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Title: Development of a Method to Elucidate Biodegradation Pathways of Chlorinated One and Two Carbon Compounds Using a Gas-Permeable-Membrane-Supported Methylotrophic Biofilm

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A gas-permeable-membrane-supported (GPMS) biofilm consisting of methylotrophic bacteria was effective in degrading chlorinated methanes, ethanes, and ethenes. The biofilm was developed on a gas-permeable fabric (Goretex, W.L.Gore & Associates, Elkton, Maryland) that divided a reactor vessel into a liquid compartment and a gas compartment. Goretex is a nylon-backed teflon mesh that allows gas transfer, but is impermeable to water. Methane and oxygen were diffused from a gas compartment, through the membrane, to the methylotrophic biofilm on the liquid side of the membrane. During the biofilm's development, methane served as the sole carbon source and electron donor, and oxygen served as the electron acceptor. Inorganic nutrients were supplied in the bulk aqueous solution. The chlorinated compounds were added to the bulk liquid. Removal of the compounds was monitored and the methylotrophic GPMS

biofilm was shown to be effective in degrading dichloromethane, 1,2-dichloroethane; and cis 1,2-dichloroethene. Rates of degradation increased in the order of chlorinated ethene, ethane, and methane. The GPMS system can be operated in either batch or continuous flow mode with similar degradation rates resulting from each process. A model was developed to predict metabolic product concentration as a function of time and retention time in batch or continuous flow reactors. No metabolic products were detected, but it is apparent that the degradation rates of any metabolites of dichloromethane or 1,2-dichloroethane are probably at least ten times greater than that of the original parent compounds.

# Development of a Method to Elucidate Biodegradation Pathways of Chlorinated One and Two Carbon Compounds Using a Gas-Permeable-Membrane-Supported Methylotrophic Biofilm

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TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# Development of a Method to Elucidate Biodegradation Pathways of Chlorinated One and Two Carbon Compounds Using a Gas-Permeable-Membrane-Supported Methylotrophic Biofilm

## INTRODUCTION AND OBJECTIVES

### Introduction and Literature Review

Contamination of the environment by solvents is an increasingly important environmental problem. A lack of care in handling in the past and indiscriminate disposal methods for industrial solvents have led to their widespread distribution. The halogenated aliphatic compounds represent one of the most important categories of industrial chemicals due to their large production volumes, wide variety of usage, dispersion in the environment, toxicological effects, and population exposure (Leisinger, 1983). Chlorinated one- and two- carbon compounds are particularly ubiquitous in groundwater due to their relatively high aqueous solubility (EPA, 1979) and their slow abiotic destruction (Leisinger, 1983). In addition, trace levels of several of these chlorinated methanes, ethanes, and ethenes are often detected simultaneously in locations without a known nearby source (Parsons et al., 1984). This may be a result of variations in effluent solvent purity and/or in-situ biotransformation (Herbert et al., 1986).

Current groundwater treatment methods for low molecular weight solvents often involve pumping followed by physical-chemical unit operations. Air stripping is commonly used for volatile organic

compounds, while removal of less volatile compounds is often achieved by activated carbon adsorption. These methods are relatively expensive and do not destroy the pollutants, but rather transfer them to another phase. Alternative treatment methods which transform the pollutant to a less toxic substance would be preferred. Since many of these chemicals are transformed by naturally acclimated microbial populations, biodegradation is an attractive alternative to physical/chemical processes.

Biodegradation of chlorinated one carbon (C1) and two carbon (C2) compounds has been observed in the laboratory (Gossett, 1984; Bouwer and McCarty, 1983; Nelson et al., 1986,1987; Rittman and McCarty, 1980; Parsons et al., 1984; Wilson and Wilson, 1985; Hensen et al., unpublished; Fogel et al., 1986; and Brunner et al., 1980). Anaerobic degradation has also been reported in soil samples from contaminated field sites (Kleopfer et al., 1985; Parsons et al., 1984). However, anaerobic degradation may produce metabolic products that are more toxic than the original contaminants. Vinyl chloride production has been observed in the degradation of several chlorinated ethenes (Barrio-Lage et al., 1986). Vogel and McCarty (1985) also noted the production of vinyl chloride in the biodegradation of tetrachloroethylene and trichloroethylene under methanogenic conditions, although they also suggested the possibility of anaerobic reduction of vinyl chloride to $CO_2$. Reports of aerobic biodegradation suggested that chlorinated aliphatic compounds are converted to $CO_2$ (Wilson, 1985; Nelson, 1986).

This research examined the biodegradation of C1 and C2 compounds

by methylotrophs. Methylotrophs are microorganisms that aerobically degrade a variety of compounds. These bacteria use single carbon compounds as electron donors and carbon sources with oxygen serving as the electron acceptor. Obligate methylotrophs are able to metabolize many compounds with the sole requirement apparently being that no carbon-carbon bonds exist (Brock et al., 1984). Facultative methylotrophs have also been isolated. These organisms cometabolize a wide variety of compounds including aliphatic, aromatic, heterocyclic, and halogenated compounds in the presence of a single carbon compound. However, one carbon compounds must serve as their primary carbon and energy source (Higgins et al., 1980). This broad degradative capacity suggests that methylotrophs could be useful in degrading many recalcitrant pollutants.

The pathway which has been postulated for methane degradation by methylotrophs is:

$$CH_4 \text{ ---> } CH_3OH \text{ ---> } CH_2O \text{ ---> } CHOOH \text{ ---> } CO_2$$

Each sequential transfer of two electrons is catalyzed by an individual enzyme. Alhough at least 10 enzymes are involved in catalyzing the successive oxidative steps and the corresponding cell production (Haber et al., 1983), the methane mono-oxygenase (MMO) enzyme that catalyzes the initial oxidation of methane to methanol is thought to be largely responsible for the broad degradative capacity of methylotrophs (Higgins et al., 1980). Inhibition in the transformation of n-alkanes to their corresponding alcohols by the presence of methanol in the nutrient media has been reported (Patel et al., 1986; Best and Higgins, 1981). However, the MMO enzyme has also been isolated from a methanol-

grown methylotroph, Methylosinus Trichosporium OB3b (Haber et al., 1983), which indicated that the MMO enzyme is produced constitutively rather than induced by the presence of substrate. These examples indicate the complexity and importance of the understanding of the biochemical processes involved in biodegradation by methylotrophs.

In addition to investigating the ultimate biodegradative removal of a particular chemical, it is important to determine the pathways of its transformation. As well as enhancing the biochemical literature concerning specific microorganisms, this information provides a better understanding of the fate of the organic compound in the environment. Treatment processes then can be augmented to promote efficient and desirable transformations. For example, aerobic environments, such as an aerated holding tank, may naturally promote production of certain metabolites while anaerobic conditions, such as groundwater aquifers, may produce different metabolites from the same parent compound. Another example is that environmental conditions that naturally enhance biotransformation of the parent compound may not be suitable to yield biodegradation of its metabolites, and could cause an accumulation of the metabolite(s) to a toxic level. In this case, it would be necessary to design additional treatment to alleviate the metabolite. Increased knowledge of biodegradative pathways also aids in determining the extent of contamination by a pollutant since it is necessary to consider the presence and concentration of any biodegradative intermediates and products as well as the amount of parent compound present.

It is often difficult to accumulate a sufficient mass of the

metabolite for its identification and pathway determination. This is particulaly true if a metabolite is degraded faster than the parent compound either by biodegradation processes or by chemical reactions such as hydrolysis. Although the chemical engineering literature provides some information on accumulation of metabolic products in industrial processes (Park et al., 1987), these applications usually involve transformations in which the metabolic products are stable and concentrations of parent compound are high. In biodegradation studies of trace organic pollutants, concentrations are very low (in the ppm or ppb range), and intermediates may be chemically unstable. For example, Colby et al. (1977) reported that their inability to find degradation intermediates of dichloromethane is due to the instability of the 1-substituted methanol derivatives that are probable intermediates in the degradation of chlorinated methanes.

One difficulty in accumulating metabolites in the laboratory is that most biodegradation studies of trace organic compounds involve suspended microbial cultures in batch-type processes. In these studies, the metabolic products may not accumulate to detectable levels. Intermediate products may appear only transiently as the parent compound degrades. Recently, the advantages of using biofilms with continuous flow reactors have been investigated (Park et al., 1983; Okita and Kirwan, 1986). Biofilm reactors alleviate the problem of cell washout at high dilution rates and can enhance bacterial strain stability. It may also be possible to accumulate metabolic products more efficiently with biofilm systems due to greater cell density in the reactor (Okita and Kirwan, 1986). Since the nature of the

groundwater environment dictates that most microbiological activity will result from bacteria attached to solids, the study of biofilms is also quite practical. By operating a biofilm process in the continuous flow mode, it is possible to vary the hydraulic retention time while maintaining an extremely long retention time for the bacteria. Therefore, the retention time for trace organic compounds can be varied and used to develop optimum conditions to isolate metabolic products. Once the reactor is operating at steady-state, the metabolic products appear at a constant concentration which is dependent on the hydraulic retention time. Maximum product accumulation has been achieved in completely-mixed biofilm systems operating in continuous flow mode (Okita and Kirwan, 1986).

