

AN ABSTRACT OF THE THESIS OF

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Title: Biodegradation of Chlorinated Methanes Using a Methylophilic/Anaerobic
Biofilm Reactor

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Kenneth J. Williamson

Chlorinated low molecular weight solvents have been discovered in a number of the nation's groundwater supplies. Remediation of these aquifers will require some form of treatment. Presently, the dominant treatment method is air stripping which results in transfer of the solvent from the aqueous phase into the atmosphere. Biodegradation is an attractive alternative because the solvents can be converted into harmless by-products.

This study explored the use of a combination of methylophilic and anaerobic bacteria to biodegrade chlorinated methanes. The objectives were to develop a reactor system capable of sustaining both methylophilic and anaerobic bacterial populations, and to evaluate the biodegradation of chlorinated methanes in this system.

A gas permeable membrane (Goretex) separated the reactor into a liquid compartment and a gas compartment. The methylophilic were provided methane

as an electron donor and oxygen as an electron acceptor. Inorganic nutrients and chlorinated methanes were supplied in the bulk solution. Methane and chloromethanes were the sole sources of carbon supplied to the reactor. The membrane was rotated as a flat plate to ensure complete mixing and to encourage growth of a biofilm of uniform thickness. The methylotrophic bacteria were grown as a biofilm on a gas permeable membrane. The anaerobic bacteria appeared to grow both as a biofilm and in suspension in the bulk solution. The chlorinated methane concentrations were measured in the headspace, gas compartment, and liquid compartment. Oxygen and methane use was also monitored. The reactor was operated in batch mode, and the biofilm was assumed to be in a quasi-steady state condition with respect to growth.

The combined methylotrophic/anaerobic biofilm system was effective in biodegrading carbon tetrachloride, chloroform, and dichloromethane. Carbon tetrachloride removal was accompanied by production of chloroform and trace amounts of dichloromethane, which is consistent with an anaerobic dechlorination pathway. Dichloromethane removal was inhibited in the absence of oxygen, which is consistent with a biological oxidation pathway. Chloroform removal under anoxic conditions was accompanied by production of dichloromethane, suggesting an anaerobic dechlorination pathway. Under aerobic conditions, the removal rate of chloroform increased nearly four fold, indicating that chloroform was biologically oxidized as well as anaerobically dechlorinated. Dechlorination of carbon tetrachloride and oxidation of dichloromethane occurred most rapidly, followed by combined dechlorination and oxidation of chloroform.

Biodegradation of Chlorinated Methanes Using a
Methylotrophic/Anaerobic Biofilm Reactor

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Stephen J. Celeste

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Professor of Civil Engineering in charge of major

Redacted for Privacy

Head of department of Civil Engineering

Redacted for Privacy

Dean of Graduate School

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Stephen J. Celeste

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Biodegradation of Chlorinated Methanes Using a Methylophilic/Anaerobic Biofilm Reactor

INTRODUCTION

Background

The occurrence of chlorinated low molecular weight solvents in surface water and groundwater supplies is an increasingly important environmental problem. Chlorinated solvents are produced in large quantities (over 2 million tons/year in the United States), are widely used, and contaminate many soils and aquifers (23). Chlorinated solvents are used in a variety of applications including metal degreasing, aerosol spraying, paint stripping, refrigeration, dry cleaning, and in the manufacture of semi-conductors (13). Accidental spills and inadequate disposal methods are largely responsible for the contamination of groundwater and soil.

Groundwater contamination by low-molecular-weight solvents is of particular concern because of their relatively high solubility, their persistence in the subsurface environment, and their potential for degradation of drinking water quality of aquifers. Many of these compounds are believed to have toxic or carcinogenic properties. Remediation of contaminated areas is necessary to prevent further dispersion in the environment.

Common treatment techniques for contaminated groundwaters include air stripping and carbon adsorption. Air stripping is often judged as an inadequate treatment method because it simply transfers the chlorinated solvents from the

aqueous phase into the atmosphere. Similarly, treatment by adsorption to activated carbon creates solid waste disposal problems since it concentrates the solvents onto a solid medium which must be disposed of by incineration or landfill. In many cases, carbon adsorption has proven uneconomical because of the low adsorptive capacity of these solvents and the high cost of activated carbon regeneration (3). Biodegradation is an attractive treatment alternative because chlorinated solvents can be converted into harmless by-products.

Biodegradation

Biodegradation of chlorinated solvents by both methylotrophic and anaerobic bacteria has been extensively reported in the literature. Transformation of trichloroethylene (TCE) to CO_2 has been observed in a pure culture of methylotrophs, mixed methylotrophic cultures, and in soil stimulated with methane and oxygen (8,11,19,21). Methylotrophs removed chlorinated methanes and ethanes from a sand column stimulated with natural gas. Dichloromethane (DCM) was removed most rapidly, followed by chloroform (CF) and carbon tetrachloride (CT). For chlorinated ethanes, 1,2 dichloroethane (1,2 DCA) was removed most rapidly, followed by 1,1 dichloroethane (1,1 DCA), 1,1,2 trichloroethane (1,1,2 TCA), and 1,1,1 trichloroethane (1,1,1 TCA). Biodegradation was reportedly the result of cometabolism of the chlorinated organics by the methylotrophic bacteria (12).

Methylotrophic bacteria grown on a gas permeable membrane removed DCM more rapidly than CF, but CT was not degraded (7).

Methylotrophs are aerobic bacteria which utilize methane as an electron donor and carbon source, and oxygen as an electron acceptor. They are found in

the narrow interface between anoxic and aerobic regions. This interface often occurs in natural environments, such as organic rich sediments where methane produced in anoxic zones diffuses into adjacent aerobic zones. The pathway for methane oxidation by methylotrophic bacteria is postulated to be $\text{CH}_4 \rightarrow \text{CH}_3\text{OH} \rightarrow \text{HCOH} \rightarrow \text{HCOOH} \rightarrow \text{CO}_2$ (18).

While a number of enzymes are responsible for the methylotrophic transformation, the most important is the methane monooxygenase enzyme (MMO). MMO initiates the conversion of methane to methanol. MMO is a non-specific enzyme which allows methylotrophs to cometabolize a wide range of chlorinated organic compounds (8,14,19). The chlorinated compounds are oxidized to CO_2 , H_2O and Cl^- . The rate of transformation is dependent on the structure of the compound and the degree of chlorination. High degrees of chlorination result in slow degradation rates (12).

While aerobic degradation results in the oxidation of the chlorinated compounds, anaerobic transformation occurs by reductive dechlorination. Anaerobic transformation of CT, CF and 1,2 dichloroethylene (1,2 DCE) to CO_2 has been observed under methanogenic conditions (3). Dechlorination of CT to CF has also occurred under denitrification conditions (4). Tetrachloroethylene (PCE) undergoes a sequential reductive dechlorination to TCE, DCE and vinyl chloride (VC) under methanogenic conditions (9,28). The reductive dechlorination pathway of CT has been proposed as $\text{CCl}_4 \rightarrow \text{CHCl}_3 \rightarrow \text{CH}_2\text{Cl}_2 \rightarrow \text{CH}_3\text{Cl} \rightarrow \text{CH}_4$ (5).

Reductive dechlorination has been shown to occur with numerous chlorinated aromatic and aliphatic compounds. However, few studies have produced quantitative results for reductive dechlorination rates. Highly

chlorinated chlorophenols were found to dechlorinate more rapidly than those with fewer chlorines, and the dechlorinated products of chlorinated aliphatics were found to accumulate (4,9,30). These results suggest that the rate of reductive dechlorination for chloroaliphatic compounds increases with increasing chlorination.

A combination of methylotrophic and anaerobic treatment may be effective in treating a wide range of chlorinated organic contaminants. The anaerobic portion of the system could be used to dechlorinate highly chlorinated compounds. The methylotrophic portion then could be used to oxidize the dechlorination products and lesser chlorinated pollutants to CO_2 , H^+ , and Cl^- . This would prevent the accumulation of lesser chlorinated compounds, as is commonly seen in strictly anaerobic treatment systems.

Goals

The goals of this study were to develop a reactor system capable of sustaining both methylotrophic and anaerobic cell populations, and to evaluate the treatment of chlorinated methanes with the combined methylotrophic/anaerobic system.

Treatment Systems

Combined methylotrophic/anaerobic treatment can be accomplished either by utilizing two reactors in series or by developing a two-layer methylotrophic/anaerobic biofilm in one reactor. A two reactor sequential treatment system would perform anaerobic treatment in the first reactor, followed by methylotrophic treatment in the second. This system would be more expensive than a combined methylotrophic/anaerobic treatment system because of the

increased construction costs of a second reactor and additional hydraulic systems. Combined methylotrophic/anaerobic treatment would also have the advantages of operational simplicity and decreased land requirements.

Figure 1 compares a traditional deep biofilm with a biofilm grown on a gas permeable membrane. With the deep biofilm, the cells at the support are lacking either the electron donor or electron acceptor. These cells may lose their ability to remain attached to the support, increasing the likelihood of cell washout. With the gas permeable membrane supported biofilm, the electron donor and acceptor are most abundant at the support. The cells at the support are likely to be in a growth phase. The sloughing of cells common in deep biofilms may be largely avoided. Since the gas permeable membrane allows substrate to be supplied both through the support and in the bulk liquid, multi-layered biofilms can be developed.

