds with water at ionized carboxyl/hydroxyl sites.

4. **Structural lability.** It can conform to changes in its chemical environment by forming intermolecular associations.

A good part of the framework of humic acid and fulvic acid is uncharged. Therefore, nonpolar synthetic organic molecules are attracted by van der Waals forces to the uncharged parts. As discussed earlier, van der Waals forces occur when two molecules are close enough in proximity to induce dipoles in one another. Such forces are quite strong due to the net effect of induced dipoles over the entire molecule. The driving force for the association of the two nonpolar entities is the incompatibility of the synthetic compound with water (Chiou, 1989; Westall, 1987). Furthermore, the van der Waals attractions between the two nonpolar entities is stronger than those between soil organic matter and water (Sposito, 1989). Therefore, synthetic organics have a tendency to partition with soil organic matter.

Soil fertility, defined as the amount and availability of elements to plants for crop production, is influenced by the presence of organic matter (Campbell, 1978). When organic matter is sufficient, an agricultural soil possesses properties (good tilth and aeration, and sufficient root and moisture penetration) that allow for easy cultivation. However, poor farming practices have depleted the organic fraction of many agricultural soils (Garcia *et al*, 1992). In recent years, composting of biological wastes has been investigated as a means of reducing waste volumes and for the production of organic fertilizers that may be applied to soil as a

conditioner (Willson, 1989).

2.2.2 Compost as a Surrogate Soil Organic Matter (SSOM)

Composting has been described as a "humus producing process" (Hill, 1975), and is sometimes referred to as a humification process. Waste materials that have been used for composting include wood shavings, sawdust, peat moss, grape marc (solid wastes from wineries), and sewage sludge and city refuse (Garcia *et al*, 1992; Inbar *et al*, 1992; N'Dayegamiye and Isfan, 1991). A source of nutrients must be supplied in proper proportions to the microorganisms so that the composting process proceeds to completion (Willson, 1989). The primary nutrients required are carbon (C), nitrogen (N), phosphorus (P), and potassium (K). In general, it is necessary to adjust the C/N ratio to 15-30 for the process to proceed. The other nutrients are required in smaller amounts and their levels do not generally require adjustment. The microbes also require a moisture content that does not exceed 40 % and an optimum pH of 6.5. Air entry is necessary since this process is aerobic. The bacteria that carry out composting are usually thermophilic; hence, a rise in temperature is indicative of microbial activity and intensity (N'Dayegamiye and Isfan, 1991; Inbar *et al*, 1990). In addition, amounts and ratios of various humic substances have proven to be useful indicators of the humification process (Inbar *et al*, 1992). As well, increases in aliphatic carbon and carboxyl groups, reductions in phenolic OH and total aromaticity as well as reductions in polysaccharide levels in humic acids have also been associated with the humification process. The reasoning for measuring and quantifying changes in humic substances during the composting process has been to develop analytical methods that allow for the prediction of compost maturity. However, such characterizations also provide useful information and can be applied to the study of chemical interactions between SOM and organic contaminants.

Equilibrium approaches to sorption assume that SOM is a dimensionless "phase" that acts like an organic solvent (Chiou, 1989). This approach assumes that sorption of NOCs is

proportional to the organic content of soil, and that the origin and nature of the organic matter is irrelevant. However, organic matter is quite variable. For example, humic acid from tropical soils is quite aliphatic. Whereas, the humic acid of temperate soils contains more carboxyl and aromatic carbon (Scow, 1993). It has been observed that sorption of NOCs decreases with increasing quantities of functional groups of SOM.

To summarize, nonpolar organic contaminants such as TCE, tend to partition with the organic matter in soil. The mechanism of uptake is unknown; hence, the term "partition" is often used to describe uptake. In conjunction with processes of sorption and desorption, organic contaminants may undergo oxidation reactions catalyzed by Fe^{3+} at the exchange sites of some clay minerals (Yong *et al*, 1997). Therefore, although nonionic contaminants are not sorbed by the mineral fraction of soil, they may nonetheless undergo chemically mediated transformations if this fraction is present. Therefore, in studying the processes of sorption and desorption of TCE by soil, it is desirable to use a surrogate soil organic matter (SSOM) that is free of mineral impurities and that has undergone a humification process similar to that which occurs in natural soil. Other studies have implemented surrogates for the study of nonionic contaminant interactions with soil. For example, Xing *et al* (1994) used lignin, collagen, chitin, cellulose, and a collagen-tannic acid mixture to study the sorption of α-naphthol. Zytner (1992) used peat moss and granular activated carbon to study adsorption and desorption of TCE. Although the results of these studies offer some interesting insights into the sorptive behaviour of NOCs, the results are not readily transferable to natural soil since the surrogate materials are not representative of the organic fraction present in natural soil. The organic fraction of soil is intricately associated with other soil minerals, and is therefore impossible to extract for further study without altering its structure (Rice and MacCarthy, 1989). Attempts to extract humin have not been successful. For example, liquid-liquid extraction of humin results in a highly dispersed organic fraction along with an inorganic impurity (Rice and MacCarthy, 1989). Concentration of this organic component may be achieved by digestion of the sample with HF and HCl to dissolve silicate materials;

however, some organic matter is lost to the acid solution and the remaining organic residue is altered by the procedure. This is not surprising since 6 M HCl is used to remove carbohydrates and proteins from humic materials (Schnitzer, 1978). Compost is an organic material that contains humic acid, fulvic acid, and humin. By controlling the starting material for the composting process, it is possible to eliminate the presence of minerals found in natural soil. Therefore, the use of compost as a SSOM is ideal for the study of NOC interactions with soil.

2.2.3 Infrared Analysis of Surface Compounds

Infrared spectroscopy (IRS) is a powerful tool in that it can provide information concerning the functional groups of a molecule (MacCarthy and Rice, 1985). This stems from the fact that certain bonds absorb IR energy at distinctive wavelengths (Fessendon and Fessendon, 1979). The IR spectra for individual molecules are unique, as well as for humic substances. In the latter case, the spectra contain relatively few absorption bands that are quite broad. This stems from the fact that the "individual" bands are actually composed of a number of overlapping bands since each functional group exists in a number of chemical environments that are characterized by slightly different absorption bands. Hence, the overlap of a number of different bands.

IRS is also useful in the study of the interactions of adsorbents and adsorbed molecules (Kiselev and Lygin, 1975). Interpretation of the IR spectra of the adsorbed molecules is based on a comparison between the spectra of the bulk molecules (*i.e.* adsorbate), the adsorbent, and the adsorbent-adsorbate complex. Such spectra have certain characteristics that include the following:

1. Weak non-specific interactions (*i.e.* van der Waals forces) between the adsorbent and adsorbing molecules result in a spectra that retains the properties of the individual spectrum.

2. Strong specific (*i.e.* ion-dipole and dipole-dipole) interactions of the adsorbent with the adsorbed molecule that results in significant alterations of the spectra.

3. A complete disappearance of the individuality of the adsorbed molecule indicates a strong interaction with the adsorbent. Such interactions are characterized by covalent bonding.

Such characteristic spectra arise due to absorption of electromagnetic radiation energy. In a molecule, vibrational and rotational motions of atoms are determined by its geometric and electron structure and are sensitive to all interactions that lead to their alteration. In the case of physical adsorption, the interaction between the adsorbent and adsorbate results in a small shift of the absorption bands of the adsorbed molecules and the surface chemical compounds. In the case of chemisorption, several absorption bands disappear from the spectrum and several new bands appear.

2.3 Soil Sterilization

2.3.1 Rationale for Sterilization

Biodegradation is often estimated by comparing chemical losses with those observed when microbial activity has been eliminated by sterilization (Madsen, 1991). Given the same initial concentrations, the difference in contaminant losses between the sterilized and inoculated tests can be attributed to biodegradation. This concept is illustrated in Figure 2.5. In the *ideal control* experiment, there will be no contaminant losses by abiotic or biotic processes; whereas in the *actual control* experiment there will be small abiotic losses due to volatilization, photodegradation, and possible sorption onto container walls. In the experiment containing the viable microorganisms, most of the contaminant losses will be due to biodegradation, but there will also be the same abiotic losses as in the *actual control* experiment. The drawback to this approach is that sterilization techniques can significantly

influence physical and chemical soil properties (Wolf *et al*, 1989).

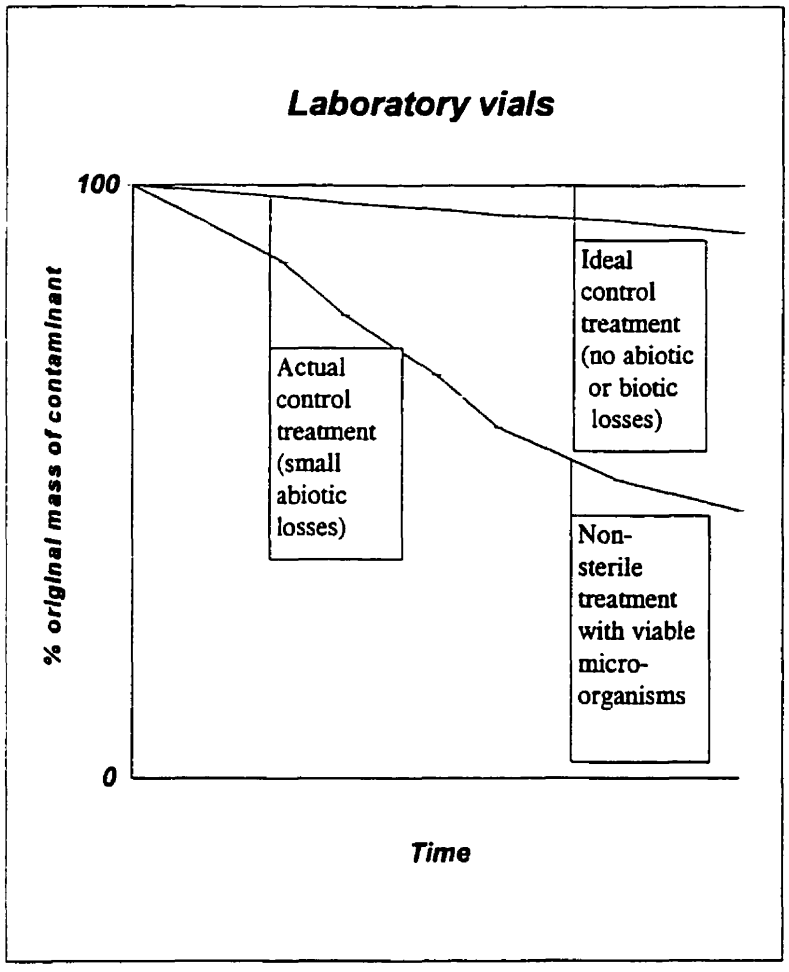


Figure 2.5. Comparison of contaminant losses by biotic and abiotic processes in sealed laboratory-incubated vials. (Adapted from Madsen, 1991).

As discussed previously, both anaerobic (Bouwer and McCarty, 1982) and aerobic (Fogel *et al*, 1986) bacteria that occur in soil are capable of degrading TCE. Therefore, in order to ensure the survival of *Desulfomonile tiedjei* and to ensure that abiotic losses of TCE are adequately estimated, it is necessary to sterilize the SOM used in the present study.

Soil sterilization has been used to exclude biological activity in order to study the

partitioning and transport behaviour of contaminants (Wolf *et al*, 1989; Lotrario *et al*, 1995), to study the biodegradability of pesticides in soil (Nègre *et al*, 1995), and also to study pure strain activity in sand or soil (Labeda *et al*, 1975; Peterson, 1962a,b; Xie and Mackenzie, 1991).

2.3.2 Methods of Sterilization

Autoclaving and the application of microwaves, x-rays, and gamma rays from a cobalt-60 source have been extensively studied for their effectiveness and influence on soil properties (Peterson, 1962b; Wolf *et al*, 1989). In general autoclaving and gamma-irradiation have proven to be effective for sterilization; furthermore, gamma-irradiation is thought to alter the chemical integrity of the soil to a lesser degree than autoclaving (McLaren, 1969). One study cited that autoclaving resulted in increases in the cation exchange capacity of a fine textured soil and this was attributed to the creation of new crystalline surfaces from amorphous Fe and Al (Xie and Mackenzie, 1991). In another study, it was observed that re-introduction of four species of soil microorganisms into soil previously sterilized by autoclaving resulted in the inhibition of growth for 19 h (*i.e.* a lag phase); whereas, the same four species grew in soil that had been sterilized by gamma-irradiation from a cobalt-60 source with no observed lag phase (Peterson, 1962a). From this, it was concluded that autoclaving may result in the production of substances which are toxic to the microorganisms (McLaren, 1969; Peterson, 1962a,b). In another study, it was suggested that the lower pH in autoclaved soil may be due to the release of organic acids from soil organic matter (Skipper and Westerman, 1973).

In addition, the use of chemical agents such as sodium azide, propylene and ethylene oxide, mercuric chloride, and chloroform have been studied for their value as sterilizing agents (Peterson, 1962a; Skipper and Westerman, 1973; Wolf *et al*, 1989). In general, the use of epoxides and sodium azide results in an increase in pH (Wolf *et al*, 1989). In the case of epoxides, this is due to reaction of the epoxide with labile H atoms. For sodium azide, the

increase in pH is due to the conversion of sodium azide to hydrazoic acid. However, residual amounts of chemicals may not only alter the chemical properties of the soil, but render it toxic thereby eliminating its usefulness in the study of pure strain activity in soil. Hence, it would appear that chemical sterilizing agents are undesirable for the present research since reductive dechlorination of TCE by *D. tiedjei* was a part of this study.

2.4 *In Situ* Bioremediation

2.4.1. Review of Existing and Emerging Technologies

As a DNAPL, TCE that has been released in the subsurface tends to migrate downward to groundwater supplies where it forms pools (Kueper and McWhorter, 1992). TCE then solubilizes in water to contaminate groundwater supplies (Anderson *et al*, 1992). Subsequently, soil becomes contaminated with TCE by two means: the downward migration of the solvent and by movement of groundwater containing dissolved TCE. Therefore, comprehensive remediation programs for sites contaminated with TCE should involve remediation of both groundwater and soil. In this last instance, the treatment method selected must be capable of removing TCE from both the aqueous and solid-sorbed phases.

For remediation of groundwater, a majority of programs that have been instituted have used the method of pump-and-treat (Mackay and Harman, 1993). In particular, this technology has been used to treat 73 % of U.S. Superfund sites (Zhang *et al*, 1995). Remediation efforts that use pump-and-treat technologies involve extraction of groundwater from the aquifer and subsequent treatment to acceptable contamination levels followed by disposal of treated water. At the surface, air stripping and carbon adsorption are generally used to remove volatile contaminants such as TCE (Mahaffey *et al*, 1992). Recently, above-ground biological treatment of contaminated groundwater has been examined as an alternative to air stripping and carbon adsorption since complete transformation of contaminants is possible

along with lower costs (Lyman *et al*, 1992). Results of a recent study which reviewed the results of various pump-and-treat remediation schemes indicate that no system was successful in completely remediating a contaminated aquifer, but that such treatments are sometimes successful in containing contaminant plumes (Mackay and Harmon, 1993). This is due in part to the free-phase DNAPLs that often exist below the water table and act as a continuous source of contamination.

In situ bioremediation of soil and groundwater containing organic contaminants is an emerging technology that is currently under review by the U.S. EPA as well as by private industry (Thomas and Ward, 1989). The advantages of such an approach include reduced costs, elimination of liability associated with the transport of the contaminated material, and complete transformation of the contaminant to less noxious products (Baker and Herson, 1994).

There are three basic types of *in situ* bioremediation treatment technologies: bioventing, biostimulation, and bioaugmentation (Baker and Herson, 1994). Bioventing involves the injection of oxygen to the soil to stimulate aerobic microbial activity. Not only does this approach result in the stimulation of indigenous microbes, it provides a means of stripping volatile chemicals from the saturated zone (Johnson *et al*, 1995). In the case of biostimuation, the indigenous microbes are stimulated by the addition of an electron acceptor (*i.e.* oxygen) and nutrients (*i.e.* nitrogen, phosphorus, and trace elements) (Thomas and Ward, 1989). Bioaugmentation involves the addition of selected bacterial cultures to the contaminated medium (Baker and Herson, 1994). Although this technique is more common to bioreactors and other *ex situ* situations, potential *in situ* applications exist. As an emerging technology, *in situ* bioaugmentation has been demonstrated in the laboratory for a number of contaminants. For example, bioaugmentation of contaminated soil with *Clostridium bifermentans* resulted in complete and rapid biodegradation of trinitrotoluene (TNT); whereas in uninoculated soil, TNT removal was not observed (Shin and Crawford, 1995).

In addition, biodegradation of 2,4,5-trichlorophenoxyacetic acid, parathion, and pentachlorophenol (PCP) has been shown to occur more quickly in soil bioaugmented with selected isolates (Forsyth *et al*, 1995).

Since it is an emerging technology, there is little documentation regarding the effectiveness of introducing organisms for subsurface bioremediation (*i.e.* bioaugmentation) (Thomas and Ward, 1993). However, it is known that properties of both the subsurface and the organisms themselves will affect their transport, survival, and capability to degrade contaminants. Bioaugmentation is considered appropriate when the required microorganism is absent in the zone of contamination (Adriens and Vogel, 1995). Once at the zone of contamination, microorganisms must compete with the indigenous microbial population for nutrients as well as escape predation (Thomas and Ward, 1993). Furthermore, it is thought that bioaugmentation for remediation of chlorinated compounds may be particularly beneficial since many microorganisms are incapable of degrading such compounds, or the period required for their adaptation may be too long. Environmental factors such as substrate concentration, pH, temperature, the presence of toxic compounds, and alternate substrates all play a role in successful remediation by introduced organisms.

However, there are mixed results with regard to the effectiveness of bioaugmentation at sites contaminated with chlorinated solvents (Thomas and Ward, 1993). For example, mineralization of PCP in contaminated soil was studied by inoculation with a PCP-degrading *Flavobacterium*. Soil obtained from one site contaminated with 298 mg/kg of PCP exhibited signs of mineralization following inoculation with the *Flavobacterium* (uninoculated samples showed no sign of mineralization). However, soil obtained from another site containing 321 mg/kg PCP exhibited the same degree of mineralization in both inoculated and uninoculated samples.

As mentioned earlier, introduction of organisms is carried out when the appropriate

organisms are not already present in the contaminated soil. Although such organisms may be natural, they may not be ubiquitous (McCarty and Semprini, 1993). Hence, the survival and growth of specialized organisms is an important consideration. Adsorption of substrates and metabolites by clay minerals is thought to affect biological activity and influence population dynamics of microbes in soil (Harter and Stotzky, 1971). Furthermore, some enzymes are extracellular and can also be adsorbed by clays. By extension, it can be said that extracellular enzymes could also be adsorbed by other soil components such as SOM. In addition, the soil surface may have an indirect influence on microbial activity by affecting a change in the composition of the medium by adsorption (Van Loosdrecht, 1990).

Godbout *et al* (1995) examined the mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) by *Pseudomonas cepacia* BRI6001L in sterile re-constituted soils (*i.e.* composed of varying amounts of sand, illite clay, and humic acid). It was concluded that the soil characteristics affected mineralization by adsorption of nutrients as well as by their influence on the concentration of the contaminant in soil water. Furthermore, survival of the bacterium increased with increasing levels of organic matter (*i.e.* humic acid). From this last observation, it was suggested that the humic acid may have acted as a secondary substrate for the bacterium.

In a study by Zaidi *et al* (1989), it was postulated from experimental results that protozoa hindered degradation of p-nitrophenol by *Corynebacterium* sp. bacteria in lake water. It was further concluded that a low molecular weight cationic inhibitor suppressed mineralization. This study concluded that predators and inhibitory agents have the potential to limit the success of inoculated bacteria in destroying organic chemicals in contaminated environments (*i.e.* in the scenario of bioaugmentation).

Goldstein *et al* (1985) reported on the successes and failures when microorganisms were added to natural environments for the destruction of organic chemicals. However, the reasons

for the inability of certain microorganisms to degrade organic chemicals in soil are unknown. In particular, the capacity of a microorganism to grow by utilizing a particular source of organic carbon does not necessarily mean that inoculation of that organism in soil will result in biodegradation. The ability of the microbe to degrade the compound is a necessary but not sufficient condition for biodegradation to occur in many different circumstances (Goldstein *et al*, 1985).

To summarize the literature related to bioaugmentation, the following are some reasons cited that can impede the ability of an introduced bacterium to perform in nature as it does in pure culture (Godbout *et al*, 1995; Goldstein *et al*, 1985; Thomas and Ward, 1993; Zaidi *et al*, 1989):

- The concentration of the compound in natural environments may be too low to support growth of the inoculated species.
- The natural environment may contain inhibitory substances.
- The added bacterium may use other organic substrates in the natural environment rather than the contaminant of interest.
- The introduced microbe must compete with the indigenous microbial population for nutrients as well as escape predation.

Hence, there are a multitude of factors that can affect the success or failure of bioaugmentation for the purposes of bioremediation.

Regardless of the method of *in situ* bioremediation, the subsurface material must be permeable enough to allow infiltration of electron acceptors, donors, and nutrients. For the

case of biostimulation and bioventing, microbes capable of degrading the contaminant must be present (Thomas and Ward, 1989). In terms of permeability, the hydraulic conductivity of the material must be 10^{-4} cm/sec or greater for successful *in situ* bioremediation. In some cases, it has been found that addition of surfactants to contaminated soil enhances biodegradation by removing contaminants from the soil surface. For example, Ghosh *et al* (1995) found that addition of a biosurfactant (rhamnolipid R1) increased the mineralization rate of 4,4'chlorinated biphenyl in a contaminated soil by more than 60 fold. In another study, it was shown that mineralization of ^{14}C-radio-labelled fluoranthene by *Sphingomonas paucimobilis* increased in the presence of a synthetic surfactant (Triton X-100) (Lantz *et al*, 1995). On the other hand, surfactants also have the ability to retard the biodegradation process, possibly due to toxicity affects (Deschênes *et al*, 1995). However, once the microorganisms have made contact with sufficient nutrients and electron donors and acceptors, the temperature and pH must be maintained to ensure the appropriate microbial activity (Baker and Herson, 1994).

One study used numerical models of plume remediation to show that *in situ* bioremediation is more efficient for TCE plumes compared to air sparging (Bridwell, 1995). It was found that *in situ* bioremediation was more efficient than air sparging in terms of duration of treatment, level of remediation, and costs. In this simulation, air sparging consisted of the injection of air and oxygen below the water table to displace the contaminant plume. *In situ* bioremediation consisted of the injection of air, substrates (CH_4 for cometabolism), and other nutrients below the water table to degrade the contaminant plume. For the dissolved phase, TCE was reduced to 58 µg/L by *in situ* bioremediation and 128 µg/L by air sparging, both after 1000 d. However, even after 2000 d, the mass of TCE in the sorbed phase in the air sparging simulation was 1.1 mg/kg compared to 50 µg/kg for the *in situ* bioremediation simulation. Based on these simulations, it was estimated that air sparging would be required for 10 to 20 years to achieve the EPA maximum contaminant limits (MCL). For *in situ* bioremediation, it was estimated that TCE levels approach the MCL following 250-500 d,

and actually reach those levels after 7 years.

In general, *in situ* bioremediation strategies applied in the field are aerobic and involve expensive oxygen delivery systems. In addition, aerobic biodegradation of TCE is a co-metabolic process that requires a secondary organic substrate such as propane, methane, or phenol to produce the enzyme monooxygenase that in turn oxidizes TCE (Fan and Scow, 1993; Fliermans *et al*, 1988; Fogel *et al*, 1986; Little *et al*, 1988; Wilson and Wilson, 1985). Injection of any one of these organic substrates into an already contaminated site could pose certain regulatory problems. As discussed earlier, anaerobic degradation of TCE occurs by reductive dechlorination and does not require the type of toxic organic substrates such as those listed above. For example, *D. tiedjei* utilizes pyruvate, an unregulated compound, as a source of carbon for reductive dehalogenation of TCE under anaerobic conditions (Cole *et al*, 1995); whereas, *Burkholderia pickettii* PK01 utilizes toluene, a regulated compound, as a source of carbon to co-metabolize TCE under aerobic conditions (Leahy *et al*, 1996). In view of the cost of oxygen delivery coupled with toxicity of secondary organic substrates that can be required for aerobic biodegradation of TCE, development of anaerobic *in situ* bioremediation strategies may be necessary to handle remediation of TCE contaminant plumes below the water table where anaerobic conditions can exist.

2.4.2 Biosurfactants for Enhanced Bioremediation

As discussed previously, sorption of contaminants such as TCE can, in some cases, interfere with its biodegradation potential. Furthermore, nonionic contaminants like TCE tend to partition with the organic fraction of soil (DiCesare and Smith, 1994). Desorption is characterized by an initial rapid phase followed by a slower phase (Pavlostathis and Jaglal, 1991). The duration of the slower desorption phase is proportional to the length of time of the contamination. This observed biphasic desorption is consistent with the intra-organic-matter diffusion (IOMD) model proposed by Brusseau and Rao (1989). According to this

model, SOM is a three-dimensional flexible matrix composed of polymer chains. Organic solutes diffuse within the interior of the matrix. Therefore, over time, contaminants can diffuse deep within the SOM structure. In terms of biphasic desorption, the initial rapid phase is likely associated with contaminants at the outermost surfaces of the SOM, and the slower second phase is likely associated with contaminants held deep within the SOM matrix.

Surfactants are defined as organic compounds that are composed of a water soluble, or hydrophilic, component and a water insoluble, or hydrophobic, component (DiCesare and Smith, 1994). When the hydrophilic component is negatively charged, the surfactant is said to be anionic. Conversely, when the hydrophilic component is positively charged, the surfactant is cationic. Finally, nonionic surfactants have no charge. At system interfaces in an aqueous environment, surfactant molecules orient themselves so that the hydrophilic end rests in the aqueous solution and the hydrophobic end orients itself toward the interface . The critical micelle concentration (CMC) is defined as the concentration at which monomers assemble to form aggregates, or micelles. Below the CMC, surfactant molecules exist as monomers. Above the CMC, the monomer concentration remains at the CMC level and any additional monomers form micelles. At concentrations greater than the CMC, the apparent solubility of nonionic contaminants is increased since the contaminant resides at the inner hydrophobic region of the micelle. Below the CMC, surfactant molecules exist as monomers. There are also some cases where the apparent solubility of a contaminant is increased below the CMC.

There are several types of synthetic and naturally occurring compounds that are known, or believed, to possess the chemical structure associated with surfactants. Surfactants are either anthropogenically derived, or are produced by microorganisms (*i.e.* biosurfactants). Although synthetic surfactants may improve the solubility of some nonionic organic contaminants, some exhibit inherent toxicity that limit their usefulness in subsurface environments (Xu *et al*, 1994); furthermore, toxicity affects may also interfere with the

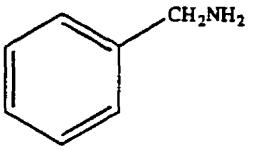
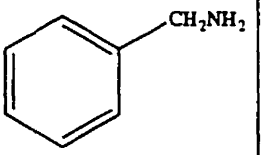
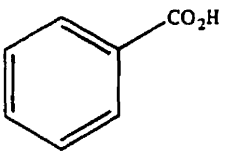
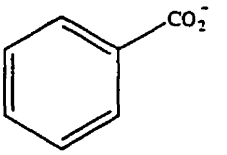
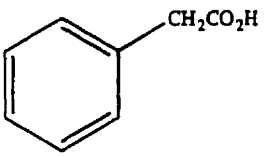
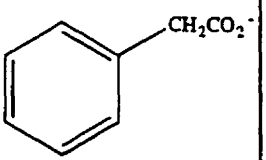
biodegradation of contaminants. In one study, it was observed that biodegradation of PAH was inhibited by a synthetic surfactant (*i.e.* sodium dodecyl sulphate) since the surfactant was preferred as a growth substrate (Tiehm, 1994). The use of biosurfactants as opposed to synthetic surfactants is appealing since they are biodegradable and hence do not pose the same environmental hazard (Scheibenbogen *et al*, 1994). Not only are biosurfactants capable of increasing the partitioning of contaminants into the aqueous phase of a soil slurry, they have also been reported to enhance the rate of mineralization of some contaminants (Providenti *et al*, 1995). In particular, the biosurfactant UG2 rhamnolipid added to a soil that had been previously contaminated with creosote was found to enhance the mineralization of radio-labelled phenanthrene by *Pseudomonas* sp. UG14r. Rhamnolipids, produced by *Pseudomonas aeruginosa*, are the most widely studied biosurfactants (Van Dyke *et al*, 1993). Although this group of biosurfactants may prove useful in the laboratory setting, application in a field setting poses some technical, and hence economical, considerations related to its production that may limit its immediate usefulness.

Another class of naturally occurring surfactants are humic acids. Wershaw (1993) proposed a model for humus that consists of an ionic, or hydrophilic, species and a hydrophobic species, much like the structure of surfactants. Furthermore, humic acid (a component of humus) has been shown to exhibit surface active properties associated with surfactants. For example, Abdul *et al* (1990) found that a humic acid solution of 29 mg/L was more effective than water in removing nonionic contaminants (*i.e.* p-xylene, ethyltoluene, *sec*-butyl toluene, and tetramethyl benzene) from a sandy aquifer material. As a naturally occurring component of soil, it has been speculated that humic acid may prove more suitable for bioremediation (Xu *et al*, 1994). In addition, if contaminated soil is amended with humic acid as a component of compost, it is possible that the organic material may act as a carbon source and thereby support biodegradation of contaminants. On the other hand, humic acid complexation with organic contaminants may reduce the availability of the contaminant for biodegradation. This was illustrated for mineralization studies involving 2,4,6-trichloro-(^{14}C)-phenol (TCP)

by *Pseudomonas aeruginosa* where the presence of humic acid (100 mg/L) reduced mineralization by 5-15 %, as compared to humic acid-free solutions (Robinson and Novak, 1994). However, in this last study, mineralization of TCP in contaminated soil was not examined; therefore, it is not possible to speculate on the value of humic acid in terms of its possible benefits in terms of bioremediation of contaminated soil.

The results obtained by Robinson and Novak (1994) are consistent with those obtained of Amador and Alexander (1988). In this last study, it was found that the presence of humic acids retarded the mineralization of low concentrations (*i.e.* 1 μg/L) of benzylamine, and the extent of mineralization decreased as the percentage of amine bound to the dissolved humic acid increased. The binding of humic acid to benzylamine was measured by dialysis. As the ratio of humic acid to benzylamine increased, the proportion of bound benzylamine also increased, and the percent of benzylamine that was mineralized decreased. Upon further investigation, it was determined that the bacteria (originally obtained from a benzylamine enrichment culture) did not preferentially degrade humic acid over benzylamine. In particular, the lag phase and doubling time were the same in both the presence and absence of humic acid. Hence, the presence of humic acid did not hinder mineralization of benzylamine by acting as a preferential source of carbon, rather it hindered mineralization by forming complexes with benzylamine. Adsorption of 90 % of the benzylamine occurred within 3-5 h and equilibrium was achieved within 72 h, and desorption occurred within 60 min. It is interesting to note that in the same study it was found that benzoic and phenylacetic acid were not adsorbed by humic acid. Figure 2.6 lists the three chemical structures and their associated basicity/acidity constants that were studied by Amador and Alexander (1988) for their ability to bind with humic acid. Also included in this Figure are the predicted ionic forms of the three compounds that are based on the acidity/basicity constants and a neutral pH. Benzylamine, which was the only compound that was found to bind with HA, remains uncharged at the pH at which the experiments were conducted. Benzoic and phenyl acetic acid, neither of which were observed to bind with humic acid, are both completely ionized

and negatively charged at pH 7.

Compound Name:	**Chemical Structure:**	**Bacisity/Acidity Constant:**	**Predicted Ionic Form at pH 7:**
Benzylamine		pK_a [1] = 9.4	
Benzoic acid		pK_a [2] = 4.17	
Phenylacetic acid		pK_a [2] = 4.28	

[1]Fessendon and Fessendon, 1979.

[2]Brown, 1975.

Figure 2.6. Predicted ionic form of three compounds studied for their ability to bind with humic acid by Amador and Alexander (1988).

2.5 Summary of Literature Review

TCE contamination of the subsurface is extensive and poses a threat to human health. Currently, pump-and-treat technologies are common methods of remediation. The above-ground treatment aspect of such remediation schemes involves air stripping, carbon adsorption, and more recently biodegradation. However, it is widely recognized that pump-and-treat is not completely successful in remediating contaminated aquifers.

Under saturated conditions, the porewater is often anaerobic barring rapid infiltration of oxygenated water. Therefore, due to the extent of contamination, *in situ* anaerobic bioremediation offers the advantage of cost effectiveness over traditional pump-and-treat systems. Bioaugmentation is an emerging *in situ* bioremediation technology that was described in this Chapter. It is thought to be appropriate for the remediation of chlorinated compounds since many microorganisms are incapable of degrading them, or the time required for adaptation may be too lengthy. However, the success of bioaugmentation and other *in situ* bioremediation strategies is dependent on a number of chemical, physical, and biological processes.

Within a saturated soil system, TCE tends to partition with the organic fraction. Many studies have indicated that partitioning behaviour is represented by the Freundlich sorption isotherm. In addition, TCE undergoes biologically mediated reductive dechlorination. Based on the literature reviewed in this Chapter, it is likely that TCE partitions with SOM under field conditions, and is consequently unavailable for reductive dechlorination. Hence, biodegradation may be dependent upon desorption kinetics.

Many of the tests reported involving sorption of NOCs, such as TCE, by soil have been implemented over short time periods that do not represent longer time periods associated with natural soils. Under such prolonged time spans, biphasic sorption has been observed,

where the sorbed phase is composed of a labile and a non-labile fraction. The non-labile fraction is more resistant to desorption compared to the labile fraction. The non-labile fraction increases with decreasing solubility, increasing organic carbon content of the soil, and increasing residence times.

The mechanism by which TCE undergoes sorption-desorption by SOM is unknown; hence, it is often referred to as a partitioning process. However, diffusion within the SOM matrix (*i.e.* IOMD) accompanied by some weak hydrogen bonding and ion-dipole interactions are likely to play a role in partitioning. Hence, as the residence time (*i.e.* aging) increases, the contaminants may diffuse farther into the SOM matrix, and this may explain the observed resistance to desorption with aging. The few studies conducted to date indicate that as aging proceeds, the resistance to desorption and biodegradation increases.

The migratory behaviour of contaminants is sometimes estimated using the octanol-water partitioning coefficient (K_{OW}). The K_{OW} has been used to estimate the chemically specific sorption parameter K_{OC}. However, since the K_{OW} does not account for variations in the SOM, such estimates have been shown to be inaccurate. Several studies have indicated that the K_{OC} is dependent on the type of SOM present in the soil. Consequently, it would appear that its value should be determined utilizing a soil that closely reflects the relevant site conditions under consideration.

In studying the interactive process of sorption, desorption, and biodegradation of TCE by reductive dehalogenation, the use of compost as a SSOM offers the advantage over some other surrogates that have been used (*i.e.* peat moss, lignin, bark chips, etc) in that it is chemically and physically representative of naturally occurring SOM. In addition, compost as a SSOM would not contain mineral impurities that may react with TCE. The need to sterilize the SSOM so as to isolate abiotic reactions with TCE was also discussed. In addition, the use of *D. tiedjei* as a model organism that exists in nature to carry out

biologically mediated reductive dechlorination offers the advantage that it is one of the most extensively studied pure strains capable of carrying out this reaction.

By studying the sorption and desorption of TCE by a sterilized SSOM, the effect of these partitioning processes on the anaerobic biodegradation of TCE by *D. tiedjei* can be assessed. As discussed in the literature, various types of surfactants have been identified as having the ability to enhance desorption of NOCs, such as TCE, from soil. If desorption is rate limiting to biodegradation of TCE, surfactants have the capability of enhancing desorption and subsequent biodegradation. On the other hand, surfactants also have the ability to retard biodegradation by either substituting as a carbon source or by enmeshment of the contaminant within a micelle formation that renders it unavailable for biodegradation. Humic acid is said to be a naturally occurring surfactant that can enhance desorption. In addition, humic acid has been shown to retard biodegradation.

Chapter 3

Methodology

The experimental work conducted for this research is comprised of four sections. The first section consists of the simulation and characterization of the SSOM, the second deals with sterilization of the SSOM, the third consists of anaerobic biodegradation of TCE by *D. tiedjei*, and the fourth is comprised of sequential desorption of TCE and *cis*-1,2-DCE from the SSOM.

3.1 Compost for SSOM

This section describes the methods employed to simulate the SSOM and the techniques used for its characterization.

3.1.1 Preparation of SSOM

The SSOM was produced by composting sphagnum moss. The material was obtained from Tourbieres Premier Limited. Initial and adjusted values of parameters that are critical for successful composting, as described in the *Literature Review*, were measured and are summarized in Table 3.1. Specifically, the C:N ratio must be between 15-30 and the pH around 6.5 for composting to proceed (Willson, 1989). From the *Initial Value* of these parameters listed in Table 3.1, it is evident that they required adjustment.

A total of 725 g of air dried sphagnum moss was set aside for composting. The pH and C:N ratio were adjusted simultaneously by adding 78.4 g ammonium carbonate to the material. The material was split and placed in two uninsulated 20 L containers fitted with 1/8 " holes at 3 " spacing on the sides and top to allow for air circulation.

The pH and temperatures of the compost and the room were monitored for nine weeks, and the contents were stirred periodically for aeration.

Table 3.1. Initial and adjusted values of critical process parameters for composting of sphagnum moss.

Parameter	Details & Method of Measure	Initial Value	Adjusted Value
pH	Measured pH of 1:25 slurry	3.9	7.5
C:N ratio	C determined by dry combustion (Jackson, 1956). N determined by acid digestion and NH_4^+ measurement (Bremner and Mulvaney, 1982).	88	20

3.1.2 Assessment of Humification

To ensure that adequate humification of the original sphagnum moss occurred over the nine weeks of composting, the temperature profile as well as the evolution of humic acid and fulvic acid were monitored. As described in the *Literature Review* of Chapter 2, composting is an exergonic biological process; hence, a rise in temperature is indicative of microbial activity and intensity (N'Dayegamiye and Isfan, 1991; Inbar *et al*, 1990). In addition, amounts and ratios of various humic substances (*i.e.* humic acid, fulvic acid, and humin) have proven as useful indicators of the humification process (Inbar *et al*, 1992).