The growth of methylotrophs is limited by the availability of their electron donor (C1 compounds) and acceptor (oxygen). In the environment, the highest methylotrophic activity occurs in the narrow aerobic-anaerobic interface (Lidstrom and Somers, 1984). At the interface, the oxygen concentration is sufficient to support the growth of methylotrophs, while dissolved methane, which is produced in the deeper anaerobic environment, is available as an electron donor. To develop an enrichment culture of methylotrophs and to take advantage of their oxidative capabilities, adequate concentrations of methane and oxygen must be provided. The concentrations of methane and oxygen are limited by their insolubility in aqueous solutions. A successful treatment process must include a method to provide an aqueous nutrient solution and gaseous growth substrates simultaneously.

To facilitate growth of a methylotrophic biofilm, a system has

been developed which utilizes a gas permeable fabric as a surface for the growth of a biofilm. In this gas-permeable-membrane-supported (GPMS) process, gases are transferred to the biofilm by diffusion through the membrane. Sufficient concentrations of the electron donor and acceptor ($CH_4$ and $O_2$) are directly available to the biofilm. Inorganic nutrients are provided in the bulk liquid. Hence, the system provides a combination of aqueous and gaseous substrates to the biofilm. Use of the GPMS system allows the selection and control of conditions that are conducive to the growth of specific organisms of interest and enhances their long term retention within the treatment system.

The GPMS methylotrophic biofilm was developed with methane as the sole carbon source. Its microbiological characteristics and abilities proved similar to suspended cultures with the advantages of biofilm systems. Methane and oxygen consumption rates and carbon dioxide production rates resembled that of Whittenbury et al., 1970. However, the reactor bulk liquid remained clear. Ely (1986) successfully treated dichloromethane and trichloromethane in a batch process with this biofilm. Carbon tetrachloride was not degraded. No metabolites were detected in the reactor.

The experiments reported here involve an extension of the use of the methylotrophic GPMS biofilm. The reactor can be operated in either batch or continuous flow mode. Batch studies are performed to determine general biodegradation rates for methylene chloride, (DCM), 1,2-dichloroethane, (DCA), and cis-1,2 dichloroethene, (DCE). The model is applied to predict the concentration of metabolic products as

a function of time and retention time. A continuous flow experiment then is conducted with operation at the predicted optimum retention time. Results from the continuous flow experiments indicate the rates of degradation of the metabolic products and, therefore, provide information concerning the aerobic degradation pathway used by methylotrophs.

Though most reports of aerobic biodegradation of chlorinated C1 and C2 compounds claim complete mineralization or the inability to detect volatile metabolites, there have been observations of some metabolic products. In the degradation of 1,2-dichloroethane for example, Yokoto et al. (1986) reported the production of 2-chloroacetate. Fogel et al. (1985) found production of 2-chloroethanol corresponding to the removal of this substrate. Other investigators have discovered increased enzyme activity or increased concentrations of enzymes specific for the degradation of other possible metabolic products indicating their presence even at trace or transient level (Janssen et al., 1984, 1985, Stucki et al., 1983). Since these metabolites are quite susceptible to biodegradation (Patel et al., 1980), the application of the predictive model to the GPMS system will allow the determination of a minimum biodegradation rate of the metabolites.

Objectives

The objectives of this research are to:

1. Demonstrate the use of the GPMS system for biodegradation of chlorinated methanes, ethanes and ethenes by methylotrophs.

2. Extend the use of the GPMS system to a continuous flow process as well as batch operation.

3. Develop a model which uses degradation kinetics to predict optimum reactor configurations for accumulating metabolites.

4. Characterize degradation pathways of chlorinated one- and two-carbon compounds by methylotrophs by determining degradation kinetics for metabolic products.

# MODEL DEVELOPMENT

The model was developed by applying mass balances for a parent compound (P) and its metabolic product (M) in either a completely-mixed batch reactor (CMB) or a completely-mixed flow reactor (CMFR). Although these simple models are not applicable to the determination of quantitative kinetic constants for biodegradation reactions, they are useful in isolating metabolic products and in determining conditional constants for a specific reactor system. The following assumptions were made in developing the model:

Assumptions

1. Biodegradation is the primary removal mechanism of the parent compound from the system.
2. Biodegradation can be described by a first-order rate equation.
3. The mass of active microorganisms that degrade the parent compound remains constant throughout the test period.
4. The molar ratio of parent degradation to metabolite production is 1:1.

To justify the first assumption, nonbiological removal mechanisms such as volatilization, sorption, hydrolysis, and photolysis cannot produce significant removal in the GPMS system. Since some chlorinated compounds are very volatile, it is necessary to account for their total mass present in the system. This is achieved by monitoring the mass of chlorinated compounds in the gas and headspace compartments as well as the liquid.

The second and third assumptions are interrelated. Although kinetic parameters for biofilm degradation should be determined using biofilm kinetics, simple batch and continuous flow models can be applied in order to estimate substrate removal efficiencies. These rates are conditional and are specific to the reactor and its operating conditions. A typical model describing biodegradation kinetics is expressed by the Monod expression as:

$$r_{su} = \frac{v\ S\ B}{K_m + S} \tag{1}$$

where:

$r_{su}$ = substrate utilization rate (mol S/(ℓ day))

$v$ = maximum substrate utilization rate (mol S/(g cell day))

$S$ = substrate concentration (mol/ℓ)

$B$ = population density (g cell/ℓ)

$K_m$ = half-velocity coefficient (mol S/ℓ)

$t$ = time (day)

For the first-order assumption to be valid, there must be a constant, $k_p$, which incorporates several parameters, i.e.:

$$k_p = \frac{v\ B}{K_m + S} \tag{2}$$

$k_p$ = first-order degradation rate constant for the parent compound, ($hr^{-1}$)

If $k_p$ is constant for varying substrate concentrations, the half-saturation coefficient must be much greater than substrate concentration so that:

$$k_p \cong \frac{v\ B}{K_m} \tag{3}$$

For Eq. (3) to be valid, the parameters v, $K_m$, and B must be constants. In the third assumption, a constant population density is assumed since the experiments are conducted over a short time period in relation to the average cell age in the biofilm. Thus, for biodegradation experiments involving very low substrate concentrations and well-developed biofilms, the first order assumption is valid.

A one-to-one molar ratio of metabolite production to parent compound degradation, as stated in the fourth assumption, implies that degradation involves removal of or substitution of a substituent group on the parent compound.

Batch Model

Mass Balance for the Parent Compound. Completely mixed batch reactors (CMB) have no flow into or out of the reactor, and the rate of change of the mass of the parent compound depends only on the rate of degradation of the compound. This is expressed as:

$$\begin{bmatrix}\text{change in the mass of the}\\ \text{parent compound with time}\end{bmatrix} = \begin{bmatrix}\text{mass of the parent}\\ \text{compound degraded with time}\end{bmatrix}$$

$$\forall \frac{dP}{dt} = -k_p P \forall \tag{4}$$

where:

$\forall$ = volume of the liquid in the reactor, ($\ell$)

P = concentration of the parent compound in the reactor, (mol/$\ell$)

t = time, (hr)

Integration of Eq. (4) yields the following equation:

$$P = P_0 \exp(-k_p t) \quad (5)$$

where:

$P_0$ = initial concentration of the parent compound, (mol/ℓ)

Mass Balance for the Metabolite. The rate of change of the metabolite mass in the batch reactor is the sum of two reactions: production by degradation of the parent compound and removal by degradation of the metabolite. Due to the competing processes, the metabolite concentration is a function of time and often has a maximum value. Assuming a 1:1 molar ratio of the parent compound to metabolite, the rate of change of the metabolite concentration with time is:

$$\begin{bmatrix}\text{change in the mass of the metabolite with time}\end{bmatrix} = \begin{bmatrix}\text{mass formed by degradation of the parent compound with time}\end{bmatrix} - \begin{bmatrix}\text{mass lost by degradation of the metabolite with time}\end{bmatrix}$$

$$\forall \frac{dM}{dt} = k_p P \forall - k_m M \forall \quad (6)$$

where:

$M$ = concentration of the metabolite in the reactor, (mol/ℓ)

$k_m$ = first-order degradation rate constant for the metabolite, ($hr^{-1}$)

Combining the expression for the concentration of the parent compound, (Eq. (5)) and Eq. (6) yields:

$$\frac{dM}{dt} = k_p P_o \exp(-k_p t) - k_m M \quad (7)$$

or:

$$\frac{dM}{dt} + k_m M = k_p P_o \exp(-k_p t) \tag{8}$$

Equation (8) is a first order differential equation in which both the metabolite and parent compound concentrations are a function of time. This equation is solved using an integration factor, exp ($k_m t$). Eq. (8) becomes:

$$\exp(k_m t) \frac{dM}{dt} + \exp(k_m t) k_m M = k_p P_o \exp\left[(k_m - k_p) t\right] \tag{9}$$