An existing bench-scale gas permeable membrane reactor was modified to allow for the growth of the methylotrophic and anaerobic populations. Chlorinated methanes (CT, CF, and DCM) were selected as the compounds to be treated. Removal of the chlorinated methanes and metabolite production was monitored by gas chromatography. Experiments were devised so that biodegradation due to the methylotrophic population could be separated from that of the anaerobic population. The methylotrophic bacteria were grown as a biofilm on a gas permeable membrane. The membrane (Goretex) is impermeable to water, but allows gases to pass through in either direction. It was used to separate the reactor into a gas compartment and a liquid compartment. Methane and oxygen were provided to the gas compartment and allowed to diffuse through

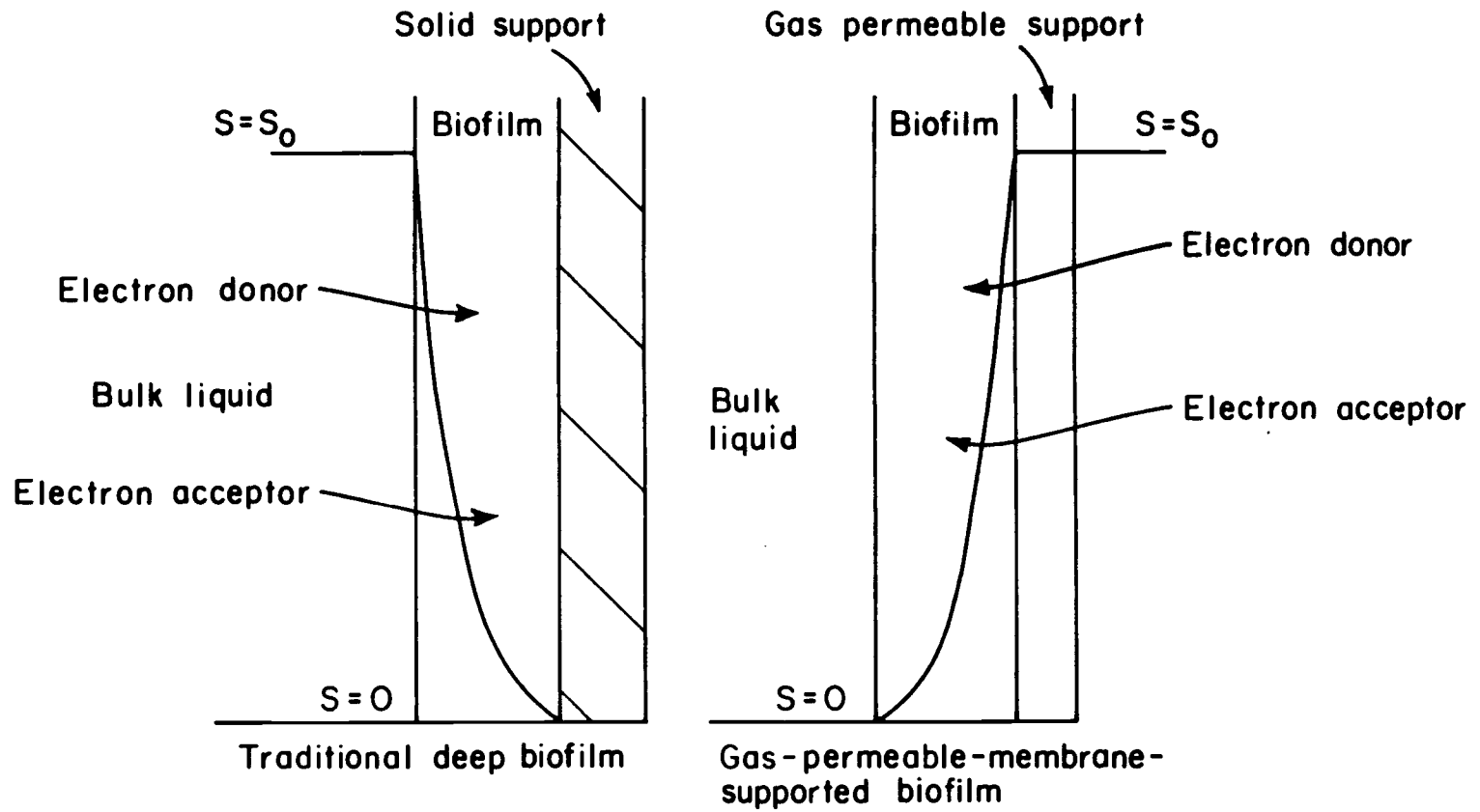


Figure 1. Comparison of a traditional deep biofilm grown on a solid support and a biofilm grown on a gas permeable membrane.

the membrane to the biofilm. As the oxygen was depleted, an anoxic zone formed above the methylotrophic bacteria, allowing for the growth of an anaerobic cell population. Figure 2 portrays methylotrophic and anaerobic cell populations grown on a gas permeable membrane. Biodegradation of CT, CF, and DCM was investigated under combined aerobic and anoxic conditions and under strictly anoxic conditions.

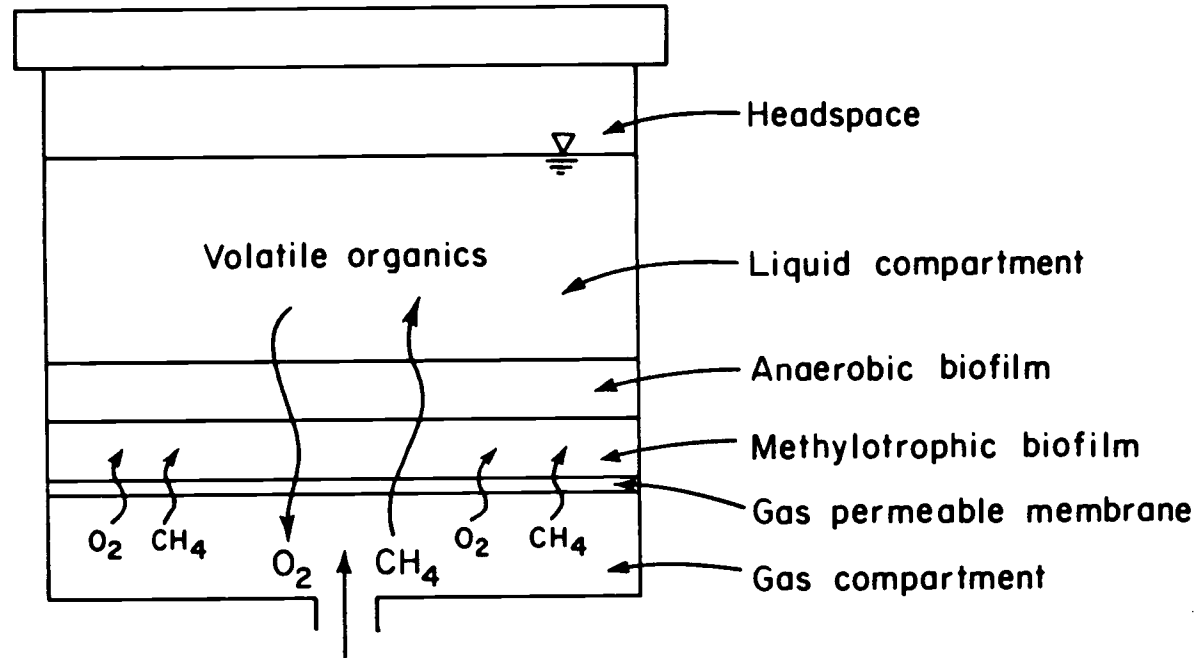


Figure 2. Methylotrophic and anaerobic cell populations grown on a gas permeable membrane.

MATERIALS AND METHODS

Reactor

The gas-permeable-membrane-supported (GPMS) biofilm reactor (Figure 3) was constructed of cylindrical glass beaded process pipe (Ace Glass, Vineland, NJ) with an inside diameter of 15.2 cm. An aluminum plate with a Teflon lined steel flange fitting (sealed with silicone vacuum grease) provided the top and bottom of the column. The column was separated into gas, liquid, and headspace compartments. A gas permeable membrane (Goretex, W.L. Gore & Associates, Elkton, MD) was clamped to the gas compartment structure. The membrane material consisted of a 216 μm thick sheet of polytetrafluoroethylene (PTFE) with 0.2 μm diameter pores between layers of Teflon and nylon.

A carbon-ceramic pump seal allowed for the rotation of the gas compartment and gas permeable membrane. The membrane was rotated at 60 RPM. The rotation of the gas permeable membrane served to keep the liquid completely mixed and to promote the growth of a biofilm of uniform thickness.

Ports were included for sampling of the headspace, gas compartment, and liquid compartment. The sampling ports utilized either Teflon/silicone or rubber septa.

Methane and oxygen were supplied to the gas compartment in a 50:50 volume/volume ratio via a gas drier and a diffuser. A low pressure regulator was used to furnish the gases at a pressure equal to the pressure exerted on the membrane by the overlying water in the liquid compartment to support the