3.1.2.1 Humic and Fulvic Acid Extraction

Humic acid and fulvic acid were extracted from the sphagnum moss and the compost, or the SSOM, by an alkali-acid washing procedure described by Schnitzer (1982). The material (2 g) was first washed with a 0.1 N NaOH solution under a nitrogen atmosphere for 24 h. The resulting supernatant containing dissolved fulvic acid and humic acid was acidified to pH 2 with 6 N HCl to precipitate the humic acid. The precipitated humic acid was removed by centrifugation and the weights of the humic acid and fulvic acid fractions were determined by weight difference. All extractions were conducted in triplicate.

3.1.2.2 Temperature Profile

The temperatures of the room and the compost were monitored for comparison with a mercury thermometer. The thermometer was placed in the mid-section of the compost for 10 min before each measurement.

3.1.3 Characterization of SSOM

The SSOM was characterized with respect to its surface properties that may affect sorption. In particular, the cation exchange capacity (CEC), anion exchange capacity (AEC), specific surface area (SSA), pH, and a quantitative analysis of functional groups were determined.

3.1.3.1 CEC, AEC, SSA, and pH

The CEC and AEC were determined as a function of pH for the SSOM according to the method of Hendershot *et al* (1993). This method of determining pH dependent CEC is quite detailed, and it is recommended that the interested reader examine the original protocol for full details. However, a brief description of this method is provided here. The SSOM was

saturated a number of times with dilute $Ca(NO_3)_2$ (*i.e.* 0.05 M and 0.005 M) solutions so as to saturate the exchange sites with Ca^{2+} cations. For pH adjustment, specific amounts of 0.1 M HNO_3 and 0.05 M $Ca(OH)_2$ were added, in duplicate for each target pH value. Following each washing, the supernatant was removed by filtration with a vacuum pump. After the last $Ca(NO_3)_2$ washing, the supernatant was set aside for pH measurement. The exchangeable Ca^{2+} was displaced by adding a concentrated KCl (1.0 M) solution to the saturated SSOM. Following sufficient agitation, the supernatant was removed by filtration and set aside for analysis of displaced Ca using a Varian GTA-95 atomic absorption spectrophotometer.

SSA was measured in triplicate according to the ethylene glycol-monoethyl ether (EGME) method of Cihacek and Bremner (1979) .

3.1.3.2 Quantification of Functional Groups

As described in the *Literature Review*, functional groups of SOM are important to the soil reactivity since they contribute to the exchange capacity and the binding of organic compounds (Schnitzer, 1978). Originally developed for the characterization of brown coals in Australia (Brooks and Sternhell, 1958), the tests described in Table 3.2 have been used more recently in the characterization of SOM (Schnitzer, 1982). These tests were performed in triplicate for the present study so as to characterize the functionality of the SSOM.

3.2 Sterilization of SSOM

Both gamma-irradiation and autoclaving were compared for their ability to effectively sterilize the SSOM. The efficiency of gamma irradiation, at varying intensities, was compared to that of autoclaving. Using the most effective method tested, sterilized SSOM was characterized with respect to its surface properties (*i.e.* as evidenced by the cation exchange capacity (CEC), specific surface area (SSA), pH, infrared spectra, and TCE

sorption isotherms) which were then compared with those of non-sterilized SSOM. The methodologies involved in this exercise are detailed in this section.

Table 3.2. Tests for characterization of functional groups (Brooks and Sternhell, 1958).

Functional Group	Description of Test
Total Acidity	The total ion-exchange capacity is determined by measuring the chemisorption of $Ba(OH)_2$.
Carboxylic (CO_2H) Acid Group	Calcium acetate is used to determine the carboxyl functional group by the following reaction: $2RCOOH + Ca(CH_3COO)_2 \rightarrow (RCOO)_2Ca + 2CH_3COOH$ The amount of acetic acid generated is proportional to the carboxylic acid groups present.
Hydroxyl (OH) Group	Hydroxyl groups are estimated by acetylation of the hydroxyl groups. The amount of acetyl groups introduced is measured by titration with 0.1 N NaOH to the phenolphthalein endpoint. The hydroxyl groups initially present are equivalent to the acetyl groups introduced.
Phenolic (OH) Group	The phenolic groups are estimated in the following way: Total Acidity (meq) - Carboxyl Groups (meq) = Phenol Hydroxide (meq)
Alcohol (OH) Group	Alcohol groups are estimated as follows: Total Hydroxyl (meq) - Phenolic Hydroxyl (meq) = Alcohol Hydroxyl (meq)

3.2.1 Gamma-Irradiation and Autoclaving

For sterilization by gamma-irradiation, material was sent to the Canadian Irradiation Centre (Laval, Quebec) where it was treated with a cobalt-60 source to deliver total dosages of 25 and 50 kGy of gamma rays in two separate runs. The SSOM was autoclaved at 121°C and 15 lb/in^2 for 1 h, followed by 24 h incubation at 35 °C. This process was repeated two (2x) and three (3x) times. In both methods of sterilization, SSOM was initially dried in the oven overnight at 105 °C.

The effectiveness of the sterilization method was evaluated by estimating the viable bacteria, or colony forming units (CFU), by plate counts within 24 h of sterilization. First, approximately 0.15 g SSOM was blended with 9 mL of a 0.1 % sodium pyrophosphate solution, according to the method of Balkwill and Ghiorse (1985). The slurries were homogenized aseptically for 5 min at 10,800 rpm using a Kinematica mixer to produce a sufficiently dispersed mixture. Serial dilutions of this suspension were spread-plated in triplicate onto a most probable number (MPN) media for all sterilization treatments tested. However, for gamma-irradiation at 50 kGy and autoclaving 3x, the survival of viable fungi was also evaluated by spread-plating the above suspension onto potato-glucose agar amended with 0.033 g/L rose bengal and 0.03 g/L streptomycin sulphate (Wollum, 1982). All plated agar media were incubated in an anaerobic jar for 2 weeks at 35 °C.

3.2.2 Effect of Sterilization Method on Surface Properties

3.2.2.1 CEC, AEC, SSA, and pH

As in the previous section that describes the characterization of compost, the CEC and AEC were determined as a function of pH according to the method of Hendershot *et al* (1993). The SSA was measured according to the ethylene glycol-monoethyl ether (EGME) method of Cihacek and Bremner (1979).

The pH of the SSOM was measured in distilled water at a soil:solution ratio of 100 on a weight basis (*i.e.* 0.17 g SSOM in 17 mL of water). The pH was recorded following 1 h when the reading was constant.

3.2.2.2 Infrared Spectrophotometry (IRS)

The sterilized and unsterilized SSOM were analyzed by IRS to detect differences in surface functionality. Sample preparation (pressing KBr powder and dry sample to form a pellet) was conducted according to Page (1982). A PYE Unicam PU95 infrared spectrophotometer was used to scan the KBr pellets containing the SSOM samples. Analysis of IR spectra and peak assignments was done according to Bellamy (1985), Inbar *et al* (1992), Kiselev and Lygin (1975), Page (1982), and Yong *et al* (1994).

3.2.2.3 TCE Sorption

These sorption tests involved the sorption of TCE from ultra pure water. In the sorption tests that were conducted in conjunction with biodegradation, the aqueous medium was a reduced nutrient medium (*i.e.* the cold anaerobic assay, or CAA) instead of ultra pure water. The *Methodology* followed for the sorption tests conducted in the reduced nutrient medium (*i.e.*

in conjunction with the biodegradation tests) are described subsequently in this Chapter.

In particular, sorption of TCE by sterilized (gamma-irradiated at 50 kGy) and non-sterilized SSOM was assessed after 24 h of agitation. This time period was selected since it was observed in a previous experiment that sorption had stabilized at 24 h over the 74 h time period tested. TCE (99 % + ACS reagent) was obtained from the Aldrich Chemical Company, Inc. For sorption tests, 0.15 g SSOM was combined with 17 mL TCE solution (made-up using ultra pure water) in glass amber vials crimped with Teflon coated septa. The initial TCE concentration ranged from about 2 to 130 mg/L in duplicate. Following 24 h agitation, the TCE remaining in the supernatant was analyzed by a Perkin-Elmer Sigma 2000 gas chromatograph (GC) headspace analyzer outfitted with a DB-petrol 100 column (100 m x 0.25 m x 0.25 μm) and a flame ionization detector (FID). The supernatant was retrieved by a GC syringe (*i.e.* 40-500 μL, depending on the concentration) and diluted in 10 mL of ultra pure water in 20 mL headspace vials, and chlorobenzene was used as an internal standard. Since the Teflon coated septa were not punctured at the start of the sorption tests, and the experiment ceased following puncture of the septa, unnecessary losses of TCE by volatilization were avoided.

3.3 Biodegradation of TCE

This section describes details of the TCE biodegradation tests utilizing the pure strain bacterium, *D. tiedjei*. For tests involving sterilized SSOM, the method of sterilization implemented was gamma-irradiation at 50 kGy since this proved to be the most efficient method in terms of eradicating bacterial and fungal activity, as described in the *Results & Discussion* in Chapter 4.

3.3.1 *D. tiedjei*

The *D. tiedjei* culture was received from Professor J. Tiedje of Michigan State University. Upon receipt of the culture, a gram stain was conducted, according to the methods of Chan *et al* (1993), and as expected the bacteria were gram negative bacilli. Furthermore, there were no variations in morphology and cell wall type, which is consistent with a homogeneous culture.

3.3.2 Selection of Medium

Prior to commencing TCE biodegradation experiments, a set of experiments were conducted to determine the growth medium that produced the highest biomass concentration. For this purpose, growth media described by Cole *et al* (1995) and DeWeerd *et al* (1990) were compared since the former was reported to yield higher rates of dechlorination. Details describing preparation of both media are summarized in *Appendix A*. A strict anaerobic technique was used in the preparation of both media.

The basis of comparing biomass concentration was the protein assay using the bicinchonic acid (BCA) method (Smith *et al*, 1985). According to this method, the peptide bonds of proteins chelate with copper ions in alkaline solution (Hanson and Phillips, 1981). The protein concentration is proportional to the blue colour that forms due to chelation and is then measured colorimetrically by UV spectrophotometry using known standards. Furthermore, since protein makes up 15 % of the total weight of an *E. coli* cell on a wet weight basis (Lehninger, 1982), a proportionality constant exists between the protein and total cell concentrations.

Following cell growth for two months at 37°C in the dark, samples were prepared in replicates of four for protein analysis by concentrating cells in a volume of 2% of the

original, as described in section 3.3.3 that follows. Based on the results of these tests, the medium described by Cole *et al* (1995) was used to harvest cells for all subsequent biodegradation tests.

3.3.3 Determination of Incubation Time

Prior to implementing TCE biodegradation experiments, it was necessary to determine the time required for incubation at 37°C such that sufficient biodegradation of TCE occurs. These experiments were carried out in the absence of sterilized SSOM.

D. tiedjei was grown on the growth medium described by Cole *et al* (1995) for two months in the dark at 37°C. Microcosms were prepared according to the protocol described in the study just cited, using strict anaerobic technique in a laminar flow biological hood so as to maintain aseptic conditions. In particular, the cultures were centrifuged at 10,000 rpm and 20°C for 10 min under an N_2:CO_2 (80:20) atmosphere and resuspended in 2 % of the initial volume in a cold anaerobic assay (CAA). The CAA consisted of 50 mM NaCl, 10 mM sodium pyruvate, 10 mM HEPES (N-2[hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], hemisodium salt), and 0.001 mg/L resazurin along with 1 mM cysteine-HCl and 1 mM Na_2S in order to reduce the medium. Sterilized controls containing no *D. tiedjei* were prepared so as to estimate abiotic TCE losses that occurred during subsequent incubation. Samples were analyzed for TCE, *cis*-1,2-DCE, and *trans*-1,2-DCE following 0, 3, 15.5, 22.5, 74, and 115 h of incubation at 37°C in a Haake water bath (model W26). All experiments were conducted in replicates of four. To minimize losses by volatilization, the TCE (20 µL of a 1000 mg/L stock solution) was added to the open vials containing the CAA, and the vials were quickly crimped with Teflon coated septa (Supelco). This manipulation was carried out under an N_2:CO_2 (80:20) atmosphere.

3.3.4 Preparation of Microcosms with SSOM for Aging Affect

In the presence of SSOM, 0.12 g of sterilized SSOM was combined with about 9 mL of CAA, determined by weight difference, in 10 mL serum bottles under an N_2:CO_2 atmosphere. The contents were quickly amended with 20 μL of a 1000 mg/L TCE stock solution to make up a final concentration of approximately 2 mg/L. After which, the bottles were crimped with Teflon coated septa (Supelco). TCE losses from the vials to the surrounding environment were minimized as much as possible since the Teflon coated septa were not pierced during this manipulation. The bottles were agitated on a Canlab test tube rocker for aging times of 2 d, 14 d, and 30 d. Following which, the contents were inoculated with 1 mL of resuspended cells, and the sterile controls were amended with 1 mL of CAA (reduced nutrient medium). The pierced septa were replaced under an N_2:CO_2 (80:20) atmosphere. Since biodegradation was estimated by subtracting the mass of TCE remaining in the inoculated vials from the mass of TCE remaining in the sterile controls, losses due to volatilization were taken into account. The concept of this method of estimating biodegradation was illustrated in Figure 2.5 of the *Literature Review*. The sterile controls were essentially sorption experiments. It is not anticipated that the manipulation of replacing the pierced Teflon coated septa with unpierced-sterile Teflon septa resulted in significant losses by volatilization since such losses were found to be insignificant in the transfer of SSOM slurries from serum bottles to headspace vials (as described in subsequent section 3.3.6.1). Where this last manipulation took more time than the simple act of replacing the pierced septa. Samples were prepared in a similar manner (without TCE and SSOM) for protein analysis.

All inoculated vials and sterilize controls were incubated at 37°C for the time period (*i.e.* 115 h, as described in *Results & Discussion*, chapter 5) that was determined in the previous set of experiments to be sufficient for TCE biodegradation.

Figure 3.1 depicts a flow diagram that illustrates the sequence of aging of TCE with the

SSOM for 2 d, 14 d, and 30 d, followed by inoculation with resuspended cells or CAA and incubation for 115 h at 37°C.

Following 115 h of incubation, vials were placed on crushed ice and subsequently analyzed for chlorinated species within a few hours. Replicates of eight were implemented for each condition. As described in section 3.3.6.1 that describes the analytical protocol, replicates of four were analyzed for aqueous TCE and DCE isomers, and replicates of four were analyzed for total concentrations of TCE and DCE isomers.

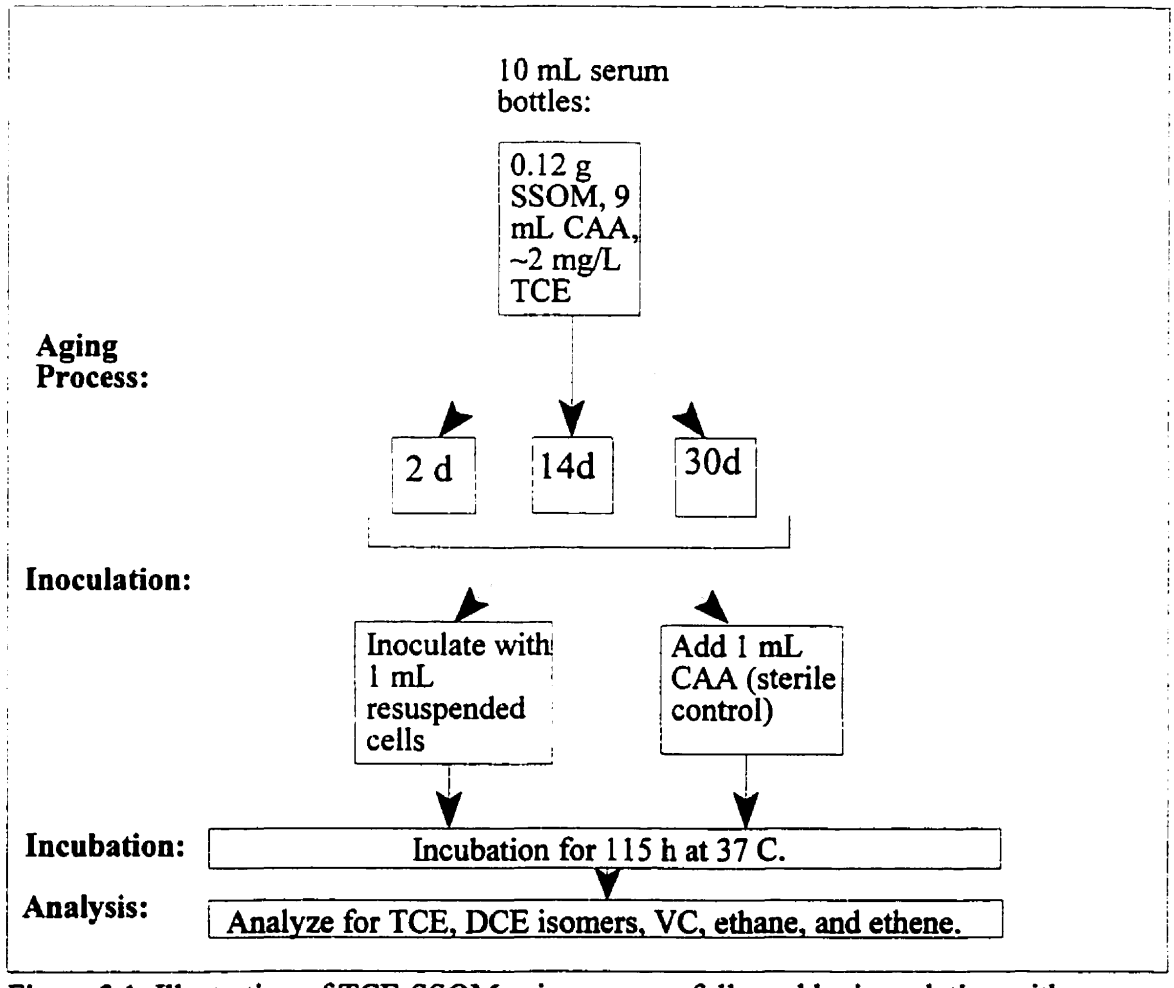


Figure 3.1. Illustration of TCE-SSOM aging process followed by inoculation with resuspended *D. tiedjei* cells.

3.3.5 Preparation of Microcosms with and without SSOM for Long-Term Biodegradation

The experiments described in this section were designed to determine whether *D. tiedjei* is capable of utilizing the SSOM itself as a carbon source. The microcosms were set up in a manner similar to those just described, except that the TCE-SSOM system was not aged prior to inoculation. As was described for the experiments in sections 3.3.3 and 3.3.4, the Teflon coated septa were not pierced during the sample preparation. In replicates of twelve, microcosms were prepared with SSOM, TCE, and live cells. Twelve replicates were also prepared with live cells but without the SSOM. Sterilized controls were also prepared in replicates of twelve containing SSOM and twelve in the absence of SSOM, where both sets contained TCE. Sterilized controls were included so that abiotic losses (*i.e.* volatilization, sorption onto container walls, etc.) may be taken into account.

After 115 h of incubation at 37°C, each vial was re-spiked with 0.0072 μmol of 3-fluorobenzoate to make up a total concentration of 0.72 μM 3-fluorobenzoate, and the septa were replaced. This was the same 3-fluorobenzoate concentration that was initially added to the growth medium, and it was repeated so as to re-induce enzyme production in cells that may have lost this ability. The same approach was taken by Fatherpure and Tiedje (1994) for dechlorination of PCE where 3-chlorobenzoate was added to groundwater contaminated with PCE fed to a biofilm reactor containing a consortium enriched with *D. tiedjei*. The 3-chlorobenzoate served to induce enzyme production that had been otherwise lost.

With enzyme production re-established with the pulse feed of 3-fluorobenzoate after 115 h incubation, all vials were placed in the water bath for further incubation of 235, 451, and 571 h (*i.e.* 10 d, 19 d, and 24 d, respectively). Replicates of four were analyzed for total quantities of TCE and *cis/trans*-1,2-DCE.

Figure 3.2 depicts a flow diagram that illustrates the overall set-up of the long term biodegradation experiments designed to determine whether *D. tiedjei* uses SSOM as a carbon source.

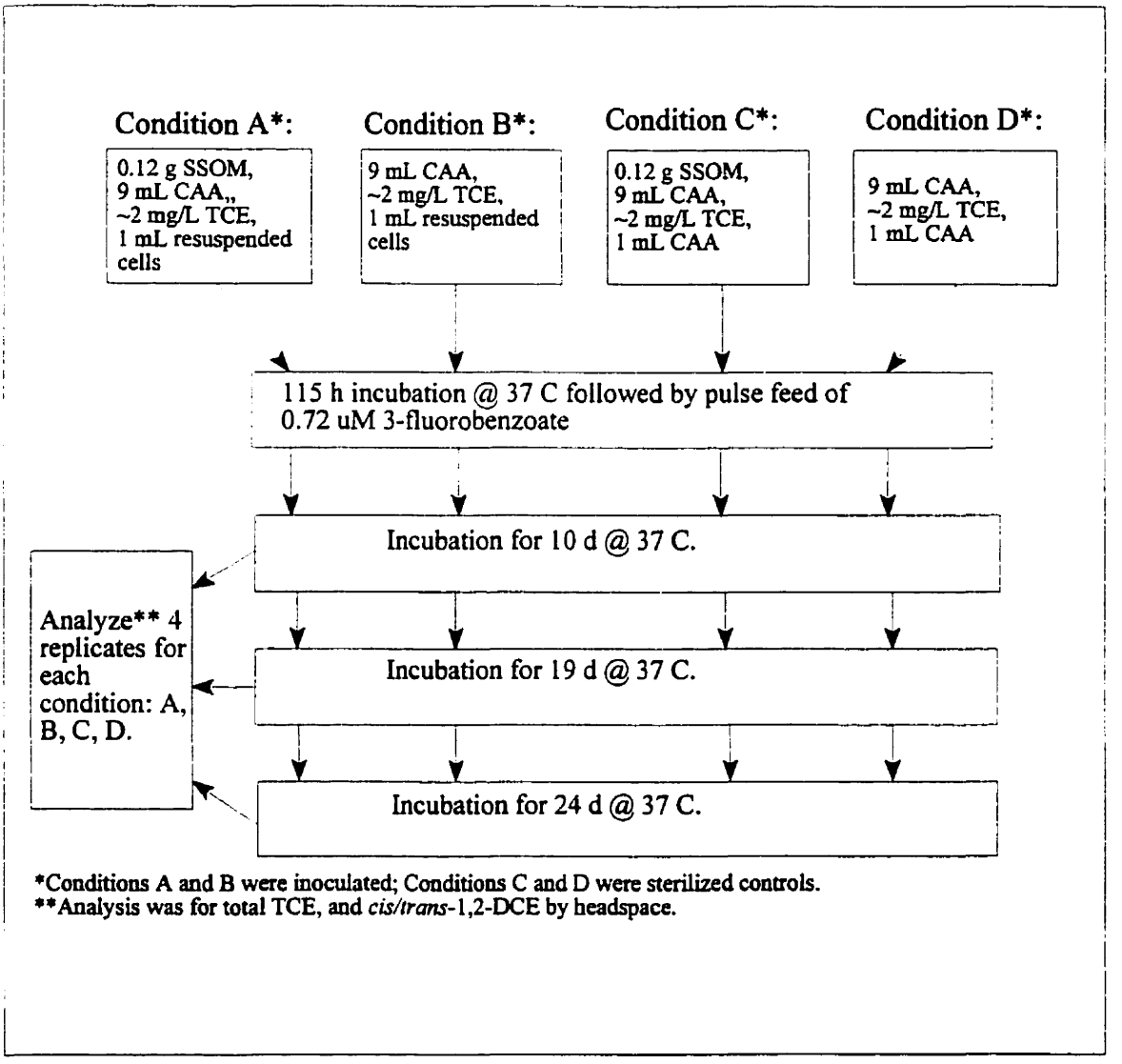


Figure 3.2. Illustration of long-term biodegradation tests to determine whether *D. tiedjei* utilizes SSOM as a carbon source.

3.3.6 Analytical Methods

This section describes the various analytical protocol utilizing gas chromatography (GC) that were implemented to quantify amounts of chlorinated ethenes and some other non-chlorinated products of biodegradation. As well, the GC mass spectroscopy (GCMS) technique that was used to identify an unknown compound produced by biodegradation is described.

3.3.6.1 Quantitative Analysis of TCE and DCE Isomers

The analytical protocol that was developed for analysis of TCE and DCE isomers in the aqueous and solid-SSOM phase is based on the method that is presently used by the Laboratory Services Branch of the Ontario Ministry of the Environment (Ontario Ministry of the Environment, 1990).

In brief, all analyses were carried out in 20 mL headspace vials with crimper type silicone rubber/Teflon coated septa, supplied by Supelco. Instrument conditions are summarized in Table 3.3.

For analysis of both aqueous and soil suspensions, the 10 mL serum bottles were placed on crushed ice, so as to minimize volatilization during transfers. For aqueous samples, vials were opened and transferred to 20 mL headspace vials.

For soil suspensions, TCE and the DCE isomers were measured in the aqueous phase alone as well as in the aqueous plus sorbed phase (*i.e.* total concentration). The aqueous phase was analyzed (in replicates of four) by removing 3 mL of the supernatant using a glass GC syringe and combining it with 7 mL ultra pure water in a 20 mL headspace vial. The rest of

the sample was discarded. The total amounts of TCE and *cis/trans*-1,2-DCE (*i.e.* aqueous phase plus sorbed phase) were analyzed (also in replicates of four) by transferring the sample contents (*i.e.* supernatant plus SSOM) to 20 mL headspace vials. The amount of contaminant sorbed by the SSOM was calculated as the difference between the aqueous phase and the total concentration.

Table 3.3. Instrument conditions for headspace autosampler.

Instrument:	Perkin-Elmer Sigma 2000
Column:	DB-petrol 100
Column Length/diameter/film thickness:	100 m x 0.25 mm x 0.25 μm
Detector:	FID
Carrier Gas:	Helium (48 psi)
Temperature:	50°C for 5 min, 5°C/min up to 200°C
Sample equilibrium time:	60 min
Injection temperature:	200°C

Tests were also conducted to determine losses by volatilization of TCE and *cis*-1,2-DCE during the transfer from the 10 mL serum bottles to the 20 mL headspace vials. Results indicated that there were no significant losses (95 % confidence level) of either TCE or *cis*-1,2-DCE during the process of transferring both the aqueous phase alone and the SSOM suspension from the serum bottles to the headspace vials. In replicates of three, TCE and *cis*-1,2-DCE solutions were prepared in both the presence and absence of the SSOM (as described in section 3.3.4 for aging, but aging was not implemented here). Simultaneous to this, the same quantities of the 1000 mg/L TCE and *cis*-1,2-DCE stock solutions were added directly to 10 mL of ultra pure water in the headspace vials. Hence, the measured concentrations of TCE and *cis*-1,2-DCE transferred from the serum bottles (with and without

SSOM) were compared to those obtained when no transfer occurred (*i.e.* when stock solutions were added directly to the headspace vials).

Chlorobenzene (Aldrich Chemical Company Inc.) was used as an internal standard (*i.e.* 10 μL of a 1000 mg/L stock solution) for analysis of all standards and samples.

Standard TCE and *cis/trans* 1,2-DCE solutions were prepared as follows. A stock solution consisting of 3,000 mg/L of TCE and *cis/trans*-1,2-DCE was first prepared. In particular, 234 μL of TCE (neat) and 238 μL of *cis/trans*-1,2-DCE were dissolved in 50 mL of methanol in a 115 mL serum bottle and the remaining head space was filled with ultra pure water. The serum bottle was crimped and used for preparation of all standards that were prepared fresh and in duplicate each time samples were analyzed. Every time that a set of standards was prepared, the pierced Teflon septa of the 3,000 mg/L TCE, *cis*-1,2-DCE, and *trans*-1,2-DCE stock solution was replaced. In 2 mL GC vials, the following standards were prepared from the stock solution with ultra pure water: 0, 15, 25, 100, and 200 mg/L. The standard solutions were further diluted 100 fold by combining 100 μL with 10 mL of ultra pure water in 20 mL head space vials. Before crimping with Teflon coated septa, 10 μL of a 1000 mg/L chlorobenzene stock solution was added as an internal standard. Therefore, the final concentrations of TCE, *cis*-1,2-DCE, and *trans*-1,2-DCE in the standard solutions analyzed by head space were 0, 0.15, 0.25, 1.00, and 2.00 mg/L. The detection limits were determined according to the methods of Glaser *et al* (1981), and were found to be 8 μg/L for TCE, *cis*-1,2-DCE, and *trans*-1,2-DCE.

3.3.6.2 Quantitative Analysis of VC, Ethene, and Ethane

For quantitative analysis of VC, ethene, and ethane, the experiments described in section 3.3.4 (Figure 3.1) were repeated in replicates of eight for total analysis, and eight for analysis of the supernatant. For total analysis, the slurries were transferred to 20 mL headspace vials,

which were crimped with Teflon coated septa (Supelco). The vials were then heated for 30 min at 85°C , and 250 μL gas samples were taken and manually injected into a Perkin Elmer Sigma 2000 GC fitted with a 6 ft packed column (1 % SP-1000 on 60/80 carbopack kB, Supelco), and an FID detector. The detection limits, according to the methods of Glaser *et al* (1981), were determined to be 10 μg/L for VC, ethene, and ethane.

3.3.6.3 Qualitative Analysis

GCMS was used in an attempt to identify an unknown compound from biodegradation that was found in chromatograms arising from microcosms, prepared according to section 3.3.4, that contained SSOM and that were inoculated with live *D. tiedjei* cells. This unknown compound was absent in microcosms that did not contain SSOM. Furthermore, the unknown compound appeared regardless of whether or not TCE was present.

A Varian Saturn II GCMS with purge and trap was used. The column used was a DB-624, 60 m long, 0.32 mm I.D., and had a coating thickness of 0.25 μm.

3.3.6.4 Dissolved Organic Matter (DOM)

To measure the DOM in the supernatant as a function of aging, the aging experiments were repeated in the absence of TCE. Following 2, 14, and 30 d aging the supernatant was removed and the DOM was measured as the total organic carbon (TOC) in replicates of four. These experiments were conducted separately in the absence of TCE so that the measured TOC would be entirely humic in nature.

The supernatant containing the DOM was filtered through 0.22 μm membrane filter paper, and the TOC was measured using a TOC analyzer (O-I-Corporation, model 700).

3.4 Sequential Desorption

Sorption of TCE and *cis*-1,2-DCE were from the CAA solution, and the initial concentrations of TCE and *cis*-1,2-DCE were approximately 2 mg/L. The SSOM slurries were left to equilibrate for the same aging times implemented for biodegradation: 2 d, 14 d, and 30 d. The samples were prepared in the same way as described in Figure 3.1 (*i.e.* following the *aging process* and prior to the *inoculation* step). As was done in the previous experiments, the Teflon coated septa were not pierced so as to reduce avoidable losses of TCE and *cis*-1,2-DCE from the system by volatilization. Following aging, total and aqueous amounts of TCE were measured by headspace, as described in section 3.3.6.1. All of the desorption experiments were conducted under aseptic conditions.

For the set of desorption tests, the initial condition of aging was the same as it was for the biodegradation tests that also examined the affect of aging. The method of desorption employed was essentially sequential in nature, and is very similar to the procedure followed by Kan *et al* (1994). For clarity, this procedure is schematically depicted in Figure 3.3. The driving force for desorption in these experiments was the concentration gradient between the solid-sorbed and aqueous phases, much as one would expect in a saturated soil system where groundwater flow constantly occurs.

For desorption, 4 mL of supernatant was removed by piercing the septa with a syringe. The 4 mL of supernatant that was removed was replaced with 4 mL of fresh CAA. This was done in replicates of four. The volume of the supernatant that was removed and the CAA added were both determined by weight difference. Immediately following addition of 4 mL of the CAA, the vials were placed on crushed ice for 30 min, and the pierced septa were replaced with unpierced, sterile Teflon coated septa so as to minimize losses due to volatilization. Direct losses by volatilization from changing the septa were not measured, but based on the fact that losses during transfer from the serum bottle to headspace vials were insignificant,

it is unlikely that changing the septa resulted in significant losses by volatilization. The slurries were agitated on a Canlab test tube rocker for 24 h, and 4 mL of supernatant was again removed and replenished with 4 mL fresh CAA as was just described. This was repeated four more times with 24 h of agitation between each removal for a total of six sequential desorption steps. The supernatant removed for the six desorption steps, plus the soil slurry remaining at the end were analyzed for TCE and *cis*-1,2-DCE by the headspace method. Chlorobenzene (10 μg of a 1000 μg/L stock solution) was used as an internal standard. Each 4 mL volume of supernatant was combined with 6 mL of ultra pure water, to yield a total volume of 10 mL, and was analyzed by the headspace method described previously. The final slurries (*i.e.* each containing 0.12 g sterile SSOM and ~10 mL of supernatant since 4 mL of fresh CAA was added following the sixth desorption step) were analyzed by headspace. The whole desorption procedure described above was carried out under aseptic conditions so as to eliminate biotic losses that could be misinterpreted as irreversible sorption.

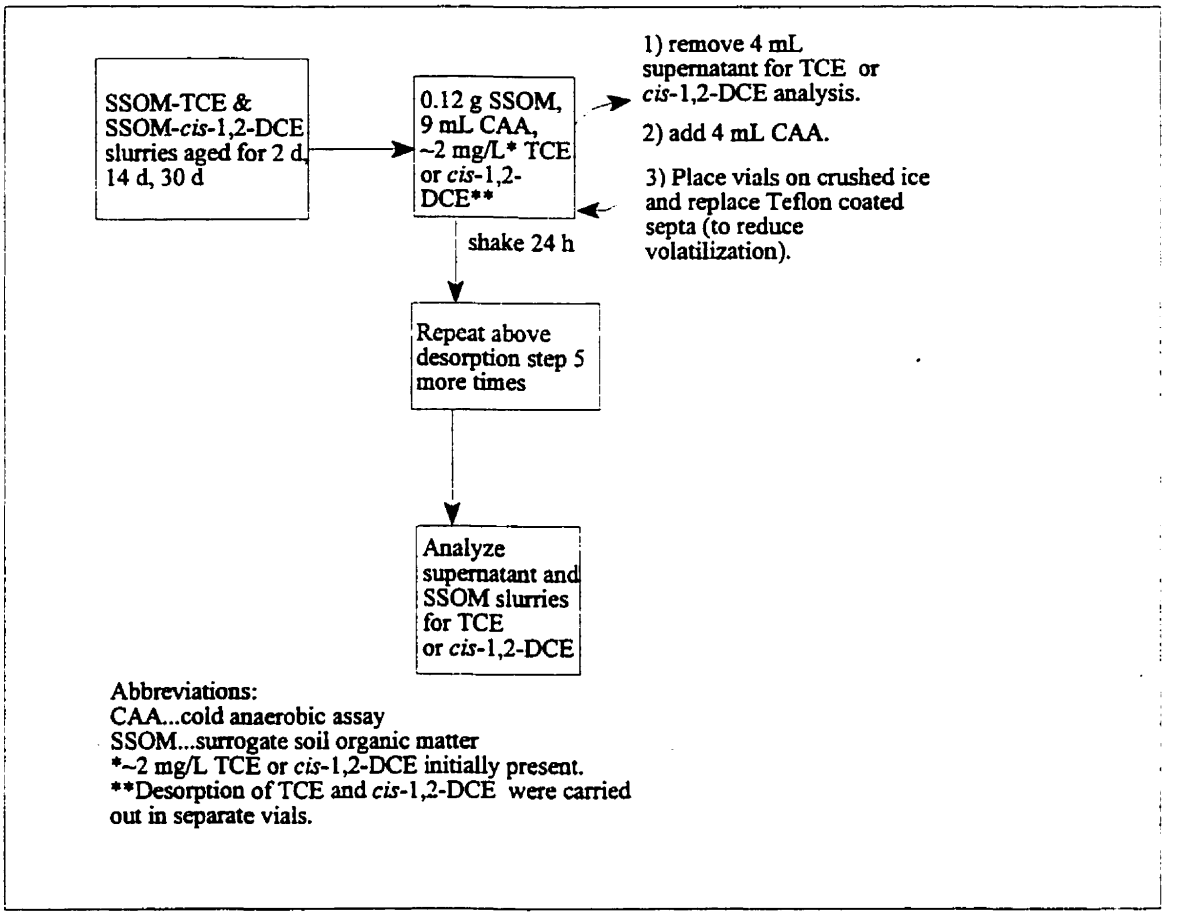


Figure 3.3. Sequential desorption of TCE and *cis*-1,2-DCE aged with SSOM for 2 d, 14 d, and 30 d.

3.5 IR Analysis of TCE-SSOM Complex

An additional experiment was conducted to obtain the IR spectra of the TCE-SOM sorption complex. In particular, 0.17 g of sterile SSOM was combined with 30 µL of a 1,000 mg/L TCE stock solution in a 3 mL GC vial. The vial was crimped, wrapped in tin foil, and left to equilibrate for 2 d. Following this, a sample was prepared as a KBr pellet for IR analysis, as described in section 3.2.2.2. The remainder of the solid sample was combined with 10 mL of ultra pure water in a 20 mL headspace vial and analyzed for total TCE according to the protocol described in 3.3.6.1.

Peak assignments were made according to the methods of Bellamy (1985), Inbar *et al* (1990, 1992), and Schnitzer and Preston (1986).

3.6 Statistical Analysis

The confidence intervals for the ensemble means and variance were calculated according to the methods of Himmelblau (1970). In particular, the following equations were used:

Ensemble Mean: $$\overline{X} = \frac{1}{n} \Sigma X_i$$

where, n.....total number of observations.

X_i.....ith independent variable.

Sample Variance: $$S_{\overline{X}}^2 = \frac{\Sigma (X_i - \overline{X})^2}{n-1}$$

Confidence Interval: $$\overline{X} + t_{1-\frac{\alpha}{2}} S_{\overline{X}} > \mu_{\overline{X}} \geq \overline{X} - t_{1-\frac{\alpha}{2}} S_{\overline{X}}$$

where, $\alpha = 0.05$ for a 95 % confidence level.

$\mu_{\overline{X}}$.....expected value of X.

$t_{1-\frac{\alpha}{2}}$ .....random variable.