The left side of the equation can be factored as follows:

$$\exp(k_m t) \frac{dM}{dt} + k_m M \exp(k_m t) = \frac{d}{dt} M \exp(k_m t) \tag{10}$$

Inserting Eqn. (10) into Eqn. (9) and integrating yields:

$$M \exp(k_m t) = \frac{k_p P_o \exp\left[(k_m - k_p) t\right]}{k_m - k_p} + C \tag{11}$$

Since:

$$M = M_o \text{ at } t = 0, \tag{12}$$

$$C = M_o - k_p P_o/(k_m - k_p) \tag{13}$$

Inserting the value for the constant of integration from Eqn. (13) into Eqn. (11) yields:

$$M \exp(k_m t) = \frac{k_p P_o \exp\left[(k_m - k_p)t\right]}{(k_m - k_p)} + M_o - \frac{k_p P_o}{(k_m - k_p)} \tag{14}$$

Therefore:

$$M = \frac{k_p P_o \left[\exp(-k_p t) - \exp(-k_m t)\right]}{(k_m - k_p)} + M_o \exp(-k_m t) \qquad (15)$$

Thus the concentration of the metabolite in the reactor at any time is dependent on the initial parent compound concentration, the initial metabolite concentration, and the rates of degradation of the parent compound and metabolite. Equations (5) and (15) are used to model the concentration of the parent compound and the metabolite in a batch reactor with time and initial metabolite concentration equal to zero, $M_o = 0$ (Figure 1).

The maximum concentration of the metabolite exists when dM/dt is equal to 0. Setting dM/dt equal to zero in Eqn. (8) yields:

$$0 + k_m M_{max} = k_p P_o \exp(-k_p t_{max}) \qquad (16)$$

or:

$$M_{max} = \left(\frac{k_p}{k_m}\right) \left[P_o \exp(-k_p t_{max})\right] \qquad (17)$$

where: $M_{max}$ = maximum metabolite concentration (mol/$\ell$)

$t_{max}$ = time at which $M_{max}$ occurs (hr)

Thus, the solution for $M_{max}$ requires knowledge of the time at which the maximum metabolite concentration occurs. This can be calculated by differentiating Eqn. (15) with respect to time, setting the differential equal to zero and solving for the expression for $t_{max}$:

$$\frac{dM}{dt} = 0 = \left[\frac{k_p P_o}{k_m - k_p}\right]\left[\exp(-k_p t)(-k_p) - \exp(-k_m t)(-k_m)\right]$$

$$+ M_o \exp(-k_m t)(-k_m) \qquad (18)$$

$$t_{max} = \frac{1}{(k_m - k_p)} \ln\left[\left(\frac{k_m}{k_p}\right)\left[1 - \frac{M_o(k_m - k_p)}{k_p P_o}\right]\right] \qquad (19)$$

A graphical representation of the concentrations of the parent and metabolite as a function of time where the metabolite degrades faster than the parent compound is shown in Figure 1. In this case, the value for $k_m$ was assumed to be twice that of $k_p$. The reason for the transient metabolite response is that at the start of the batch degradation, (before X1), sufficient parent degradation has not occurred to create high concentrations of the metabolite. To the right of the apex of the metabolite curve, the rate of degradation of the parent compound (or production of the metabolite) decreases to the point that metabolite degradation exceeds metabolite production. If a lower analytical limit exists below which the metabolite can no longer be detected (for example Y′), then a limited period of time will exist during which the metabolite concentration will be measurable (from X1 to X2) (Figure 1). Thus, metabolite production may go undetected if the reactor contents are not monitored during this time.

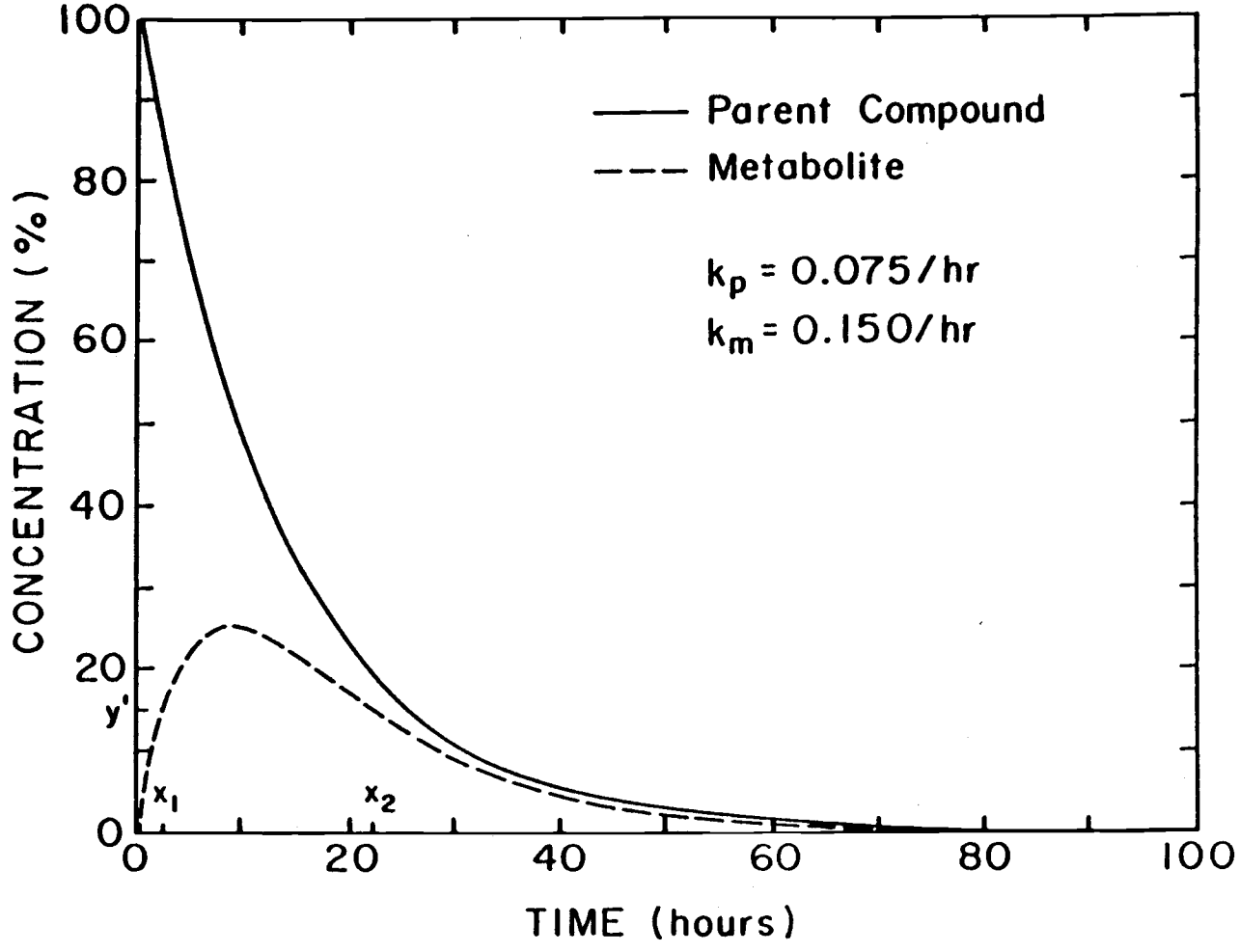


Figure 1. Predicted concentrations of a parent compound and its metabolite as a function of time in a batch reactor when the metabolite is degraded at twice the rate of the parent compound.

Continuous Flow Model

Mass Balance for the Parent Compound. The rate of change in mass of the parent compound in a continuous flow reactor is a result of both biodegradation and flow into and out of the reactor. It is expressed as:

$$\begin{bmatrix}\text{change in mass of parent compound with time}\end{bmatrix} = \begin{bmatrix}\text{mass of parent compound entering reactor for a given time}\end{bmatrix} - \begin{bmatrix}\text{mass of parent compound exiting reactor for a given time}\end{bmatrix}$$

$$- \begin{bmatrix}\text{mass of compound degraded for a given time}\end{bmatrix}$$

$$\forall \frac{dP}{dt} = QP_o - QP - k_p P\forall \tag{20}$$

where:

Q = volumetric flow rate of reactor influent and effluent, ($\ell$/hr)