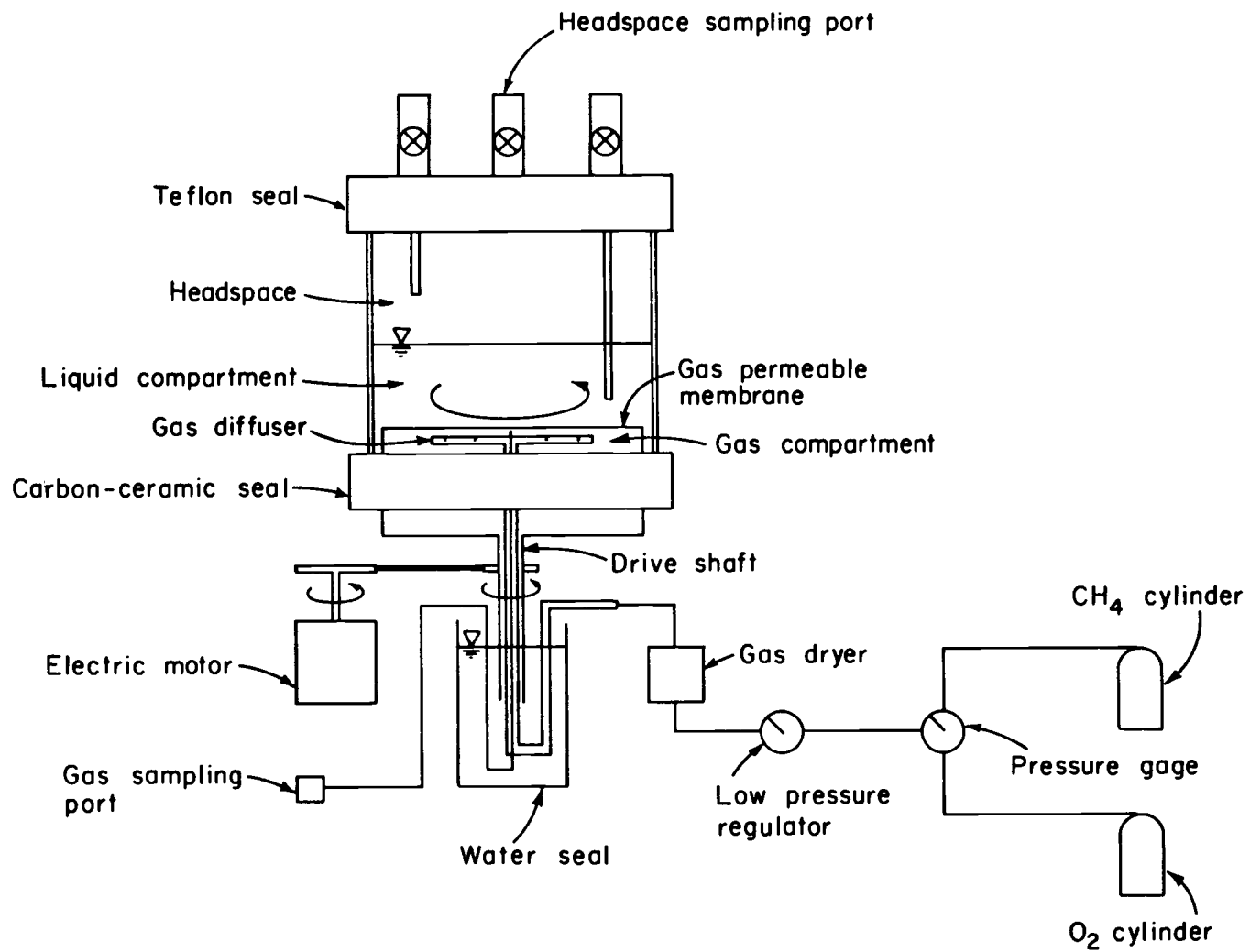


Figure 3. Reactor configuration.

membrane. The gas compartment was sealed with a water seal.

The reactor could be operated in either batch or continuous flow modes. In this study, the reactor was operated in batch mode. The reactor was filled with nutrient solution, and the chlorinated methanes were injected through the headspace sampling port.

Bacterial Seed

The original seed was isolated from thickened trickling filter effluent in 1986 from the Corvallis, Oregon wastewater treatment plant. The bacterial seed was taken from a biofilm that had been used to treat DCM, CF, CT, 1,2 DCA, and cis 1,2 DCE (7). The bacterial seed was applied to the gas permeable membrane, and allowed to attach for several days, and then methane, oxygen and feed solution provided. The bacterial population was allowed to increase in size for eight weeks before experimentation began.

Nutrient Media

The nutrient media was originally formulated by Whittenbury et al (1970) and modified by Ely et al (1986). It consisted of 488.4 mg/L MgSO_4 ; 101.3 mg/L CaCl_2 ; 4.1 mg/L EDTA Disodium Salt $\cdot 2\text{H}_2\text{O}$; 1000 mg/L KNO_3 ; 272 mg/L KH_2PO_4 ; 276.4 mg/L $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; 3 mg/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; 0.7 mg/L $(\text{NH}_4)_2\text{HPO}_4$; and 5 mL/L trace element solution. The trace element solution contained 500 mg/L EDTA Disodium Salt $\cdot 2\text{H}_2\text{O}$; 200 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 10 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 3 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 30 mg/L H_3BO_3 ; 20 mg/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.8 mg/L CaCl_2 ; 2.5 mg/L $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$; and 3.0 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$. The nutrient feed solution was neutralized to pH 7 with 1 M NaOH.

Analytical Methods

The concentrations of chlorinated compounds were measured using a Hewlett Packard 5890A gas chromatograph with a flame ionization detector coupled to a Hewlett Packard 3392A integrator. A 1/4 inch OD glass column, 8 feet in length and packed with 1% SP-1000 and 60/80 Carbopack B was used to separate the compounds. The temperature program used was: 70°C for 3 minutes, 8°C per minute to 100°C, 100°C for 9 minutes, 40°C per minute to 220°C, and then 220°C for 1 minute. One hundred μL liquid samples and 250 μL gas samples were injected directly into the gas chromatograph.

The concentrations of methane, oxygen, nitrogen, neon, and carbon dioxide were determined with a Fisher 25V gas partitioner. Neon was used as a volatile tracer. Neon concentrations were monitored to detect leakage of gases from the reactor.

Rate Constants

First-order rate constants were determined graphically. The natural log of the initially measured mass divided by the measured mass at time T was plotted against time. A regression was run to determine the slope of this line. The slope is the first-order rate constant.

RESULTS

Six batch runs were performed exploring the biotransformation of chlorinated methanes in the methylotrophic/anaerobic biofilm reactor. Run 1 explored the transformation of CT under aerobic conditions. In Run 2, the reactor was kept anoxic in order to determine the role of anaerobic bacteria in degrading CT. Runs 3 and 4 investigated the transformation of CF and DCM under both anoxic and aerobic conditions. The role of heterotrophic bacteria in the removal of CT was evaluated in Run 5. In Run 6, the O₂ concentration was elevated to increase methylotrophic activity and determine its effect on CT removal.

Degradation of Carbon Tetrachloride Under Aerobic Conditions

In Run 1, 40.3 μmol of CT were injected into the reactor at time zero (Figure 4). The initially measured (t = 1.5 hours) mass of CT was 31.6 μmol. Seventy percent of the initially measured CT was removed after 96 hours. The first-order removal rate constant for CT was estimated as 0.016 hr⁻¹ (r² = 0.66, n = 30, s = 0.0008). CF was first detected in the liquid compartment at 1.5 hours and increased to a maximum of 3.7 μmol at 96 hours (Figures 4 and 5). Trace levels of DCM were detected in the liquid compartment after 82.1 hours. Since the neon concentration was constant, it was concluded that no significant leaks in the apparatus were present.

After injection into the reactor, CT partitioned between the gas compartment, headspace, and liquid compartment (Figure 6). CT diffused through the liquid compartment and the gas permeable membrane and was first detected in the gas compartment after 3.8 hours. As shown in Figure 7, O₂ in the gas

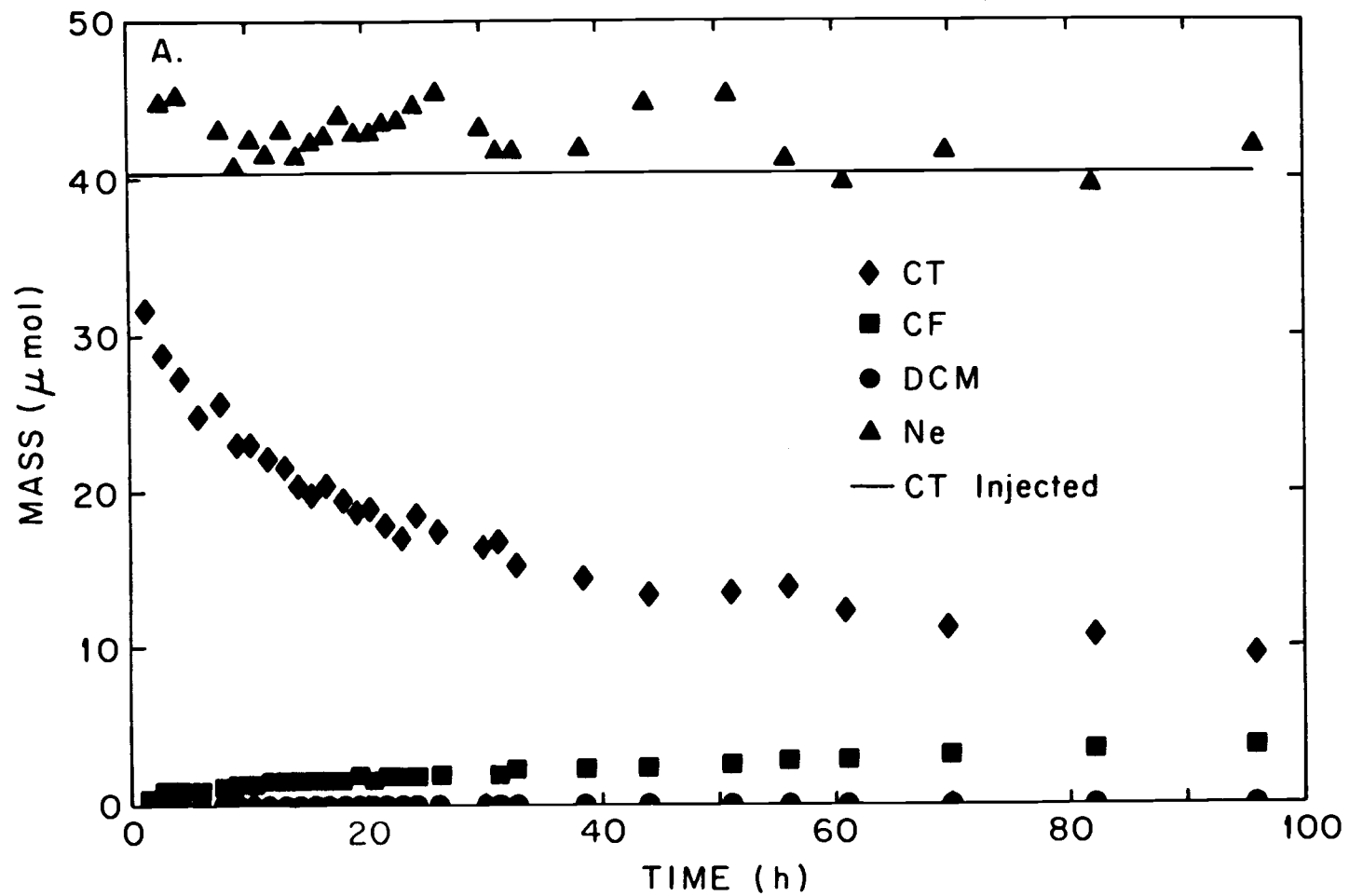


Figure 4. Run 1: Degradation of carbon tetrachloride under aerobic conditions.