For the characterization of the SSOM of Chapter 4, the number of observations (*i.e.* n) was three in many cases. It is recognized that the validity of the calculated confidence interval is limited due to the small number of observations. Consequently, in such instances the minimum and maximuma values are also reported. For the biodegradation experiments of Chapter 5, the number of measurements included in the calculations were eight. Due to the small number of observations, the reported 95 % confidence intervals should be interpreted with caution.

Chapter 4

Results and Discussion: Simulation, Characterization, and Sterilization of SSOM

4.1 Introduction

The results and discussion pertaining to the simulation, characterization, and sterilization of composted sphagnum moss that was used as a SSOM in this study are presented in this Chapter. Furthermore, these results have been published in the journal Soil Science & Plant Analysis (Sheremata, Yong, and Guiot, 1997). As discussed previously, the composted sphagnum moss was used to represent the organic fraction in soil for the sorption, desorption, and biodegradation experiments of the subsequent two chapters.

4.2 Compost for SSOM

4.2.1 Assessment of Humification

The humic fraction of natural soils arises from the degradation, or humification, of plants and animals (Schnitzer, 1978). Furthermore, the natural process of humification is simulated in the process of composting; where, a rise in temperature is indicative of microbial activity and intensity. Furthermore, it has been suggested that humic acid and fulvic acid are products of this oxidation process. Hence, in this section humification of the composted material is established in terms of temperature elevations and humic and fulvic acid evolution that occurred.

4.2.1.1 Temperature and Biological Activity

The increase in temperature (indicated in Figure 4.1) relative to room temperature is evidence that composting, an exergonic biological process, occurred (N'Dayegamiye and Isfan, 1991; Inbar *et al*, 1990). The temperature difference between the room and the compost increased following three weeks of composting, and remained that way for the subsequent six weeks. The rise in relative temperature is indicative of the activity of mesophilic bacteria that degraded, or humified, the sphagnum moss. Compared with temperature rises reported in the literature, the increase was not high as compared with larger composting piles. Particularly, for a composting pile with dimensions of 2.2x5.4x1.5 m, the temperature increased to a steady 50°C (N'Dayegamiye and Isfan, 1991). However, the small size of the composting pile of the present study (30 cm by 70 cm) likely resulted in greater rates of heat transfer to the surrounding environment since the apparatus was not insulated. It should be re-iterated that the objective of the composting experiment was to simulate a surrogate soil organic matter, and not to carry out composting *per se*. Furthermore, in natural soil the temperature at which plant material is decomposed is considerably lower than 50°C. Particularly, it has been reported that the maximum rate of plant residue decay in soil occurs at temperatures between 30 and 35°C (Alexander, 1977). Hence, it can be concluded that the temperature of 31°C that was observed during the composting process (Figure 4.1) is sufficient to bring about humification of the sphagnum moss. More importantly, the temperature elevation observed in this experiment is more representative of the temperatures at which such biological processes can occur in soil.

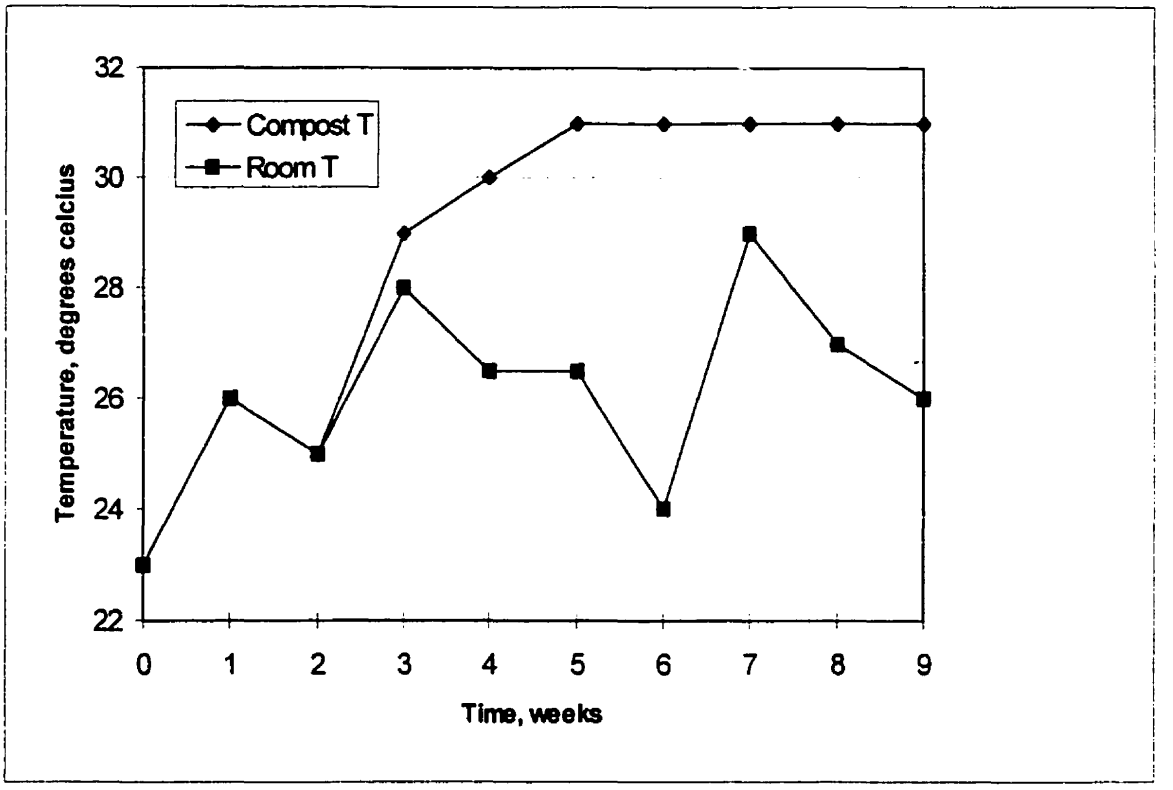


Figure 4.1. Temperature of compost compared to ambient room.

4.2.1.2 Humic Acid and Fulvic Acid Evolution

The proportions of humic acid, fulvic acid, and humin tend to change as composting proceeds. Particularly, the levels of humic acid and fulvic acid increase and the level of humin decreases as humification proceeds (Inbar *et al*, 1990). This is consistent with the suggestion that the process of humification proceeds as humin is converted to humic acids that in turn are converted to fulvic acids, as outlined previously in section 2.2.1. Table 4.1 summarizes the initial and final levels of humic acid and fulvic acid for sphagnum moss and the SSOM.

From the evolution of both humic acid and fulvic acid following composting, it is evident that the SSOM was humified compared to the original sphagnum moss since it had a greater proportion of humic acid and fulvic acid. In conjunction with the elevated temperatures that

occurred, it can be concluded that humification of the material occurred during the composting process.

Table 4.1. Humic acid and fulvic acid extracted from the sphagnum moss and the SSOM.

Material	Amount Extracted (mg/g)					
	Humic Acid			Fulvic Acid		
	Mean	Minima	Maxima	Mean	Minima	Maxima
sphagnum moss (initial)	113±37[†]	100	124	212±41	200	220
SSOM (final)	692±40	677	703	310±15	305	315

[†]95 % confidence intervals calculated for three observations.

4.2.2 Characterization of SSOM

4.2.2.1 pH Dependent CEC and AEC

As expected, the CEC increased with pH; however, the AEC remained relatively unchanged as the pH increased (Figure 4.2). To understand why this occurred and its potential influence on the binding capability of TCE and *cis*-1,2-DCE by the SSOM, it is useful to examine the functional groups of the SSOM. The characteristics of the pH dependent CEC and AEC are related to the functional groups of the SSOM in the following two sections.

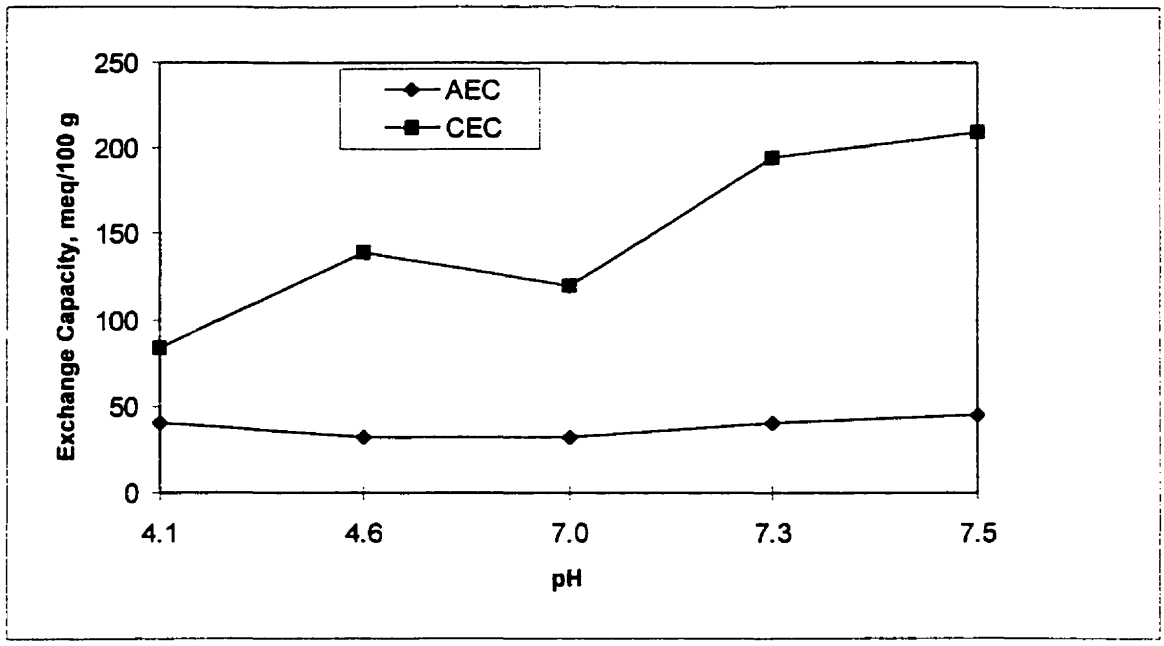


Figure 4.2. pH dependent CEC and AEC of SSOM.

4.2.2.2 Oxygen-Containing Functional Groups of SSOM

As mentioned previously, the organic fraction of soil contains functional groups that are important to the soil reactivity since they contribute to the exchange capacity and the binding of organic compounds (Schnitzer, 1978). The properties of the compost, or SSOM, produced for this study were characterized in terms of the following surface oxygen containing functional groups: carboxylic (*i.e.* CO_2H), phenolic (*i.e.* ArOH), and alcoholic (*i.e.* ROH). The quantities of the three functional groups in the SSOM, as determined by chemical methods described in the previous chapter, are summarized in Table 4.2.

As outlined in the calculations detailed in Appendix B, the oxygen-containing functional groups are acidic and hence will loose a proton and, depending on their pK_a, will become negatively charged at a certain pH. The more acidic the functional group, the lower the pH at which the functional group deprotonates and becomes negatively charged. As can be seen

from Table 4.2, at a pH of 6.7 (*i.e.* pH at which all experiments involving sorption, desorption, and biodegradation reported on in this thesis were conducted), the carboxyl group is completely deprotonated, whereas, the phenol and alcohol groups are un-ionized.

Table 4.2. Quantification of functional groups present in SSOM and concentration of each group that is ionized at pH 6.7.

Functional Group	meq/100 g[1]	pK_a[2]	Concentration ionized at pH 6.7, meq/ 100g[3]
carboxylic (*i.e.* CO_2H)	23±7.8[4]	4.2	23
phenolic (*i.e.* ArOH)	230±6.0	10	0.12
alcoholic (*i.e.* ROH)	203±9.9	18	0
Total Amount	456±13.8		23

[1]chemically determined as described in section 3.1.3.2.
[2]pK_a's reported by Fessendon and Fessendon (1975).
[3]See Appendix B for calculations.
[4]95% confidence intervals calculated for average of three observations.

From Table 4.2, it is evident that only a small proportion of the measured oxygen-containing functional groups ionize to yield a negative charge. Specifically, only the carboxylic groups fully ionize, and the phenolic and alcoholic groups remain virtually unionized. According to the calculated degree of ionization of the chemically-measured functional groups, the total CEC should be 23 meq/100 g. This calculated CEC originates from the carboxylic acid functional groups. However, when compared to the CEC measured at the same pH obtained by the method described by Hendershot *et al* (1993) (Figure 4.2), the calculated CEC is quite low (i.e. 23 compared to 120 meq/100 g from Figure 4.2). Schnitzer and Preston (1986) observed a similar discrepancy between functionality obtained by ^{13}C NMR and with the same chemical methods used in the present study. As noted in the study just cited, the

chemical estimates of the carboxylic groups were 1.1 to 2.5 times lower due to lack of reactivity and steric hindrance[1]. In addition to this, chemical measurements of the phenolic groups were thought to be high due to inclusion of carboxylic groups[2]. Therefore, if steric hindrance was a factor that contributed to the under estimation of the carboxylic acid functional groups and over estimation of the phenolic acid functional groups, then the pH dependent CEC of Figure 4.2 is a better estimate of the ionized oxygen-containing functional groups since the CEC was estimated with K^+ ions.

However, regardless of the actual distribution of the three oxygen-containing functional groups in Table 4.1, a knowledge of the pK_a's (Table 4.1) and the pH (*i.e.* 6.7) at which the experiments were conducted can provide useful information as to the possible chemical interactions that may occur between TCE, *cis*-1,2-DCE and the SSOM used in the present study. As indicated in Table 4.1, essentially all of the phenolic and alcoholic functional groups remain un-ionized, or neutral at pH 6.7. In addition, the carboxylic acid groups are totally ionized, and hence appear to be major contributors to the CEC. As outlined in the *Literature Review* of this thesis, hydrogen bonding between the chlorine atoms of TCE and the hydrogen ion in the oxygen containing functional groups of humic substances is possible. Furthermore, according to the results in Table 4.2, the oxygen containing functional groups that are likely to maintain their hydrogen ion at pH 6.7 are the phenols and alcohols.

Since chlorine is basic (*i.e.* has the ability to donate electrons) and the phenols are acidic, the possibility of hydrogen bonding exists. The likelihood of such interactions is also supported

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[1]Recall from Table 3.2 that calcium acetate was used to determine the carboxylic acid functional groups.

[2]Recall also from Table 3.2 that the phenolic groups are estimated as the difference between the total acidity (as measured by $Ba(OH)_2$ chemisorption) and the carboxylic groups. The acetate ion reacted with the carboxylic acid.

by the fact that TCE and *cis*-1,2-DCE are small compared with other organic contaminants, and hence steric hindrance would likely be less of a factor. In addition, the hydrogen atom(s) of chlorinated ethenes can potentially form ion-dipole interactions with the ionized carboxylic acid functional groups. Individually, hydrogen bonds and ion-dipole interactions are only a few kcal/mol (Pignatello, 1993); therefore, the strength by which small molecules such as TCE and *cis*-1,2-DCE can be held by SSOM is not expected to be particularly significant. Figure 4.3 indicates a proposed partial structure for FA (Schnitzer, 1978) with possible associations with a TCE molecule based on the calculated dissociations of the oxygen containing functional groups that were quantified in this study (Table 4.2).

Due to the low bond energies involved with hydrogen bonding and ion-dipole interactions, it is not anticipated that the forward (*i.e.* sorption) and reverse (*i.e.* desorption) reactions are rate limiting (Pignatello, 1993).

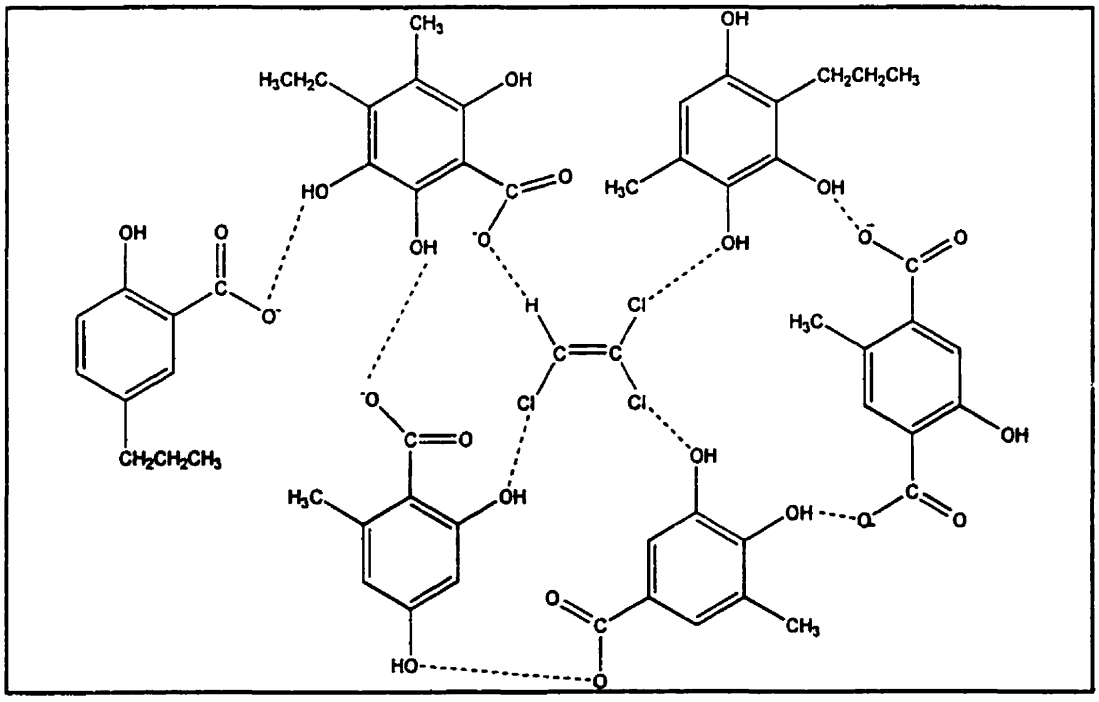

Figure 4.3. Partial chemical structure of fulvic acid (adapted from Schnitzer, 1978) with potential bonding associations with TCE with the calculated dissociations of the oxygen containing functional groups that were quantified in the SSOM (Table 4.2).

4.2.2.3 Nitrogen-Containing Functional Groups of SSOM

As can be seen from Figure 4.2, the AEC is relatively constant over the pH range tested. Positive charge in aquatic humic substances has been associated with secondary-amine[3] functional groups (Aiken, 1985). However, little is known about the chemical structure of the major components of the nitrogen fraction in SOM, and most of the literature in this area is focused on the types and concentrations of amino acids and amino sugars (Schnitzer, 1985). Of the amino acids identified in both tropical soil and soils formed under cool, temperate, conditions, the following four basic (*i.e.* positively charged) amino acids have been identified: arginine, histidine, lysine, and ornithine. The structures of these basic amino acids are given in Figure 4.4. The arrow indicates the amino group that becomes protonated to yield a net positive charge. Since the isoelectric points of these basic amino acids ranges from 9-10 (Fessendon and Fessendon, 1978), one can expect a constant positive charge over the pH range that the AEC was measured if such amino acids comprise the bulk of the nitrogen compounds in the SSOM. However, such characterizations were beyond the scope of this research. Although little is known about the chemical structure of nitrogen in soil, it is likely that the AEC of the SSOM used in the present study originates either from the four amino acids listed in Figure 4.4, or a similar structure. This is based on the fact that the positive charge (*i.e.* AEC) is constant over a broad range in pH. This would not occur with other nitrogen containing functional groups. However, chemical tests for quantification of the nitrogen containing functional groups in soil were not found in the literature. Hence, these groups were not quantified in the present study.

Ion-dipole interactions between the protonated amino groups (that likely contribute to the AEC) and the electronegative chlorine atoms of TCE are also possible. However, since the

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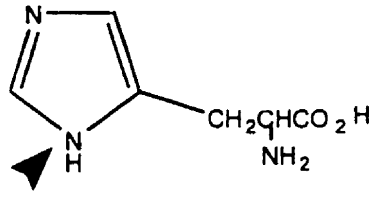
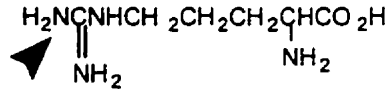
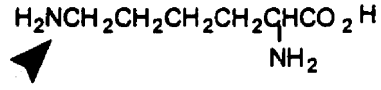
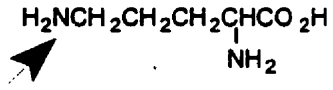
[3]An amine is an organic compound containing trivalent nitrogen atoms bonded to one or more carbon atoms. In a secondary-amine, the nitrogen is attached to two carbon atoms.

AEC is almost four times less than the CEC (*i.e.* 33 and 120 meq/100 g, respectively from Figure 4.2) at the pH at which all the experiments of this study were conducted, there would appear to be a greater likelihood of TCE interaction with the oxygen-containing functional groups that make-up the CEC, as depicted in Figure 4.3.

Isoelectric Point:	Compound Name:	Formula :
nr	Histidine	
10.76	Arginine	
9.74	Lysine	
nr	Ornithine	

Arrow indicates location of positive charge at pH < IEP.

nr: not reported.

Figure 4.4. Basic amino acids, and some reported isoelectric points, that have been isolated from soil (Fessendon and Fessendon, 1979; Schnitzer, 1985).

4.3 Sterilization of SSOM

Sterilization of the SSOM was necessary in order to eliminate the presence of viable bacteria and fungi that may compete with the pure strain bacterium, *D. tiedjei*, used for dechlorination of TCE in this study. Sterilization was also necessary for the experiments involving sorption and desorption of TCE and *cis*-1,2-DCE by and from SSOM. Following the composting process, the SSOM was rich in microorganisms that were involved in its humification. Therefore, in order to ensure that TCE losses were purely abiotic, an effective method of sterilization was sought. Otherwise, biotic losses could be mistakenly attributed to abiotic processes, and the resulting partition coefficients would be overestimated. However, the method of sterilization must minimize both chemical and structural changes that could also affect sorption.

Autoclaving and gamma-irradiation were assessed for their ability to eliminate the number of viable bacteria and fungi in the SSOM, and also for their influence on chemical and physical properties of the SSOM. Other chemical agents, such as propylene oxide, mercuric chloride, and sodium azide were not considered for this study. Particularly, propylene oxide is known to react with the carboxylic and phenolic hydroxide groups in SOM (Wolf *et al*, 1989). Mercuric chloride is highly toxic and difficult to work with. Sodium azide is converted to hydrazoic acid in soil and results in pH increases (Wolf *et al*, 1989). Since the charge of SSOM is highly pH dependent, as can be seen by results in Figure 4.2, changes in pH could potentially affect the surface properties of the material.

The two sterilization methods selected (*i.e.* autoclaving and gamma-irradiation) were compared in terms of their efficiency. Based on these results, the most efficient method was then implemented. The surface properties of the sterilized SSOM were then compared to those of the unsterilized SSOM.

4.3.1 Efficiency of Sterilization Method

The efficiency of the two sterilization methods are summarized in Table 4.3. Initially, the SSOM was irradiated at 25 kGy by gamma-irradiation since it has been reported in the literature that 10 kGy to 60 kGy is sufficient for sterilization of soil (Lotrario *et al*, 1995; Wolf *et al*, 1989). In addition, it has been reported that autoclaving two times is sufficient in eliminating viable bacteria in soil samples (Wolf *et al*, 1989). However, from Table 4.3, it is evident that such treatments were not sufficient for the SSOM. Hence, the intensity of gamma-irradiation was increased from 25 to 50 kGy, and autoclaving was increased from two to three times. In addition to numbers of viable bacteria (*i.e.* colony forming units, or CFUs) and viable fungi were also assessed for the more aggressive sterilization techniques employed. From the results in Table 4.3, it is evident that gamma-irradiation at 50 kGy was the most effective sterilization method tested. Although autoclaving three times was not as effective as gamma-irradiation at 50 kGy, it was more effective than the latter at 25 kGy and autoclaving two times.

Table 4.3. Effect of sterilization method on microbial populations of SSOM.

Method of Sterilization	Bacteria (CFU/g)			Fungi (CFU/g)
	Mean	Minima	Maxima	
gamma-irradiation, 25 kGy	13,534±6,055[1]	11,278	15,038	not measured
Autoclaving, two times	961±734	720	1200	" "
gamma-irradiation, 50 kGy	0	0	0	0
Autoclaving, three times	752±0	752	752	0

[1]95 % confidence intervals for three observations.

4.3.2 Effect of Sterilization Method on Surface Properties

4.3.2.1 SSA and pH

In order to assess the effect of sterilization on the physical and chemical properties of the SSOM, the specific surface area (SSA) of untreated and gamma-irradiated (50 kGy) materials were measured and compared (Table 4.4). The SSA of the sterilized SSOM was less than that of the unsterilized material (at a 95 % confidence level). From the literature, the reported results of the effect of gamma-irradiation on the SSA of soil are conflicting. Lotrario *et al* (1995) found that gamma-irradiation at 50-60 kGy of a natural soil containing 3.5 % organic matter resulted in a 16 % decrease in the SSA compared with unsterilized soil; however, the total amount of organic matter was not reduced by either method of sterilization. On the other hand, Wolf *et al* (1989) found that gamma-irradiation of a soil containing 1.42 % organic carbon did not significantly reduce the SSA.

The method used to determine the SSA in the present study was based on the sorption of a mono layer of ethylene glycol monoethyl ether (EGME) (Cihacek and Bremner, 1979). It is possible that the reduction in SSA observed in the SSOM following gamma-irradiation was the result of a collapse of the micropores incurred during the sterilization process. This would need to be verified by comparing scanning electron micrographs of irradiated and non-irradiated SSOM, but such a technique was not available for this study. However, it was observed that the SSOM appeared to be in a more desiccated condition following gamma-irradiation as compared to autoclaving. Hence, restrictions in porous diameters may have impeded contact between EGME and the SSOM surface.

If inadequate contact was a factor for the observed difference in the SSA, this artifact could not have been avoided due to the nature of the EGME method for SSA determination. In particular, the method of Heilman *et al* (1965) with the modifications described by Cihacek

and Bremner (1979), is described briefly as follows. According to this method, soil samples are air-dried and combined with 3 mL of reagent grade EGME in a vacuum desiccator over an EGME-$CaCl_2$ solvate (to control the EGME vapour pressure at the soil interface). The weight of the samples are measured at 24 hour intervals (followed by evacuation of the dessicator using a vacuum pump) until a constant weight is achieved. The weight of EGME at equilibrium is equal to a monolayer of EGME adsorbed by the soil and is therefore proportional to the soils' SSA. In particular, 0.000286 g of EGME adsorbs as a monolayer per m^2 of soil (Carter *et al*, 1965). Hence, according to the methodology for SSA determination, varying the contact time is not an option since the time of contact is determined by equilibrium adsorption of EGME. Furthermore, the EGME method is the most accepted protocol for SSA determination of soil (Cihacek and Bremner, 1979).

Table 4.4. SSA and pH of untreated and gamma-irradiated SSOM.

Treatment	SSA, m^2/g			pH
	Mean	Minima	Maxima	
Unsterilized SSOM	168.5 ± 14.2^1	164.4	172.5	6.4
gamma-irradiated (50 kGy) SSOM	133.4 ± 37.3	144.0	122.8	6.3

[1]95 % confidence interval for three observations.

The pH of the unsterilized SSOM suspension showed little variation from that of the irradiated material (Table 4.4).

4.3.2.2 pH Dependent CEC and AEC

Although there were no differences in the pH between the non-irradiated and irradiated materials, there were differences in the pH-dependent CEC. In the procedure for pH-dependent CEC outlined by Hendershot *et al* (1993), a fixed amount of acid or base was added for pH adjustment followed by agitation in an end-over-end shaker overnight. Results for pH dependent CEC and AEC for non-irradiated SSOM are presented again for comparison with those obtained for irradiated SSOM (Figures 4.5 and 4.6). Although an equivalent amount of acid or base was added to both non-sterilized and irradiated SSOM, the non-sterilized SSOM exhibited a sharp increase in CEC at pHs greater than 7 that was not observed for irradiated SSOM. Therefore, it would appear that irradiation of the SSOM resulted in an increase in CEC at pHs less than 7. Above pH 7 there were no differences in the CEC.

At pH 6.7, the CEC of irradiated SSOM was larger than that of non-irradiated SSOM (*i.e.* ~200 vs ~125 meq/100 g, respectively). In addition, the AEC of both materials was relatively similar (*i.e.* ~40 meq/100 g). This observation is somewhat contradictory to the observed decrease in SSA for irradiated SSOM described in the previous section. In general, as the SSA increases, the CEC of soil also increases (Yong *et al*, 1992). Since the SSOM used in this study is a fibrous as opposed to a particulate material, adequate contact may not have been achieved in the tests conducted for the SSA determination. However, as mentioned in the previous discussion, due to the nature of the SSA test, alteration of the contact time would not have resolved this perceived difficulty.

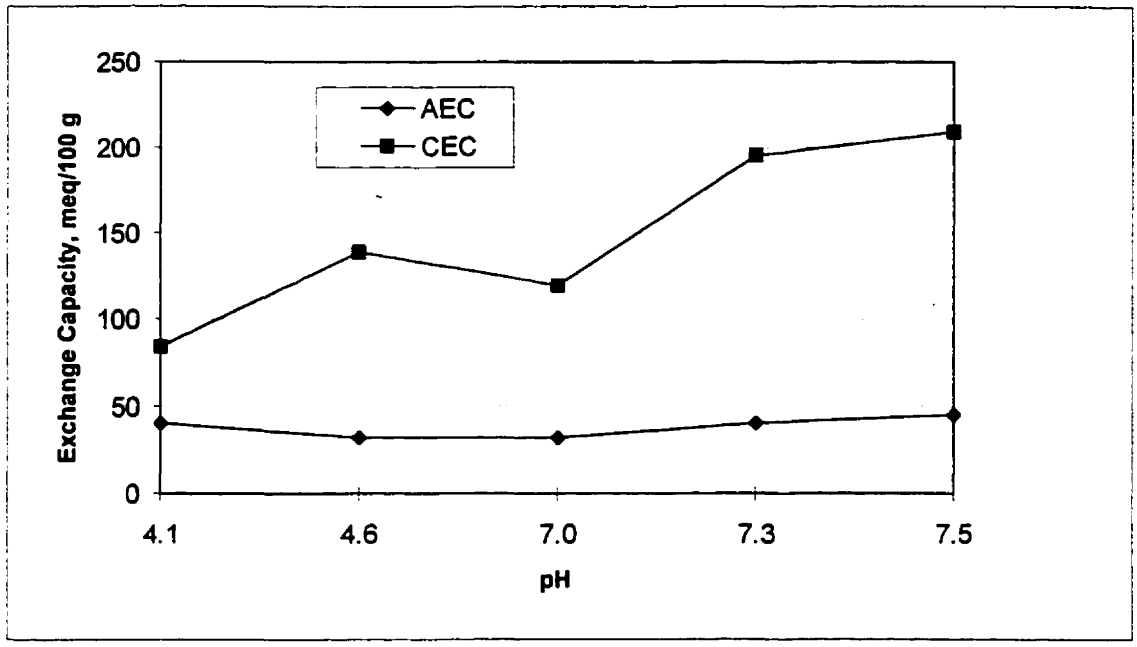


Figure 4.5. CEC and AEC of non-irradiated SSOM.

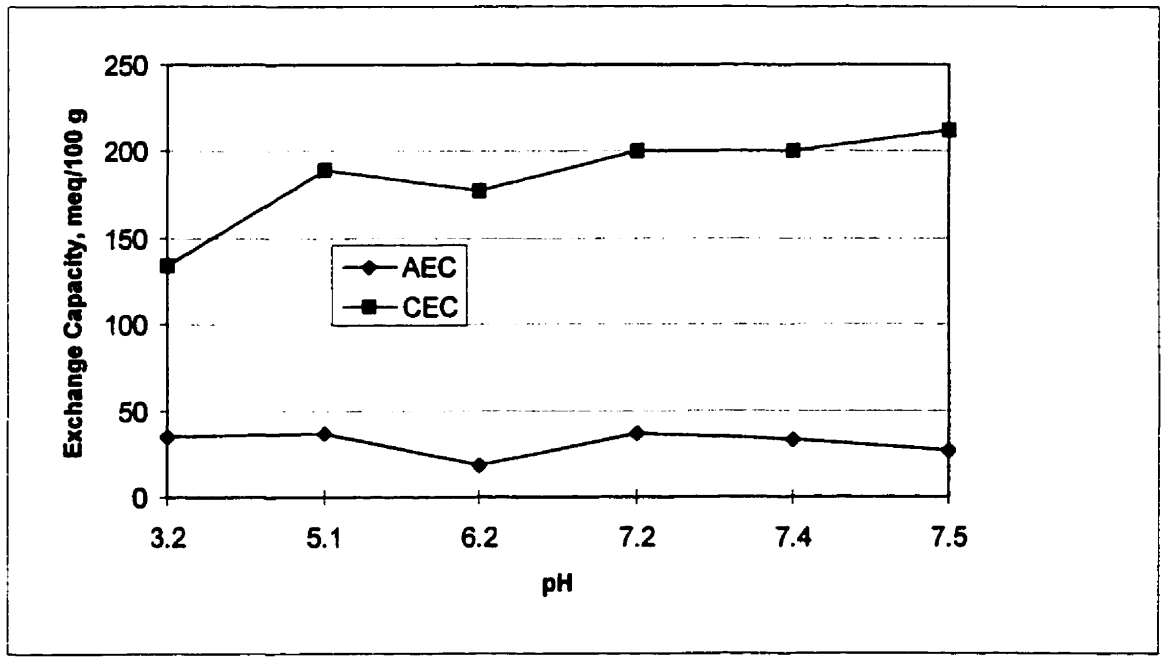


Figure 4.6. CEC and AEC of irradiated SSOM (50 kGy).

4.3.2.3 IR Analysis of Gamma-Irradiated and Non-Sterilized SSOM

To further investigate the effect of gamma-irradiation on surface properties, the IR spectra of the two materials were compared (Figure 4.7), and the structural similarities of the two materials are evident. Furthermore, the absorbance bands were similar to those previously published for other composted materials in the literature (Inbar *et al*, 1990, 1992; Schnitzer and Preston, 1986). In particular, there is a strong broad peak at 3400 cm^{-1} from hydrogen bonded OH stretching, a sharp peak at 2920 cm^{-1} and a shoulder at 2850 cm^{-1} from aliphatic C-H stretching, evidence of a shoulder at 1720 cm^{-1} from C=O of carboxyl or ketonic origin, a broad peak at 1620 cm^{-1} from aromatic C=C, hydrogen bonded C=O of carbonyl origin, a medium peak at 1510 cm^{-1} from aliphatic C-H bending, a peak at 1430 cm^{-1} from aliphatic C-H aromatic ring stretch, a peak at 1160 cm^{-1} from aromatic ring bending, and peaks around 1020-1100 cm^{-1} from the C-O stretch of polysaccharides. Therefore, not only was the IR transmission behaviour of irradiated and non-sterilized SSOM similar, they were also similar to those of other composted materials reported in the literature. In summary, it can be said that sterilization of SSOM did not have an effect on surface properties as evidenced by IR transmission behaviour.

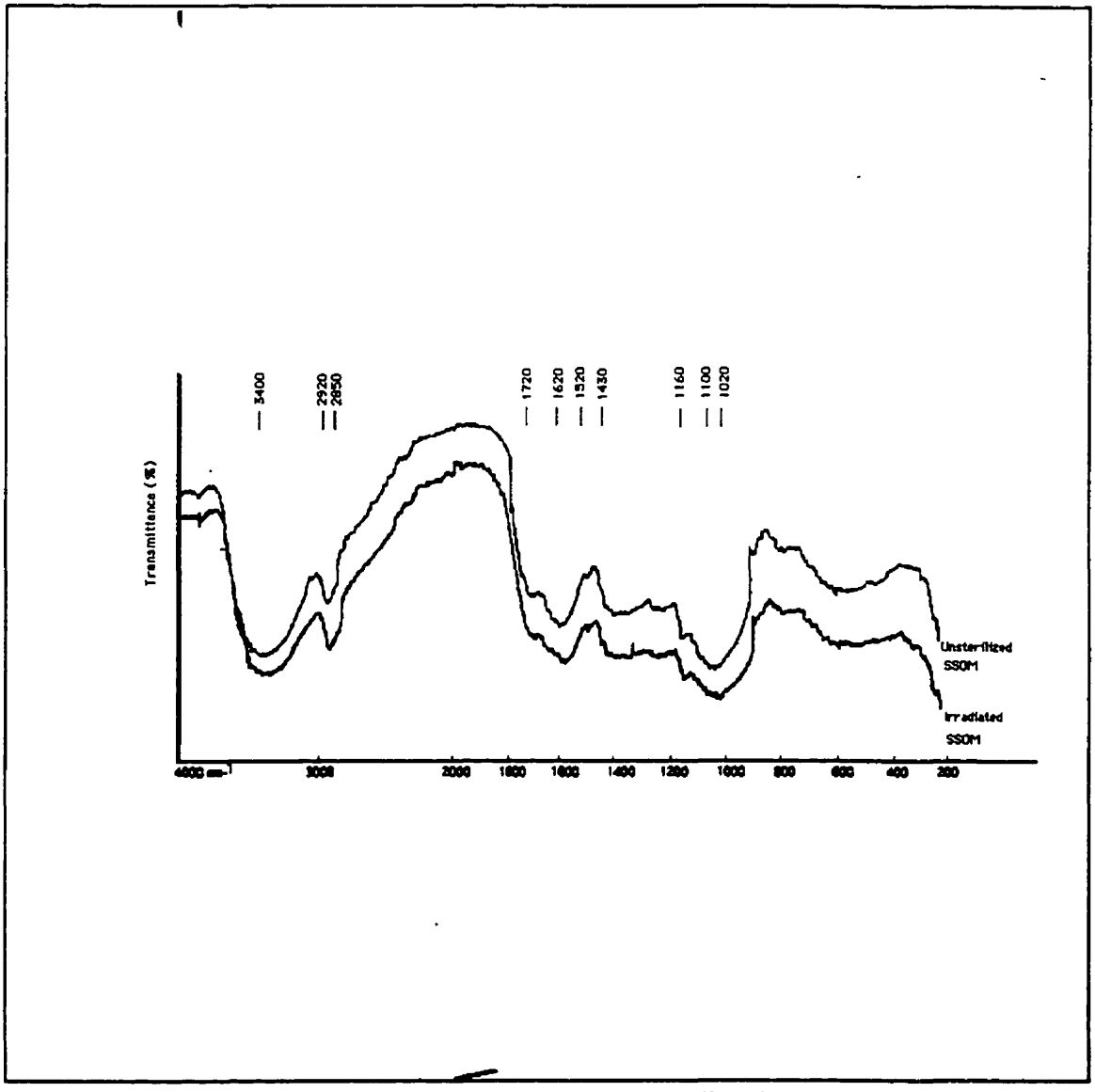


Figure 4.7. IR spectra of irradiated (50 kGy) and non-irradiated SSOM.