At steady state, the mass of the parent compound remains constant. Setting eq. (18) equal to zero and rearranging yields:

$$P = \frac{P_o}{1 + k_p \tau} \tag{21}$$

where the hydraulic retention time, $\tau$, represents:

$$\tau = \frac{\forall}{Q} \tag{22}$$

Mass Balance for Metabolite. The rate of change of the metabolite mass in a continuous flow reactor is the sum of the two biochemical reactions plus the rate at which metabolite enters the reactor in the influent minus the rate at which the metabolite leaves the reactor in the effluent. This is expressed as:

$$\begin{bmatrix}\text{change in mass of metabolite with time}\end{bmatrix} = \begin{bmatrix}\text{mass of metabolite entering the reactor for a given time}\end{bmatrix} - \begin{bmatrix}\text{mass of metabolite exiting the reactor for a given time}\end{bmatrix} + \begin{bmatrix}\text{mass of metabolite formed by degradation of parent compound for a given time}\end{bmatrix}$$

$$- \begin{bmatrix}\text{mass of metabolite degraded for a given time}\end{bmatrix}$$

$$\forall \frac{dM}{dt} = QM_o - QM + k_p P\forall - k_m M\forall \tag{23}$$

Assuming no metabolite is introduced in the feed:

$$QM_o = 0 \tag{24}$$

At steady state the mass of metabolite remains constant. Therefore, Eq. (23) can be set equal to zero. Inserting Eq.(24) into Eq. (23) and rearranging yields:

$$P = \frac{QM + \forall Mk_m}{k_p \forall} \tag{25}$$

Using Eq. (22), the parent compound concentration can be expressed alternately as a function of retention time:

$$P = \frac{M\ (1 + \tau k_m)}{\tau k_m} \tag{26}$$

Eq. (26) describes the parent compound concentration based on the metabolite concentration, the rate constants, and the retention time. Eq. (21) expresses the parent compound concentration as a function of the initial parent concentration. Therefore, in order to determine the concentration of metabolite at steady-state, it is necessary to set Eq. (26) equal to Eq. (21). Rearranging this expression yields:

$$M = \frac{P_o\ \tau k_p}{(1 + \tau k_p)(1 + \tau k_m)} \tag{27}$$

The retention time yielding the maximum metabolite concentration was determined by setting the derivative of Eq. (27) with respect to retention time equal to zero:

$$\frac{dM}{d\tau} = 0 = P_o \left[k_p - \tau^2 k_p^2 k_m\right] \tag{28}$$

Equation (28) can be solved to give the residence time where the metabolite concentration will be greatest:

$$\tau_{max} = \left[\frac{1}{k_p k_m}\right]^{1/2} \tag{29}$$

Therefore, the optimum residence time to accumulate the maximum metabolite concentration is a function only of kinetic constants of biodegradation of the parent compound and metabolite. The magnitude of the metabolite concentration depends on the influent parent compound concentration, the retention time, and the degradation rate constants.

The parent and metabolite concentrations are shown in Figure 2 as a function of retention time. If a lower analytical limit (Y') is again assumed, there exists a limited range of retention times in the continuous flow reactor which will result in the detection of metabolites. At short retention times (below X1), the rate of degradation of the parent compound is insufficient to produce a high metabolite concentration. At long retention times, sufficient parent compound degradation occurs, but the metabolite is also rapidly degraded. An advantage of the continuous flow process as compared to the batch process is that once steady state has been achieved, the concentration of the metabolite should remain constant. If the retention time is within the range of X1 to X2, samples should contain detectable concentrations of the metabolite.

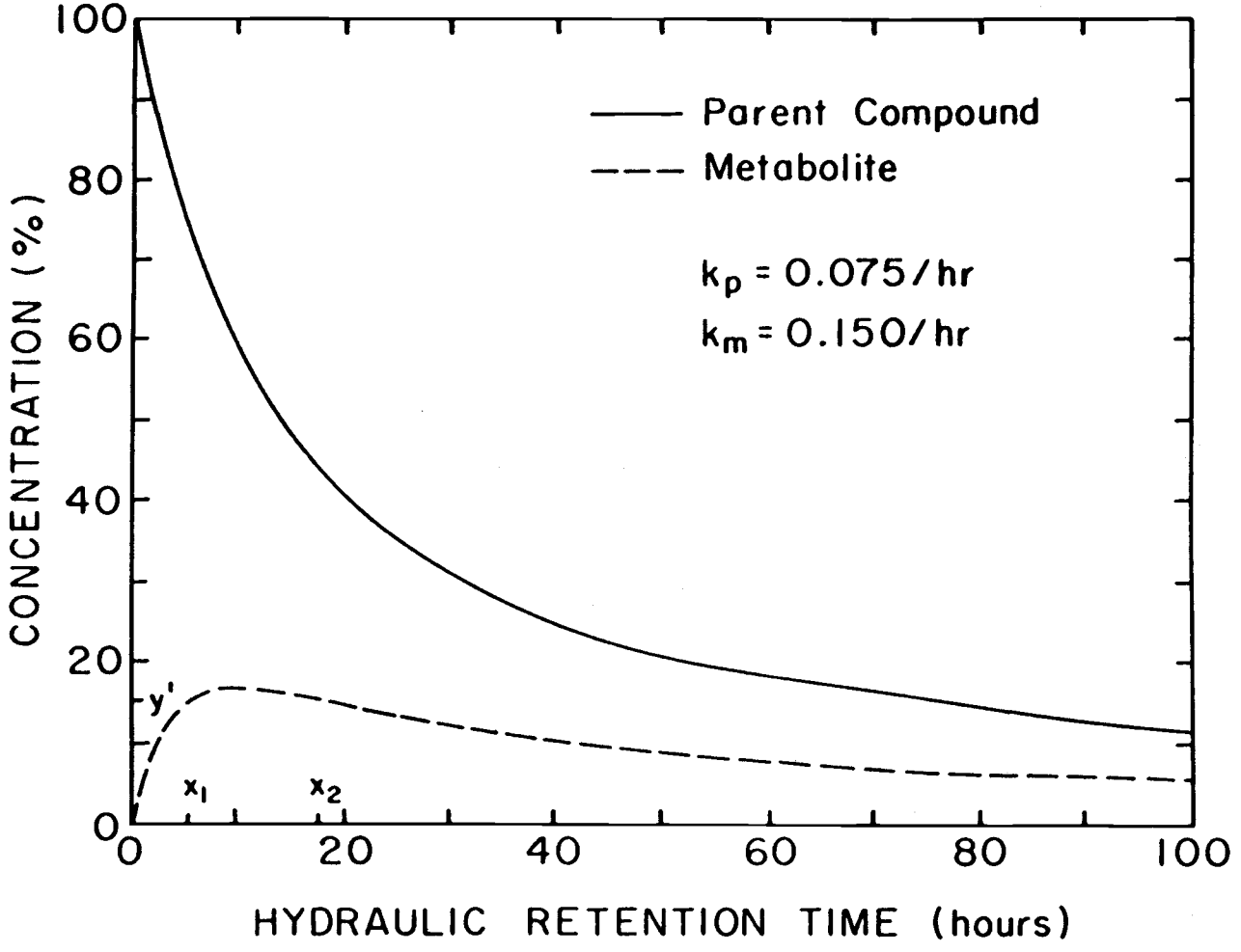


Figure 2. Predicted concentrations of a parent compound and its metabolite as a function of time in a continuous flow reactor when the metabolite is degraded at twice the rate of the parent compound.

# EXPERIMENTAL MATERIALS AND METHODS

## Experimental Design

The research was conducted in four phases. In the first phase, the reactor was operated in a batch mode to evaluate first-order removal rates for the chlorinated alkanes and alkene. These rates were used to select retention times for continuous flow tests in Phase II. The intent of the continuous flow tests was to optimize the possibility of detecting and identifying metabolites. A batch test was performed in Phase III to evaluate the removal rate of a potential metabolite, 2-chloroethanol. Phase IV was a control experiment conducted in batch flow without a biofilm. The purpose of this experiment was to determine the magnitude of the chemical and physical removal mechanisms, including sorption of the chemicals to the reactor surface, photolysis, and hydrolysis.

## System Design

Reactor. The general reactor configuration used for batch and continuous flow experiments is shown in Figure 3. The reactor was constructed of three cylindrical sections of Kimax beaded process pipe (10.2 cm diameter). Each joint was sealed with Teflon-lined stainless-steel flanges and sealed with Silicone vacuum grease. The reactor was divided into gas and liquid compartments by a gas permeable membrane installed across the lower joint. The membrane material was a nylon backed Teflon laminated fabric manufactured by W.L. Gore & Associates, (Elkton, Maryland) and commonly called