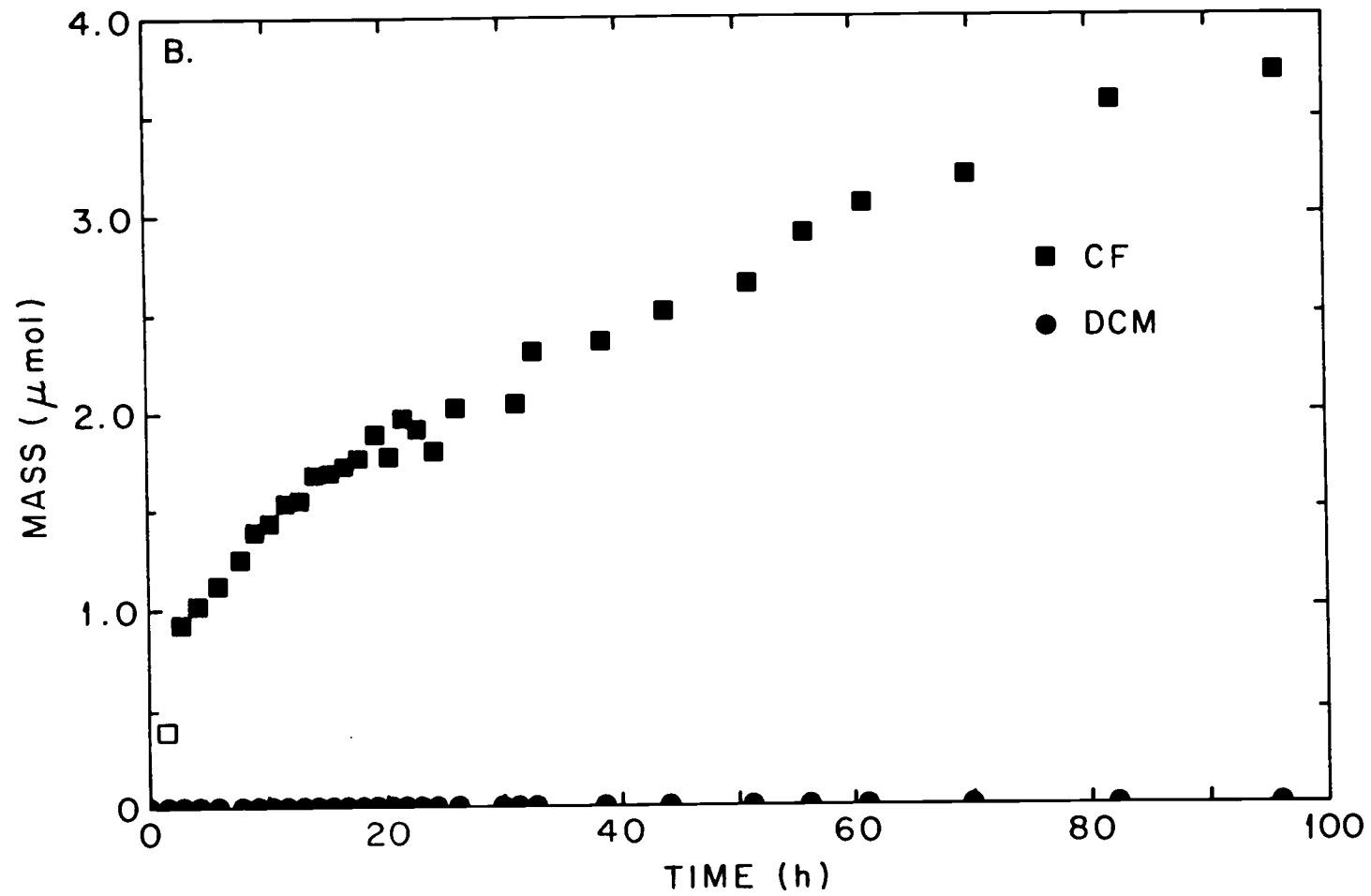


Figure 5. Run 1: Expansion of chloroform and dichloromethane production curves.

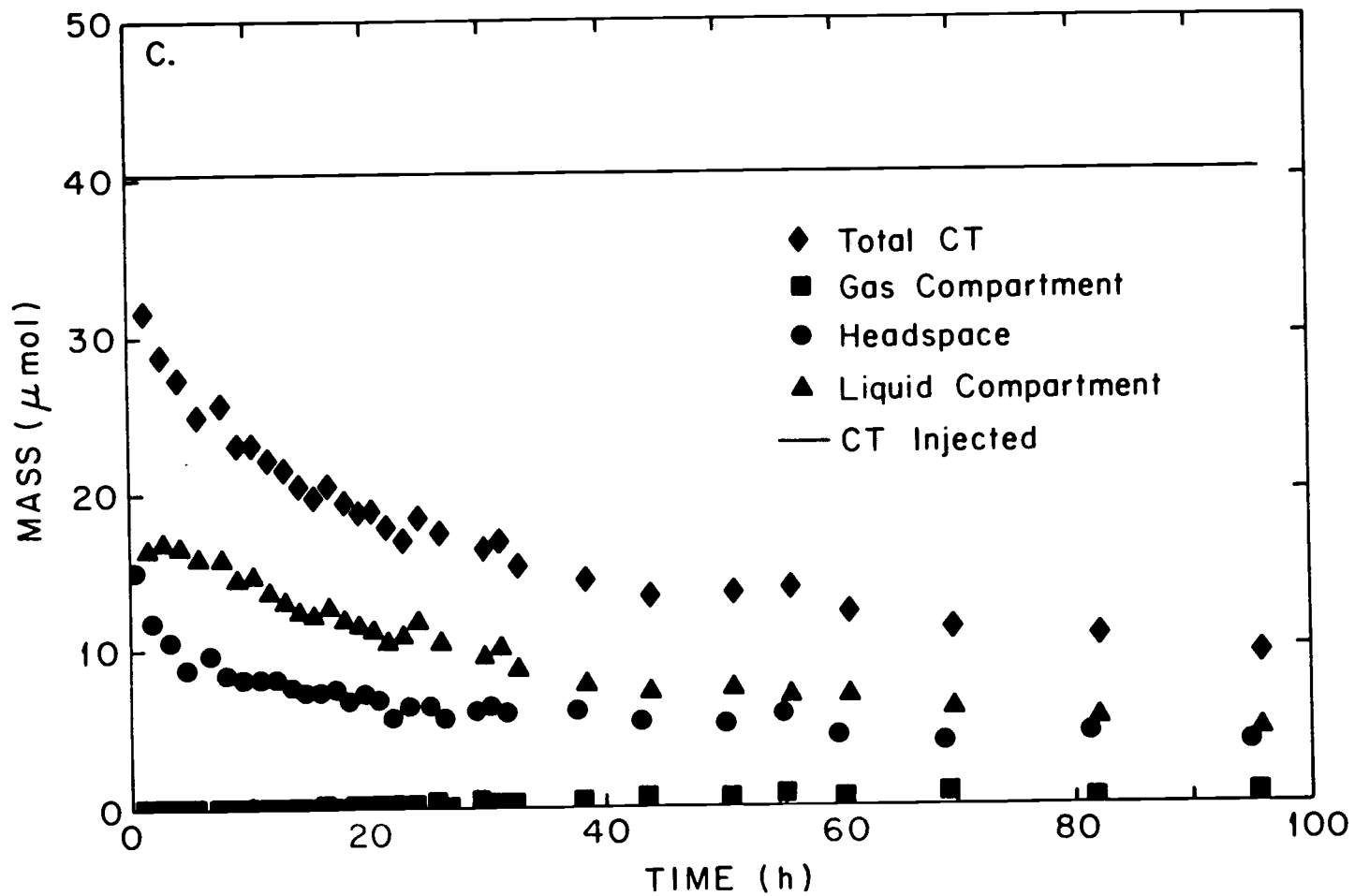


Figure 6. Run 1: Distribution of carbon tetrachloride in gas, headspace, and liquid compartments.