4.3.2.4 TCE Sorption

To further determine if the process of sterilization had any effect on the ability of the SSOM to interact with contaminants by sorptive processes, TCE sorption from water by both materials was studied at initial concentrations that ranged from 2 to 130 mg/L.

However, as described in the Methodology, it was first necessary to determine the length of time necessary to achieve apparent equilibrium with respect to sorption, beyond which there was little or no sorption. These results are presented in Figure 4.8. By inspection it is evident that beyond 24 h, there was little or no additional sorption. Therefore, in the subsequent experiment, sorption of TCE was conducted over 24 h to determine if gamma-irradiation had an effect on sorption.

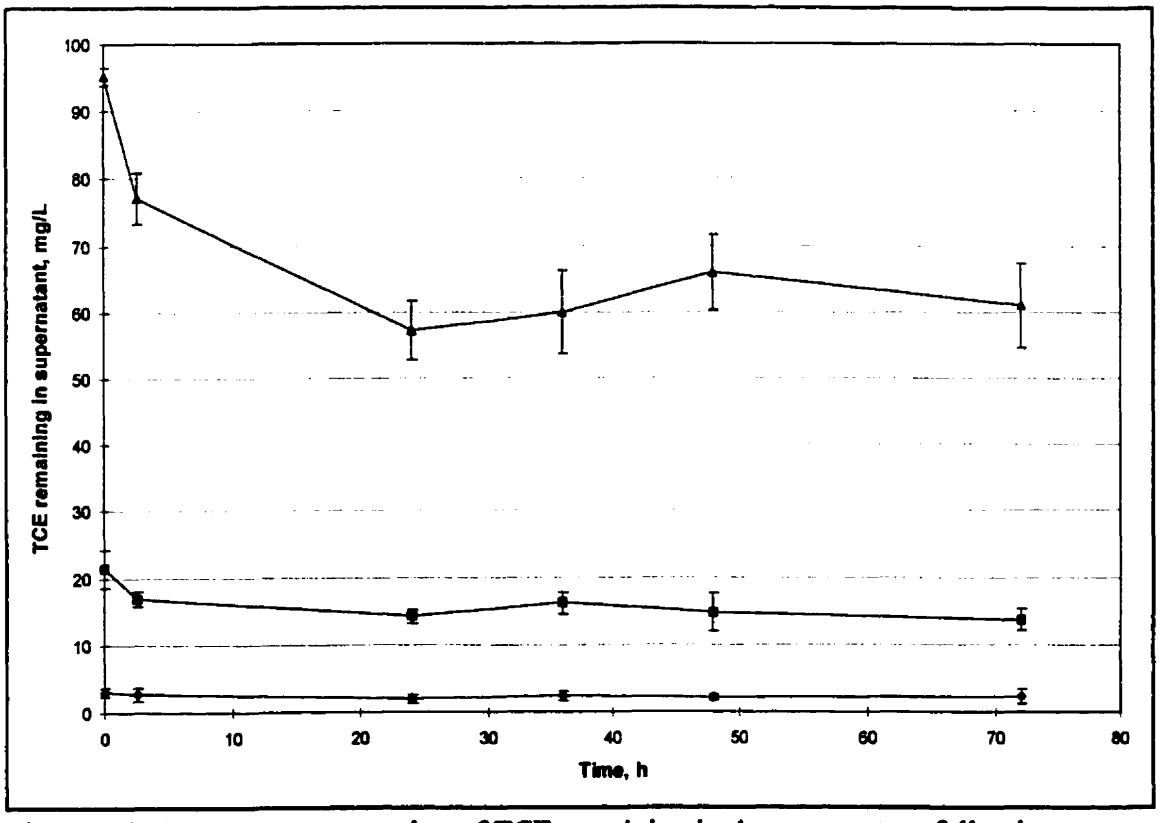


Figure 4.8. Aqueous concentration of TCE remaining in the supernatant following sorption as a function of time (95 % confidence intervals are shown).

From the sorption data in Figure 4.9, it is evident that there is little difference between the sorption of TCE by irradiated and non-irradiated SSOM.

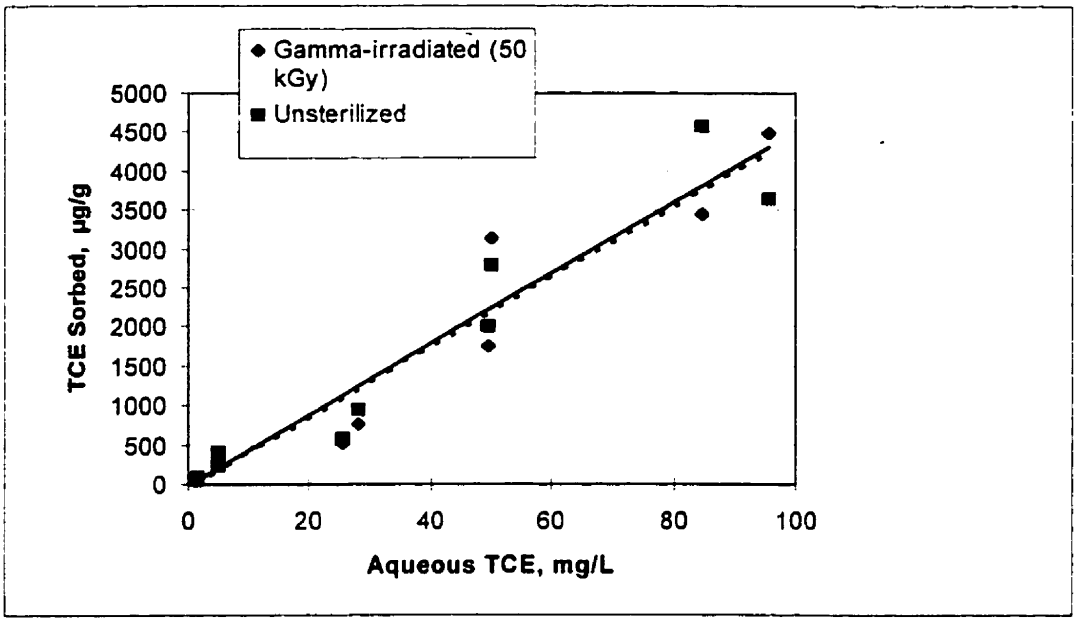


Figure 4.9. Sorption of TCE by gamma-irradiated SSOM (50 kGy) and non-irradiated SSOM.

Therefore, although there are differences in the SSA and CEC between the two materials, the surface properties, as indicated by IR spectra and sorption of TCE, were essentially the same. On this basis, the irradiated SSOM was used for the sorption, desorption, and biodegradation studies of TCE and *cis*-1,2-DCE. Results and accompanying discussion regarding this work is presented in the subsequent two chapters.

4.4 Summary of Results and Discussion

The following is a summary of the results and discussion related to the simulation, characterization, and sterilization of composted sphagnum moss as a SSOM:

- Sphagnum moss composted over a 9 week period was found to be humified and is consequently more representative of the organic fraction found in natural soil as compared to some surrogates used by others (Xing *et al*, 1994; Zytner, 1992; Zytner *et al*, 1989).

- Gamma-irradiation at 50 kGy was necessary to sterilize the SSOM (*i.e.* composted sphagnum moss) so as to eliminate biological activity that may be misinterpreted as irreversible sorption and that may also compete with *D. tiedjei*.

- Sterilization had no effect on the ability of SSOM to sorb TCE or on IR transmission behaviour.

- The reactivity of the SSOM with TCE appears to originate from the interaction of chlorine atoms with the un-ionized phenolic and alcoholic functional groups by hydrogen bonding and/or with the positively charged amino groups by ion-dipole interactions.

Chapter 5

Results and Discussion:

TCE Biodegradation

5.1 Introduction

In this chapter, the results from biodegradation of TCE by *D. tiedjei* in sterilized SSOM are discussed. Biodegradation was studied as a function of aging (*i.e.* the time that TCE was resident in the SSOM prior to inoculation with *D. tiedjei*). Since the availability of TCE for biodegradation was a function of partitioning between the aqueous and solid-sorbed phases, the results of TCE sorption are discussed prior to those of TCE biodegradation (both as a function of aging). In addition, long-term biodegradation experiments were conducted to determine the ability of *D. tiedjei* to utilize SSOM as a source of organic carbon.

Although described in the *Methodology* of Chapter 3, an overview of the biodegradation experiments is summarized in Figure 5.1. The three sets of experiments that were conducted are depicted in this diagram along with the corresponding section numbers in which they are discussed (*i.e.* sections 5.2, 5.3, and 5.4). However, it should be emphasized that this schematic diagram does not contain details that are provided in the *Methodology*. Particularly, the sterilized controls that were conducted in parallel with the aging experiments (section 5.3), as well as experiments that were conducted in the absence of SSOM (section 5.4) are both described in greater detail in the *Methodology* (*i.e.* Figures 3.1 and 3.2, respectively).

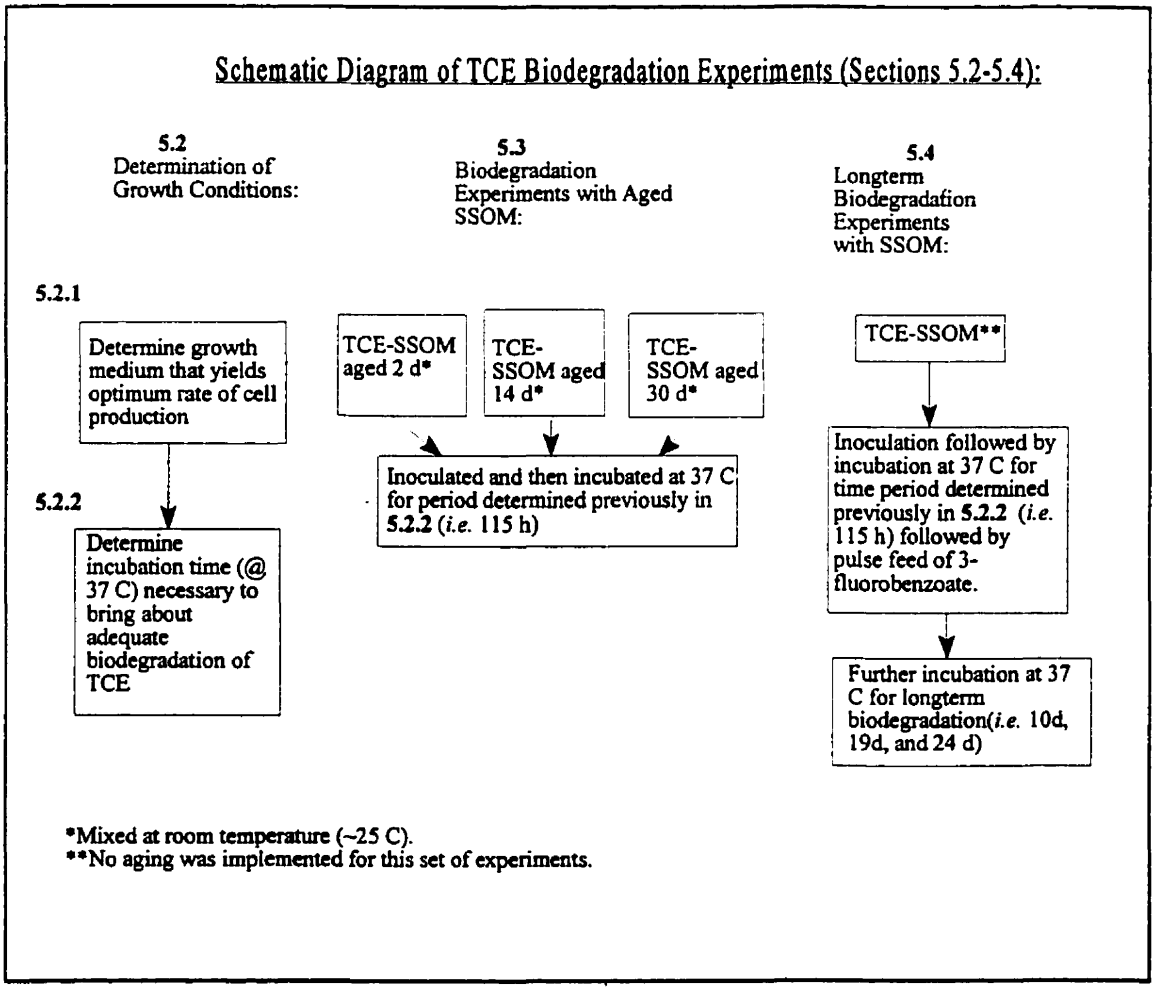


Figure 5.1. Schematic diagram of TCE biodegradation experiments.

As described in the *Literature Review* of Chapter 2, TCE undergoes the following microbially mediated reductive dechlorination under anaerobic conditions:

$$TCE \rightarrow cis\text{-}1,2\text{-}DCE + trans\text{-}1,2\text{-}DCE + 1,1\text{-}DCE \rightarrow VC \rightarrow ethene + ethane \qquad (12)$$

Cole *et al* (1995) reported *cis*- and *trans*-1,2-DCE as the product of PCE and TCE dehalogenation by *D. tiedjei*; but, the presence or absence of lesser-chlorinated ethenes by analytical methods was not confirmed. Furthermore, complete mineralization of TCE to CO_2 does not occur by *D. tiedjei* (J. Tiedje, personal communication May 10, 1996). In another study, an enrichment containing *D. tiedjei* in a biofilm reactor converted TCE to *cis*- and *trans*-1,2-DCE, and VC was never detected (Fatherpure and Tiedje, 1994). In natural environments, PCE and TCE can be converted to DCE and VC, and sometimes there is total dechlorination to ethene or ethane; however, of the DCE isomers 1,1-DCE is the least significant intermediate, and *cis*-1,2-DCE usually predominates over *trans*-1,2-DCE (Bouwer, 1993). Therefore, for the TCE biodegradation experiments, *cis*- and *trans*-1,2-DCE were measured in all cases, and for some experiments VC, ethene, and ethane were also measured. The rationale for the measurement of VC, ethene, and ethane is that the medium utilized in the present study is relatively novel. In particular, it contains 3-fluorobenzoate for induction of the dehalogenase enzyme that has been used in only one other study published to date (Cole *et al*, 1995). Furthermore, as was mentioned above, in this last study, even though the medium was improved, only *cis*- and *trans*-1,2-DCE were measured following biodegradation. Therefore, due to the improvements in the medium, and since there are presently no reported studies that have examined TCE dechlorination by *D. tiedjei* in soil, it was thought prudent to analyze for VC, ethene, and ethane.

The carbon balance that relates the TCE that is biodegraded to the products of reductive dechlorination is given by the following equation:

$$(N_{TCE})_{Abiotic} - (N_{TCE})_{Biotic} = N_{cis\text{-}1,2\text{-}DCE} + N_{trans\text{-}1,2\text{-}DCE} + N_{1,1\text{-}DCE} + N_{VC} + N_{ethene} + N_{ethane} \quad \textbf{(13)}$$

Where, $(N_{TCE})_{Abiotic}$ is the TCE remaining in the sterilized controls following incubation, moles.

$(N_{TCE})_{Biotic}$ is the TCE remaining following biodegradation by *D. tiedjei* following incubation, moles.

$N_{cis\text{-}1,2\text{-}DCE}$, $N_{trans\text{-}1,2\text{-}DCE}$, $N_{1,1\text{-}DCE}$ are the DCE isomers that are formed by partial TCE dechlorination, moles.

N_{VC} is the vinyl chloride formed from dechlorination of DCE isomers, moles.

N_{ethene}, N_{ethane} are products of complete TCE dechlorination, moles.

By taking the difference, $((N_{TCE})_{Abiotic} - (N_{TCE})_{Biotic})$, the disappearance of TCE by abiotic processes is taken into account when calculating the amount of TCE that disappeared as a result of microbial reductive dehalogenation. It is anticipated that abiotic losses are predominantly associated with aging, handling, and volatilization during incubation at 37°C. For TCE, these losses are estimated from the sterilized controls (*i.e.* abiotic losses). Total abiotic losses measured in the sterile controls were assumed to be the same as those that occurred during biodegradation since handling was identical in both cases. However, for the products of dechlorination, this estimation of abiotic losses was not possible. Hence, the sum of the products from the reductive dechlorination of TCE (*i.e.* $N_{cis\text{-}1,2\text{-}DCE} + N_{trans\text{-}1,2\text{-}DCE} + N_{1,1\text{-}DCE} + N_{VC} + N_{ethene} + N_{ethane}$) is expected to be less than the estimated amount of TCE that was biodegraded (*i.e.* $(N_{TCE})_{Abiotic} - (N_{TCE})_{Biotic}$).

By rearranging equation (13), the following expression can be written:

$$1 > x_{TCE} = \frac{(N_{cis\text{-}1,2\text{-}DCE} + N_{trans\text{-}1,2\text{-}DCE} + N_{1,1\text{-}DCE} + N_{VC} + N_{ethene} + N_{ethane})_{Biotic}}{((N_{TCE})_{Abiotic} - (N_{TCE})_{Biotic})} \quad (14)$$

Where x_{TCE} is the fraction of TCE that is converted to products of reductive dechlorination. In this study, x_{TCE} is less than unity since abiotic losses of the products of reductive dehalogenation (*i.e.* the numerator) cannot be accurately estimated.

5.2 Determination of Growth Conditions

This section describes the results that pertain to the determination of growth conditions required to bring about adequate biodegradation of TCE. In particular, the optimum growth medium as well as the incubation time required to bring about a reasonable degree of TCE degradation were determined.

5.2.1 Growth Medium

Growth of *D. tiedjei* is quite slow, therefore determination of optimum conditions for growth was desired so as to harvest an adequate supply of cells for the biodegradation experiments within a reasonable amount of time. Two media were compared for their ability to achieve this goal. As described in the *Methodology*, the media developed by DeWeerd *et al* (1990) and by Cole *et al* (1995) were compared. The use of 3-fluorobenzoate as an improved inducer of enzyme activity described by Cole *et al* (1995) was implemented in the medium described by DeWeerd *et al* (1990). Following transfer and incubation of *D. tiedjei* for two months in the Cole and DeWeerd growth media,

results of protein analysis indicated that the Cole medium yielded approximately 22 % more growth by weight, as compared to the DeWeerd medium (Table 5.1).

Table 5.1. Protein yield for Cole and DeWeerd media.

Medium	Protein Concentration (mg/L)		
	Mean	Minima	Maxima
Cole	212 ± 19.1 [1]	194	225
DeWeerd	165 ± 12.7	157	172

[1]95 % confidence interval for four observations.

In particular, an average of 212 mg/L of protein was obtained with the Cole medium as compared to 165 mg/L of protein measured for the DeWeerd medium. By comparing the average protein concentration using the t-test, as described by Himmelblau (1970), it can be said with 95 % confidence that the Cole medium yielded more biomass than the DeWeerd medium. Based on these results, the Cole medium was used for all subsequent harvesting of *D. tiedjei*.

5.2.2 Determination of Incubation Time for Biodegradation of TCE

The results of biodegradation of TCE by *D. tiedjei* are presented in Figure 5.2 for an average protein concentration of 189.64 ± 13 mg/L (95 % confidence interval). Biodegradation of TCE was evidenced by the disappearance of TCE accompanied by the appearance of *cis*-1,2-DCE, a major metabolite of TCE degradation (Mohn and Tiedje, 1992). From the results, it is evident that in the first 40.5 h there was little or no biodegradation. However, at some time between 80 and 115 h of incubation, degradation of TCE is apparent by the disappearance of TCE accompanied by the appearance of *cis*-1,2-DCE. Based on these results, all subsequent biodegradation experiments were conducted with 115 h of incubation at 37°C to ensure adequate biotransformation of TCE.

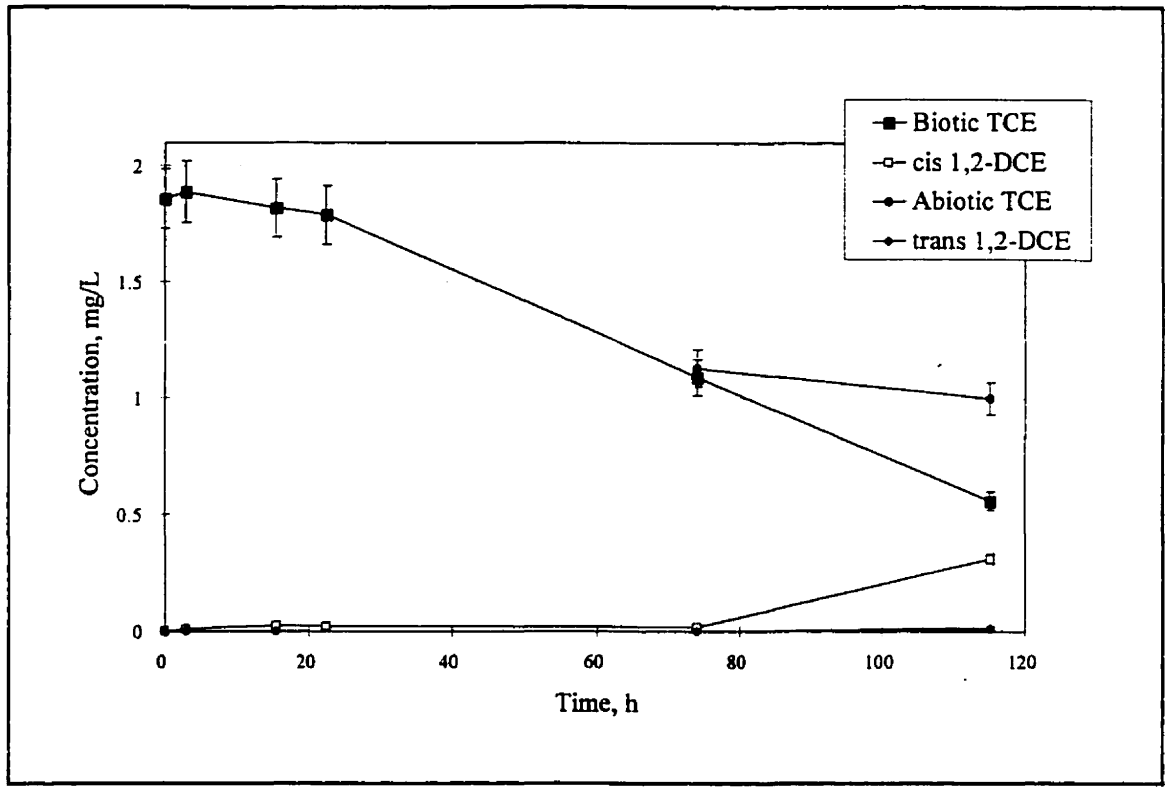


Figure 5.2.. TCE degradation by *D. tiedjei* over 115 h (95 % confidence intervals shown for all points, but some are hidden by symbols).

5.3 Biodegradation Experiments With Aged SSOM

5.3.1 Sorption of TCE

As described in the literature review section, the Freundlich adsorption isotherm[1] is commonly used to describe sorption of TCE by soil (Carter and Weber, 1994; Grathwohl, 1990; Xing *et al*, 1996; Zytner, 1992). The procedure for determining the constants (*i.e.* K and n) consists of combining a specific soil:solution ratio with six different initial concentrations of the contaminant (Lyman *et al*, 1990). After shaking for 48 h in order to

[1]Equation 3 of chapter 2.

achieve "equilibrium", the concentrations of contaminant in the sorbed and aqueous phases are determined analytically. The values of x/m (the amount sorbed, μg/g) are plotted versus C (the "equilibrium" aqueous phase concentration, mg/L). However, as was also described in the *Literature Review*, such short term studies do not adequately describe the non-labile, or non-equilibrium, sorption kinetics that occur in natural systems (Pignatello, 1993). Therefore, longer contact times are required to adequately describe sorption. However, by inspection of Figure 5.3, it is evident that total losses of TCE occurred during the 30 d time course of the sorption experiments. Such losses were likely due to volatilization since the experiment was conducted under sterile conditions (hence eliminating biotic losses) and since TCE is quite volatile[2]. As was pointed out in the *Methodology*, the Teflon coated septa that were in place during aging were not pierced. Therefore, losses of TCE observed were unavoidable and occurred as a result of aging. This is evident by the trend in Figure 5.3, where it can be seen that the total concentration of TCE decreases as a function of aging. Furthermore, Teflon coated septa are accepted and approved for handling of TCE, *cis*- and *trans*-1,2-DCE, 1,1-DCE, and many other volatile compounds, for analysis of soil contaminated with the above mentioned volatiles by the Ontario Ministry of the Environment (1990). Therefore, since the total concentration of TCE decreased, equilibrium conditions were not attained for this system. As well, the level of dissolved organic matter (DOM) increased with time (Figure 5.4). Since the SSOM is a humified material and the DOM was measured in the absence of TCE, the DOM is humic in nature (*i.e.* humic acid and fulvic acid). The DOM will act to reduce the sorption of a contaminant by soil possibly by increasing the solubility of the contaminant in solution or by competitive adsorption (Lyman *et al*, 1990). Furthermore, as the concentration of dissolved humic substances increases, sorption decreases (Rebhum *et al*, 1996). Therefore, a second variable, aside from volatilization, existed that will cause a shift from equilibrium conditions.

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[2]Henry's Law constant for TCE is 544 atm. Since this is greater than 160 atm, it is considered to be volatile (Nyer, 1993).

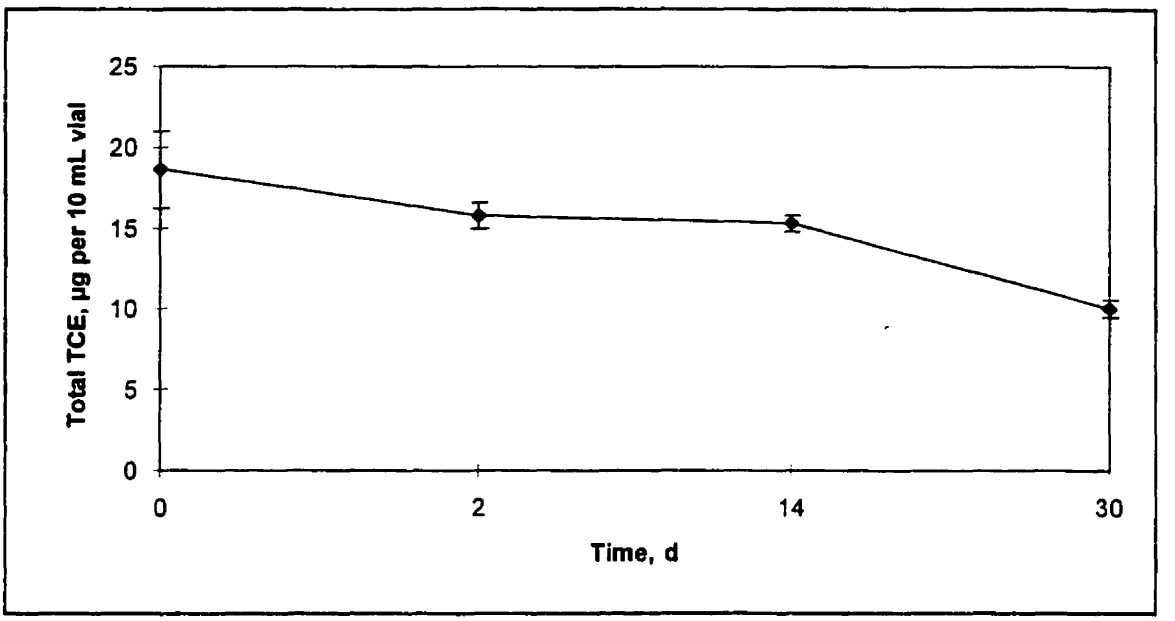

Figure 5.3 Total TCE remaining (*i.e.* aqueous and sorbed) following 2 d, 14 d, and 30 d of aging that was implemented for sorption tests (95 % confidence intervals shown).

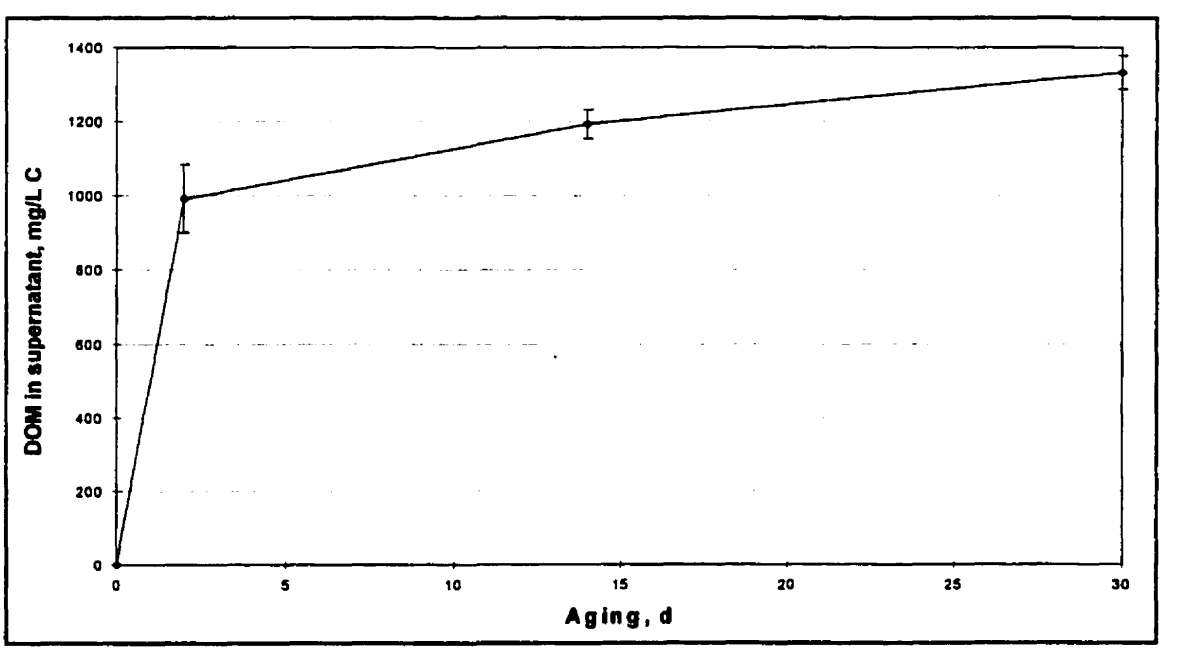

Figure 5.4 DOM in supernatant as a function of aging (95 % confidence intervals shown).

Equilibrating the reduced nutrient medium (*i.e.* CAA) with the SSOM prior to the addition of the TCE may have resulted in a more uniform level of DOM. However, there is no guarantee that this precautionary measure would have ultimately resulted in uniform levels of DOM since the TCE itself may aid in leaching organic matter from the SSOM into solution. This is a distinct possibility due to the solvation properties of TCE (Swoboda-Colberg, 1995). Following "pre-equilibration" of the SSOM with the CAA to attain uniform DOM levels, the subsequent process of aging the TCE with the SSOM from 2 to 30 d would again have resulted in varying levels of DOM.

Recognizing that such processes (*i.e.* volatilization of TCE from the system and leaching of humic and fulvic acids from the SSOM into the aqueous phase) rendered the system in a state of "quasi equilibrium" at best, the values of x/m versus C were plotted in Figure 5.5 in order to obtain an estimate of the Freundlich partitioning coefficient K[3]. By fitting the amount of TCE sorbed, x/m, and the aqueous TCE concentration, C, to the Freundlich sorption isotherm, the sorption coefficient K was estimated as 30.9 mL/g. From this the K_{OC} can be estimated[4]. The K_{OC} estimated in this way, as well as those estimated from the sorption experiments that were conducted to determine the effect of gamma-irradiation on TCE sorption (Figure 4.9), were compared to those that have been reported in the literature for TCE (Table 5.2). The K_{OC} obtained from data in Figure 4.9 are about two times greater than those obtained from the data in Figure 5.5. There are several differences in the conditions of the two sets of sorption experiments that likely contributed to the differences in sorption. The first is the likely difference in the DOM between the two sets of experiments. The sorption experiments in Figure 4.9 were carried out over 24 h, so the DOM was probably less than those values associated with the aging experiments (*i.e.* DOM of 933 to 1333 mg/L of C for

[3]From equation 3 in Chapter 2.

[4]From equation 2 in Chapter 2.

aging of 2 to 30 d, respectively). In addition, the losses by volatilization in aging experiments were greater than those that occurred after only 24 h.

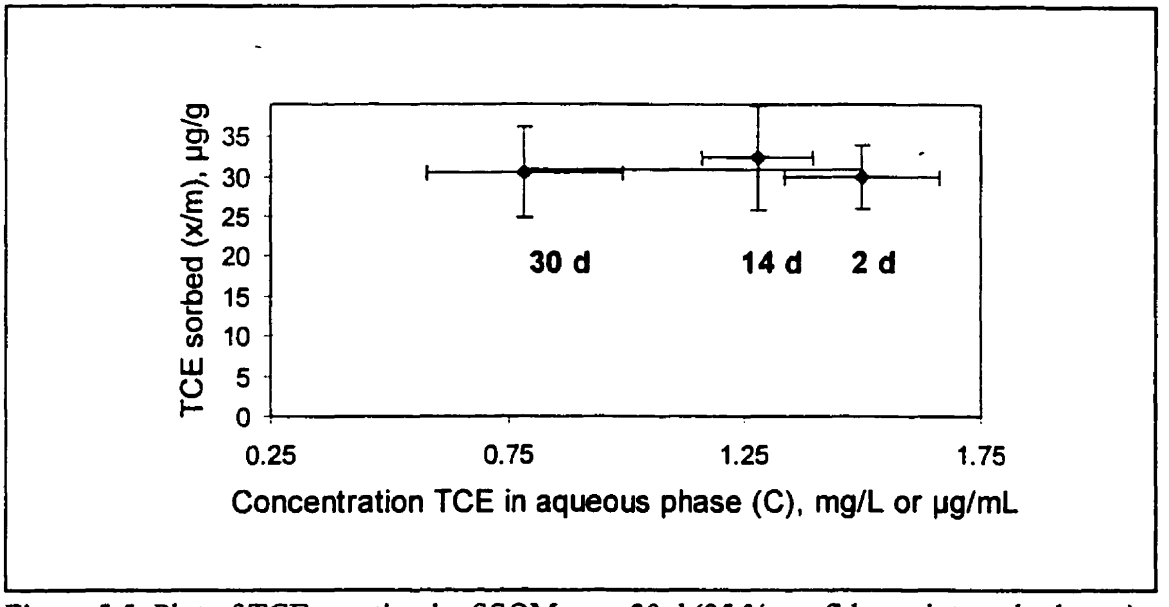

Figure 5.5. Plot of TCE sorption by SSOM over 30 d (95 % confidence intervals shown).

The effect of DOM on partitioning is illustrated by the difference in partitioning coefficients of the experiments in Figure 4.9 and those obtained by Zytner (1992) for peat moss (Table 5.2). In particular, K_{OC}'s of 117 mL/g (sterile) and 104 mL/g (non-sterile) were obtained for SSOM. Whereas, Zytner (1992) found a K_{OC} of 189 mL/g for the peat moss. Peat moss, like sphagnum moss, is an unhumified material. Hence, peat moss would be expected to have lower levels of DOM (*i.e.* humic and fulvic acids are thought to be products of humification) compared to the composted sphagnum moss (*i.e.* SSOM). The humic acid, a component of DOM, would be expected to reduce TCE sorption (Abdul *et al*, 1990; Xu *et al*, 1994).

It should be noted that the K_{OC}'s determined for TCE with a number of natural soils by Grathwohl (1990) that were discussed in the *Literature Review* (section 2.1.4) were not included in Table 5.2 since sorption by wet soil was from the vapour phase and not the

aqueous phase.

Therefore, although it is recognized that the system under study was not in a state of equilibrium, the estimated K_{OC} from Figure 5.5 is about 4 times smaller than the value reported for peat moss by Zytner (1992). However, it may be that the system studied by Zytner (1992) was also probably not in a state of equilibrium. As pointed out by Pignatello and Xing (1996), during the second slower stage of sorption, experimentally observed changes in the solution-phase concentration may not be apparent over short time periods due to random analytical errors. From Figure 5.3, it can be seen that there are considerable losses by volatilization from the aging process. In addition, from Figure 5.4 it can be seen that the DOM also increased with aging. Both of these factors (*i.e.* presence of DOM and losses from volatilization) likely resulted in a reduction in the partitioning coefficient (Lyman *et al*, 1990).

From Figure 5.5, it is evident that the amount of TCE sorbed, x/m, did not vary significantly with the aqueous TCE concentration. This was despite the fact that the total amount of TCE decreased from approximately 16 to 10 μg (per 10 mL vial) between 2 and 30 d of aging (Figure 5.3). However, from Figure 5.5, it can be seen that the aqueous TCE also decreased with aging, probably a result of volatilization. Since unpierced Teflon coated septa were used, this volatilization from aging was unavoidable. Furthermore, from the confidence intervals that are shown in Figures 5.3 and 5.5, it is evident that these decreases are statistically significant.

Table 5.2. Estimated K_{oc}'s compared to those reported in the literature along with % OC of the corresponding materials.

Source	Material	K_{oc} (mL/g, or L/Kg)	% OC
Present study	Sterile SSOM (Figure 5.5)	57	54
Present study	Sterile SSOM (Figure 4.9)	117	54
Present study	Non-sterile SSOM (Figure 4.9)	104	54
Zytner (1992)	Sandy loam	50	1
Zytner (1992)	Organic top soil	115	11.74
Zytner (1992)	Peat moss	189	49.42

With decreasing aqueous TCE levels, one would expect desorption of TCE from the solid sorbed phase owing to a change in the concentration gradient between the aqueous and solid sorbed phases. However, since the level of TCE in the sorbed phase remained relatively constant with aging (Figure 5.5), it appears that the solid phase TCE was non-labile in nature and resisted desorption. As discussed in the *Literature Review*, as aging of a contaminant in soil increases, the sorbed contaminant becomes non-labile and less amenable to both desorption and biodegradation (Karickhoff, 1980; Pignatello, 1993). Such resistance was not observed by Zytner (1992) where desorption was conducted following aging for 48 h; hence, in the present study, the observed resistance may stem from aging. It would appear that the results in Figure 5.5 are representative of retarded desorption resulting from 30 d aging of TCE with the SSOM.