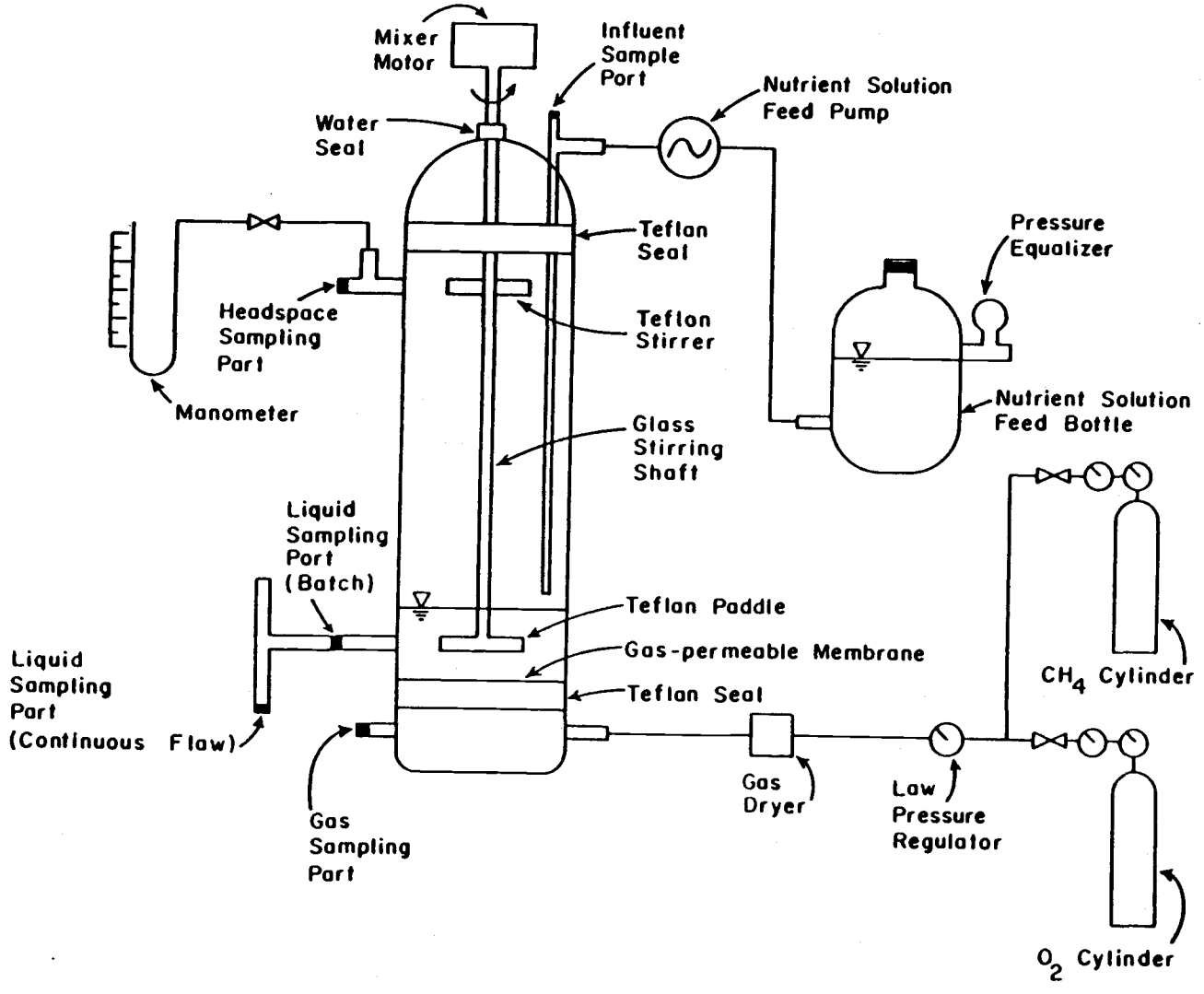


Figure 3. Reactor configuration.

Goretex.

Ports were installed for sampling the gas compartment and headspace gases as well as for providing influent and effluent liquid nutrient solution and influent gases to the gas compartment. Sampling ports were equipped with double rubber septa. Reactor liquid and headspace gases were continuously mixed by Teflon impellors attached to a glass stirrer and powered by an electric motor operating at 60 RPM. The stirrer connection was sealed with a water seal. All sampling ports and other connections to the reactor were sealed with a Silicone sealant to alleviate any losses of volatile compounds. Gases in the lower gas compartment were mixed with a Teflon coated magnetic stirring rod. The reactor was incubated at 30$^{o}$C.

Methane and oxygen were supplied in a 50/50 volume mixture to the gas compartment at a constant pressure of 14 cm of water. Pressure was regulated by a low pressure regulator (Matheson Gas Products, Inc., Newark, CA) and gas consumption was determined by the decrease in pressure in the gas storage tanks. Headspace gas pressure was monitored with a manometer attached to the heaspace sampling port and controlled by a Teflon-lined stopcock.

Nutrient feed solution was introduced to the reactor through an influent port located at the top of the reactor. During batch tests, the liquid influent port was sealed from the headspace with a water seal and stainless steel clamp. The effluent port was equipped with a rubber septum through which liquid samples were withdrawn. Chlorinated compounds dissolved in a distilled water

stock solution were injected with a 50 ml syringe to the liquid through this septum.

For continuous flow experiments, a glass sidearm was appended to the effluent port to control the liquid level in the reactor. Chlorinated compounds were dissolved in the feed solution. The feed solution was stored in the incubator and introduced to the reactor by a calibrated liquid pump (Fluid Metering Inc., Oyster Bay, N.Y.). A 0.25 cm diameter glass tube was extended from the influent port to a position approximately 1.25 cm above the liquid level to prevent stripping of the volatile compounds from the feed during the continuous flow experiments.

Bacterial Seed. The biofilm was developed from a seed of the biofilm used by Ely (1986). His original seed consisted of thickened trickling-filter effluent from the Corvallis, Oregon wastewater treatment plant and thickened sludge from a bench-scale anaerobic digestor in operation at the Oregon State University Environmental Engineering Laboratory. Enrichment for methylotrophs was accomplished by providing methane as the principle carbon source and maintaining a constant flow of nutrient feed to wash out undesired organisms. A "paste" obtained by filtering the material that was removed from the original Goretex membrane was allowed to dry for several hours onto a new Goretex membrane. Nutrient solution and gases then were provided to enrich for methylotrophic bacteria.

Nutrient Media. The nutrient media (Ely, 1986) was comprised of 488.4 mg/ℓ $MgSO_4$; 101.3 mg/ℓ $CaCl_2$; 4.11 mg/ℓ EDTA Disodium Salt;

1000 mg/ℓ $KNO_3$; 0.5 ml/ℓ Trace Elements Solution; 272 mg/ℓ $KH_2PO_4$; 284.4 mg/ℓ $Na_2HPO_4$; 3.0 mg/ℓ $FeCl_3 \cdot 6H_2O$; and 1.0 mg/ℓ $(NH_4)_2HPO_4$ which were dissolved in 1 ℓ of distilled water. The trace elements solution contained 500 mg EDTA Disodium Salt; 200 mg $FeSO_4 \cdot 7H_2O$; 10 mg $ZnSO_4 \cdot 7H2O$; 3 mg $MnCl_2 \cdot 4H_2O$; 30 mg $H_3BO_3$; 20 mg $CoCl_2 \cdot 6H_2O$; 0.75 mg $CaCl_2$; 2.45 mg $Ni(NO_3)2 \cdot 6H_2O$; and 3 mg $Na_2MoO_4 \cdot 2H_2O$ in 1 ℓ of distilled water. The distilled water contained 30 to 40 ppb copper, but care was taken to eliminate all other copper from the nutrient solution.

Analytical Methods

Concentrations of chlorinated compounds were determined using a Hewlett-Packard Model 5890A gas chromatograph equipped with a Hewlett-Packard Model 3392A integrator. A 1/4-inch glass column, 8 feet long and packed with 60/80 Carbopack B with 1% SP-1000, was used for the separation. Nitrogen, flowing at a rate of 40 ml/min, was used as the carrier gas. Hydrogen and air flows to the flame ionization detector were 20 ml/min and 200 ml/min, respectively. Gas and liquid samples were withdrawn from the reactor with 50 uL and 500 uL Pressure-Lok gas-tight syringes, respectively, and injected directly into the GC for analysis. Injection volumes were 500 uL for gas samples and 50 uL for liquid samples. The temperature program used was: 45°C for 1.5 minutes, 20°C per minute to 150°C, 2 minutes at 150°C, 25°C per minute to 200°C, and 5 minutes at 200°C. The injection port temperature was 200°C and the flame ionization detector temperature was 250°C.

# RESULTS

## Batch Experiments

Two batch tests were performed examining removal of dichloromethane (DCM) and dichloroethane (DCA) in the first, and dichloroethene (DCE) in the second. The masses of the chlorinated compounds in each compartment (liquid, gas, and headspace) as well as the total mass present are shown in Figures 4, 5, and 6. An immediate substantial decrease in mass of chlorinated compounds in the liquid was observed in all cases. Since this decrease corresponded to a substantial increase in the mass of compound in the gas and headspace, it is thought to result from volatilization.

The concentration of chlorinated compounds in the liquid phase as a function of time is shown in Figures 7 and 8. Due to the initial volatilization, the initial concentration used in calculating first-order degradation constants was taken at the point after the initial volatilization which occurs 1.7 hours after injection of DCM and DCA (Figure 7) and 2.5 hours after injection for DCE (Figure 8).

The data in Figures 7 and 8 were linearized by a natural log transformation of the liquid concentration data, (based on Equation 5), as shown in Figures 9 and 10. The slopes determined by linear regression yielded overall degradation rate constants, $k_p$, as listed in Table 1.