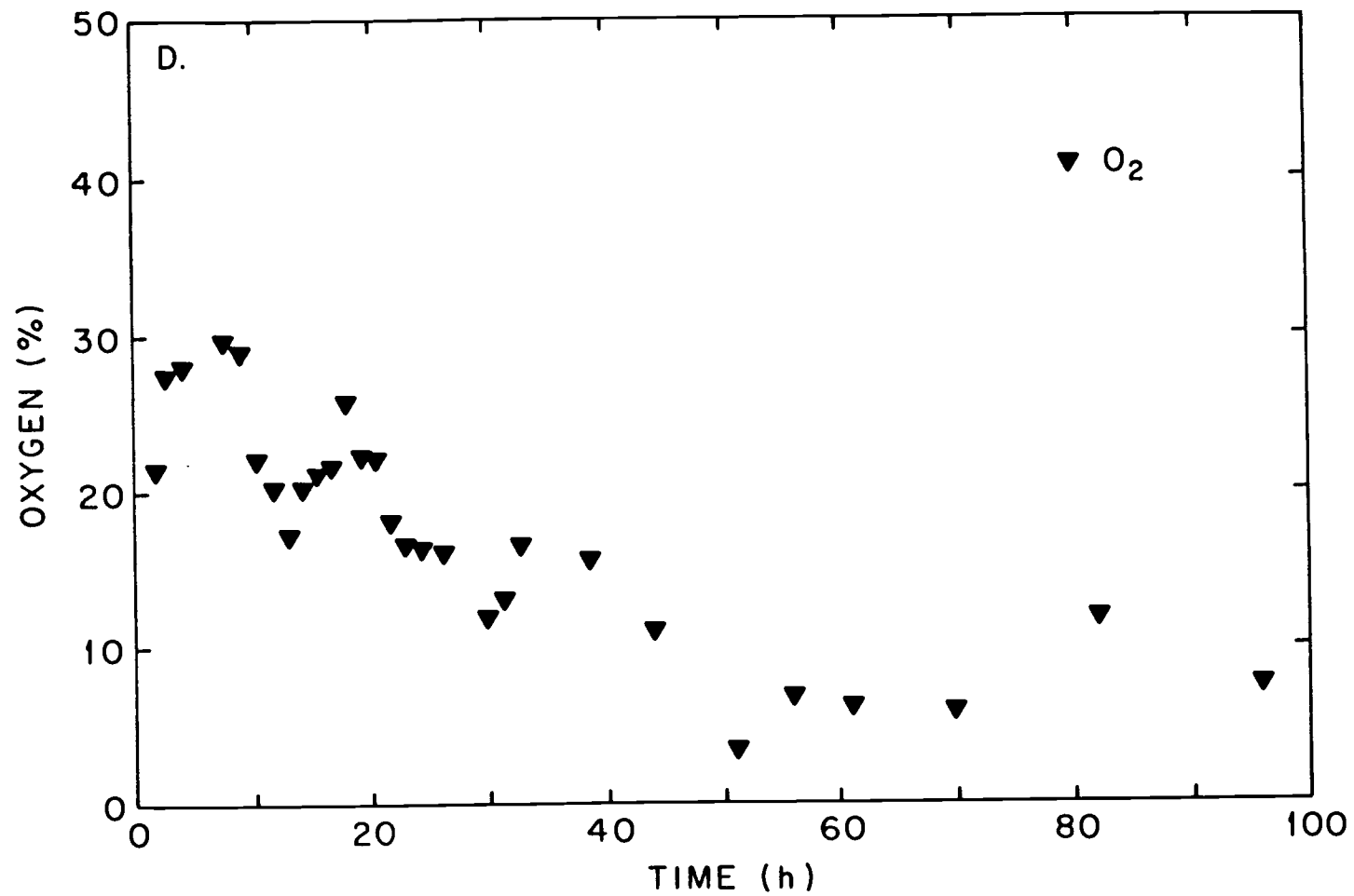


Figure 7. Run 1: O_2 use.

compartment was depleted over time since the reported methylotrophic stoichiometry of O_2 to CH_4 is a molar ratio of 1.7:1, and the gases are supplied at a constant molar ratio of 1:1 (18).

Degradation of Carbon Tetrachloride Under Anoxic Conditions

In Run 2, the reactor was kept anoxic to inhibit methylotrophic and heterotrophic activity and examine the role of the anaerobic cell population. The gas compartment contained only CH_4 . 40.3 μmol of CT were injected into the reactor of which 55% of the initially measured CT was removed after 50 hours (Figure 8). The first-order CT removal rate constant was estimated as 0.011 hr^{-1} ($r^2 = 0.83$, $n = 10$, $s = 0.001$). 2.2 μmol of CF were present at the start of Run 2 and 3.2 μmol were present after 50 hours. The initially measured CF may have been left over from the previous run. DCM was detected in measurable quantities at 12.7 hours, 50 hours, and reached a maximum of 0.16 μmol at 28.7 hours. Trace quantities of DCM were detected throughout the rest of the run. The neon curve showed that no significant leakage occurred.

Degradation of Chloroform Under Sequential Anoxic and Aerobic Conditions

The roles of the anaerobic and methylotrophic cells in the removal of CF were determined in Run 3 by operating the reactor under anoxic conditions followed by aerobic conditions (Figure 9). Initially, the gas compartment contained only CH_4 . At 50.8 hours, the gas compartment was purged with the CH_4 and O_2 mixture to bring the O_2 and CH_4 concentrations to 50% each. Information concerning neon and O_2 concentrations was unavailable after 71 hours.

43.5 μmol of CF were injected into the reactor. The initially measured CF

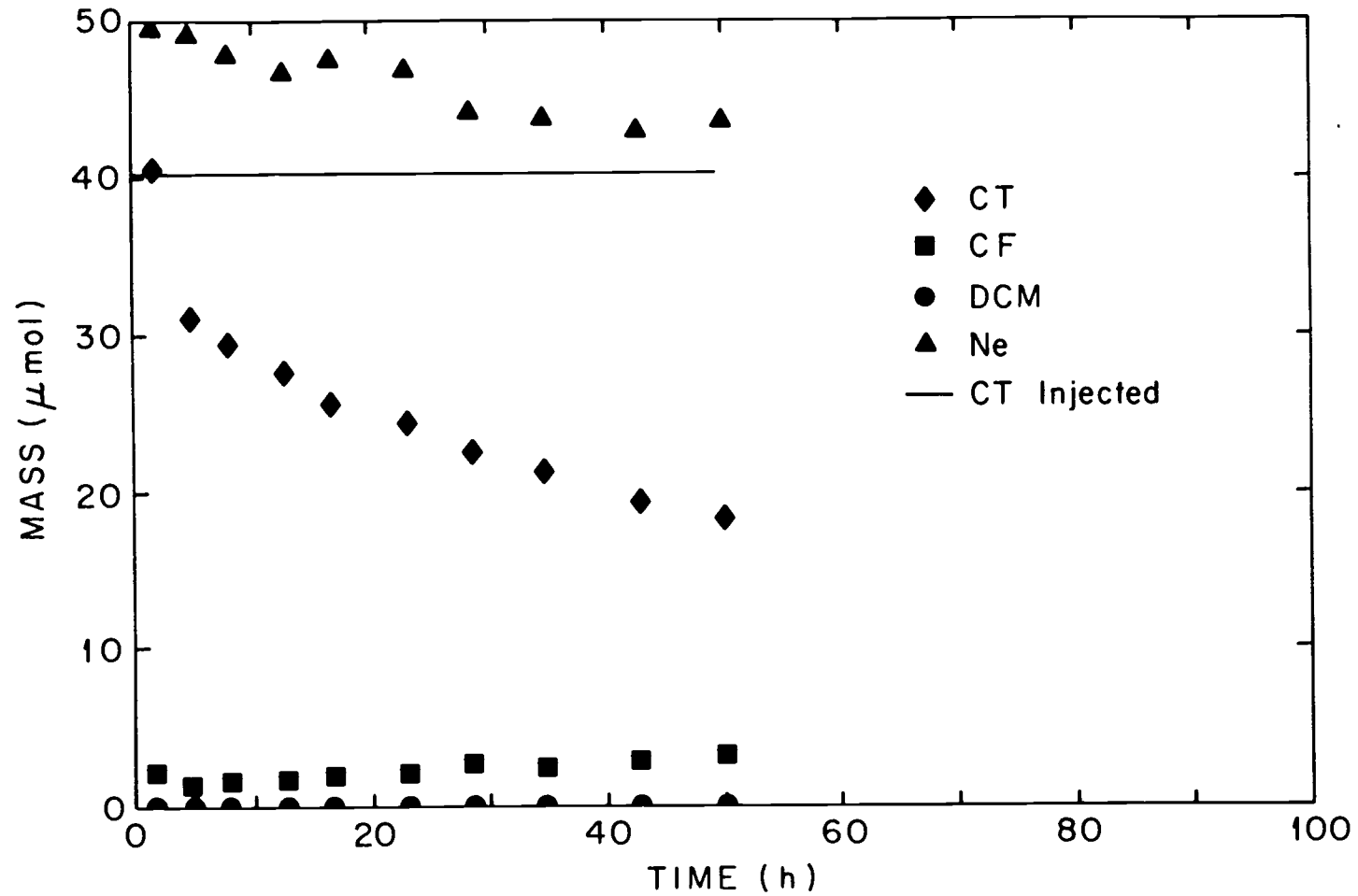


Figure 8. Run 2: Degradation of carbon tetrachloride under anoxic conditions.