The retarded desorption observed is consistent with the pore diffusion (PD) model discussed in the *Literature Review* of Chapter 2. According to this model, as the aging of TCE and

SSOM increases, TCE molecules diffuse within the SSOM structure. However, there is an inconsistency with this model as applied to the TCE-SSOM system. In particular, the PD model assumes a fixed-pore system. This is inconsistent with the model of humus proposed by Wershaw (1993). According to this model, humus exhibits hydrophobic (nonpolar) and hydrophilic (polar) behaviour. In solution, aggregates form and they consist of a hydrophobic interior and a hydrophilic exterior. The aggregates are said to be held together by virtue of hydrophobic interactions. The hydrophobic interiors are said to have freedom of movement and are liquid in nature. This notion is inconsistent with the PD model that assumes rigid pores.

However, the polymeric model proposed by Wershaw (1993) is consistent with the intra-organic matter diffusion (IOMD) model proposed by Brusseau *et al* (1991). According to this model, TCE molecules diffuse within the hydrophobic interior of SSOM. Presumably, as the contact time increases, the TCE molecules diffuse farther within the hydrophobic interior. In addition to this, TCE is capable of interacting with the oxygen and nitrogen containing functional groups that are likely to exist at the hydrophilic exterior of the aggregates. Such interactions between the SSOM and TCE were discussed previously[5]. In particular, hydrogen bonding between the chlorine atoms of TCE and the hydrogen atom of the phenolic functional groups (likely located on the exterior hydrophilic surface of the aggregates) and ion-dipole interactions between the hydrogen atom of TCE and the ionized carboxylic acid functional group are likely. Also, ion-dipole interactions between protonated amino groups and electronegative chlorine atoms was cited as a possible interaction between SSOM and TCE. Such interactions are consistent with the adsorption bond energy (ABE) model that was also discussed in Chapter 2. Furthermore, if a TCE molecule is enmeshed within a matrix of dissolved humic acid, by interactions just described, the large polymeric humic acid molecules themselves may form strong interactions with solid SSOM by the cumulative

[5]Sections 4.2.2.2 and 4.2.2.3 of Chapter 4.

effect of hydrogen bonding. Due to the high levels of DOM (Figure 5.4), this is a distinct possibility.

As well, the SSOM, being a complex fibrous mixture of humic and non-humic material, likely possesses a pore structure of sorts. Hence, the PD model may apply in this context. As TCE diffuses within these pores, it likely sorbs onto the surface by mechanisms of hydrogen bonding and ion-dipole interactions just described. This is consistent with the sorption retarded pore diffusion (SRPD) model.

Hence, it would appear that no single model is adequate in describing rate-limited desorption of TCE from the SSOM complex that is apparent from the fact that the quantity of sorbed TCE remained constant despite the fact that the aqueous phase TCE decreased from volatilization over 30 d of aging. Rather, a combination of the four models[6] that describe the origins of retarded desorption likely applies. Desorption from aged complexes will be discussed in more detail in a subsequent section entitled *Sequential Desorption* in Chapter 6.

5.3.2 Biodegradation of TCE Aged With SSOM

As indicated in the schematic diagram describing the TCE biodegradation experiments (Figure 5.1), the soil suspensions were inoculated with *D. tiedjei* following the three times (*i.e.* 2 d, 14 d, and 30 d) that the TCE and SSOM were aged. Hence, the partitioning of TCE between the solid and aqueous phases depicted in Figures 5.5 and 5.6 were the initial conditions for biodegradation. Figure 5.7 depicts the total concentration of TCE biodegraded. On the x-axis, zero aging time corresponds to the case involving TCE degradation in the absence of SSOM. On the y-axis, the total amount of TCE degraded

[6]Depicted in Figure 2.2, chapter 2.

corresponds to the amount of TCE biodegraded (*i.e.* left hand side of equation (13) of this Chapter) by *D. tiedjei*. The units of μg/L correspond to the mass of TCE biodegraded (in μg) per total volume of the batch reactor (*i.e.* 10 mL).

The protein concentrations for the aging experiments are summarized in Table 5.3. Since the concentrations were similar (95 % confidence, as determined by the t-test), the density of cells used for inoculation in this set of experiments was also similar. Hence, the relative differences in biodegradation are comparable.

Table 5.3. Protein concentrations from biodegradation of TCE following aging.

Aging with SSOM, d	Protein, mg/L			Number of Observations
	Mean	Minima	Maxima	
0 (*i.e.* no SSOM)	189.64±13.5[†]	173.59	225.41	10
2	175.92±4.0	164.53	195.02	4
14	182.00±13.7	173.59	190.00	4
30	178.50±14.3	169.50	187.85	4

[†]95 % confidence intervals.

The amounts of TCE degraded as a function of aging are summarized with results of t-tests that were conducted at both 90 and 95 % confidence levels in order to compare the averages, according to methods of Himmelblau (1970). This statistical analysis is summarized in Table 5.4.

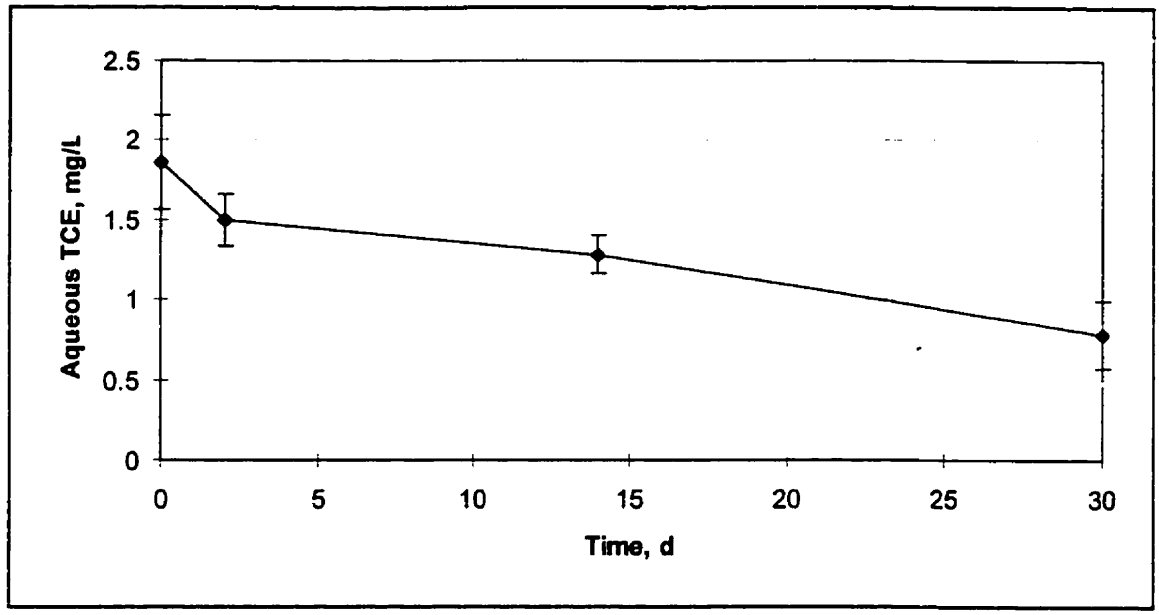

Figure 5.6. TCE in aqueous phase following sorption (95% confidence intervals shown).

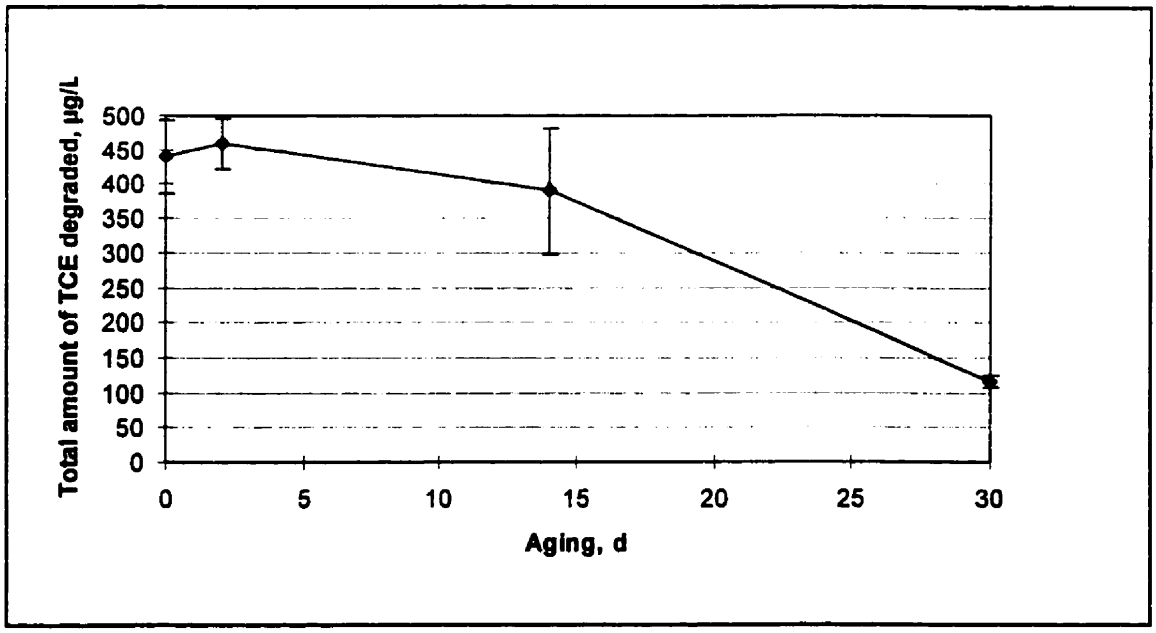

Figure 5.7. Total concentration of TCE biodegraded with aging (95 % confidence intervals shown).

Table 5.4. Comparison of average masses of TCE biodegraded as a function of aging using the t-test (90 and 95 % confidence levels).

Aging with SSOM, d	Average Mass of TCE biodegraded, µg/L	Results of t-test to compare averages	
		90 % confidence level	95 %confidence level
0 (*i.e.* no SSOM)	440‡	0 and 2 d aging:	0 and 2 d aging:
		0 d (440) = 2 d (459)	0 d (440) =2 d (459)
2	459‡	2 and 14 d aging:	2 and 14 d aging:
		2 d (459) > 14 d (390)	2 d (459) = 14 d (390)
14	390‡	14 and 30 d aging:	14 and 30 d aging:
		14 d (390) >30 d (116)	14 d (390) > 30 d (116)
30	116‡		

‡eight observations were used to calculate the mass of TCE biodegraded.

There was no difference in biodegradation between the case involving no SSOM and the TCE-SSOM aged for 2 d (95 % level of confidence). With 90 % confidence, it can be said that there was a slight decrease in biodegradation from 2 to 14 d aging; however, the same cannot be said with 95 % confidence. Beyond 14 d aging, the degree of biodegradation markedly falls off. This trend was despite the fact that the initial level of TCE, in terms of the total amount present (Figure 5.3) and also in terms of the amount present in the aqueous phase (Figure 5.6), decreased with aging. In general, as the concentration of a contaminant increases, so does its toxicity to the degrading organism and this can result in a reduction in the rate and extent of biodegradation (Troy, 1994). As described in the *Literature Review*, surfactants have been reported to have inhibitory effects on biodegradation (Tiehm, 1994). Toxic effects of DOM (*i.e.* humic and fulvic acids) on biodegradation were not found in the literature, and this possibility was not investigated in the present study. However, it is thought that humic acid may be less toxic than synthetic surfactants (Xu *et al*, 1994). Furthermore,

it is questionable that the DOM had a toxic effect on *D. tiedjei* if one considers the long-term biodegradation study that was conducted both with and without SSOM. These results are presented in a subsequent section in this Chapter (section 5.4). However, the results do not indicate toxicity of SSOM or DOM to *D. tiedjei*. On the contrary, results of long-term degradation indicate that either the SSOM or DOM are beneficial to the dechlorination activity of this bacterium.

Furthermore, sorption can in some cases, enhance bioremediation by reducing the concentration, and hence toxicity, of the contaminant in the aqueous solution (Apajalahti and Salkinoja-Salonen, 1984). Sorption of toxic halogenated substrates such as TCE and PCE has been identified as a mechanism that can increase the rate of dechlorination by reducing aqueous levels of the toxic substrates (Mohn and Tiedje, 1992). Dechlorination activity declined at PCE concentrations that were greater than 9.8 mg/L in a reactor containing *D. tiedjei* in an anaerobic consortium (Fatherpure and Tiedje, 1994). Furthermore, for *D. tiedjei*, at cell concentrations equivalent to 100 mg/L initial protein concentration, increasing the PCP concentration from 0.792 to 2.640 mg/L had a negative effect on cell growth (Mohn and Tiedje, 1992). Oldenhuis *et al* (1991) observed acute toxicity at TCE concentrations of 2.45 mg/L for *Methylosinus trichosporium* OB3b at initial cell concentrations of 100 mg/L. However, for the present research, toxicity studies to determine the effect of TCE on *D. tiedjei* were not conducted. Hence, although there are presently no published reports regarding the toxic effects of TCE on *D. teidjei* activity in the literature, from the studies just cited, it is possible that there would be a decline in dehalogenation activity with increasing concentrations of TCE. Consequently, an increase in biodegradation, or at least a stable degree of biodegradation, with decreasing contaminant levels in the aqueous phase would be expected. However, despite the fact that the initial level of TCE in the aqueous phase decreased with aging, the total amount of TCE degraded also decreased.

According to the conceptual model[7] proposed by McCarty (1988) to describe the interaction between sorption and biodegradation, the contaminant in the aqueous phase is available for biodegradation and the solid-sorbed phase contaminant is unavailable for biodegradation. Furthermore, the sorbed contaminant must undergo desorption before it can be degraded. If this model applies to the present case, then sorption of TCE by the SSOM did not appear to result in limited biodegradation since the amount of TCE present in the aqueous phase was greater than the amount degraded (Figure 5.8). Furthermore, with the reduced TCE concentration, and hence potential toxicity, in the aqueous phase (Figure 5.8), one would expect the degree of biodegradation to increase or remain constant. However, the total mass of TCE that was biodegraded decreased as the initial level of TCE in the aqueous phase decreased. Since the amount of TCE degraded was always less than what was available in the aqueous phase, it would appear that sorption of TCE by the SSOM did not hinder biodegradation. Furthermore, if the aqueous phase TCE was more "available" to *D. tiedjei* for biodegradation, then it would be expected that there would be an increase in the total amount of TCE biodegraded with decreasing amounts of aqueous phase TCE with aging since toxicity affects were reduced. However, this was not observed.

The TCE that partitioned with the SSOM should not have posed a limit to biodegradation, according to the model of McCarty (1988). Nonetheless, the presence of SSOM and aging resulted in a reduction in the overall biotransformation of TCE. This may have been the result of DOM that contained humic acid and fulvic acid. To further understand the potential effects of DOM on biodegradation, it is useful to examine the results of a study conducted by Amador and Alexander (1988) that was discussed in the *Literature Review*. In this last study it was found that benzylamine (an NOC like TCE) binding with humic acid resulted in reduced mineralization. The degree of binding, as measured by dialysis, increased as the ratio of humic acid to benzylamine increased. The increase in binding also resulted in a

[7]Depicted in Figure 2.4 of chapter 2.

corresponding decrease in mineralization. Amador and Alexander (1988) also found that benzoic acid and phenyl acetic acid did not bind at all with humic acid. Using acidity constants reported in the literature, it was estimated that these last two compounds are negatively charged at pH 7. Hence, it is likely that TCE can bind with humic acid (a component of DOM) since it is also neutral. However, it was not possible to measure TCE binding with DOM since the dialysis equipment that is necessary to measure binding of dissolved components (Amador and Alexander, 1988; Hintelmann *et al*, 1997) was not available for the present study. As well, due to the extreme volatility of TCE, such an apparatus would require modification to minimize volatilization losses. Such a study was beyond the scope of this work. However, it is worthy of further study.

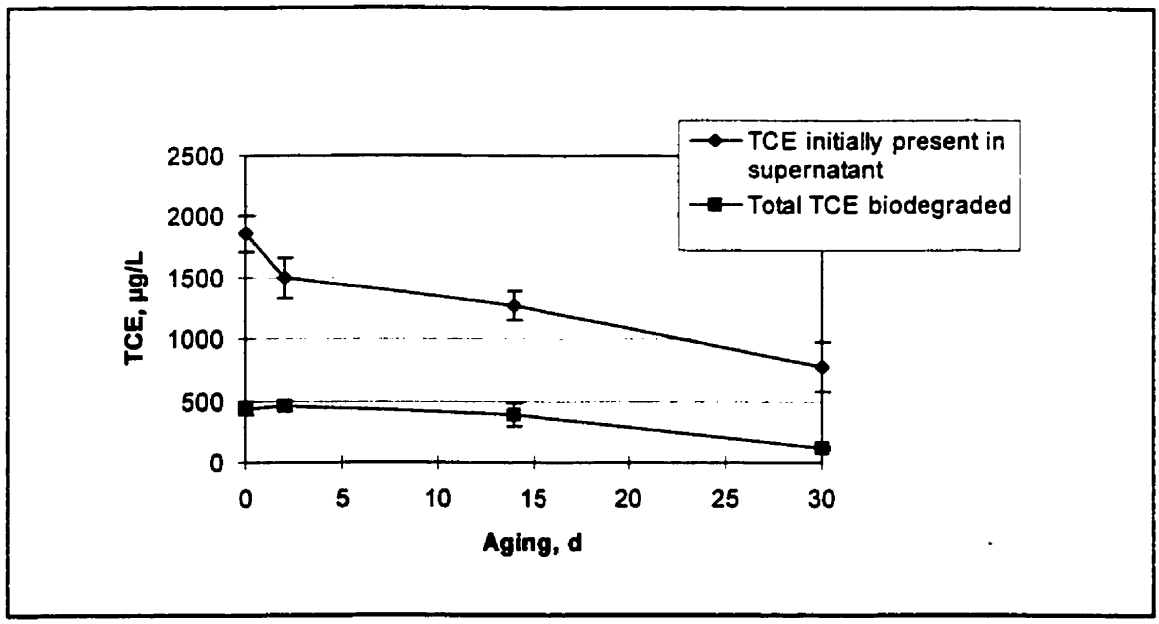

Figure 5.8. TCE initially present in the supernatant versus total TCE biodegraded (95 % confidence intervals shown).

If TCE binds with the DOM and if this rendered the TCE unavailable for biodegradation by *D. tiedjei*, then increasing the ratio of DOM:TCE should have resulted in increased binding and a corresponding decrease in biodegradation, as was observed by Amador and Alexander (1988). Figure 5.9 shows the ratio of DOM:TCE in the supernatant versus aging and the amounts of TCE that were biodegraded. The ratio of DOM:TCE in the supernatant increased

with aging (from 5 to 15.5 mg C[8] per mg TCE for 2 to 30 d aging, respectively) and the corresponding total quantity of TCE biodegraded decreased (from 459 to 116 μg/L for 2 to 30 d aging, respectively). Between 0 and 2 d aging, where the DOM:TCE ratio increased from 0 to 5, there was no significant difference in the amount of TCE degraded. Between 2 and 14 d aging, where the DOM:TCE ratio increased from 5 to 8.5, there was a slight decrease in the amount of TCE degraded (at 90 % confidence level, Table 5.4). Whereas, between 14 and 30 d aging, where the DOM:TCE ratio increased from 8.5 to 15.5, there was a significant decrease in the amount of TCE degraded (at a 95 % confidence level, Table 5.4). These results are consistent with what was observed by Amador and Alexander (1988), particularly as the ratio of DOM (*i.e.* humic acid) to aqueous NOC (*i.e.* benzylamine) increases, the biodegradation of the NOC decreases due to binding of the NOC with DOM. Although it is possible that a similar phenomenon occurred in the present experiments (*i.e.* binding of TCE and DOM), as was just mentioned, it was not possible to measure the extent of the binding by the dialysis technique in the present study. If in fact the decrease in TCE biodegradation with aging was caused by its binding with DOM, then the sudden decrease in the amount of TCE degraded between 14 and 30 d aging may be related to a critical micelle concentration (CMC) of DOM. As described in the *Literature Review*, humic and fulvic acids, that make up DOM, act as surfactants. Furthermore, as surfactants they would possess a CMC, above which nonionic contaminants would exist at the inner hydrophobic region of the micelle. If this were the case, TCE in the aqueous phase would be unavailable to *D. tiedjei*.

The possible binding of TCE and DOM is consistent with the observation made by Sposito (1989) that humic and fulvic acids are quite reactive due to their functionality, macromolecular nature, hydrophobicity, and structural lability. Further studies are required to determine the extent of the effect of DOM on TCE biodegradation. In particular, it would

[8]TOC.

be necessary to conduct TCE biodegradation studies in aqueous solution with increasing concentrations of DOM.

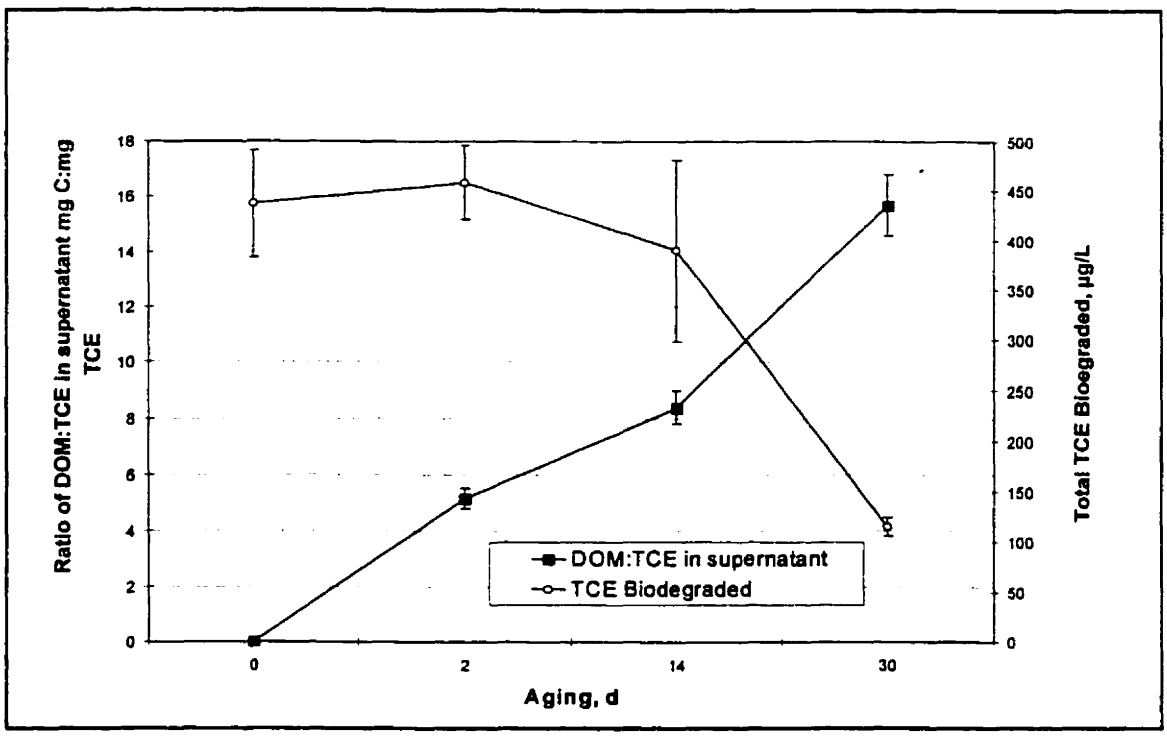

Figure 5.9. Ratio of DOM to TCE in the supernatant and the total quantity of TCE that was biodegraded as a function of aging (95% confidence intervals shown).

At 2 d aging, 933 mg/L C existed in the aqueous phase and rose to approximately 1333 mg/L following 30 d aging. The DOM of uncoloured water in natural environments usually contains 2 to 10 mg/L C (Malcom, 1993). Organically coloured streams may have DOM that ranges from 3 to more than 50 mg/L C, shallow groundwaters have DOM ranging from 0.2 to 15 mg/L C, and in the western U.S., some groundwaters that are highly coloured (referred to as "trong" waters) due to contact with lignin, have DOM levels in excess of 1000 mg/L. Hence, the levels of DOM that were measured in the present study are significantly greater than what may be encountered in many natural groundwater environments; however, they may be representative of porewater in soils containing significant quantities of organic carbon.

From Figure 5.10, it is evident that heating the TCE-SSOM slurries under sterile conditions did not result in significant losses of TCE from the solid-sorbed phase after 2 and 30 d aging (at 14 d aging there was a slight decrease in sorption). However, certain observations can be made concerning the distribution of TCE within the batch reactor. In particular, as was indicated in the *Methodology* of Chapter 3, the batch reactors were filled with CAA, SSOM, and TCE at the start of the aging experiments so as to minimize the head space. With time, it was observed that a meniscus of head space formed as the SSOM became wetted. From this meniscus, volatilization of TCE across the Teflon coated septa likely occurred, as evidenced by the decline in the total and aqueous quantities of TCE with aging (Figures 5.3 and 5.6, respectively). This was despite the fact that the septa were not pierced. The process of volatilization was likely complex due to the existence of SSOM. Based on a description of the volatilization of organic contaminants from soil by Lyman *et al* (1990), it is likely that there were three main distribution/transport processes in the batch reactors containing the CAA, SSOM, and TCE. These include the transport of TCE between the following: the solid-sorbed phase and the aqueous phase, the aqueous phase and the gas-meniscus phase, and the gas-meniscus phase and the surrounding atmosphere (*i.e.* across the unpunctured Teflon coated septa to the surrounding environment). The concentration of TCE in the aqueous phase, or the desorbed state, controlled the vapour density of TCE in the gas phase (*i.e.* in the meniscus). In turn, the vapour density controlled the rate of volatilization to the surrounding atmosphere. The partitioning of TCE with SSOM reduced the amount of TCE available to partition between the aqueous phase and the gas phase. Weakly adsorbed contaminants may have volatilized quickly; however, if the contaminant was incorporated within the soil, the total rate of volatilization would have decreased. This is illustrated from the results of one study where it was found that the vapour density of dieldrin decreased with increasing fractions of organic matter in the soil (Lyman *et al*, 1990).

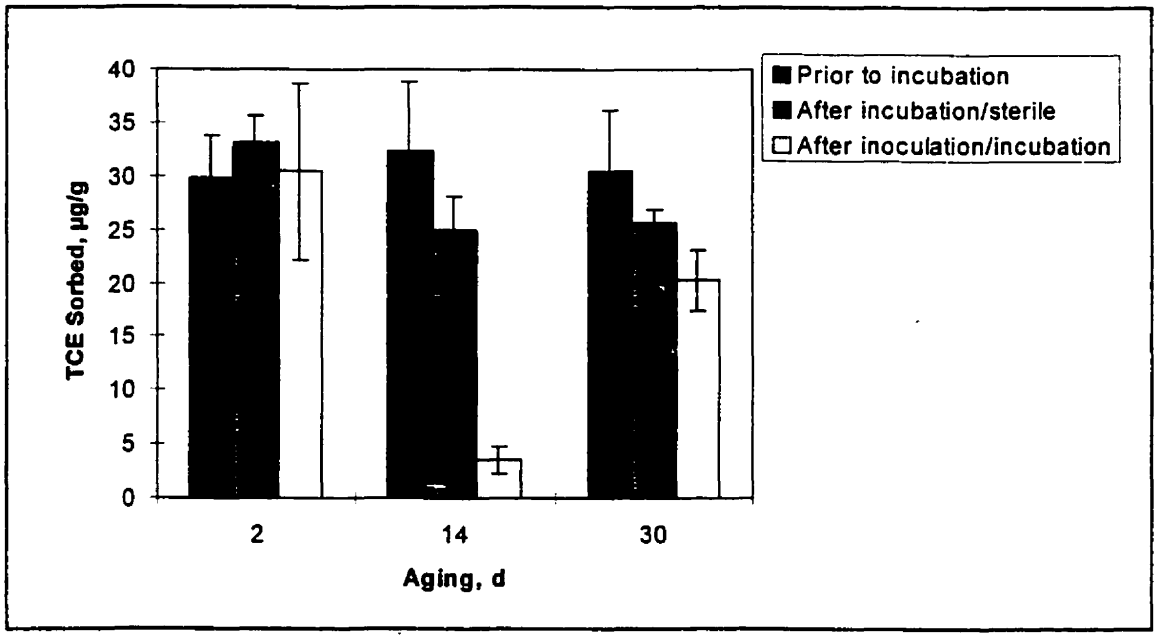

Figure 5.10. TCE sorption initially (*i.e.* prior to incubation), following incubation under sterile conditions (*i.e.*, 115 h heating at 37 °C), and following inoculation and incubation (*i.e.* heated for 115 h at 37°C) (95 % confidence intervals shown).

In general, an increase in temperature will result in an increase in the equilibrium vapour density that would result in volatilization of the contaminant to the atmosphere and transfer of contaminant from the solid-sorbed phase to the aqueous phase (*i.e.* desorption). However, from Figure 5.10, it can be seen that heating the batch reactors for 115 h at 37°C did not result in a significant reduction in solid-sorbed phase TCE (*i.e.* before and after incubation under sterile conditions). In general, it is difficult to distinguish sorption and desorption as separate processes (Yong *et al*, 1992). However, it is unlikely that additional sorption occurred over the 115 h of incubation time since the level of sorbed TCE prior to incubation was stable over the 30 d time period that was studied. Therefore, since desorption was the only mass transfer process that was likely to have occurred between the solid and aqueous phases it can be said that since TCE partitioning was relatively stable following incubation, desorption was negligible. Furthermore, from Figure 5.7 it can be seen that for 2 d aging, the greatest amount of TCE was biodegraded. Since for 2 d aging, TCE partitioning remained

relatively constant even after biodegradation, it can be concluded that TCE was biodegraded from the aqueous phase. It is interesting to note that even with the "removal" of TCE from the aqueous phase by biodegradation, desorption of TCE from the solid-sorbed phase did not occur. Therefore, for 14 d aging, the reduction in TCE from the solid-sorbed phase was likely the result of biodegradation directly at the SSOM surface. At 30 d aging, the solid-sorbed phase TCE decreased only slightly with biodegradation. Hence, biodegradation of solid-sorbed phase TCE following 30 d aging was very slight. To further examine the effects of aging on TCE biodegradation from the solid-sorbed and aqueous phases, it is useful to examine the results as they are represented in Figures 5.11 and 5.12.

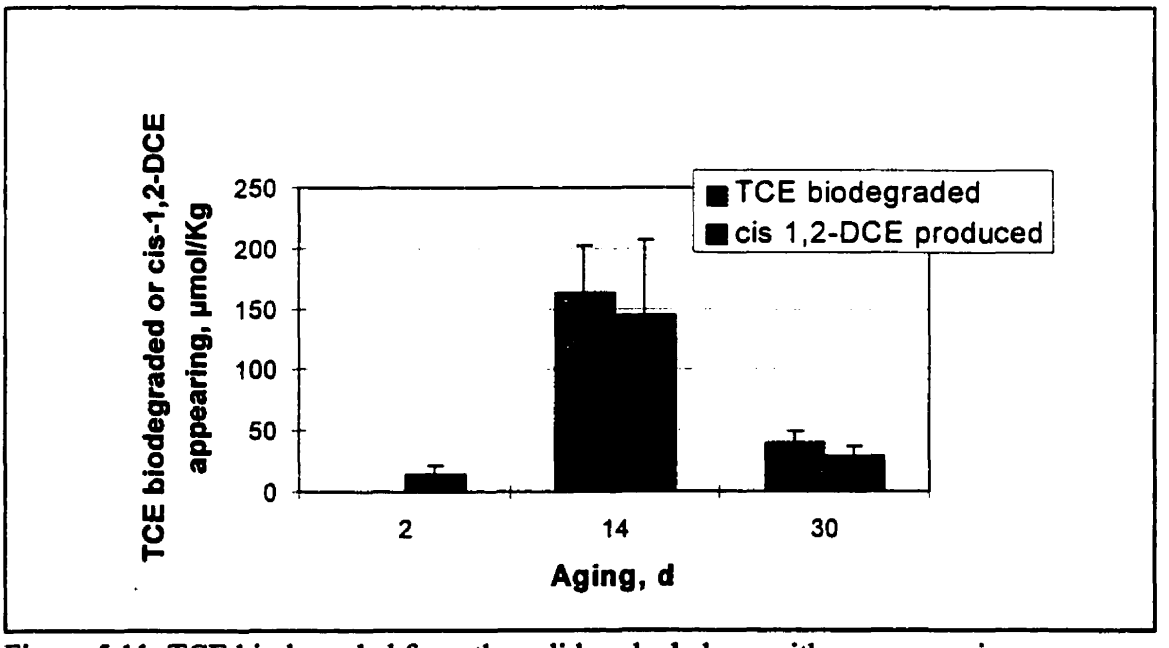

Figure 5.11. TCE biodegraded from the solid-sorbed phase with accompanying appearance of *cis*-1,2-DCE in the solid-sorbed phase (95% confidence intervals shown).

Figure 5.11 depicts the TCE that was biodegraded in the solid-sorbed phase and the *cis*-1,2-DCE that appeared also in the solid-sorbed phase as a function of aging. The amount of TCE biodegraded in the solid-sorbed phase was calculated by taking the difference between the TCE that was partitioned with the SSOM in the sterilized controls and the TCE that was partitioned with the SSOM following biodegradation. Figure 5.12 depicts the amounts of

TCE biodegraded in the aqueous phase as a function of aging. Similar to Figure 5.11, the amounts that were degraded were calculated as the difference between the TCE in the aqueous phase of the sterilized controls and the TCE in the aqueous phase following biodegradation. The amount of *cis*-1,2-DCE is that which appeared in the aqueous phase following 115 h of incubation.

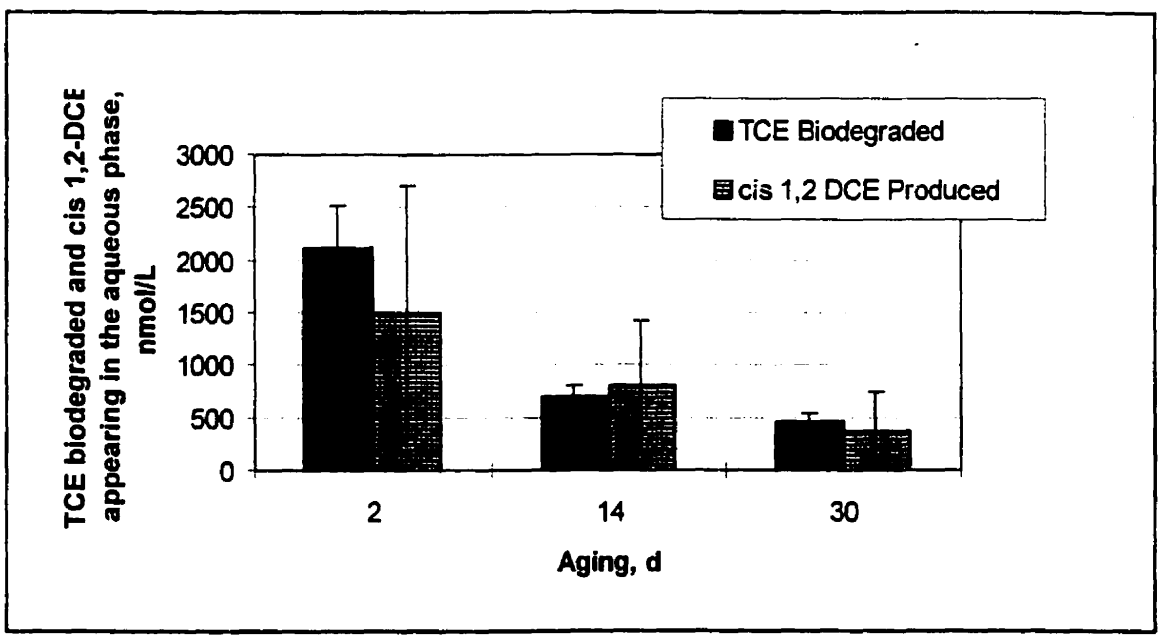

Figure 5.12 TCE biodegraded from the aqueous phase with accompanying appearance of *cis*-1,2-DCE in the aqueous phase (95% confidence intervals shown)

At 2 d aging, there was no conversion of solid-sorbed phase TCE to *cis*-1,2-DCE (Figure 5.11), and from Figure 5.12, it can be seen that biodegradation of TCE occurred predominantly from the aqueous phase. Therefore, aqueous phase TCE was preferentially biodegraded for the case of 2 d aging. However, at 14 d aging, TCE was preferentially degraded from the solid-sorbed phase (Figures 5.11 and 5.12) despite the fact that TCE was presumably in a less labile state in the solid-sorbed phase compared to 2 d aging. At 30 d aging, biodegradation of both solid-sorbed phase TCE and aqueous phase TCE occurred (Figures 5.11 and 5.12). As aging proceeded, biodegradation of TCE in the aqueous phase declined. This decrease in biodegradation may have been due to the increased ratio of

DOM:TCE in the aqueous phase that occurred with aging (Figure 5.9). As discussed previously, the binding of non-ionic contaminants such as TCE with DOM can result in a reduction in biodegradation; however, since it was not possible to measure such binding directly for TCE in this study, such an affect can only be inferred. At 14 d aging, the solid-sorbed phase TCE was predominantly biodegraded, presumably because the aqueous phase TCE was bound by DOM and hence was less available for degradation. At 30 d aging, neither the aqueous phase TCE nor the solid-sorbed phase TCE was biodegraded to any considerable extent. Solid-sorbed phase TCE became non-labile between 14 and 30 d presumably due to the diffusion of TCE deeper within the micropores as a result of the aging process. It has been postulated that as diffusion of contaminants within soil occurs with time (aging), the contaminants are less available to microorganisms since a considerable part of the pore volume consists of pores with effective diameters less than 1.0 μm (Alexander, 1995). Furthermore, pore sizes that are less than 20 nm are abundant. Alexander (1995) further concluded that such pores would not accommodate even the smallest bacterium. To summarize thus far, it would appear that as the DOM increased, biodegradation of aqueous phase TCE decreased due to binding with DOM, and the biodegradation of solid-sorbed phase TCE decreased with aging probably due to diffusion of TCE farther within the SSOM matrix.