The initial total mass of DCM in the system was 26.1 umoles. After approximately 48 hours, 3.8 umoles remained in the reactor

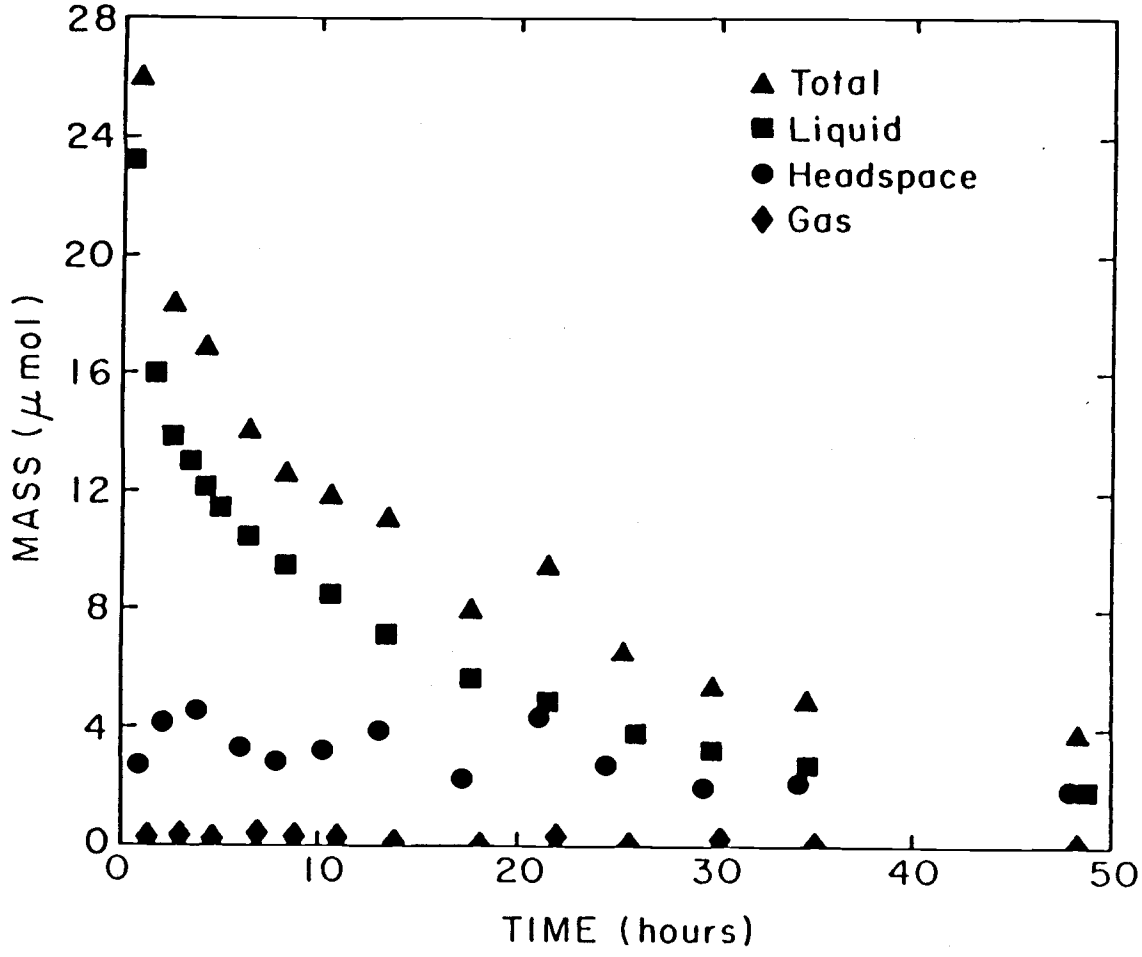


Figure 4. Mass balance for batch degradation of dichloromethane.

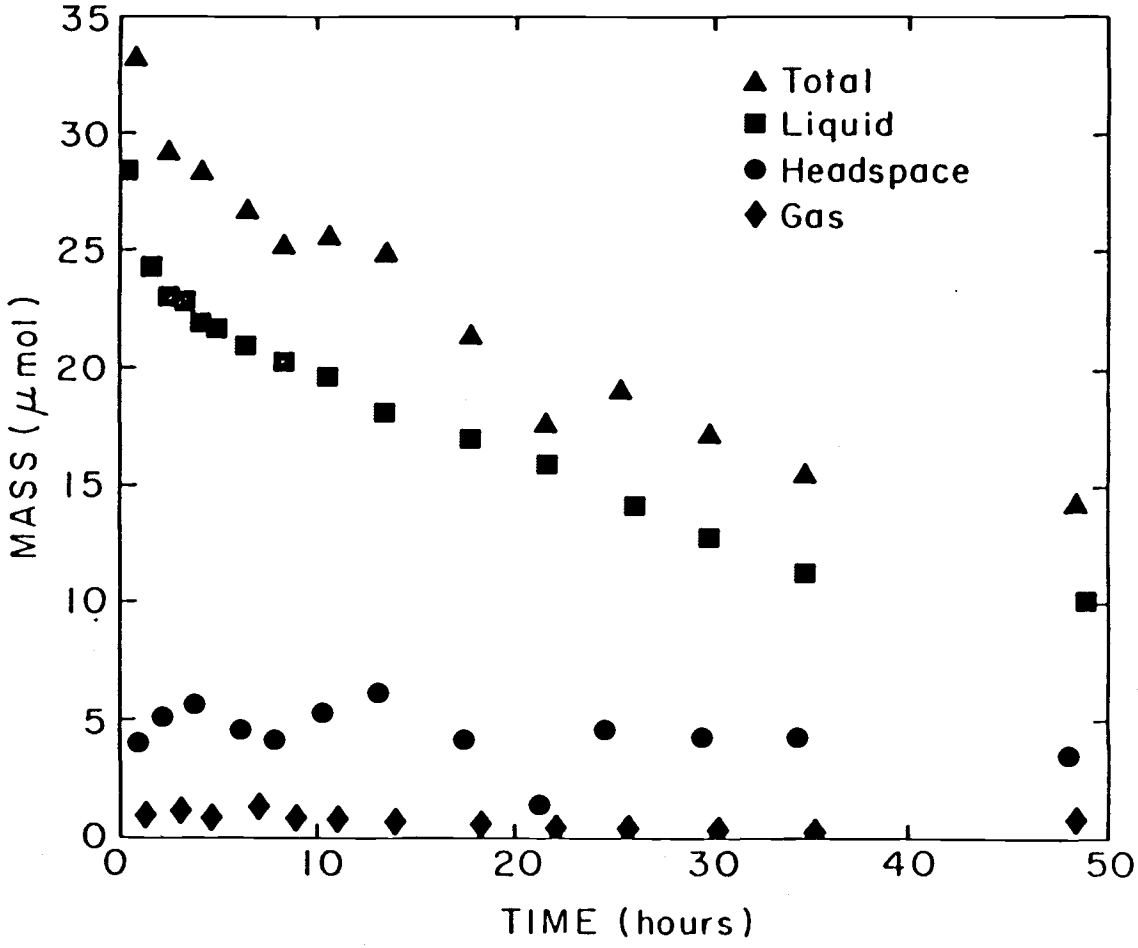


Figure 5. Mass balance for batch degradation of 1,2-dichloroethane.