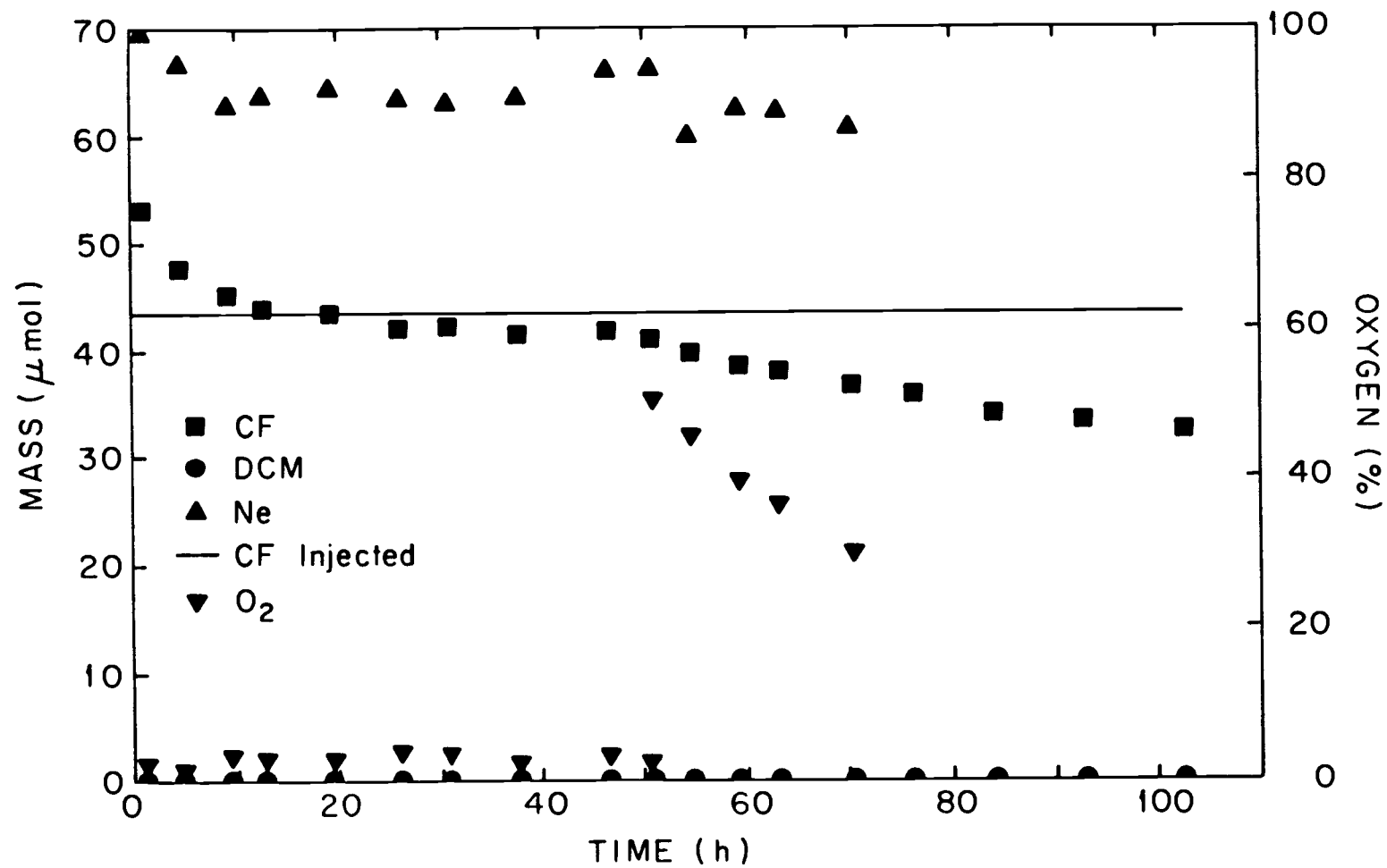


Figure 9. Run 3: Degradation of chloroform under sequential anoxic and aerobic conditions.

mass was 53.2 μmol , this discrepancy may have been due to an error in injecting the CF. Thirty nine percent of the initially measured CF was removed over the 102.6 hour run; 17% of the CF was removed over the first 12.7 hours. Six percent was removed during the time period starting at 12.7 hours and ending when the O_2 was added to the gas compartment at 50.8 hours. The first-order removal rate constant was 0.0013 hr^{-1} ($r^2 = 0.81$, $n = 7$, $s = 0.0001$) during this anoxic period. Sixteen percent was removed from 50.8 hours to 102.6 hours. During this time the first-order rate constant increased to 0.0050 hr^{-1} ($r^2 = 0.96$, $n = 9$, $s = 0.0002$). DCM was detected in measurable quantities from 46.5 hours to the end of the run. The maximum value was 0.3 μmol at 76.1 hours. The O_2 concentration in the gas compartment remained low until it was purged at 50.8 hours. The O_2 concentration fell from 50% to 30% during the time period from 50.8 hours to 70.3 hours. The neon curve indicated that leakage was not a major removal mechanism.

Degradation of Dichloromethane Under Sequential Anoxic and Aerobic Conditions

The roles of the anaerobic and methylotrophic cells in the removal of DCM was determined in Run 4 by operating the reactor under anoxic conditions followed by aerobic conditions (Figure 10). Initially, the gas compartment contained only CH_4 . At 45.5 hours, the gas compartment was purged with the CH_4 and O_2 mixture, raising the O_2 and CH_4 concentrations to 50% each. 46.9 μmol of DCM were initially injected into the reactor. The initially measured mass of DCM was 63.7 μmol , this discrepancy may have been due to an error in injecting the DCM. Fifty two percent of the initially measured DCM was removed over the 98.4 hour run. Thirteen percent of the DCM was removed over

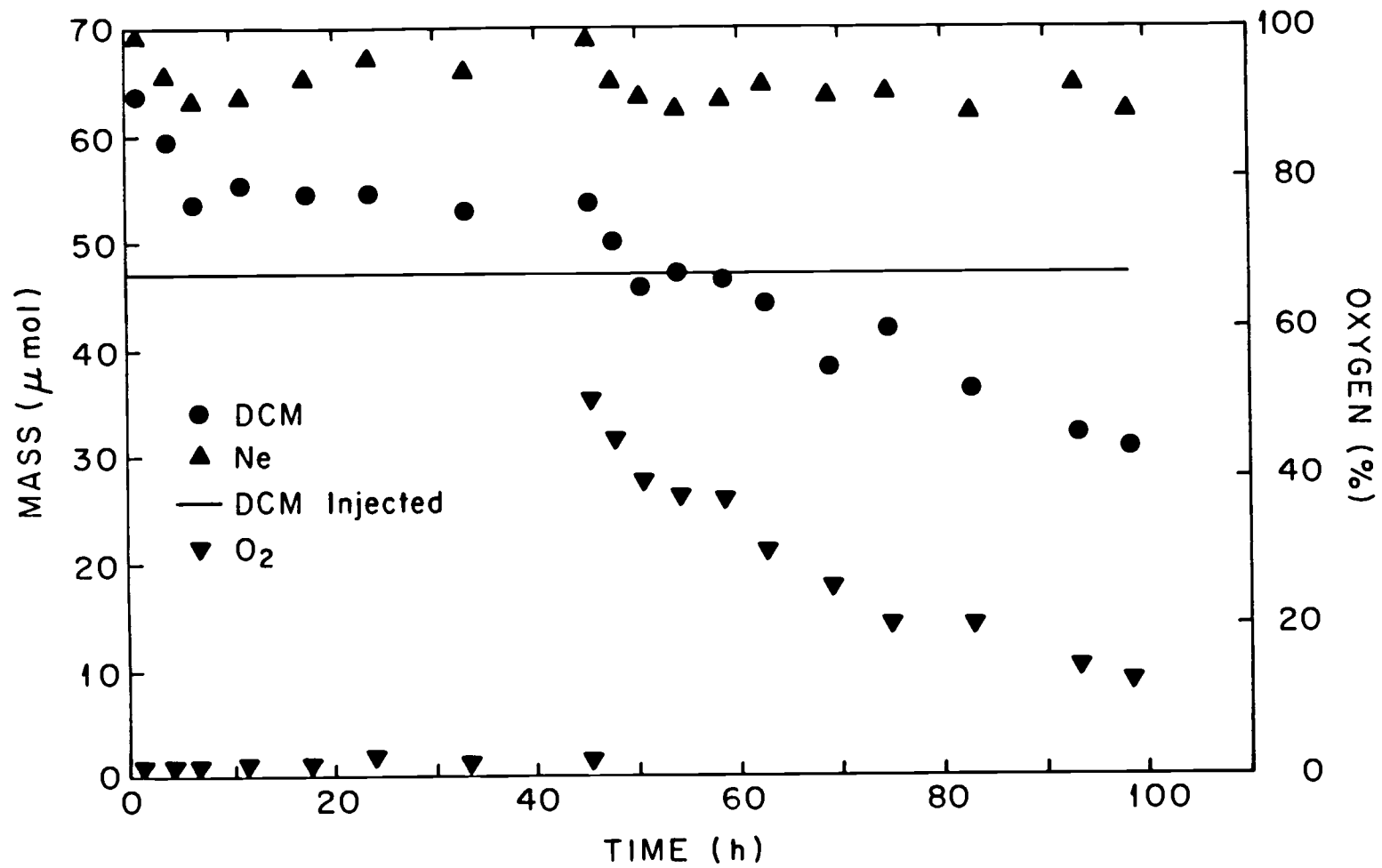


Figure 10. Run 4: Degradation of dichloromethane under sequential anoxic and aerobic conditions.

the first 11.2 hours. Less than 3% was removed during the time period between 11.2 hours and 45.5 hours. The first-order removal rate constant during this period was 0.0008 hr^{-1} ($r^2 = 0.66$, $n = 5$, $s = 0.0002$). Thirty six percent of the DCM was removed while the gas compartment was aerobic, from 45.5 hours to 98.4 hours. The first-order rate constant was 0.011 hr^{-1} ($r^2 = 0.89$, $n = 10$, $s = 0.0007$) during this time period. The O_2 concentration in the gas compartment fell from 50% to 12.5% during the time period starting at 45.5 hours and ending at 98.4 hours. The neon curve indicated that leakage was not a significant removal mechanism.

Degradation of Carbon Tetrachloride Under Acetylene Inhibition

Run 5 was designed to ascertain the role of aerobic heterotrophs in the transformation of CT. Acetylene is a known inhibitor of methylotrophic activity, but does not affect aerobic heterotrophic bacteria (13,15,18). A comparison of Run 5 to the results of a run which had an aerobic gas compartment and no acetylene would indicate the effect of heterotrophic activity.