Biodegradation of solid-sorbed TCE following 14 d aging is consistent with results reported by Guerin and Boyd (1993) where it was reported that *Pseudomonas putida* had access to labile-sorbed naphthalene and the labile-sorbed phase became non-labile following one year of aging. Guerin and Boyd (1993) also found that sorbed phase naphthalene was unavailable to a gram negative isolate from a petroleum-contaminated soil designated Np-Alk. From this, it was concluded that solid-phase contaminant availability is strain dependent.

Results for 30 d aging, where solid-sorbed phase TCE was more resistant to biodegradation were consistent with the results obtained by Hatzinger and Alexander (1995). In this last

study, it was found that aging of phenanthrene in a natural soil for 72 d resulted in a drop in the maximum mineralization rate from 11.5 % per d for unaged soil to 7.2 % per d. In the study by Guerin and Boyd (1993) the organic carbon content of the soils varied from 0.76 to 5.36 %. In the study of Hatzinger and Alexander (1995) the soil investigated had an organic carbon content of 0.40 %. These values were significantly lower than the 54 % organic carbon content of the SSOM used in the present study (Table 5.2). According to the conceptual representation of the two-compartment model of sorption kinetics (Figure 2.1 of the *Literature Review*), as the organic carbon content of a soil increases, so will the non-labile fraction of the sorbed phase. Hence, one would expect non-labile sorption to occur with SSOM at shorter aging times than the soils implemented in the above two studies because the organic carbon content was much greater (*i.e.* 54 % for SSOM compared to a range of 0.4 to 5.36 % for the other two studies).

The reduction in aqueous phase TCE biodegradation at 30 d aging can be explained in terms of the surfactant-like behaviour of humic acid. In particular, humic acid, a component of DOM, has been described as a natural surfactant (Wershaw, 1993). It is possible that between 14 and 30 d aging, the concentration of DOM reached the CMC which then rendered the TCE as unavailable to *D. tiedjei*. Or, hydrogen bonding between the chlorine atoms of TCE and the phenolic and alcohol functional groups of DOM occurred in a similar manner as described for solid SSOM in the last Chapter. This may have rendered the TCE as unavailable to the dehalogenase enzyme of *D. tiedjei* due to steric hindrance imposed by DOM. The supposition that DOM limited the biodegradation of TCE in the aqueous phase at 14 and 30 d aging is supported by the results of Robinson and Novak (1994). In this last study, it was reported that 100 mg/L of humic acid reduced mineralization of C^{14} labelled 2,4,6-trichlorophenol by *Pseudomonas aeruginosa* by 5-15 %, as compared to solutions that were free of humic acid.

The following is a summary of findings from Figures 5.11 and 5.12 combined with

observations made by others in the literature:

1. At lower concentrations of DOM and short aging times (*i.e.* 2 d), the aqueous phase TCE was preferentially biodegraded over the sorbed phase TCE, and there was a corresponding increase in the aqueous phase *cis*-1,2-DCE.

2. It would appear that the aqueous phase TCE became less available for biodegradation as the DOM increased with aging. As a neutral compound, TCE may bind with humic acid in the DOM and become unavailable for biodegradation, as has been observed for mineralization of nonionic benzylamine with humic acid (Amador and Alexander, 1988). In particular at 14 and 30 d aging, the biodegradation of TCE from the aqueous phase and the appearance of *cis*-1,2-DCE also in the aqueous phase fell off sharply compared to 0 d aging (*i.e.* 0 g/L C) and 2 d aging (*i.e.* 933 mg/L C).

3. At 14 d aging, when the DOM increased to 1200 mg/L C and TCE biodegradation in the aqueous phase decreased, there was an increase in the amount of TCE biodegraded from the solid-sorbed SSOM phase that was accompanied by a corresponding appearance of *cis*-1,2-DCE also in the solid-sorbed phase. These results indicate that as the aqueous phase TCE became unavailable, presumably from binding with DOM, there was a fraction of the solid-sorbed phase TCE that was available for biodegradation.

4. As aging proceeded, the solid-sorbed phase fraction also became unavailable for biodegradation, as is demonstrated in Figure 5.12 for 30 d aging. As well, the aqueous phase TCE remained unavailable, presumably due to its binding with DOM (*i.e.* 1333 mg/L C at 30 d aging). This may be explained in terms of the mass transfer models that were described in the *Literature Review* of Chapter 2. In particular, according to the SRPD model, with time TCE may have diffused farther within the pore structure of the SSOM where it may have formed the following associations: a) hydrogen bonds between its chlorine atoms and the

hydrogen atoms of the phenolic and alcoholic functional groups of the SSOM, b) ion-dipole interactions between its chlorine atoms and the protonated nitrogen containing functional groups, and c) ion-dipole interactions between the hydrogen atom of the TCE and the ionized carboxylic acid functional group of SSOM. The rationale for the above mentioned associations between TCE and SSOM was outlined previously in Chapter 4 (section 4.2.2.2) where the characterization of SSOM in terms of its oxygen-containing functional groups was described.

5. In all of the aging experiments, *trans*-1,2-DCE, 1,1-DCE, VC, ethene, and ethane were not detected in either the aqueous or solid phases of the SSOM slurries. Fatherpure and Tiedje (1994) examined dechlorination of PCE and 3-chlorobenzoate by an anaerobic consortium containing *D. tiedjei*. This study found no evidence of VC, but the presence or absence of ethane and ethene were not confirmed by analytical methods. In an earlier study, Fatherpure *et al* (1987) examined PCE dechlorination by *D. tiedjei* in a methanogenic consortium that utilized 3-chlorobenzoate to induce enzyme activity. In this earlier study, conversion of PCE to TCE was only studied, and lesser chlorinated ethenes, ethene, and ethane were not measured. In the most recent study of TCE dechlorination by *D. tiedjei* (Cole *et al*, 1995), an improved medium containing 3-fluorobenzoate instead of 3-chlorobenzoate was used to induce enzyme activity. In this last study, despite the improvements in the medium, only *cis*- and *trans*-1,2-DCE were examined as products of reductive dechlorination. Other studies have indicated that PCE and TCE can be dechlorinated to ethene and ethane by an anaerobic consortium (Freedman and Gossett, 1989). Therefore, since studies involving PCE and TCE dechlorination by *D. tiedjei* did not actively analyze for the lesser chlorinated products, except for the study by Fatherpure *et al* (1994) that analyzed for VC, and since formation of ethene and ethane has been observed by others in the reductive dechlorination of PCE and TCE (Freedman and Gossett, 1989), the formation of these products from the dechlorination of TCE by *D. tiedjei* was a possibility. As well, the present study is the first to use the improved medium containing 3-fluorobenzoate (Cole *et al*, 1995) in soil. In addition, the

SSOM itself may have acted as an additional source of carbon. Therefore, it was decided to analyze for all possible products of reductive dechlorination of TCE.

In summary, aging resulted in reduced availability of TCE by producing a non-labile fraction of TCE in the solid-sorbed phase as well as by leaching DOM from the SSOM. The latter appears to have limited the availability of aqueous phase TCE by binding to it. However, in order to ascertain this, a method of measuring the binding of DOM with aqueous phase TCE must be developed and implemented in order to study this affect on biodegradation. Traditional dialysis techniques, that were not available in the present study, would need to be modified to prevent volatilization.

Little is known of the mechanism by which *D. tiedjei* reductively dehalogenates 3-chlorobenzoate and other chlorinated compounds such as TCE. To re-iterate some of what was discussed earlier in the *Literature Review* of Chapter 2, 3-chlorobenzoate induces dechlorination activity that in turn results in PCE and TCE dechlorination (Cole *et al*, 1995). Rates of dechlorination were reported to be low when 3-chlorobenzoate was used to induce dechlorination in previous studies since 3-chlorobenzoate itself was dechlorinated. However, 3-fluorobenzoate can also induce dechlorination and rates have been reported to be higher since no energy is spent on dehalogenation of 3-fluorobenzoate. Presumably, both benzoates induce the same enzyme system. The 3-chlorobenzoate reductive dehalogenase (*i.e.* enzyme responsible for reductive dechlorination) is an integral membrane protein of the cytoplasmic membrane[9] and is involved with energy metabolism of the cell (Ni *et al*, 1995). Hence, it is likely that reductive dehalogenation of TCE occurred at or near the cytoplasmic membrane, as opposed to external to the cell by an extracellular enzyme. Hence, TCE in the solid-sorbed phase at 14 d aging appears to have been more available to the bacteria for biodegradation

[9]The cytoplasmic membrane is located beneath the cell wall and is the site of enzyme activity and transport of molecules both into and out of the cell (Pelczar *et al*, 1993).

than was the case for 30 d aging since the TCE was not held deep within the pore structure at 14 d aging. Furthermore, DOM may have de-activated the reductive dehalogenase enzyme, or as mentioned previously, binding of TCE by DOM may have impeded contact of TCE with the enzyme at the cytoplasmic membrane. However, a more definitive explanation is not possible at this point in time since the enzyme system of *D. tiedjei* is not completely understood.

5.3.3 Proposed Conceptual Model

At this stage, it is appropriate to propose a conceptual model that describes the TCE biodegradation in the present set of circumstances that is consistent with the experimental data discussed thus far. Such a model is presented in Figure 5.13. It incorporates the mechanisms for rate-limited sorption described previously by Pignatello (1993) that were presented in Figure 2.2 of Chapter 2. This proposed model assumes that *D. tiedjei* degrades TCE at or near the cell surface, as described by Ni *et al* (1995). As discussed extensively throughout this thesis, mechanisms of rate-limited sorption would also be expected to have an effect on the availability of TCE for biodegradation.

The following four mechanisms depicted in Figure 5.13 are consistent with the experimental data and would be expected to hinder biodegradation of TCE to *cis*-1,2-DCE by *D. tiedjei:*

1) Diffusion of TCE within the SSOM pore water by PD (*i.e.* "1" in Figure 5.13) would render solid phase TCE unavailable for biodegradation by *D. tiedjei* with prolonged aging.

2) Diffusion of TCE within the SSOM by IOMD (*i.e.* "3" in Figure 5.13) would render the contaminant unavailable to the bacteria for degradation. IOMD would also increase with aging.

3) SRPD (*i.e.* "4" in Figure 5.13) would retard diffusion of TCE from within the SSOM complex and would consequently retard desorption. Retarded desorption of TCE would also be expected to hinder biodegradation.

4) Enmeshment of TCE within the DOM would render it unavailable to *D. tiedjei* for biodegradation (*i.e.* "6" in the Figure). As DOM is leached from the SSOM with increased aging, (Figure 5.4), binding with aqueous phase TCE likely increases. Enmeshment of TCE by fulvic acid (*i.e.* a component of DOM) was previously described in Chapter 4 (section 4.2.2.2) and is depicted in Figure 4.3. This likely resulted in reduced biodegradation of TCE in the aqueous phase.

Although the first three mechanisms for rate-limited sorption just described (*i.e.* PD, IOMD, and SRPD) all likely resulted in hindered biodegradation of TCE with increased aging, it is difficult to distinguish between them experimentally.

The proposed model also describes the biodegradation of TCE that was observed. Particularly, TCE in the aqueous phase, not bound to DOM, was preferentially degraded by *D. tiedjei* to *cis*-1,2-DCE (*i.e.* "5" in Figure 5.13). For 2 d aging, when DOM was at its lowest level (Figure 5.4), TCE was predominantly converted to *cis*-1,2-DCE in the aqueous phase. TCE in the solid sorbed phase was likely bound by hydrogen bonding and ion-dipole interactions (as described in Chapter 4) to the functional groups at the SSOM surface. At the outermost surfaces of the SSOM structure, TCE bound in this manner appears to be available to *D. tiedjei* for dehalogenation to *cis*-1,2-DCE that remains bound to the surface. As described previously, at 14 d aging, TCE was mainly degraded from the solid-sorbed phase, and the *cis*-1,2-DCE that formed remained in the solid sorbed phase. These results are consistent with the ABE mechanism of sorption and biodegradation depicted in Figure 5.13 (*i.e.* "2" in the Figure).

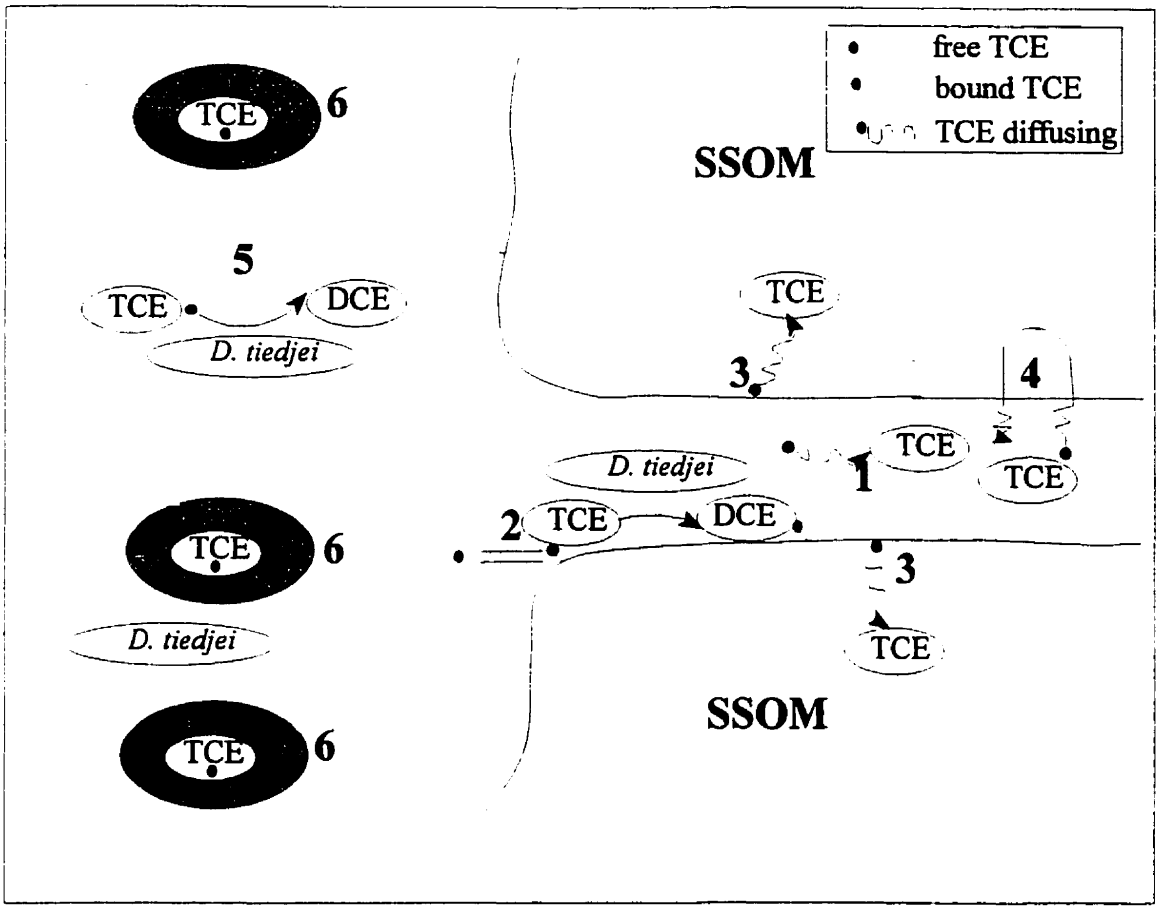

Figure 5.13. Proposed conceptual model for TCE biodegradation as influenced by sorption by SSOM and binding with DOM. Legends: (1) pore diffusion in porewater (PD); (2) adsorption bond energy (ABE); (3) intra-organic matter diffusion (IOMD); (4) sorption-retarded pore diffusion (SRPD); (5) TCE biodegraded in aqueous phase to *cis*-1,2-DCE by *D. tiedjei*; and (6) TCE that is unavailable to *D. tiedjei* due to binding/enmeshment with DOM.

According to the proposed model, as aging progresses, DOM increases and correspondingly, the TCE enmeshed within the DOM also increases. As well, TCE sorbed by rate-limited sorption processes (*i.e.* PD, IOMD, and SRPD) also increases with aging. Hence, at prolonged aging times, TCE becomes unavailable for biodegradation by increased binding with DOM (*i.e.* "6" in Figure 5.13) and sorption within the SSOM complex (*i.e.* "1", "3", and "4" in Figure 5.14). This is consistent with what was observed following 30 d aging, where both aqueous and sorbed TCE degradation declined (Figures 5.11 and 5.12).

5.4 Long-Term Biodegradation of TCE with SSOM

Long-term biodegradation of TCE by *D. tiedjei* in the presence and absence of SSOM was studied. This set of experiments was conducted on the basis of results obtained from the aging experiments and from the preliminary experiments conducted in the absence of SSOM. In particular, from GC analysis it was found that in the presence of SSOM an unknown compound was always formed following biodegradation. Table 5.5 tabulates the conditions under which the unknown compound was detected and where it did not occur. From this, it can be seen that the appearance of the unknown compound only occurred in combinations involving *D. tiedjei* and SSOM and that the presence of TCE had no effect on its appearance. Therefore, it is likely that the unknown compound was a product of SSOM biodegradation by *D. tiedjei*. Consequently, long-term biodegradation tests (*i.e.* over a 24 d incubation time as opposed to 115 h in the previous experiments) were conducted in the presence and absence of SSOM to determine if *D. tiedjei* could sustain itself on the SSOM and maintain dechlorination activity for longer periods than was observed in the previously described experiments.

Mass spectrometry is commonly used in conjunction with gas chromatography (GCMS) to identify unknown organic compounds (McMurry, 1984). This technique was used in the present study in an attempt to identify the unknown compound. Results of this investigation are summarized in Table 5.6.

In general, GCMS can be used to determine the molecular weight of a compound. As well, structural information about unknown compounds can be obtained by measuring the masses of fragments produced when high-energy molecules fly apart. The compound under question is bombarded with high energy electrons. When struck by an electron, an organic molecule becomes ionized, and the resulting high-energy molecule fragments. The positively charged fragments pass through a strong magnetic field and are sorted and ratios are then recorded.

The library of fragmentation patterns of known compounds are contained in a database, and the fragmentation pattern of the unknown compound is compared to those in the database and is identified when a perfect match occurs.

Table 5.5. Tabulation of conditions where the unknown compound did and did not appear.

Combination of components in batch reactors (all incubated for 115 h at 37°C).			Appearance of unknown compound
D. tiedjei	TCE	SSOM	
✔	✔	✔	✔
✔	✗	✔	✔
✔	✔	✗	✗
✔	✗	✗	✗
✗	✔	✗	✗
✗	✗	✔	✗
✗	✔	✔	✗
✗	✗	✗	✗

✔: Component present, or observed.
✗: Component not present, or not observed.

Table 5.6. GCMS identification of unknown compound.

Molecular weight of unknown compound	172
Possible Chemical Formula	$C_8H_2ON_4$
Possible identification from fragmentation patterns (GCMS library search of database)	a. Diazene, butyl (1-(2,2-dimethylhydrazino) ethyl))- b. 1-Butanamine, N-methyl- c. N,N-Dimethyl-1-propanamine

For the unknown compound under consideration in the present study, a perfect match did not occur in the database. However, the three compounds identified in Table 5.6 gave the closest match. All of these compounds contain a nitrogen functional group. In particular, two of the compounds contain an amine functional group. As discussed in a study by Hawari *et al* (1992) amines have been used as additives to enhance photodechlorination. It is intriguing to consider that a metabolite (*i.e.* the unknown compound) from the biodegradation of SSOM by *D. tiedjei* in some way enhanced dechlorination of TCE.

From the results in Figure 5.14, it is evident that the presence of SSOM resulted in a greater degree of TCE dechlorination. As well, the accelerated dechlorination activity that occurred with SSOM was sustained for longer periods than for batch reactors that did not contain SSOM. As in previous graphs, the amount of TCE that was biodegraded was calculated as the difference between TCE measured in the sterile controls and in the inoculated samples. The *cis*-1,2-DCE that appeared was that which was measured directly and was not corrected for volatilization losses.

Between 10 and 19 d incubation time, the quantity of TCE that was biodegraded and *cis*-1,2-DCE that was produced were relatively stable. Between 19 and 24 d incubation time, there was a two-fold increase in TCE biodegradation. From this, it is likely that there was a sudden increase in microbial activity. Possibly between 10 and 19 d, the bacteria were in a lag phase of growth where they were active but possibly adapting to their new SSOM environment. Between 19 and 24 d, the bacteria may have entered into an exponential growth phase where there was balanced growth and cells were nearly uniform in terms of their metabolic activity.

In the absence of SSOM, the quantity of TCE that was degraded was relatively stable between 10 and 24 d. This is indicative of the fact that the CAA itself did not contain sufficient nutrients to support TCE biodegradation. However, in the presence of SSOM, there

was approximately ten times more TCE biodegraded than there was in the absence of SSOM between 10 and 19 d incubation. This was very likely due to utilization of the SSOM as a nutrient source. This is supported by two factors: 1) the appearance of the unknown compound only in the presence of SSOM and 2) the doubling of TCE biodegradation between 19 and 24 d. With respect to the first factor, the unknown compound was likely a product (*i.e.* a metabolite) of biodegradation. As described earlier, if the unknown compound is an amine, it may enhance dechlorination. With respect to the second factor, doubling of TCE biodegradation between 19 and 24 d could not have occurred if the nutrients from the CAA were the sole source of nutrients for *D. tiedjei*. Hence, it is more than likely that *D. tiedjei* utilized either the DOM or the SSOM itself as a source of nutrients. It should also be re-iterated that, on the basis of the results in Figure 5.14, it is not likely that the reduced biodegradation that occurred with aging was a result of the toxicity of the DOM on the *D. tiedjei*. If one considers the origin of the SSOM, then it is entirely possible that this material was an adequate source of nutrients for the *D. tiedjei*. With 54 % organic carbon (Table 5.2), the SSOM had undergone the aerobic process of composting of sphagnum moss by mesophilic bacteria (N'Dayegamiye and Isfan, 1991; Inbar *et al*, 1990). Although the SSOM was sterilized by gamma-irradiation prior to inoculation with *D. tiedjei*, the bacteria may have benefited from metabolites or enzymes produced by the aerobic bacteria during composting. Since *D. tiedjei* is probably a syntroph (Mohn and Tiedje, 1992), it should not be surprising that it thrived on the SSOM that was humified and hence contained products of the microbial activity of the bacteria that carried out composting. In particular, reductions in total aromaticity and polysaccharide levels in conjunction with increases in aliphatic carbon as a result of composting activity has been reported by others (Inbar *et al*, 1992). Furthermore, alkanes are aliphatic and are biodegraded more rapidly than aromatic hydrocarbons (Mitchell, 1974). Hence, *D. tiedjei* may have thrived on the aliphatic carbon produced during composting of sphagnum moss to produce SSOM.

In addition to the nutrient quality of SSOM, *D. tiedjei* may have benefited from the surface

of SSOM. It has long been recognized that the presence of surfaces can both positively and negatively influence microbial metabolism and growth rates (Van Loosdrecht *et al*, 1990). It is thought that adhesion of microorganisms to surfaces in itself does not have an effect on metabolism. Rather, it is likely that the environment surrounding the cell is modified by the surface. There have been a number of studies that have utilized various soils and bacteria to determine the influence of surfaces on microbial activity in soils, and the review presented by Van Loosdrecht *et al* (1990) provides a comprehensive examination of this topic. However, further investigations involving both *D. tiedjei* and SSOM would have to be implemented in order to draw definitive conclusions regarding the present system.

In Figure 5.14, the average conversion of TCE to *cis*-1,2-DCE in the presence of SSOM was 30.6 %, 27.0 %, and 40.0 % for 10 d, 19 d, and 24 d incubation time, respectively. At this point, the practical advantage of converting TCE to *cis*-1,2-DCE should be elaborated upon. TCE is more amenable to anaerobic dechlorination (Fatherpure and Tiedje, 1994), but complete detoxification of TCE is best achieved with a coupled anaerobic/aerobic reactor system (Kuang *et al*, 1994). Based on results in Figure 5.14, the potential use of SSOM (or similar composted material) as a nutrient for *D. tiedjei* in a biological reactor coupled with an additional aerobic treatment for complete mineralization would appear to be a potential application of the present research.

In terms of *in situ* bioremediation of contaminated soils rich in natural organic matter, bioaugmentation using *D. tiedjei* may act to reduce the inherent threat posed by TCE since *cis*-1,2-DCE is considered to be less of a threat to human health compared to TCE (Pontius, 1996). In particular, according to the Safe Drinking Water Act in the U.S., the MCL[10] of *cis*-1,2-DCE and TCE are 0.07 and 0.005 mg/L, respectively. Furthermore, the MCLG[11] for *cis*-

[10]Maximum contaminant level.

[11]Maximum contaminant level goal.

1,2-DCE and TCE are 0.07 and zero mg/L, respectively. Therefore, there are technical (*i.e.* utilization of *D. tiedjei* in a coupled aerobic/anaerobic reactor system) as well as regulatory advantages in converting TCE to *cis*-1,2-DCE. According to the U.S. EPA, the best available technology for the removal of both TCE and *cis*-1,2-DCE includes air stripping (Pontius, 1996). Therefore, conversion of TCE to *cis*-12,-DCE will not necessarily preclude the more traditional technique of air stripping for above surface groundwater treatment.

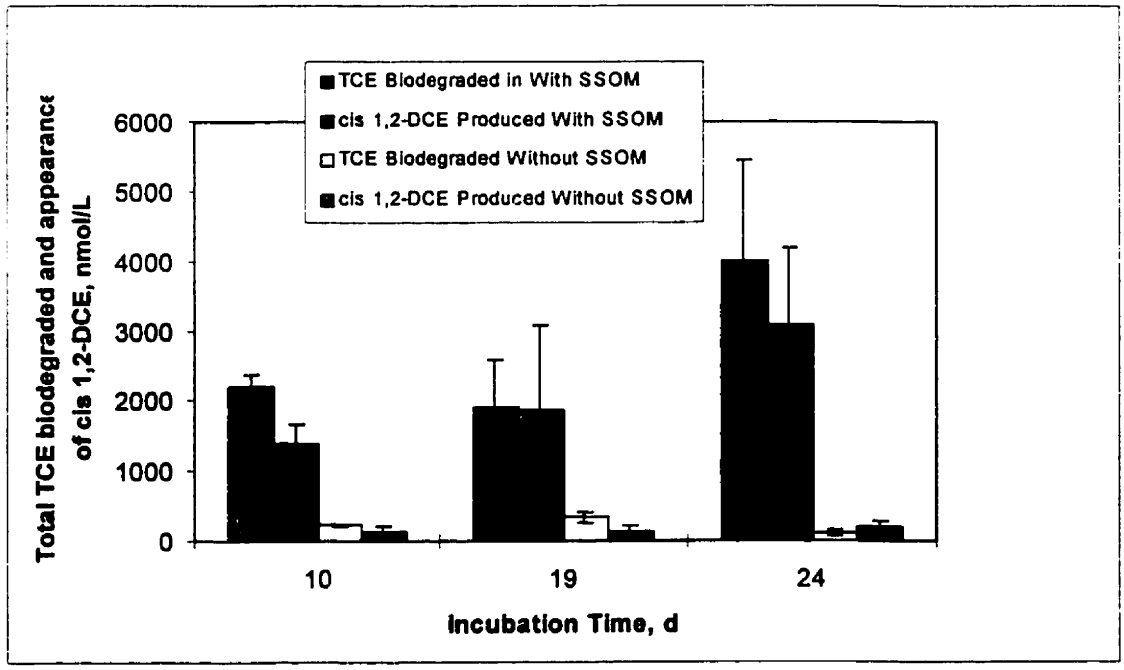

Figure 5.14. Long-term biodegradation of TCE in the presence and absence of SSOM (95 % confidence intervals shown).

The design of the present set of long-term biodegradation experiments differed from the experiments involving TCE aged with SSOM (section 5.3). In particular, the TCE-SSOM slurry was not aged prior to inoculation with *D. tiedjei*. Hence, TCE did not have the opportunity to sorb within the SSOM complex or to become enmeshed within the DOM prior to its contact with *D. tiedjei*. Therefore, the effect of aging was not included in the experimental design. As well, the batch reactors were re-induced with 3-fluorobenzoate following the first 115 h of incubation (Figure 5.1).

5.5 Summary of Results and Discussion

- Aging appears to have hindered TCE biodegradation from the aqueous and the solid-sorbed phase. In the former, aging resulted in leaching of DOM from the solid SSOM phase that probably bound with TCE thereby rendering it unavailable to the dehalogenase enzyme that is fixed in the cytoplasmic membrane of *D. tiedjei*. In the latter case, aging probably resulted in diffusion of TCE deeper within the SSOM structure thereby rendering it unavailable to *D. tiedjei*.

- Long-term biodegradation of SSOM freshly contaminated with TCE revealed that *D. tiedjei* was capable of obtaining energy from SSOM and sustaining itself for at least 24 d. At 24 d, dechlorination of TCE to *cis*-1,2-DCE was about 20 times greater in the presence of SSOM as compared to the case where SSOM was absent. These results would appear to rule out the possibility that DOM was toxic to *D. tiedjei* in the aging experiments mentioned in the previous point.

- *D. tiedjei* produced a compound, with a possible chemical formula of $C_8H_2ON_4$ and a formula weight of 172, only in the presence of SSOM. The significance of this product is not well understood, but it is likely a metabolite from SSOM biodegradation.

- Sorption of TCE by SSOM was 2 to 4 times lower than what has been reported in the literature by Zytner (1992) for peat moss. This is likely due, in part, to the presence of DOM that is known to increase the solubility of NOCs such as TCE (Lyman *et al*, 1990).

- *D. tiedjei* biotransformed TCE to *cis*-1,2-DCE with no further reductions to VC, ethene, or ethane. As well, there was little or no appearance of *trans*-1,2-DCE and

no appearance of 1,1-DCE.

- Aging of TCE with SSOM from 2 to 30 d resulted in a 75 % reduction in biotransformation of TCE to *cis*-1,2-DCE.

Chapter 6

Results and Discussion:

Sequential Desorption and IRS Analysis

6.1 Introduction

This chapter discusses the results of experiments that were conducted for the sequential desorption of TCE and *cis*-1,2-DCE from SSOM as a function of aging. In addition, results from IRS analysis of the SSOM-TCE complex that was conducted to determine possible bonding mechanisms are discussed.

As was seen by the results presented in the last chapter, the availability of solid-sorbed TCE for biodegradation by *D. tiedjei* decreases with aging, presumably due in part to diffusion of the contaminant deeper within the SSOM complex. Therefore, an understanding of the desorptive behaviour of TCE and *cis*-1,2-DCE from SSOM is critical for the purposes of predicting the availability of these contaminants for biodegradation. However, since the sequential desorption also mimics mass transfer that can occur as a result of pump-and-treat technologies, these results are also beneficial for the assessment of the effect of aging on the efficiency of this decontamination technology.

The analysis of the TCE-SSOM complex by IRS was originally carried out with the intention of describing the possible bonding mechanisms that can occur between TCE and SOM. Since the desorption behaviour is in part governed by the strength of bonding that occurs, these results are presented in conjunction with those for sequential desorption.

6.2 Sequential Desorption of TCE with Aging

As depicted in Figure 3.3 of the *Methodology*, sequential desorption is carried out such that an incremental reduction in the aqueous phase TCE concentration over time results in desorption. Such desorption has been described as a diffusion process that may or may not be hindered by adsorption (Pignatello, 1993). As was discussed previously, heating the SSOM-TCE soil suspensions at 37°C for 115 h (*i.e.* sterile controls) resulted in volatilization of aqueous phase TCE from the batch reactors but did not result in desorption, as evidenced by the fact that there was no reduction in the sorbed phase TCE (Figures 5.3, 5.5, and 5.6). It was postulated that the solid-sorbed phase TCE may be non-labile and resistant to desorption as a result of aging.

If diffusive desorption controlled the TCE partitioning between the solid-sorbed and aqueous phase for the biodegradation experiments discussed in the previous chapter, then partitioning that occurred following biodegradation should be similar to that which occurred during the course of the sequential desorption. From Figure 6.1, it can be seen that for 2 d aging, the amount of TCE that was sorbed by SSOM following biodegradation was greater than that which occurred at the same aqueous phase concentration during sequential desorption. These results are consistent with what was said earlier. In particular, as the aqueous phase TCE was degraded, desorption of TCE did not occur as would be expected due to desorption resulting from the imposed concentration gradient. This may have been the result of the elevated temperatures during incubation that increased the pore diameters of the SSOM that in turn increased diffusion of TCE further within the SSOM complex. To summarize, at 2 d aging, sequential desorption did not predict the TCE partitioning that occurred between the aqueous and solid-sorbed phase following biodegradation. This is likely due to the fact that mass transfer processes that occurred during sequential desorption did not occur during biodegradation.

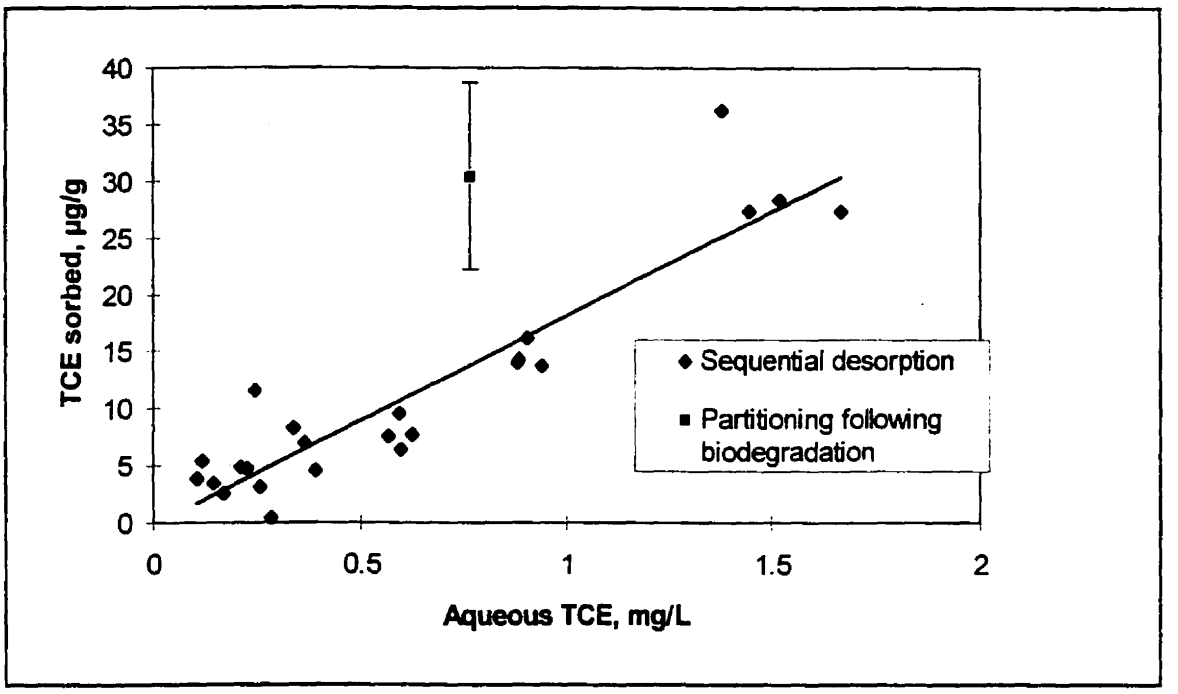

Figure 6.1. Sequential desorption of TCE from SSOM following 2 d aging. Also shown is the partitioning following biodegradation for 2 d aging with 95 % confidence interval.

At 14 d aging, sorbed TCE following biodegradation was less than what would be expected from the TCE partitioning that occurred during the course of sequential desorption (Figure 6.2). This is consistent with the supposition that biodegradation of solid-sorbed TCE occurred. From Figure 6.2, one would expect that when TCE in the solid-sorbed phase decreased due to biotransformation by *D. tiedjei*, there would be additional sorption of TCE from the aqueous phase if diffusive mass transfer was a controlling factor. However, since biotransformation occurred at the surface, the biomass itself may have created a barrier to diffusive sorption of TCE by SSOM. As described by Van Loosdrecht *et al* (1990), microbial colonization at a solid-liquid interface can result in a biofilm. Attached microorganisms may produce polymers or polysaccharides in order to develop a surface film. If this occurred, it may have resulted in a barrier to sorption of TCE from the aqueous to the solid-sorbed phase. Colonization of *D. tiedjei* at the SSOM surface may also have occurred during the long-term

biodegradation experiments that were described in the last chapter. Since *D. tiedjei* obtained metabolic advantage from SSOM, it is also possible that it did so by forming colonies at the surface.

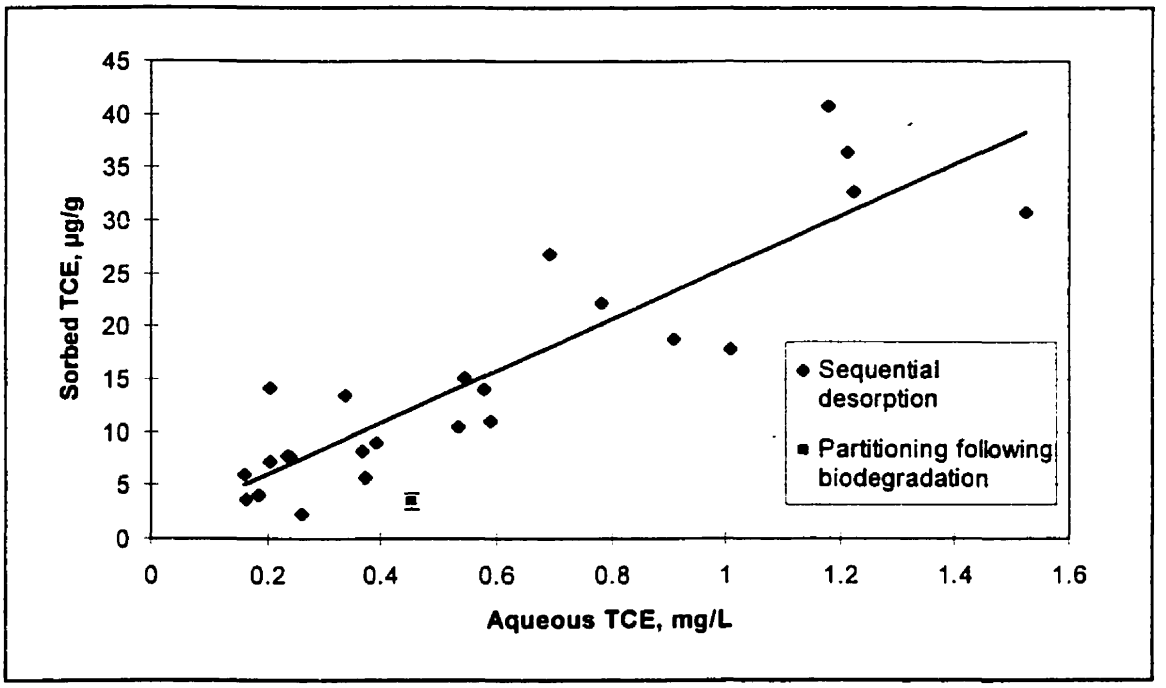

Figure 6.2. Sequential desorption of TCE from SSOM following 14 d aging. Also shown is partitioning following biodegradation for 14 d aging with 95 % confidence interval.