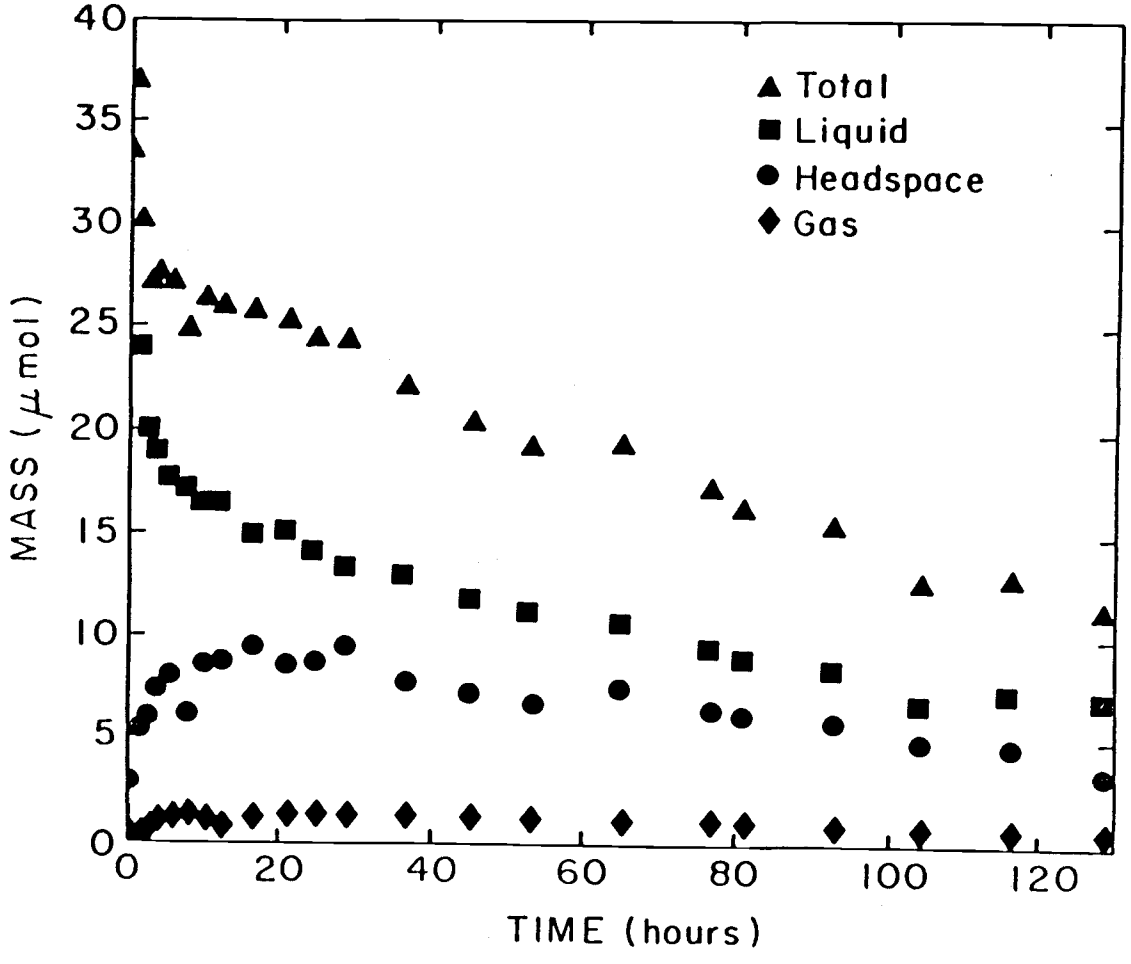


Figure 6. Mass balance for batch degradation of cis 1,2-dichloroethene.

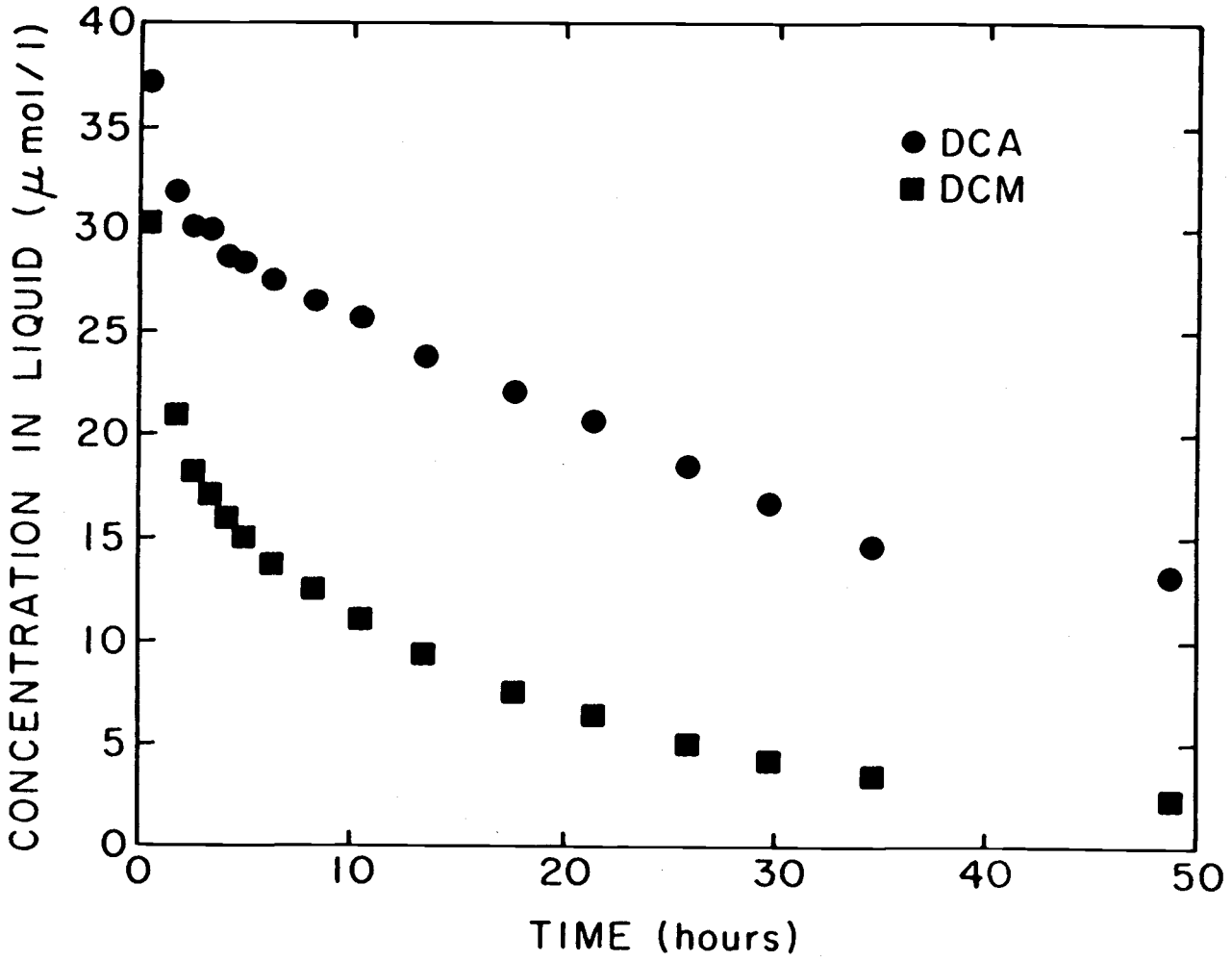


Figure 7. Liquid concentrations of dichloromethane and 1,2-dichloroethane during batch degradation.

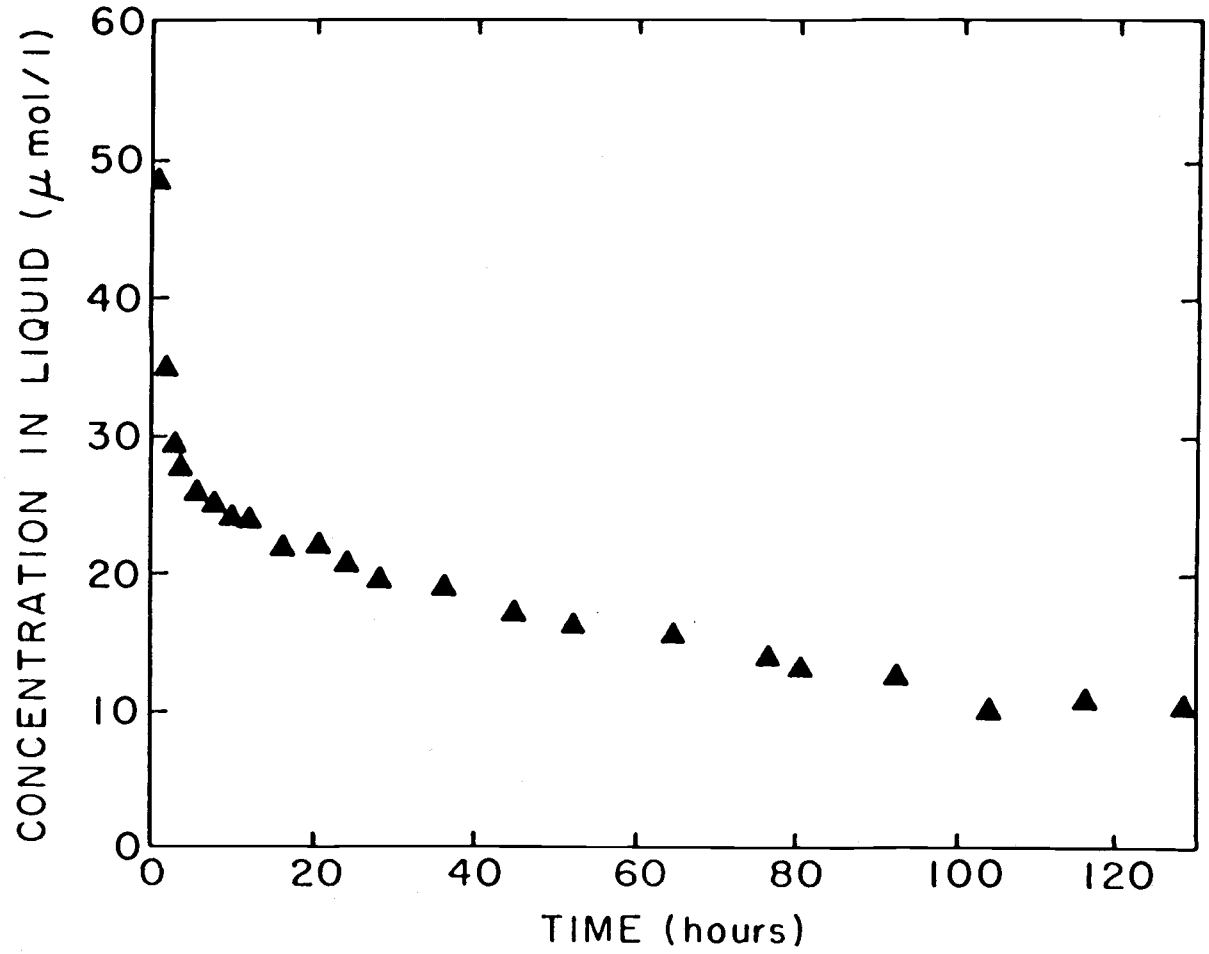


Figure 8. Liquid concentration of cis 1,2-dichloroethene during batch degradation.