Acetylene was supplied to both the headspace and the gas compartment at a concentration of approximately 20% of the headspace and gas compartment volumes. $40.3 \mu\text{mol}$ of CT were injected into the reactor. Fifty one percent of the initially measured CT was removed after 49 hours, while 63% was removed after 93 hours (Figure 11). The first-order removal rate constant was estimated as 0.013 hr^{-1} ($r^2 = 0.91$, $n = 17$, $s = 0.0005$). The curve appears to level off at about $12 \mu\text{mol}$ of CT. CF was first detected after 4.2 hours and increased to a maximum of $2.9 \mu\text{mol}$ at the end of the 93 hour run. Traces of DCM were detected in the last half of the time period. The neon curve showed that leakage

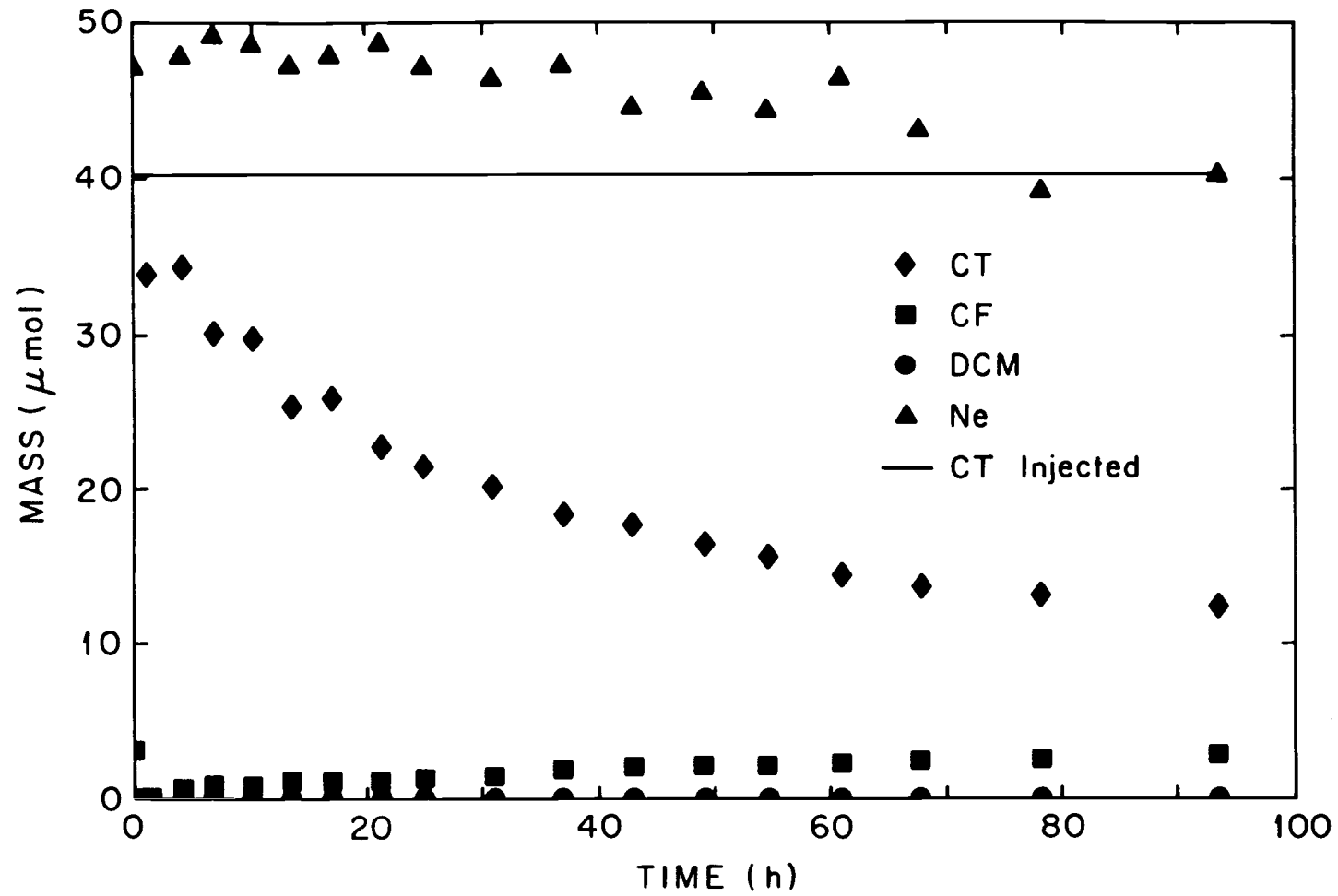


Figure 11. Run 5: Degradation of carbon tetrachloride under acetylene inhibition.

was insignificant.

Degradation of Carbon Tetrachloride Under Varying O₂ Concentrations

The role of methylotrophs in the degradation of CT was uncertain. In Run 6, the gas compartment was purged with the O₂ and CH₄ mixture periodically to see if CT removal could be enhanced by sustaining a higher O₂ concentration and therefore, a potential increase in methylotrophic activity. The gas compartment was purged with the CH₄ and O₂ mixture four times during the 100 hour run: at the start of the run, at 23.4 hours, at 48.4 hours, and at 73.7 hours. The first-order removal rate constant over the entire time period was estimated as 0.011 hr⁻¹ ($r^2 = 0.77$, $n = 12$, $s = 0.0008$).

DISCUSSION

This study was organized to determine the biological mechanisms responsible for the removal of chlorinated methanes. The runs allowed for the separation of methylotrophic, heterotrophic and anaerobic processes. Methylotrophic activity was inhibited with acetylene, allowing for the observation of anaerobic and aerobic heterotrophic activity. Aerobic activity was inhibited by purging O₂ out of the reactor, making it possible to monitor strictly anaerobic activity.

Biodegradation was concluded to be the dominant removal mechanism in these studies. Other possible non-biological removal mechanisms considered were leakage, chemical degradation, photodegradation, and sorption. Neon was used as a volatile tracer, and showed that leakage was not significant. Chemical degradation was probably insignificant since the compounds of interest have known chemical half-lives on the order of years (27). Photodegradation was eliminated by excluding light. Sorption was unavoidable, but appeared to reach equilibrium within a few hours of the beginning of each run.

First-order rate constants were calculated for the removal curves of CT, CF, and DCM. The first-order model fit the CF and DCM curves well, but a third-order model more accurately described CT removal. The first-order model was chosen for all compounds so the removal rate constants could be easily compared.

Studies have shown that CT is resistant to methylotrophic biodegradation, but can be treated anaerobically. Run 1 showed that CT removal under aerobic conditions was accompanied by production of CF, which is the metabolic product

of CT in a dechlorination pathway (3,4). In Run 2, anoxic conditions were used to inhibit the methylotrophs and heterotrophs. In Run 6, the gas compartment was resupplied with O₂ several times. The increase in available O₂ should have increased methylotrophic activity compared to Runs 1 and 2 if O₂ was the limiting factor. However, the enhanced O₂ concentration did not improve CT removal. Run 1 exhibited a first-order removal rate constant for CT of 0.0164 hr⁻¹, compared to 0.0107 hr⁻¹ in Run 2 and 0.0110 hr⁻¹ in Run 6. The rate constant of Run 2 (anoxic gas compartment) is nearly identical to the rate constant of Run 6 (highest concentration of O₂ in gas compartment). Since the removal of CT was unaffected by changes in methylotrophic activity, CT is believed to undergo anaerobic dechlorination to CF. Sorption of CT to the bacteria is probably represented by the measured rapid initial removal in all runs.

Analysis of CF is somewhat more complex than CT because it was being degraded and produced simultaneously. CF production was first detected in Run 1. Runs 2, 5, and 6 showed that CF production was unaffected by an anoxic gas compartment, the addition of acetylene, or by periodically resupplying O₂ to the gas compartment. This implies that CF production, like CT removal, is an anaerobic process. CF production should parallel CT removal, since any CF formed is the result of CT dechlorination.

CF removal was examined in Run 3. The rapid initial loss of CF (0 to 10 hours) was attributed to sorption. The relatively slow removal of CF and production of DCM under anoxic conditions suggest that CF was dechlorinated to DCM. The removal rate constant for CF increased nearly 4 fold, from 0.0013 hr⁻¹ to 0.0050 hr⁻¹ when O₂ was added to the gas compartment. This implies that both

the methylotrophic population and the anaerobic population participated in the removal of CF, but the methylotrophic bacteria removed CF more rapidly.

DCM, like CF, was both produced and degraded in the methylotrophic system. Only small quantities of DCM were detected in this study. The transformation of CF in Run 3 yielded the largest amount of DCM, indicating that CF is dechlorinated to DCM. Runs 2, 5, and 6 showed that DCM production during CT removal was not noticeably altered by an anoxic gas compartment, the addition of acetylene, or by resupplying the gas compartment with O₂.

DCM removal was investigated in Run 4. The rapid initial loss of DCM (0 to 10 hours) was probably due to sorption. Virtually no DCM was removed when the gas compartment was anoxic. The removal rate constant increased nearly 14 fold, from 0.0008 hr⁻¹ to 0.0110 hr⁻¹, after O₂ was added. This suggests that the removal of DCM is strictly a methylotrophic process. It is uncertain why no buildup of DCM was observed during the transformation of CF and CT under anoxic conditions. Perhaps there was enough O₂ present to allow the methylotrophic cells to oxidize DCM slightly faster than CF was being dechlorinated.

Acetylene was used to inhibit methylotrophic activity in Run 5 and anoxic conditions were used to inhibit all aerobic activity in Run 2. Since Runs 2 and 5 yielded similar CT removal rate constants (0.0107 hr⁻¹ and 0.0133 hr⁻¹, respectively) and heterotrophic activity should have been possible in Run 5, heterotrophic activity was deemed insignificant.

At the completion of this research, the reactor was dismantled and examined. A population of methylotrophic bacteria, approximately 6 cm in

diameter, appeared to be attached to the center of the circular gas permeable membrane. Upon removal of the gas permeable membrane, it was observed that water from the water seal had condensed in the gas compartment. Methylotrophic bacteria were present in the condensed water, upon the inside walls, and on the bottom side of the gas permeable membrane as shown in Figure 12. This bacterial growth precluded any attempt at developing a biofilm model, and also severely limited the size of the bacterial methylotrophic population on the gas permeable membrane by consuming much of the available O_2 . The methylotrophic population inside the gas compartment probably aided in the removal of CF and DCM since these volatile compounds could pass through the gas permeable membrane. Had the pollutant been a non-volatile compound, such as a chlorinated phenol, the methylotrophic bacteria inside the gas compartment would have inhibited pollutant removal by limiting the size of the methylotrophic population on the gas permeable membrane.