To summarize, at 14 d aging, biodegradation of solid-sorbed phase TCE resulted in TCE partitioning between the aqueous and solid-sorbed phase that was lower than what would be expected on the basis of sequential desorption. This is consistent with biodegradation of TCE at the SSOM surface, and the resulting biofilm at the SSOM surface may have posed a barrier to mass transfer from the aqueous to the solid sorbed phase that would be expected to occur due to the resulting concentration gradient.

For 30 d aging, the partitioning that existed following biodegradation was not significantly different than that which was observed for sequential desorption (Figure 6.3). This is not surprising since the total amount of TCE that was biotransformed at 30 d aging was low

(15.8 % TCE was biotransformed) and occurred from both the aqueous and solid-sorbed phases (Chapter 5). Therefore, at 30 d aging, sequential desorption was capable of predicting TCE partitioning following biodegradation probably only because such a small quantity of TCE was actually biodegraded. However, it should be noted that when interpreting sorption and desorption isotherms, constant temperature is assumed (Benefield *et al*, 1982). Since the partitioning that resulted following biodegradation occurred following incubation at 37°C, and that which occurred from sequential desorption occurred at about 25°C, there are limitations as to the extent to which comparisons can be made.

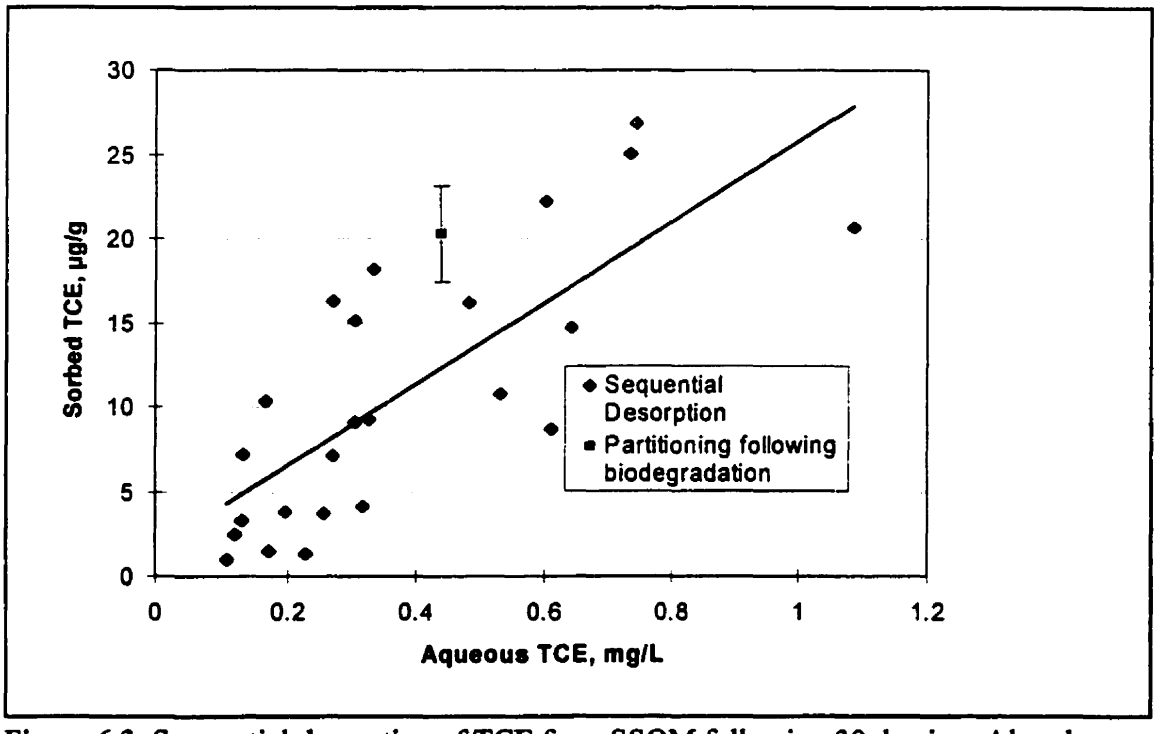

Figure 6.3. Sequential desorption of TCE from SSOM following 30 d aging. Also shown is the partitioning following biodegradation also for 30 d aging with 95 % confidence interval.

By comparing the sequential desorption isotherms obtained following the three aging times, it is evident that desorption behaviour was not radically different. By removing the

supernatant and replenishing it with uncontaminated medium much as would be expected in soil by groundwater fluxes, TCE desorbs from SSOM to the same extent up to 30 d aging. However, as evidenced by biodegradation patterns identified in the last chapter, the availability of TCE for biodegradation decreased with aging. Therefore, the availability of TCE for biodegradation was not limited by desorption. Rather, it would appear that the presence of DOM in the supernatant limited the availability of TCE for biodegradation at 14 and 30 d aging. Furthermore, since aqueous phase TCE was not biodegraded at 30 d aging, the concentration gradient necessary for desorption did not exist. The observation that at 14 d aging solid-sorbed phase TCE was more available for biodegradation compared to that at 30 d aging was likely the result of TCE being more physically available to the bacterium since the dehalogenase enzyme is thought to exist at the cytoplasmic membrane of *D. tiedjei* (Ni *et al*, 1995).

The Freundlich desorption constants were determined for each aging time using simple linear regression. A form of the Freundlich desorption equation (6)[1] that was utilized by Zytner (1992) was used to treat the data in the present study. However, it was necessary to modify this equation in order to apply it to the present study. In the desorption study conducted by Zytner (1992), the supernatant was completely removed and replaced with de-ionized water. Over a period of time, the supernatant was sampled to determine the TCE released from the sorbed phase. The values of TCE released, x_d/m, were calculated from this. Therefore, K_d was the equilibrium desorption constant, or the amount of TCE released, and a relative decrease in K_d would correspond to an increase in resistance to desorption. However, in the present study, sequential desorption was carried out where a fraction of the supernatant was removed and replenished with uncontaminated medium. The slurry was left to equilibrate for 24 h, and the resulting partitioning was measured. This extraction was repeated six times. Therefore, the K_d estimated in the present study represents a sorption coefficient for TCE that

[1]From *Literature Review* of Chapter 2.

is resistant to desorption, hence a relative increase in K_d would correspond to an increase in resistance to desorption. Table 6.1 contains the Freundlich desorption constants that were estimated for the three aging times, and it can be seen that K_d increases with aging. This signifies that TCE was increasingly resistant to desorption with aging. The value of n_d was close to unity for each aging time, but it also decreased slightly with aging. As n_d decreased, $1/n_d$ increased, and according to equation (6), the fraction of TCE that was resistant to desorption also increased.

Table 6.1. Summary of TCE desorption data fitted to Freundlich desorption isotherm.

Aging, d	Degrees of freedom	Regression coefficient, r	K_d (mL/g)	n_d
2	22	0.741	15.30	1.17
14	22	0.854	24.06	1.10
30	22	0.743	29.13	0.88

However, overall, the level of TCE that remained in the solid-sorbed state of the SSOM at the end of sequential desorption did not vary with aging. Therefore, truly non-labile sorption of TCE by SSOM was not achieved even after 30 d aging. If non-labile sorption of TCE had occurred, then there would have been bi-phasic desorption, as described by Karickhoff (1980) and discussed in more detail in the *Literature Review* of Chapter 2. The results of sequential desorption would indicate that aging periods of greater than 30 d should be implemented in order to study the effect of non-labile TCE sorption on biodegradation. The residence times employed by Pavlostathis and Mathavan (1992) of 2.5, 5.5, and 15.5 months should be employed in further studies since they were found to result in a non-labile fraction of TCE that was resistant to desorption (*i.e.* 10, 32, and 45 % fractions were resistant, respectively). However, as was apparent from previous results, 30 d aging did result in

hindered biodegradation of TCE by *D. tiedjei*, as compared to shorter aging times.

6.3 Sequential Desorption of *cis*-1,2-DCE with Aging

This section describes the results obtained from the sequential desorption of *cis*-1,2-DCE from SSOM. These experiments were conducted to determine if aging resulted in an increased resistance of *cis*-1,2-DCE to desorption. The relevance of this set of experiments is as follows. It is known that *cis*-1,2-DCE is a major product of reductive dehalogenation of both PCE and TCE under passive conditions in the subsurface (Kleopfer *et al*, 1985) and it has been found to accumulate in groundwater (Gregi & Université Laval, 1993). Hence, sorptive-desorptive behaviour of this compound is relevant to its mobility in soil as well as its availability for further biodegradation. As well, in a coupled anaerobic/aerobic bioremediation scenario, the sorptive-desorptive behaviour of *cis*-1,2-DCE as a function of aging could be critical to the aerobic segment of the process where *cis*-1,2-DCE would be converted to CO_2.

In the Figures describing sequential desorption of *cis*-1,2-DCE from SSOM which follow, the initial levels of *cis*-1,2-DCE that were sorbed by SSOM at the start of the sequential desorption experiments are the data points at the extreme right of the x-axis. As the contaminant was removed in the supernatant, the amount of the contaminant that was sorbed decreased along with the concentration in the aqueous phase. At 2 d aging (Figure 6.4), the initial level of *cis*-1,2-DCE that was sorbed by SSOM was greater than that which occurred for 14 d aging (Figure 6.5) and 30 d aging (Figure 6.6). From these Figures, it is evident that *cis*-1,2-DCE was less amenable to sorption with aging. In fact, after 30 d aging (Figure 6.6), *cis*-1,2-DCE appears to have been predominantly in the desorbed state. This is contrary to what was observed for TCE, where it was found that TCE was more resistant to desorption with aging. This may stem from a difference in the interaction between the two chlorinated compounds and the SSOM and DOM. Overall, sorption of *cis*-1,2-DCE by SSOM decreased

with aging. In Figure 5.4, it was seen that the DOM increased from about 933 mg/L after 2 d aging to about 1333 mg/L after 30 d aging. As the organic matter leached from the SSOM, desorption of *cis*-1,2-DCE may have resulted. Humic acid was found to be more effective than water in removing NOC from a sandy aquifer material (Abdul *et al*, 1990). However, as was pointed out earlier (Figure 6.1, 6.2, and 6.3), this behaviour was not observed for TCE. From Figure 6.7, it can be seen that losses by volatilization were not significantly different from what was observed for TCE (Figure 5.3). Therefore, the reduced sorption of *cis*-1,2-DCE, as compared to TCE, with aging was not likely the result of desorption arising from the concentration gradients imposed by volatilization. Rather, the difference between sorptive and desorptive behaviour of TCE and *cis*-1,2-DCE was more likely due to chemical differences between the two contaminants. The *cis*-1,2-DCE appears to have had a greater affinity for the aqueous phase, containing DOM, as opposed to the solid-sorbed phase. This is consistent with the reported aqueous solubilities for TCE and *cis*-1,2-DCE (*i.e.* 1100 and 3500 mg/L, respectively) (Nyer, 1993).

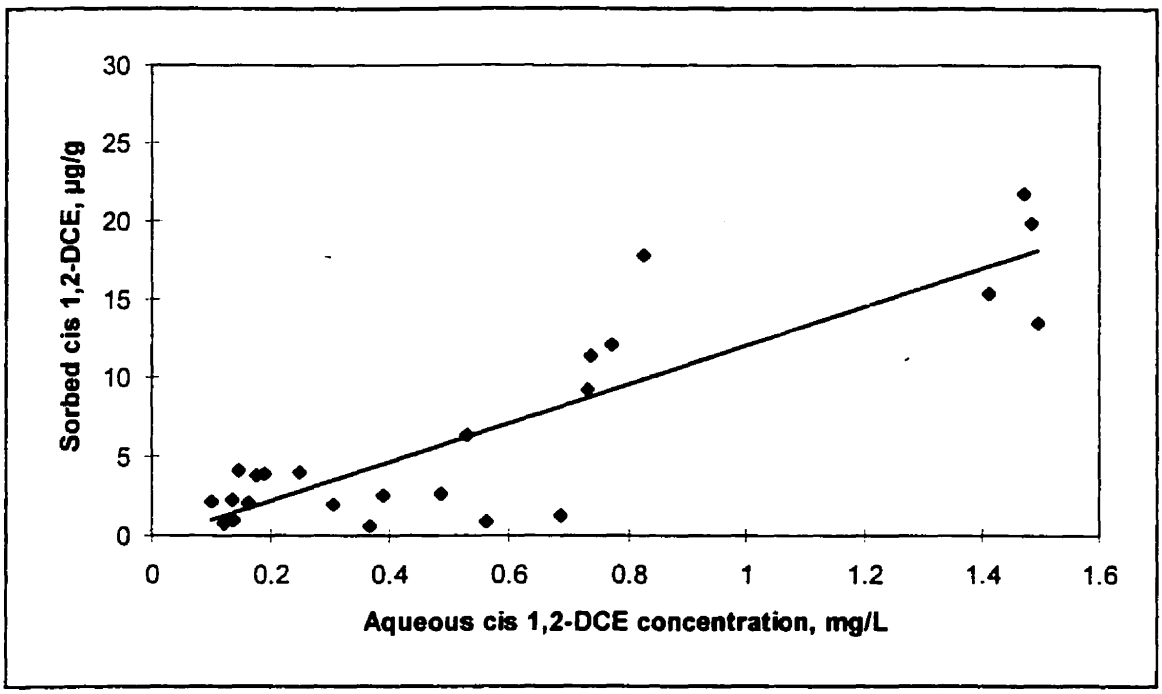

Figure 6.4. Sequential desorption of *cis*-1,2-DCE from SSOM following 2 d aging.

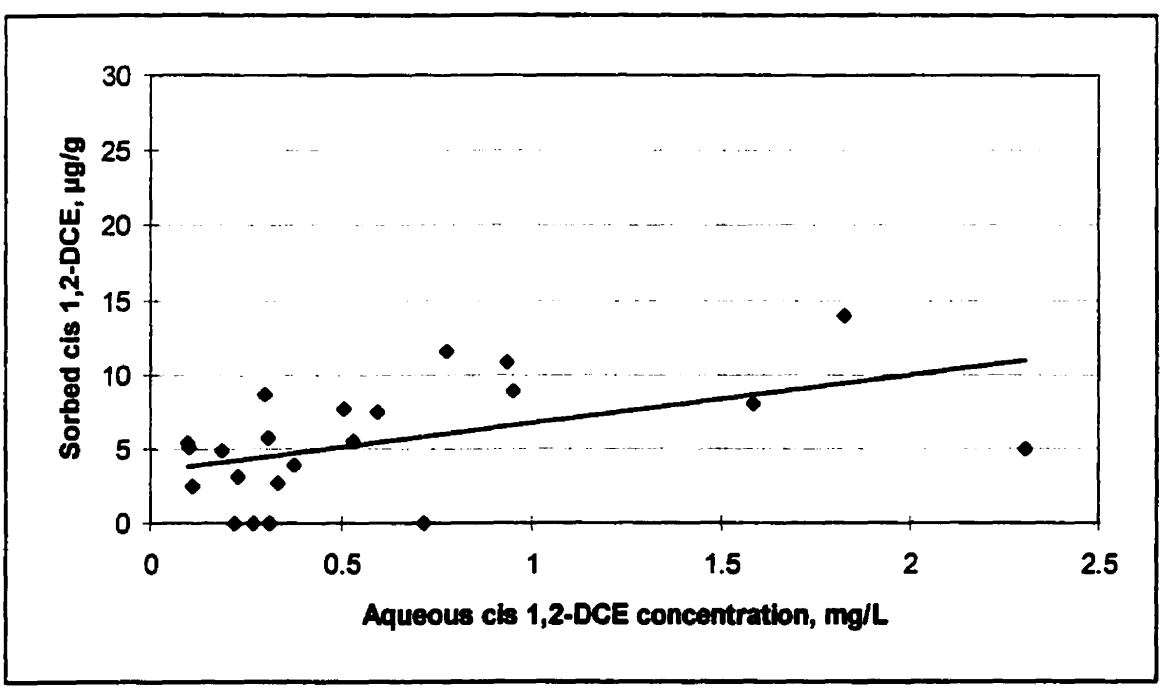

Figure 6.5. Sequential desorption of *cis*-1,2-DCE from SSOM following 14 d aging.

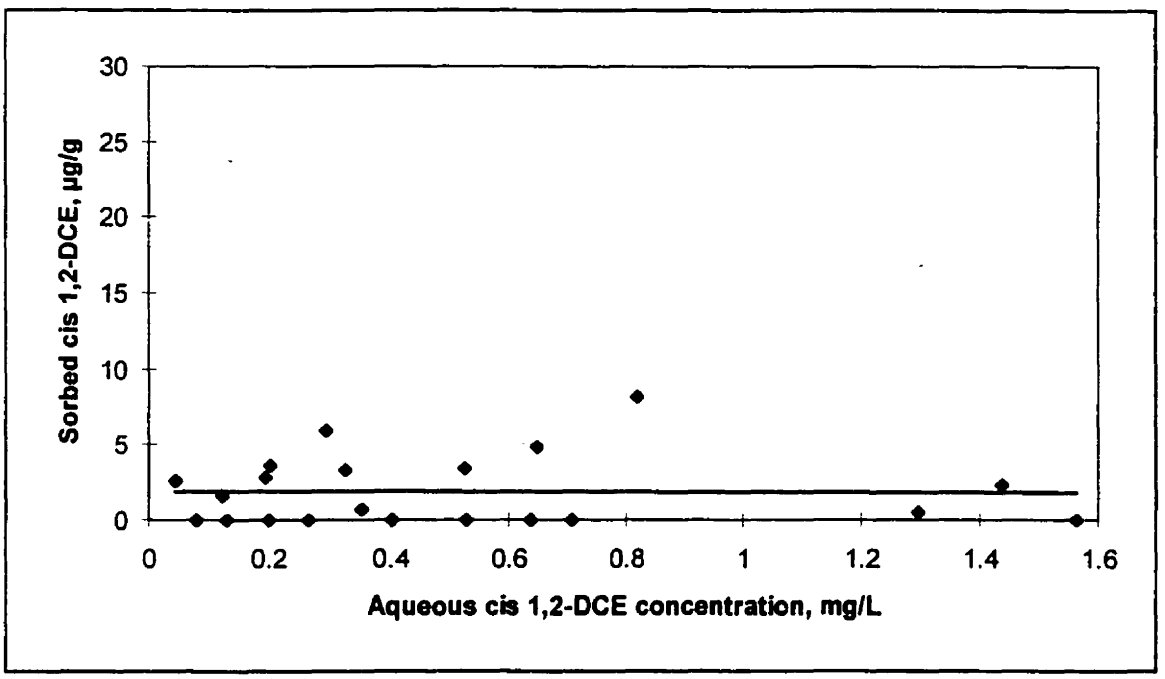

Figure 6.6. Sequential desorption of *cis*-1,2-DCE from SSOM following 30 d aging.

Table 6.2. Summary of *cis*-1,2-DCE desorption data fitted to Freundlich desorption isotherm.

Aging, d	Degrees of freedom	Regression coefficient, r	K_d (mL/g)	n_d
2	22	0.690	8.69	1.17
14	20	0.190	2.71	1.49
30	21	0.010	0.14	-28.59

The Freundlich desorption constants (K_d) for *cis*-1,2-DCE as a function of aging are summarized in Table 6.2. The resistance of *cis*-1,2-DCE to desorption decreased with aging,

as evidenced by the fact that K_d decreased with aging. In addition, the changes in the constant n_d with aging are also consistent with a decrease in resistance to desorption.

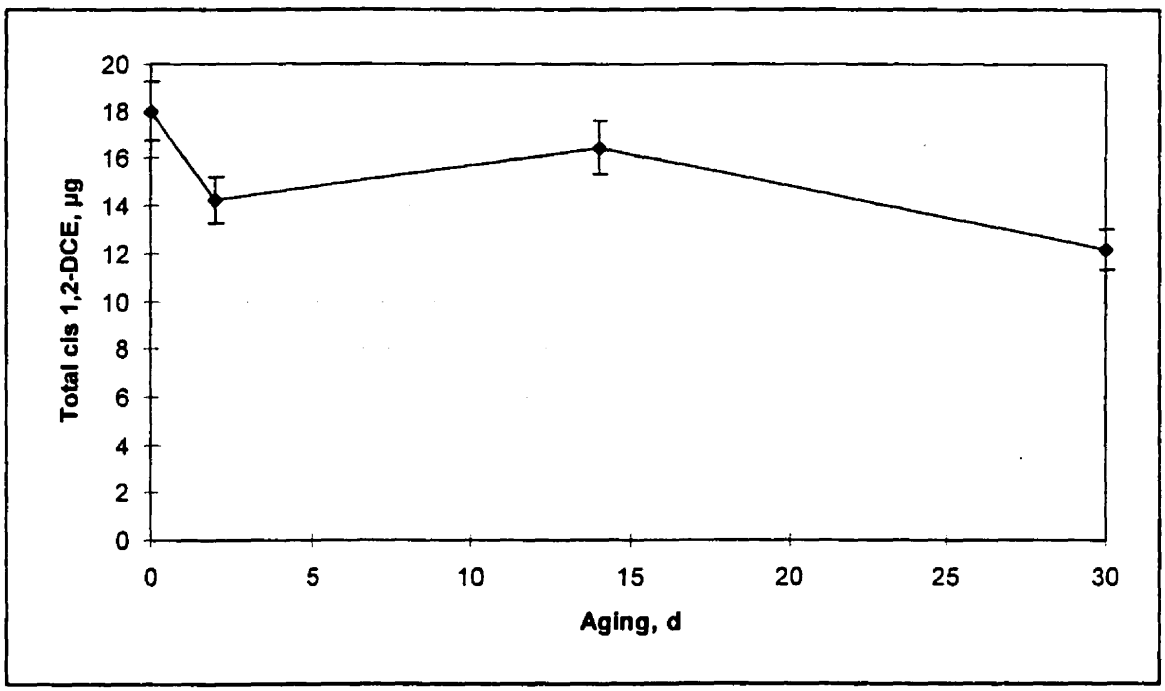

Figure 6.7. Total *cis*-1,2-DCE remaining (*i.e.* aqueous and sorbed) following sorption by SSOM over 30 d (95 % confidence intervals shown).

As outlined in the *Literature Review*, the fate of DCE isomers in soil is not well understood (Fogel *et al*, 1986). Literature pertaining to the study of the sorptive-desorptive behaviour of *cis*-1,2-DCE was not found in the course of the present study. Hence, comparison of the data obtained in the present study with those from other laboratories was not possible. However, based on the results of the present study, it would appear that *cis*-1,2-DCE is potentially more mobile than TCE in soil. This is consistent with the fact that the octanol-water partition coefficient, K_{OW}[2], of *cis*-1,2-DCE (*i.e.* 5.0) is less than that of TCE (*i.e.* 240) (Nyer, 1993). However, as was discussed in the *Literature Review* (section 2.1.4), the K_{OC}

[2]Defined by equation (9) in Chapter 2.

that is estimated from the K_{OW} of the organic compound is limited since the nature of the organic matter in the soil will affect its value (Gerstl and Kliger, 1990; Grathwohl, 1990; Xing *et al*, 1994). Hence, it appears desirable to predict the retention of contaminants by soil using the soil, or soil type, in question as opposed to estimations based on K_{OW}.

The K_{OC} for *cis*-1,2-DCE was estimated from sorption data for 2, 14, and 30 d of aging prior to the onset of desorption. In particular, the constants n and K for the Freundlich sorption isotherm (equation (3) on page 13) were determined by linear regression analysis of the log transformed equation. The K_{OC} was then determined using equation (2) (page 13). Results of these K_{OC} estimations are summarized in Table 6.3. The K_{OC} estimated from the sorption data (*i.e.* using the Freundlich sorption isotherm according to Method A in Table 6.3) was three times less than the K_{OC} estimated from the K_{OW} (*i.e.* Method B in Table 6.3). This is consistent with the observations of others (Gerstl and Kliger, 1990; Grathwohl, 1990; Xing *et al*, 1994) that K_{OW} is not necessarily an accurate means of predicting retention of organic contaminants by soil. Using the same equation that correlates K_{OC} and K_{OW}, for TCE the K_{OC} was estimated as 148.0 [mL/g]. However, from the sorption data presented in Chapter 5, the K_{OC} for TCE was estimated to be 57.0 [mL/g] (see Table 5.2 on page 118). Therefore, the K_{OC}'s estimated from the K_{OW}'s of TCE and *cis*-1,2-DCE were approximately three times greater than those which were estimated from the two sets of sorption data. Therefore, although the K_{OW} may be useful for comparing the relative retention of contaminants by soils and sediments, actual retention should only be estimated from sorption data obtained with soil or sediment from the contaminated site in question. The discrepancy between these two methods of estimating K_{OC} has been reported for TCE (Grathwohl, 1990), but until now this has not been reported upon for *cis*-1,2-DCE.

Table 6.3. K_{OC} estimation for *cis*-1,2-DCE using a) the Freundlich sorption isotherm in conjunction with sorption data for 2, 14, and 30 d aging and b) the octanol-water partition coefficient K_{OW}.

Method A:	Method B:
K_{OC} estimated from sorption data:	K_{OC} estimated from K_{OW}:
Freundlich sorption isotherm to estimate K (equation (3) on page 13): $x/m = KC^{1/n}$ **(3)** Estimation of K_{OC} (equation (2) on page 13): $K_{OC} = \frac{K}{\%OC} \times 100$ **(2)**	Equation that relates K_{OC} and K_{OW} (equation (10) page 27): $\log K_{OC} = a \log K_{OW} + b$ **(10)**
Constants K and n: ♦ Linear regression of log transformed equation (3): K = 0.486 [mL/g] n = 2.1 regression coefficient, r = 0.883 %OC = 54 %	Constants a, b, and K_{OW}: ♦ a = 1.00 b = -0.21 Karickhoff *et al* (1979) ♦ K_{OW} = 5.0 Nyer (1993)
K_{OC} = 0.900 mL/g	**K_{OC} = 3.08 mL/g**

The results of sequential desorption of TCE and *cis*-1,2-DCE would suggest that the chlorine atoms are more instrumental than the hydrogen atoms in forming associations with SSOM. This suggestion is consistent with results reported in the literature in relation to the Freundlich partitioning coefficients for PCE and TCE with peat moss (Zytner *et al*, 1989; Zytner, 1992) and also with the relative values of K_{OW}, except for VC, given in Table 2.2 (pg. 29) of the *Literature Review*. The reported Freundlich partition coefficient, K, for PCE was found to be greater than that of TCE for peat moss (*i.e.* 264 compared to 93.4 mL/g, respectively) thereby indicating that PCE has a greater tendency to partition with organic matter than TCE. Therefore, hydrogen bonding between the chlorine atoms of PCE, TCE, and DCE isomers and the hydrogen ion of the phenolic acid functional groups of the SSOM (as depicted in Figure 4.3 of Chapter 4 for TCE) would appear to play an important role in the partitioning of such chlorinated compounds with soil. As the number of chlorine atoms decreased, the tendency for partitioning also decreased in the studies just cited and in the present study. Vogel (1993) has also observed that, in general, as the degree of chlorination of a compound increases, its tendency to partition with soil increases. With the characterization of the functional groups of the SSOM, this study offers a possible mechanistic explanation for this observation.

To further investigate the possible bonding characteristics of TCE with the SSOM, IR analysis of the TCE-SSOM complex was investigated. These results and potential implications are described in the next section.

6.4 IR Analysis of TCE-SSOM Sorption Complex

As described in the *Literature Review*, IR analysis can be used to study the interactions of adsorbents and adsorbed molecules (Kiselev and Lygin, 1975). As applied to the present study, this was achieved by comparing the IR spectra of the bulk molecules (*i.e.* TCE neat), the adsorbent (*i.e.* SSOM), and the adsorbent-adsorbate complex (*i.e.* SSOM-TCE). The IR spectrum of SSOM and the SSOM-TCE complex (aged for 2 d) are depicted in Figure 6.8. Figure 6.9 contains the IR spectrum of TCE (neat). This last spectrum was obtained from the literature (Pouchert, 1975) since the available equipment for IR analysis was not outfitted for liquid analysis.

The spectrum of TCE shows relatively few but distinctive peaks in comparison to the SSOM spectra. These include a sharp peak at 3106 cm^{-1} from C-H stretching, a peak at 1587 cm^{-1} from C=C stretching variations, and three strong peaks between 790 and 960 cm^{-1} that are likely from C-Cl stretching. Although the stretching of C-Cl usually exhibits strong peaks between 600 and 800 cm^{-1}, several chlorine atoms attached to the same or adjacent atoms, usually have higher C-Cl frequencies (Bellamy, 1958).

Peak assignments were already described for SSOM in relation to Figure 4.7 of Chapter 4, so they will not be repeated in this Chapter. However, from Figure 6.8, it is evident that the distinctive C-Cl stretching of TCE (Figure 6.9) were not apparent in the 790 to 960 cm^{-1} region of the SSOM-TCE spectrum. Furthermore, there are few differences between the SSOM and SSOM-TCE complex. From this, it would appear that TCE was absent from the SSOM-TCE spectra. Since TCE is quite volatile, the remaining sample that was used for IR analysis was analyzed for TCE by GC to ensure that TCE had not volatilized. Results indicate that the sample contained 10.918 µg TCE per g SSOM (*i.e.* 0.011 mg/g). Therefore, it can be concluded that the SSOM-TCE sample analyzed by IRS did indeed contain TCE despite the fact that this was not evident in the spectra. According to Kiselev and Lygin (1975),

complete disappearance of the individuality of the adsorbed molecule (*i.e.* TCE) is indicative of a strong interaction with the adsorbent (*i.e.* SSOM). However, as was described in the characterization of SSOM (section 4.2.2.2), the hydrogen bonding between the chlorine atoms of TCE and unionized protons of the oxygen containing functional groups as well as ion-dipole interactions between the hydrogen atom of TCE and the ionized oxygen containing functional group of SSOM are both relatively weak interactions. Hence, the disappearance of the individuality of TCE from the SSOM-TCE complex was likely due to diffusion of TCE within the micropores of the SSOM complex. Based on the above discussion, the pore diffusion (PD) model would appear to describe sorption of TCE by SSOM. However, combined with the discussion of the last section concerning sorption of PCE, TCE, and *cis*-1,2-DCE, it would appear that sorption increases with the number of chlorine atoms. Therefore, the sorption-retarded pore diffusion (SRPD) model presented from the literature in Chapter 2 (Figure 2.2) also likely plays a role in partitioning. These observations are consistent with the conceptual model for TCE biodegradation and sorption presented in Figure 5.14 in the last Chapter.

Another possibility is that the TCE in the TCE-SSOM complex was not detectable. However, if one considers published reports on detectable amounts of organic chemicals, levels far below 0.011 mg/g have been detected by others. The detection limit for oil and grease by IR analysis is 2×10^{-4} mg/g (Standard Methods, 1976). Since oil and grease is really a composite of compounds, one would expect an even lower detection limit for the individual compounds. Senesi and Testini (1980) found very strong peaks from 1.25 mg/g of prometone, methoprotryne, monuron, and fenuron. As well, Doner and Mortland (1969) used IR to study the interaction of benzene with a homoionic montmorillonite clay. For this study, the IR spectra of pure liquid benzene was compared to those of the montmorillonite clay (alone) and the clay that had been in contact with benzene (concentration not reported). In this last study, benzene was detectable in the clay-benzene complex even though the clay had only been in contact with benzene vapour. Hence, it does not appear likely that TCE was not apparent in

the TCE-SSOM spectrum because it was non-detectable. However, the possibility that TCE was below the detection limit cannot be entirely ruled out.

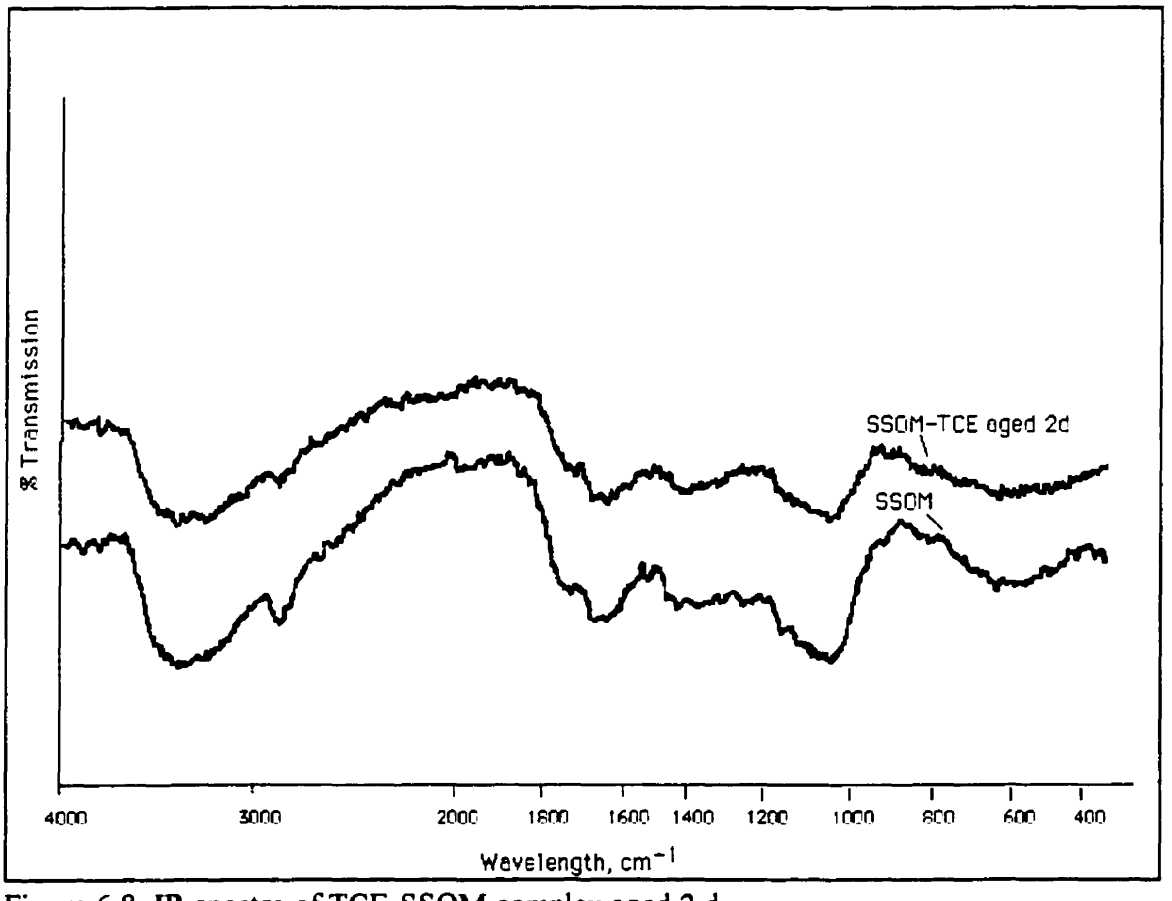

Figure 6.8. IR spectra of TCE-SSOM complex aged 2 d.

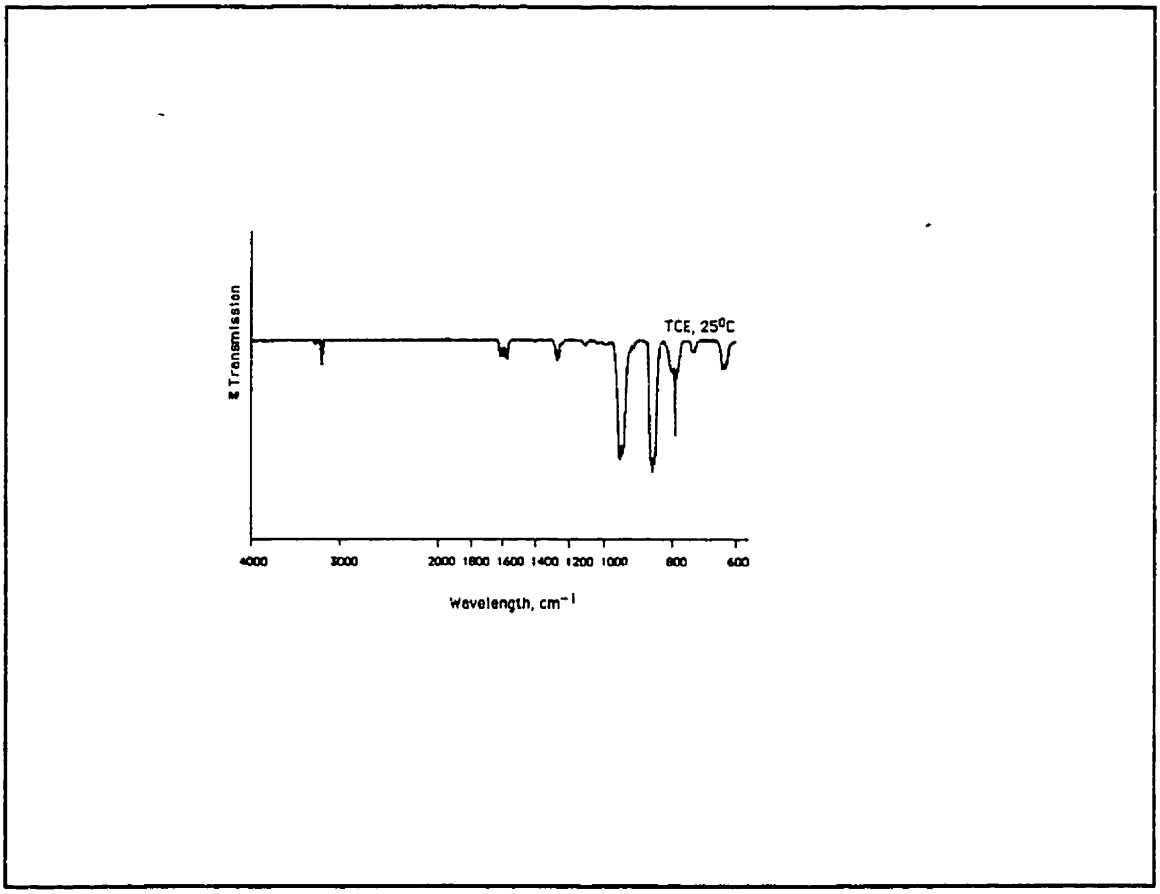

Figure 6.9. IR spectrum of TCE (adapted from Pouchert, 1975).

6.5 Summary of Results and Discussion

- TCE was slightly more resistant to desorption with increased aging times; however, biphasic desorption was not observed from 2 to 30 d aging. Therefore, 30 d aging was not sufficient to bring about non-labile sorption of TCE.

- The K_{OC}'s for TCE and *cis*-1,2-DCE estimated from sorption data using the SSOM were three times less than those estimates that were based on their respective K_{OW}'s. Therefore, it would appear that estimates of the retention of TCE and *cis*-1,2-DCE should be made on the basis of sorption data obtained using soil from the contaminated site in question. Otherwise, on the basis of results from the present study, retention of TCE and *cis*-1,2-DCE based on their K_{OW}'s could be over estimated.

- IR and GC analysis of the SSOM-TCE complex aged for 2 d indicated that TCE was absent from the outermost surface of the SSOM but was present within the SSOM structure. This is consistent with the supposition that NOCs, such as TCE, partition with the organic fraction of soil by diffusion within the SOM matrix.

Chapter 7

Summary, Conclusions, and Recommendations

7.1 Summary

7.1.1 General

TCE contamination of groundwater, soils, and sediments is extensive in Canada (Environment Canada, 1993). Under passive conditions, TCE is dechlorinated under anaerobic conditions in saturated soil following the consumption of residual oxygen by aerobic and facultative organisms (Liss and Baker, 1994). Under such conditions, 1,1-DCE, *cis*-1,2-DCE, *trans*-1,2-DCE, and VC have been shown to accumulate in the subsurface (Gregi & Université Laval, 1993; Kleopfer *et al*, 1985; Pavlostathis and Mathavan, 1992). However, 1,1-DCE is more commonly associated with the abiotic transformation of 1,1,1-trichloroethane (McCarty, 1993) as opposed to the reductive dechlorination of TCE.

In itself, pump-and-treat technologies are insufficient for remediation of chlorinated solvents such as TCE, but they can be implemented to contain contaminated plumes (Mackay and Harmon, 1993). In such instances, above-groundwater biological treatment is preferable to air-stripping due to lower costs and since they can result in complete conversion of contaminants to innocuous products (Lyman *et al*, 1992). For the case of TCE, complete detoxification can be achieved with a coupled anaerobic/aerobic biological reactor system (Kuang *et al*, 1994) or by complete reductive dehalogenation to ethene.

In situ anaerobic bioremediation is a preferable option for saturated soils since they are not amenable to oxygen delivery due to limitations in gas flow regardless of the soil's permeability (Hinchee, 1993). However, sorption of TCE by the organic fraction in soil has the potential to limit the availability of the contaminant for biodegradation, as has been

demonstrated by others (Alvarez-Cohen *et al*, 1993). In addition, more recently, it has been demonstrated that aging of a contaminant in soil further reduces its availability for biodegradation apparently due to its diffusion deeper within the soil's micropores (Alexander, 1995; Guerin and Boyd, 1993; Hatzinger and Alexander, 1995).

7.1.2 SSOM

To study the effects of TCE sorption on biodegradation within a saturated soil system, it was necessary to select a soil that would allow for the critical sorptive interactions with TCE. Since TCE tends to partition with the organic fraction in soil, and this fraction is intricately associated with minerals and is not amenable to extraction, a surrogate material was selected for this study. Other studies (Xing *et al*, 1994; Zytner *et al*, 1992) have implemented surrogates for the study of NOC interactions with soil, but the materials selected (*i.e.* lignin, collagen, chitin, cellulose, collagen-tannic acid mixture, peat moss, and GAC) are unhumified and hence do not realistically represent natural SOM. Alternatively, composting is essentially a humification process similar to that which occurs in natural soil where plants and animals are humified by biological processes that convert them to SOM (Garcia *et al*, 1992; Hill, 1975). Therefore, for the present research, composted sphagnum moss was selected as a SSOM to study the effect of aging of TCE in soil on its anaerobic biodegradation.

The increase in temperature of the material relative to the ambient temperature during the 9 weeks of composting (*i.e.* 31°C vs 24-29°C, respectively) as well as the evolution of humic acid and fulvic acid (*i.e.* 113-692 and 212-310 mg/g, respectively) were both indicative of biological activity. On this basis, composted sphagnum moss was selected as a SSOM for this research since it was shown to be humified and therefore more likely to be representative of natural SOM. However, it was first necessary to sterilize the SSOM to eliminate potential biotic transformation of TCE that could be misconstrued as irreversible sorption as well as

to eliminate competition of microorganisms that were allowed to proliferate during composting but which may have competed with *D. tiedjei* in the biodegradation experiments. In comparing autoclaving and gamma-irradiation for this purpose, it was found that the latter at 50 kGy was sufficient to eliminate viable bacteria and fungi. Although there were observed differences in the pH dependent CEC and SSA of the sterilized and unsterilized materials, the differences between the pH dependent AEC and the pH of the two materials were insignificant. More importantly, the ability of the SSOM to sorb TCE was not influenced by sterilization. The surface functionality, as determined by IRS, of the two materials was also found to be similar to those reported in the literature for other composted materials (Inbar *et al*, 1991, 1992) and soil (Schnitzer and Preston, 1986).

Hence, based on the following three points, composted sphagnum moss that was subsequently irradiated at 50 kGy from a cobalt-60 source, was utilized as a SSOM in the present research:

1) It was sterile.
2) It was sufficiently humified and thereby representative of the organic fraction in natural soil.
3) Sterilization did not alter its ability to sorb TCE.

7.1.3 Sorption

In order to study the effect of SSOM on TCE biodegradation, it was necessary to determine the partitioning under the same conditions of aging. From this, it was determined that the amount of TCE sorbed by SSOM remained constant following 2 d, 14 d, and 30 d; but the concentration of TCE in the aqueous phase had decreased with time, probably as a result of volatilization. It is difficult to distinguish between the processes of sorption and desorption (Yong *et al*, 1992); hence, it is impossible to conclude whether sorption that had occurred

with time had reversed itself due to volatilization and subsequent desorption, or whether sorption had plateaued and volatilization from the aqueous phase occurred with no resulting desorption.

It was also observed that the extent of TCE partitioning, as determined by the K_{oc}, was four times lower for the SSOM in the aging experiments as compared to that which has been reported in the literature by Zytner (1992) for peat moss. The likely cause of this was increased solubility of TCE by binding with DOM in the aqueous phase and volatilization caused by aging. The DOM in the aqueous phase was between 933-1333 mg/L C following 2-30 d aging. These levels were significantly higher than the typical ranges (*i.e.* 0.2-15 mg/L C) that have been reported for shallow groundwater (Malcolm, 1993). Although DOM in excess of 1000 mg/L C has been reported in the Western U.S., levels associated with the SSOM of the present study are the exception rather than the rule.

However, it was not possible to measure the extent of TCE binding with DOM. The reason for this is that the dialysis equipment that is required to measure binding of aqueous chemicals with dissolved humic species (Amador and Alexander, 1988; Hintelmann *et al*, 1997) was not available for this study. Furthermore, if it had been available it should be noted that extensive modifications would have been necessary due to the volatility of TCE. However, Amador and Alexander (1988) determined by direct measurement that NOCs (less volatile than TCE) tend to bind with humic acid (a constituent of DOM) and that ionized organic contaminants have no ability to bind with humic acid. Hence, it may be that TCE, an NOC, also binds with DOM. Phenolic acid functional groups were the most abundant of all of the functional groups quantified in the SSOM. Since DOM originates from SSOM, it may also contain an abundance of phenolic acid functional groups. It was shown that a majority of those groups remained un-ionized at pH 6.7, the pH at which all experiments were conducted. Hydrogen bonds between the basic chlorine atoms of TCE and the acidic proton of the phenolic acid functional groups were likely to have occurred (Smith, 1973).

It is noteworthy that PCE was found to have had a greater affinity than TCE for several organic soils and materials (Zytner, 1992; Zytner *et al*, 1989). Similarly, in the present study, TCE was shown to have a greater affinity than *cis*-1,2-DCE for sorption by SSOM. Between the studies of Zytner and the present work, it can be said that the affinity for sorption by organic matter increased as follows:

$$C_2Cl_4\,(PCE) > C_2HCl_3\,(TCE) > C_2H_2Cl_2\,(DCE\,isomers)$$

The increase in sorption was coupled with an increase in the number of chlorine atoms and a decrease in the number of hydrogen atoms. Therefore, it would appear that the chlorine atom was more instrumental than hydrogen in sorption by solid phase organic material. The above affinities of the chlorinated ethenes for soil are consistent with K_{OW}'s of the same compounds reported in the literature (Table 2.2 on page 29, Nyer 1993).

After 2 d of aging, the TCE-SSOM complex showed no visible signs of TCE when examined by IRS. However, analysis of the same TCE-SSOM complex by GC indicated an abundance of TCE (*i.e.* 11 mg/g). This observation is consistent with the adsorption of TCE by hydrogen bonding within the inner surfaces of the SSOM complex. This is compatible with a combined ABE and SRPD model of rate limited sorption put forth by Pignatello (1993). According to the ABE model, hydrogen bonding of contaminants with soil organic matter can retard desorption. The SRPD assumes that sorption of NOCs occurs within the SOM, but it does not go as far to characterize possible mechanisms of sorption.

Regardless of the mechanism by which TCE partitions with SSOM, it was demonstrated that the sorption of TCE by solid-phase SSOM was reduced by the presence of DOM. In instances where sorption of TCE has resulted in reduced availability for biodegradation, DOM may be useful in enhancing the solubility of TCE and may eventually lead to enhanced biodegradation. However, this was beyond the scope of this research and hence requires further investigation.

7.1.4 Aging and TCE Biodegradation

Aging of TCE with SSOM for 2 d, 14 d, and 30 d was implemented in order to achieve longer contact times than are commonly employed in the laboratory. Although 30 d aging is less than what occurs in field conditions, it was nonetheless sufficient to retard biodegradation.

The level of TCE biodegraded by *D. tiedjei* was always less than the initial level of TCE in the aqueous phase. Hence it appears that the sorption of TCE by SSOM was not a factor in reducing biodegradation. However, since DOM also increased with aging, the availability of TCE for biodegradation was more complex than was originally anticipated at the start of this study. At 2 d aging, where DOM was 933 mg/L, the level of biotransformation of TCE to *cis*-1,2-DCE was similar (95 % confidence level) to what occurred in the absence of SSOM (*i.e.* 459 and 440 µg/L, respectively). Therefore, the availability of aqueous phase TCE to *D. tiedjei* appeared to have been unhindered at a DOM level of about 933 mg/L. However, when the DOM increased to 1200 mg/L after 14 d aging, the biotransformation of aqueous TCE significantly decreased (90 % confidence) and biotransformation of solid-sorbed phase TCE predominated. However, after 30 d aging, both solid-sorbed phase and aqueous TCE biotransformation waned. Between 14 and 30 d, solid-sorbed TCE had likely diffused deeper within the SSOM complex, thereby limiting its availability to *D. tiedjei* that is thought to dehalogenate TCE at its cell surface (Ni *et al*, 1995). In addition, the DOM was high following 14 and 30 d aging (*i.e.* 1200 and 1333 mg/L C, respectively) and was likely the reason for the reduced biodegradation from the aqueous phase. Considering the fact that TCE biodegradation over a longer time period (*i.e.* 24 d incubation instead of 115 h, as was done for aging experiments) resulted in much greater TCE biodegradation in the presence of SSOM than in its absence, it is unlikely that DOM was toxic to the *D. tiedjei* bacterium.

Furthermore, the ability of *D. tiedjei* to function in the presence of DOM at levels of about

933 mg/L C (2 d aging) is interesting from the view that DOM may be used as a natural surfactant to enhance the solubility of NOCs that will in turn enhance *in situ* bioremediation. However, since the degree of biodegradation decreased at a DOM concentration of 1200 mg/L, further investigations regarding the effect of DOM concentration on biodegradation over a broader range are required.

This study represents one of the first that used *D. tiedjei* in soil (personal communication Prof. J. Tiedje, May 10, 1996). Recently Fantroussi *et al* (1997) published results of a study that examined the activity of *D. tiedjei* in soil. This last study examined the dechlorination of 3-chlorobenzoate, not TCE or PCE, in an unsterilized natural soil. Since surfaces have been shown to both hinder and enhance biodegradation by affecting the aqueous environment of bacteria (Van Loosdrecht *et al*, 1990), the finding that *D. tiedjei* can function in a soil environment greatly improves the candidacy of this species for bioaugmentation. Since TCE contamination exists in saturated soil environments that are limited to oxygen delivery, bioaugmentation with anaerobic bacteria offers an interesting option for bioremediation. Fatherpure and Tiedje (1994) were successful in introducing this bacterium to an anaerobic consortium in a biofilm reactor for wastewater treatment. Hence, this bacterium has demonstrated its ability to function within a microbial community.

Parallels can be drawn between the results of the present study and the fate of TCE and *cis*-1,2-DCE in soil. In particular, prolonged aging times render TCE unavailable for reductive dechlorination; but, DOM also appears to decrease its availability for dechlorination as well as to increase its solubility. Hence, soils and groundwaters contaminated by TCE that also contain high levels of DOM could result in increased mobility as well as reduced bioavailability. For this reason, it may be useful to report levels of DOM in cases of contaminated groundwater. This being a relatively simple and inexpensive parameter to measure.

7.1.5 Long-Term Biodegradation of TCE

By prolonging the incubation time from 115 h (*i.e.* for all aging tests) to 10 d, 19 d, and 24 d as well as by re-inducing dehalogenation activity with 3-fluorobenzoate, the degree of dehalogenation of TCE to *cis*-1,2-DCE increased significantly over that which occurred in the absence of SSOM. However, direct comparisons between 115 h and the longer incubation times are not possible due to the fact that the latter were re-spiked with 3-fluorobenzoate and the TCE-SSOM slurries were not aged prior to inoculation with *D. tiedjei*.

However, if one examines the TCE biotransformation behaviour in the presence and absence of SSOM, it becomes evident that *D. tiedjei* utilized the SSOM as a carbon source. The amount of TCE converted to *cis*-1,2-DCE increased 2-fold between 19 and 24 d in the presence of SSOM. In the absence of SSOM, the level of conversion was 6 to 20 times lower compared to the case involving SSOM, and there were no dramatic increases in the level of TCE biotransformation over the 10 to 24 d incubation period. As well, an unknown compound (*i.e.* possibly $C_8H_2ON_4$) with a molecular weight of 172 was produced by biodegradation of SSOM itself by *D. tiedjei*. Such biodegradation was likely the cause of the accelerated dechlorination that was observed; however, either re-induction with 3-fluorobenzoate or longer incubation times were necessary to realize such gains. As well, the SSOM itself may have provided a surface for the *D. tiedjei* to form a biofilm, that may in turn have contributed to accelerated dechlorination.

The implications of the finding that *D. tiedjei* utilized SSOM as a source of energy are the following:

1. Release of TCE bound by SOM:

Long term contamination of soil by TCE typically results in a non-labile fraction in the sorbed phase that is resistant to desorption, and it is thought that this non-labile fraction is immobile and poses little threat to surrounding groundwater (Pavlostathis and Jaglal, 1991; Pavlostathis and Mathavan, 1992).The fact that a dehalogenating microbe is capable of degrading the SOM itself presents the possibility that in field conditions, release of soil-bound TCE from degradation of SOM can be accompanied with VC formation. Hence, remediation requirements of such soils would become more pressing.

2. Improved bioremediation strategies:

The fact that the presence of a composted material such as SSOM provided energy for *D. tiedjei* that resulted in significant increases in biotransformation of TCE to *cis*-1,2-DCE offers some interesting possibilities in the area of *in situ* bioremediation and pump-and-treat technologies. *In situ* bioremediation of soils high in organic matter may be feasible by bioaugmentation with *D. tiedjei*. In such a case, the bacterium may be capable of deriving energy from the SOM, and over time convert TCE to *cis*-1,2-DCE. Since 1,1-DCE, VC, ethene, and ethane were never detected in the present work, the possibility of VC accumulation in such an *in situ* bioremediation scenario may be avoided. Since the MCL of TCE is less than *cis*-1,2-DCE (Pontius, 1996), this may be sufficient treatment in some locales. In addition, bioaugmentation of contaminated soil with *D. tiedjei* within a DOM medium may be sufficient for some soils contaminated with TCE. For pump-and-treat technologies, above ground treatment of TCE contaminated groundwater in coupled anaerobic/aerobic biological reactors with *D. tiedjei* is a possibility. In particular, a bioreactor containing composted material as an inexpensive carbon source, followed by an appropriate aerobic segment to completely convert *cis*-1,2-DCE to CO_2, is a possible treatment application of this research.

7.1.6 Sequential Desorption

Sequential desorption attempts to simulate field conditions where incremental changes in the concentration gradient between the solid-sorbed and aqueous phase that result from groundwater fluxes in turn bring about desorption of contaminants. However, due to the volatile nature of TCE and *cis*-1,2-DCE, the results of this exercise were quite variable. Predictably, variations increased with prolonged aging. Hence, improvements in the design of this experiment are required if it is to be useful in further research. However, some general points can be made concerning the results obtained with TCE and *cis*-1,2-DCE.

For TCE, aging resulted in a slight increase in resistance to desorption. However, non-labile sorption was not observed for 2-30 d aging as evidenced by the fact that most of the TCE was finally desorbed for all aging times and biphasic desorption was not observed. However, aging for 30 d did result in a fraction of solid-sorbed TCE that was resistant to biodegradation by *D. tiedjei*. Hence, desorption may be required prior to biodegradation in some *in situ* bioremediation scenarios. Furthermore, resistance to biodegradation in this case was not coupled with resistance to desorption. Hence, from this, it would appear that both desorption and biodegradation should both be assessed independently. Finally, partitioning following biodegradation was not predicted by sequential desorption. This may stem from the formation of a biofilm that impeded mass transfer or from the difference in temperature between the two sets of experiments.

The results obtained for *cis*-1,2-DCE indicate that aging resulted in a decreased tendency for sorption. This may stem from a greater affinity of *cis*-1,2-DCE for DOM compared to solid SSOM. As well, sorption of *cis*-1,2-DCE may be less than TCE due to the reduced number of chlorine atoms in the molecular structure. However, under identical conditions, results indicate that *cis*-1,2-DCE was more mobile than TCE, as evidenced by its greater affinity for the aqueous phase. This observation is consistent with reported K_{OW}'s for the two

compounds. However, as other studies have indicated, there are limitations to the extent to which K_{OW} can be used to estimate K_{OC} (Gerstl and Kliger, 1990; Grathwohl, 1990; Xing *et al*, 1994). Results of this research indicate that K_{OC}'s estimated from the K_{OW} were three times greater than those estimates that were based on sorption data for both TCE and *cis*-1,2-DCE. Therefore, retention of the products of reductive dehalogenation by soil are more accurately estimated from sorption data as opposed to their K_{OW}'s.

7.2 Conclusions

Conclusions that can be drawn from this study are as follows:

- Composted material (*i.e.* SSOM) is representative of natural organic matter in soil and is a desirable alternative to other surrogate materials that have been used to study the partitioning behaviour of NOCs in soil.

- Aging resulted in hindered biodegradation of TCE to *cis*-1,2-DCE probably due to the following: increased levels of DOM that bound with aqueous TCE and sorption within the SSOM matrix, both of which appeared to have rendered TCE unavailable to *D. tiedjei*.

- TCE resistance to biodegradation was not parallelled with resistance to desorption.

- Non-labile sorption of TCE was not achieved within 30 d thereby indicating that longer aging times need to be studied in the laboratory.

- *cis*-1,2-DCE was less amenable to partitioning with SSOM as compared to TCE; hence, it is likely to be more mobile in subsurface environments. This observation is consistent with published K_{OW}'s for *cis*-1,2-DCE and TCE.

- K_{OC}'s estimated from the K_{OW}'s of TCE and *cis*-1,2-DCE were approximately three times greater than those estimated directly from sorption data.

- *D. tiedjei* was found to be capable of converting TCE to *cis*-1,2-DCE (major product) and *trans*-1,2-DCE (very minor product). No evidence of 1,1-DCE, VC, ethene, or ethane was found. Since *cis*-1,2-DCE is considered to be less toxic than TCE, there are some interesting possibilities for the use of *D. tiedjei* for bioremediation. However, the fact that *cis*-1,2-DCE is more mobile than TCE must also be considered.

- Long-term incubation (*i.e.* 10 d≤ t ≤ 24 d) of *D. tiedjei* with TCE-SSOM complexes resulted in extensive conversion (*i.e.*up to 40 %) of TCE to *cis*-1,2-DCE. Conversion of TCE to *cis*-1,2-DCE was up to 20 times greater in the presence of SSOM as compared to the case where it was absent. These results indicate that neither SSOM or DOM were toxic to this bacterium.

- *D. tiedjei* has been shown to function and even thrive in SSOM by deriving energy from this carbon source. An unknown compound (MF:$C_8H_2ON_4$; MW: 172) was the product of SSOM biotransformation. This also identifies a potential mechanism of TCE release from soil not previously identified in the literature.

7.3 Suggestions for Further Study

Based on the summary and conclusions provided in the previous sections, the following points are worthy of further research:

- In order to further knowledge concerning *D. tiedjei* metabolism, the unknown compound that resulted from incubation of the SSOM with *D. tiedjei* should be

identified. In addition, it should be determined if this compound contributes directly to reductive dehalogenation.

- Further work concerning partitioning and biodegradation of contaminants in the presence of SSOM should be conducted at lower concentrations of DOM that were encountered in the present study so as to realistically represent levels found in groundwater.

- It should be determined whether *D. tiedjei* thrived on solid SSOM or DOM as a source of energy.

- Development of techniques to measure the binding of aqueous DOM with TCE and *cis*-1,2-DCE should be pursued.

- Aging times greater than 30 d should be employed in order to bring about non-labile sorption of TCE and to study its impact on biodegradation.

- Biodegradation of PCE by *D. tiedjei* in soil should also be studied to determine its ability to degrade this compound.

- Bioreactor design should be pursued for above surface groundwater treatment that utilizes compost as a carbon source for *D. tiedjei*.

- Investigation of the use of *D. tiedjei* for bioaugmentation of field contaminated soils should be pursued. Such a study should commence with columns rather than slurries as was done for this study. This would also address the issue of transport and adhesion of the bacterium to soil.

- Further studies with *D. tiedjei* and SSOM are required to determine if long-term biodegradation of TCE was enhanced by surface effects of the solid material.

Chapter 8

Statement of Originality

This chapter summarizes in point form the originality of the present research.

- The results of this research indicate the potential of *D. tiedjei* as a candidate for bioaugmentation. As a chlorinated solvent, bioaugmentation poses an attractive option for bioremediation of TCE since indigenous organisms may not possess the ability to degrade this persistent class of contaminants (Thomas and Ward, 1993). Prior to this work, it had not been demonstrated that this organism could dehalogenate TCE in soil. Fantroussi *et al* (1997) recently demonstrated dehalogenation activity of *D. tiedjei* in an unsterilized soil; however, the compound that was dechlorinated was 3-chlorobenzoate, not TCE. Since the bacterium used in the present study was originally isolated from an anaerobic sewage sludge (Shelton and Tiedje, 1984), studies that indicate its activity in soil are necessary, as well as studies that show that it has the ability to compete with microbes indigenous to soil, as was demonstrated by Fantroussi *et al* (1997). To complement this, the present study demonstrated the ability of *D. tiedjei* to exhibit TCE dechlorination activity within a chemical environment that may be found in soil. As was pointed out in the *Literature Review*, the capacity of an organism to grow utilizing a particular organic carbon does not necessarily mean that inoculation of that organism in soil will result in biodegradation (Godbout *et al*, 1995; Goldstein *et al*, 1985). Furthermore, chemical inhibitory agents have been cited as factors that limit the activity of organisms introduced to soil (Zaidi *et al*, 1989).

- The ability of this organism to derive energy from SSOM that in turn enabled it to dehalogenate TCE at accelerated rates was an unexpected yet significant contribution

of this research to improvements of the performance of this organism. The impact of this observation may lie in a more serious look at utilizing this organism for the purposes of *in situ* bioremediation.

- The utilization of compost as a SSOM is also unique to this study, and allows for the isolation of the chemical interaction of NOCs with SOM. Since the SSOM is representative of the organic fraction in soil, it can be used to further study the processes of sorption, desorption, and biodegradation as they are affected by this highly reactive fraction of soil. In the present study, such an approach allowed for analysis of the TCE-SSOM complex by IRS that would not have been feasible in the presence of other soil minerals.

- With aging, hindered biodegradation of TCE was observed, but this was not parallelled with hindered desorption. Therefore, a contaminant that is non-labile with respect to biodegradation is not necessarily non-labile with respect to desorption. Hence, declining availability is not necessarily related to sequestration of organic compounds by soil as described by Alexander (1995). These results suggest that although a compound may be unavailable for biodegradation, it may nonetheless be mobile. In the present study, TCE was amenable to desorption as a result of relatively weak forces (*i.e.* slight concentration gradients between the solid and aqueous phases); however, it was resistant to biodegradation.

- The sorptive/desorptive behaviour of *cis*-1,2-DCE by soil has not been studied until now. The conclusion that *cis*-1,2-DCE is more mobile than TCE can also be inferred from published K_{OW} values for these two compounds. However, the K_{OC} of *cis*-1,2-DCE estimated from its K_{OW} was three times greater than the K_{OC} that was estimated from sorption data. This observation adds credence to the claim by others (Grathwohl, 1990; Xing *et al*, 1994) that estimating contaminant mobility from K_{OW},

and not from the soil itself, can lead to inaccurate predictions.

- This study represents the first application of *D. tiedjei* to soil for the purposes of TCE dehalogenation.

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APPENDIX A

D. Tiedjei Media:

- Cole *et al* (1995) Medium.
- DeWeerd *et al* (1990) Medium

A.1 Cole *et al* (1995) Medium

Part I

1. Mineral Solution

	g/L
NaCl	1
$MgCl_2$	0.5
KH_2PO_4	0.2
NH_4Cl	0.3
KCl	0.3
$CaCl_2$	0.015

Prepare in 1L volumetric flask, but don't bring up to 1L until later.

Ref.: Cole et al, 1995.
Widdel and Pfennig, 1984.

2. Trace Element Solution

	per L
25 % (w/w) Hcl	10 mL*
$FeCl_2.4H_2O$	1.5 g
$CoCl_2.6H_2O$	190 mg
$MnCl_2.4H_2O$	100 mg
$ZnCl_2$	70 mg
H_3BO_3	6 mg
$Na_2MoO_4.2H_2O$	36 mg
$NiCl_2.6H_2O$	24 mg
$CuCl_2.2H_2O$	2 mg

*First dissolve 1.5 g $FeCl_2.4H_2O$ in 10 mL 25 % HCl.

Prepare in 1 L volumetric flask, and bring up to 1 L.

Then add 1 mL of Trace Element Solution (step 2) to Mineral Solution (step 1). Store rest of 2. Trace Element Solution at 4 °C covered in tin foil.

Ref.: Widdel and Pfennig, 1984.

3. Selenium Solution

	per L
$Na_2SeO_3.5H_2O$	6 mg
$Na_2WO_4.2H_2O$	8 mg
NaOH	0.5 g

Prepare in volumetric flask and bring up to 1 L.

Add 1 mL of Selenium Solution (step 3) to Mineral Solution (step 1). Store the rest of 3. Selenium solution at 4 °C covered in tin foil.

Ref.: Brysch et al, 1987.

4. After adding 1 mL of Trace Element Solution (step 2) and 1 mL Selenium Solution (step 3), bring up the volume of the Mineral Solution (step 1) to 800 mL with d. water and add the following:

	per L	Desired Final Conc.
Resazurin	0.1 mg	0.1 mg/L
HEPES (hemisodium salt)	2.383 g	10 mM

-Adjust pH to 7.3 with 3 N KOH.

-Boil and bubble with N_2/CO_2 (80:20)

-Cool and then add the following:

	per L	Desired Final Conc.
$NaHCO_3$	2.550 g	30 mM

-Adjust pH to 7.5 then add the following:

	per L	Desired Final Conc.
cysteine	0.1576 g	1 mM

-Bring up the volume to 1 L, but maintain headspace with N_2/CO_2 (80:20). After adding cysteine, don't bubble any more, but maintain the headspace with N_2/CO_2 (80:20).

-Dispense anaerobically into serum bottles by bubbling with N_2/CO_2 (80:20). Crimp tops securely with butyl rubber septa.

-Autoclave for 15 min. Be sure glass serum bottles are placed in steel autoclave box.

Part II

1. Prepare the following solutions for filter sterilization.

a) Vitamin solution:

	per 250 mL	Desired Final Conc.
thiamine	0.00125 g	5 mg/L
nicotinamide	0.0125 g	50 mg/L
1,4-napthoquinone	0.005 g	20 mg/L

Ref.: DeWeerd et al, 1990.

This solution may be stored at 4 °C wrapped in tin foil.

b) Pyruvate solution:

	per 10 mL	Desired Final Conc.
Pyruvic acid	2.2 g	2 M

This solution should be prepared fresh each time.

c) Sodium thionite reducing solution:

	per 100 mL	Desired Final Conc.
$Na_2S_2O_4$	0.174 g	1.74 g/L

d) Cysteine-Na_2S reducing solution:

-Place about 20 g $Na_2S.9H_2O$ onto a paper towel for about 1 h.

-Weight 10 g cysteine-HCl and add it to 200 mL d.water. Rapidly adjust the pH to 10 with freshly prepared 3 N NaOH (required about 50 mL). Dissolution is hastened by pH adjustment.

-Transfer cysteine solution to a 2 L Erlenmyer flask and start bubbling head space with N_2.

-Add 10 g of $Na_2S.9H_2O$ to the mixture, and add sufficient d.water to make up a total volume of 1 L.

-Bring the contents of the flask to a brief boil, stop heat and dispense anaerobically under N_2 gas phase. Crimp securely and autoclave (121°C-15 min) in steel autoclave box.

e) 3-Fluorobenzoate solution (Cole *et al*, 1995):

-Dissolve 0.788 g 3-fluorobenzoate in 1 L water to make a 0.00562 M solution, filter sterilize.

Solution	per10 mL Medium (Part I)	Desired Final Conc.
Vitamin solution	0.1 mL	
-thiamine		50 µg/L
-nicotinamide		500 µg/L
-1,4 napthoquinone		200 µg/L
Pyruvate solution	0.1 mL	20 mM
$Na_2S_2O_4$*	0.1 mL	0.1 mM (100 µM)
$Na_2S.9H_2O$-CysteineHCl	0.1-0.3 mL	
-Na_2S		0.4-1.2 mmol/L
-cysteine		0.63-1.89 mmol/L
3-fluorobenzoate	0.8 mL (per 45 mL medium)	100 µM

*This amount and type of reducing agent was not sufficient, as indicated by the redox indicator (resazurin). Therefore, a $Na_2S.9H_2O$-Cysteine HCl was used for reduction since it was a more effective reductant. Note-The original Cole (1995) protocol indicates the use of $Na_2S_2O_4$. Use of $Na_2S.9H_2O$-CysteineHCl is based on personal communication with Cole. The lower doses are recommended, but if necessary can go to the higher ones, only growth will be slower.

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A.2 DeWeerd *et al* (1990) Medium

Part I

1. Mineral Solution:

	g/L
PIPES	1.5
NaCl	0.8
NH_4Cl	1
KCl	0.1
KH_2PO_4	0.1
$MgCl_2.6H_2O$	0.2
$CaCl_2.2H_2O$	0.02
$NaHCO_3$	3

Prepare in 1L volumetric flask, but don't bring up to 1L until later.

Ref.: DeWeerd *et al* 1990.

2. Trace Element Solution:

	g/L
nitrilotriacetic acid*	2
$MnSO_4.H_2O$	1
$Fe(NH_4)_2(SO_4)_2.6H_2O$	0.8
$CoCl_2.6H_2O$	0.2
$ZnSO_4.7H_2O$	0.2
$CuCl_2.2H_2O$	0.02
$NiCl_2.6H_2O$	0.02
$Na_2MoO_4.2H_2O$	0.02
Na_2SeO_4	0.02
Na_2WO_4	0.02

*Add nitrilotriacetic acid to 800 mL d. water then adjust pH to 6 with 3 N KOH.

Prepare this solution in a 1L volumetric flask and bring volume up to 1 L. Adjust the pH to 7.8.

This solution may be stored at 4 °C wrapped in tin foil.

Ref.: DeWeerd et al 1990.
Tanner, 1989.

3. Add 10 mL of the Trace Element Solution (step 2) to the Mineral Solution (step 1). Then bring up the volume of the amended mineral medium to 1L with d. water. Boil solution and bubble with N_2/CO_2 (80:20).

Cool and dispense into serum bottles under N_2/CO_2 (80:20).

Autoclave medium for 15 min with serum bottles in steel autoclaave box.

Part II

1. Prepare the following solutions for filter sterilization.

a) Vitamin solution:

Same as per Cole protocol.

Ref.: DeWeerd et al 1990.

b) Pyruvate solution:

Same as per Cole protocol.

c) Sodium thionite

	per 100 mL	Desired Final Conc.
$Na_2S_2O_4$	0.870 g	8.7 mg/L

d) $Na_2S.9H_2O$-Cysteine-HCl solution:

Same as per Cole protocol.

Just prior to innoculation, aseptically add the following amounts of the above solutions to the sterilized medium prepared in Part I.

Solution	per 10 mL of medium (Part I)	Desired Final Conc.
Vitamin solution*	0.1 mL	
-thiamine		50 µg/L
-nicotinamide		500 µg/L
-1,4 napthoquinone		200 µg/L
Pyruvate solution*	0.2 mL	40 mM
$Na_2S_2O_4$**	0.1 mL	500µM
$Na_2S.9H_2O$-CysteineHCl*	0.1-0.3 mL	
-Na_2S		0.4-1.2 mmol/L
-cysteine		0.63-1.89 mmol/L

*Solutions are the same as those in Cole protocol.
**As with the original Cole protocol, this amount and type of reducing agent was insufficient, as indicated by redox indicator (resazurin). Therefore, a $Na_2S.9H_2O$-Cysteine-HCl solution was used for reduction.

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APPENDIX B

Sample Calculations for Determining Proton Dissociation for Oxygen Containing Functional Groups of SSOM:

- Carboxylic Acid Ionization.
- Phenolic Acid Ionization.
- Alcohol Group Ionization.

I. Carboxylic (CO_2H) Acid Ionization:

The ionization of aromatic carboxylic functional groups occurs according to the following equation:

$$ArCO_2H \rightleftharpoons ArCO_2^- + H^+ \quad \textbf{(B.1)}$$

From Table 4.2 in the main body of the thesis, $pK_a = 4.2$.

$$\therefore K_a = 63.1 \times 10^{-6} = \frac{[ArCO_2^-][H^+]}{[ArCO_2H]} \quad \textbf{(B.2)}$$

The fraction of functional groups that are ionized is expressed as follows:

$$\frac{[ArCO_2^-]}{[ArCO_2H]} = \frac{K_a}{[H^+]} = \frac{63.1 \times 10^{-6}}{[H^+]} \quad \textbf{(B.3)}$$

Since the pH at which all experiments were conducted was 6.7:

$$pH = 6.7 = -\log[H^+] \quad \textbf{(B.4)}$$

$$\therefore [H^+] = 19.5 \times 10^{-8} \quad \textbf{(B.5)}$$

By substituting equation (B.5) into (B.3), the fraction of the carboxylic acid functional groups that are ionized can be written as follows:

$$\frac{[ArCO_2^-]}{[ArCO_2H]} = \frac{63.1 \times 10^{-6}}{19.95 \times 10^{-8}} > 1 \quad \textbf{(B.6)}$$

Therefore, all of the carboxylic acid functional groups are ionized and hence negatively charged. From Table 4.2, the concentration of carboxylic acid groups is 23 meq/100 g; hence, according to the above calculation, 23 meq/100 g of negative charge originates from the carboxylic acid groups that are completely ionized.

II. Phenolic (ArOH) acid ionization:

The ionization of phenolic acid occurs according to the following equation:

$$ArOH \rightleftharpoons ArO^- + H^+ \qquad \textbf{(B.7)}$$

From Table 4.2, pK_a=10 for phenolic acid functional groups, and the expression for dissociation can be written as follows:

$$K_a = 10.0 \times 10^{-11} = \frac{[ArO^-][H^+]}{[ArOH]} \qquad \textbf{(B.8)}$$

By substituting the value of pK_a and (B.5) into equation (B.8), the fraction of phenolic acid groups ionized can be written as follows:

$$\frac{10.0 \times 10^{-11}}{[H^+]} = \frac{10.0 \times 10^{-11}}{19.95 \times 10^{-8}} = 5.0 \times 10^{-4} \qquad \textbf{(B.9)}$$

Therefore, according to equation (B.9) and the quantity of phenolic acid groups from Table 4.2, 0.115 meq/ 100 g of these groups are ionized and negatively charged.

III. Alcohol (ROH) group ionization:

The ionization of alcohol functional groups occurs according to the following reaction:

$$ROH \rightleftharpoons RO^- + H^+ \qquad \textbf{(B.10)}$$

From Table 4.2, pK_a=18 , so the fraction of groups ionized is expressed as:

$$\frac{1 \times 10^{-18}}{19.95 \times 10^{-8}} = 5.00 \times 10^{-12} \qquad \textbf{(B.11)}$$

Hence, none of the alcohol functional groups are ionized.

The results of these calculations are summarized in Table 4.2 in the thesis.