The nature of the anaerobic population is largely unknown. Anaerobic bacteria appeared to grow on top of the methylotrophic biofilm and also in suspension in the bulk solution. No external carbon source or electron donor was provided. The nutrient feed solution contained 1000 mg/L KNO_3 , so NO_3^- was a probable electron acceptor, allowing denitrification to occur. The electron donor and carbon source may have been produced by the methylotrophic bacteria. The oxidation of CH_4 by methylotrophs creates a number of intermediary products such as methanol, formic acid, and formaldehyde. The addition of an external carbon source could probably increase the size and effectiveness of the anaerobic population, but may inhibit the methylotrophs.

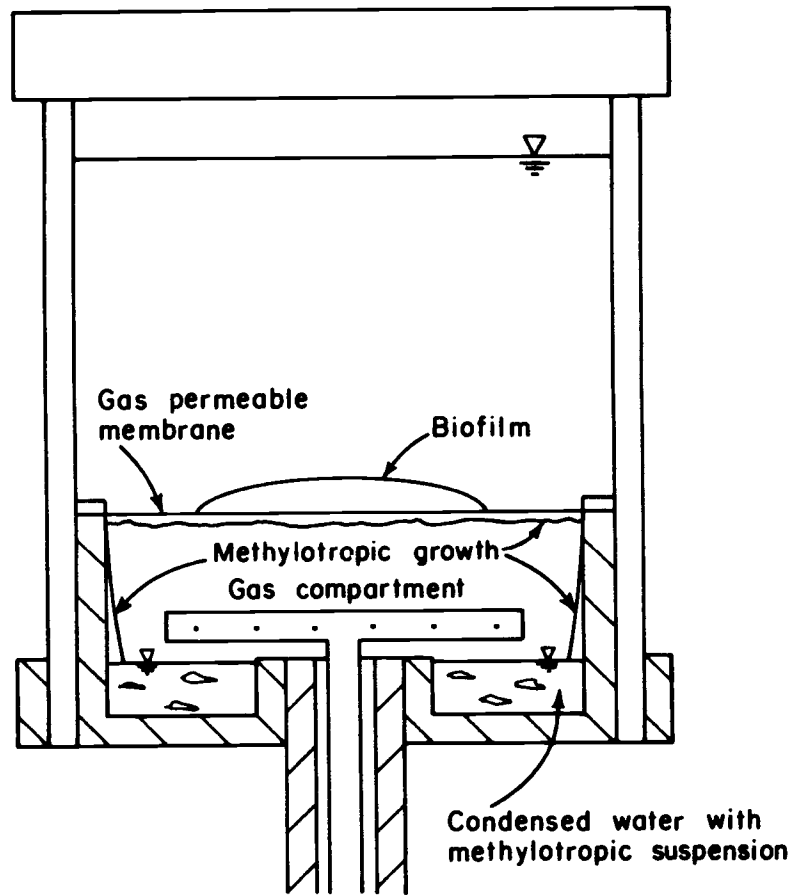


Figure 12. Locations of bacterial growth in the methylotropic/anaerobic biofilm reactor.

The proposed transformation pathway is shown in Figure 13. CT probably acts as an electron acceptor and is anaerobically dechlorinated to CF. CF appears to follow two pathways, in that it can be oxidized by methylotrophic bacteria to CO_2 , Cl^- , and H^+ , or it can act as an electron acceptor and be anaerobically dechlorinated to DCM. DCM appears to be primarily oxidized by methylotrophic bacteria to CO_2 , Cl^- , and H^+ .

The methylotrophic/anaerobic biofilm reactor system dechlorinated CT and oxidized DCM at approximately equal rates. The average first-order removal rate constant for the four CT degradation runs was 0.0129 hr^{-1} , while the rate constant for DCM removal was 0.0110 hr^{-1} . CF was degraded about one-half as fast as DCM, with a first order rate constant of 0.0050 hr^{-1} .

This study suggests that highly chlorinated compounds are most effectively treated with anaerobic bacteria, while lesser chlorinated compounds are more easily degraded by methylotrophic bacteria. The highly chlorinated compounds would be dechlorinated by the anaerobic population, while the metabolic products of these compounds and lesser chlorinated compounds would be oxidized by the methylotrophic population. Based on the successful treatment of chlorinated methanes, this system could prove effective in degrading a wide range of chlorinated aliphatic and aromatic compounds.

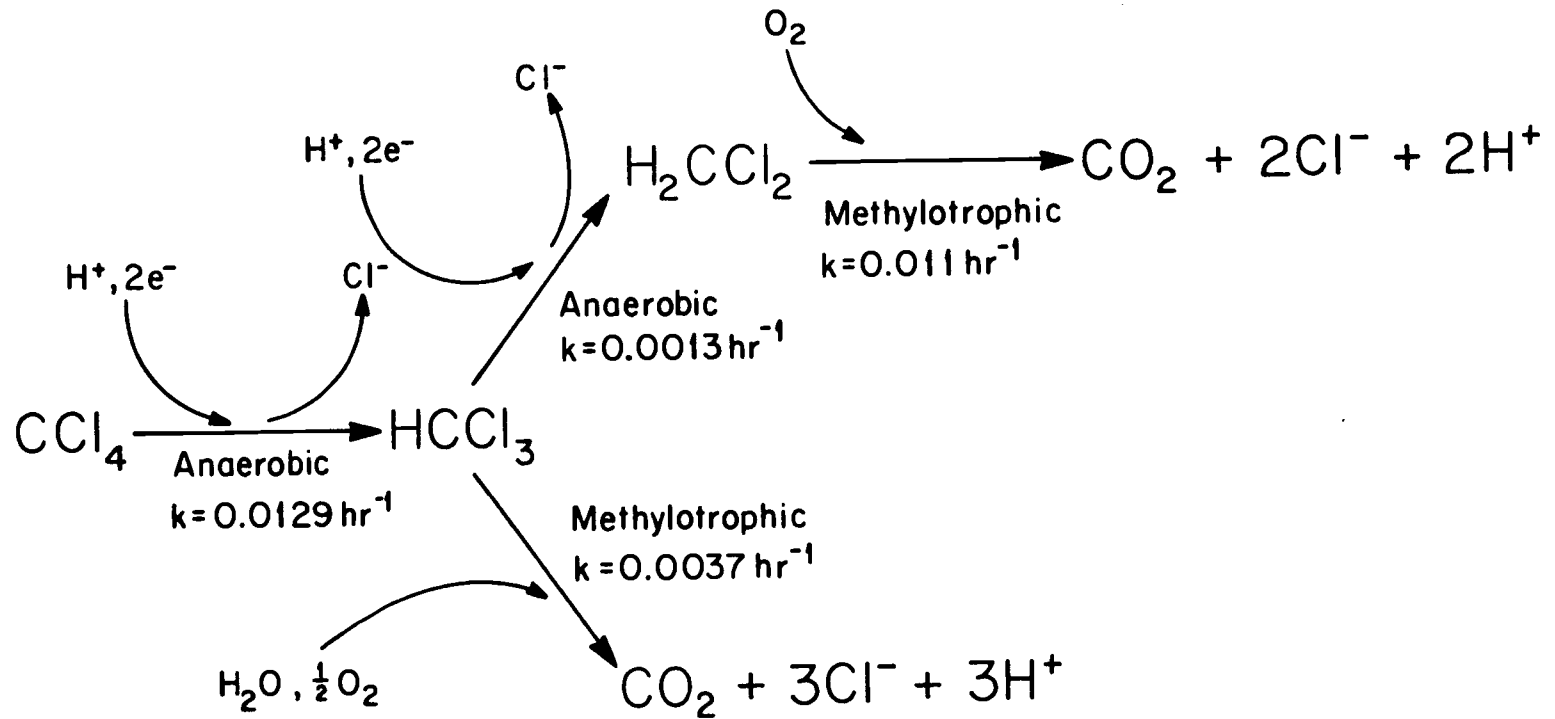


Figure 13. Proposed degradation pathway for carbon tetrachloride.

CONCLUSIONS

Based on the results of this study, the following conclusions are made:

1. It is possible to grow a combination of methylotrophic and anaerobic bacteria on a gas permeable membrane.
2. The methylotrophic/anaerobic biofilm system can degrade low concentrations of CT, CF, and DCM in aqueous samples.
3. The major removal mechanism for CT was dechlorination to CF. DCM was oxidized to CO_2 , Cl^- , and H^+ . CF was both dechlorinated to DCM and oxidized to CO_2 , Cl^- , and H^+ .

RECOMMENDATIONS FOR FURTHER RESEARCH

This research has demonstrated that the methylophilic/anaerobic biofilm system is effective in treating chlorinated methanes. Further research should be directed towards determining whether the system can degrade other groundwater pollutants such as chlorinated aromatics, pesticides, and additional chlorinated aliphatics.

The system could be improved by adjusting the $O_2 : CH_4$ ratio. Increasing the O_2 concentration could make the methylophilic bacteria more effective in degrading chlorinated compounds.

The anaerobic cell concentration could be increased with the addition of an external substrate such as methanol or acetate. The higher cell concentration should increase dechlorination rates. However, the added substrate may inhibit methylophilic bacteria, so a number of different substrates and substrate concentrations should be investigated.

The effectiveness of the methylophilic/anaerobic biofilm reactor could be improved with a few modifications. The condensation of water in the gas compartment could be avoided with a change of the gas compartment structure or by replacing the water seal with another type of seal. A gas recycle system could enhance methylophilic activity by removing CO_2 and keeping the O_2 concentration constant. Sterilizing the gas compartment prior to the start of a run may help keep methylophilic bacteria from growing in the gas compartment, thereby allowing more O_2 and CH_4 to reach the methylophilic bacteria on the gas permeable membrane.

The system could be modeled to determine kinetic constants. These constants might be useful in scaling up for the design of treatment systems.

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