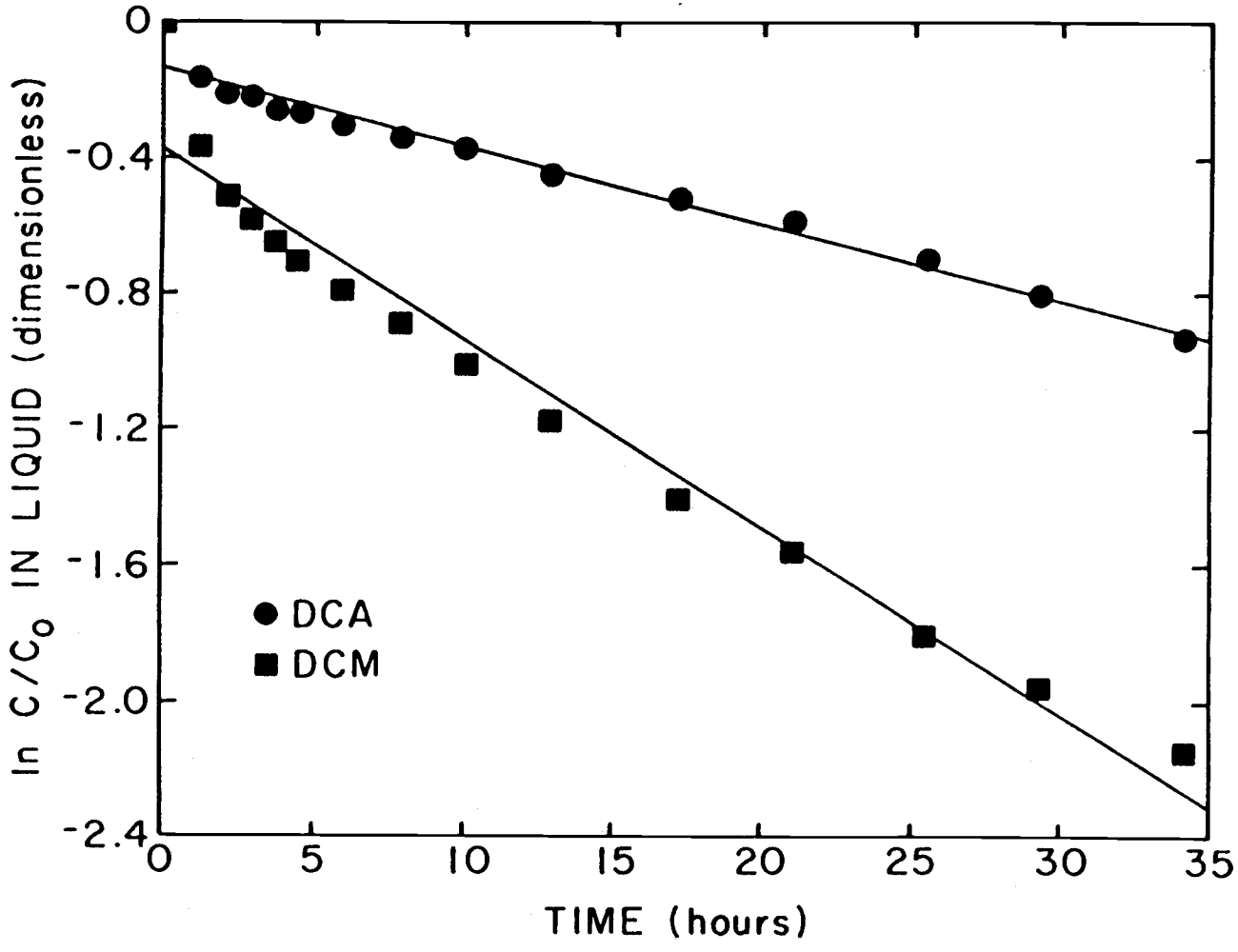


Figure 9. Linearized liquid concentrations of dichloromethane and 1,2-dichloroethane during batch degradation.
Slope = $-k_p$ $k_p = 0.056\ hr^{-1}$ (Dichloromethane)
$k_p = 0.023\ hr^{-1}$ (1,2-dichloroethane)

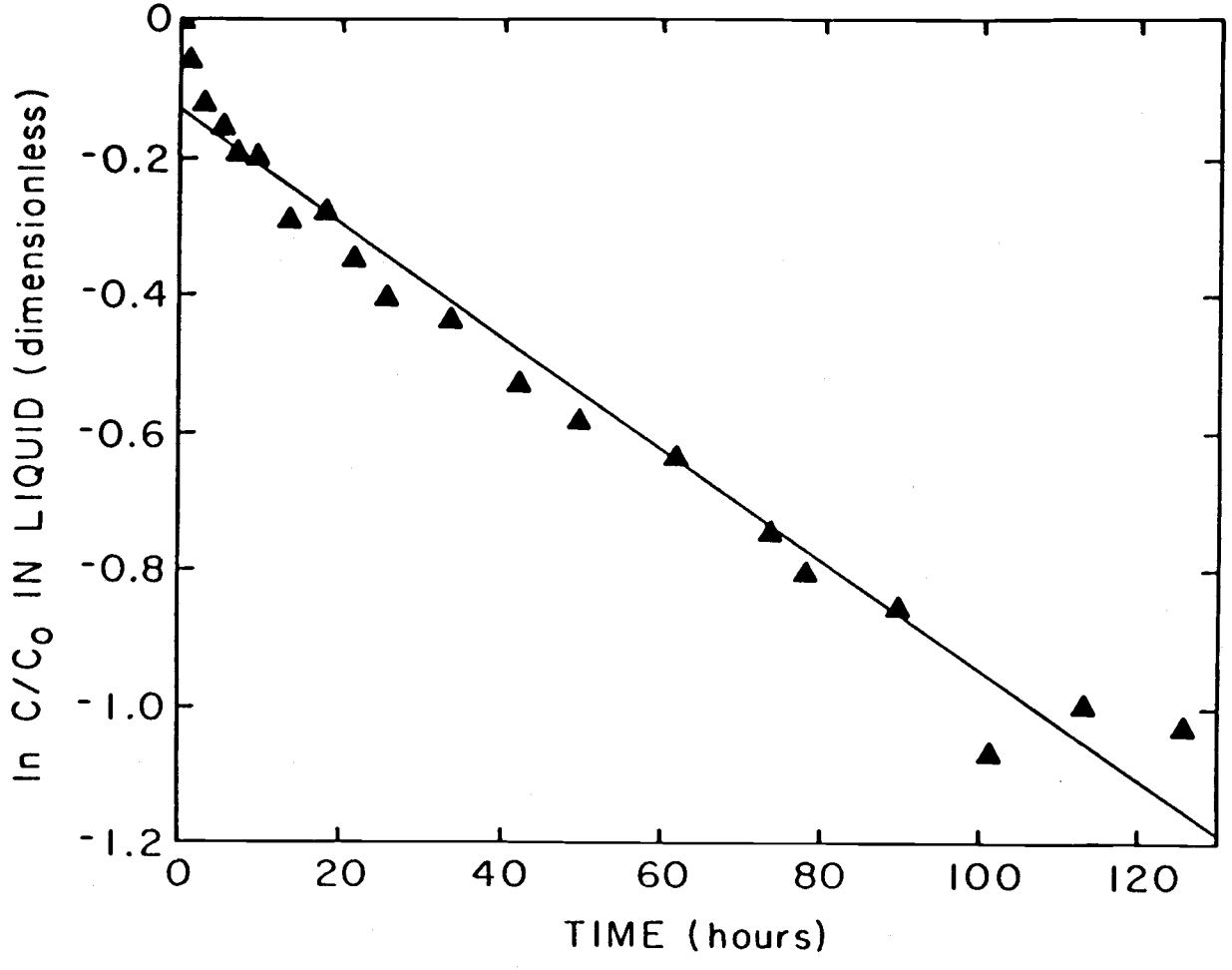


Figure 10. Linearized liquid concentrations of cis 1,2-dichloroethene during batch degradation.
Slope = $-k_p$ $k_p$ = 0.008 $hr^{-1}$

TABLE 1

Generalized Reaction Rate Constants ($hr^{-1}$)

| Compound | Batch | Continuous Flow |
|---|---|---|
| Dichloromethane | 0.056 | 0.059 |
| 1,2-Dichloroethane | 0.023 | 0.022 |
| cis 1,2-Dichloroethene | 0.008 | nm |
| 2-Chloroethanol | 0.224 | nm |

nm Not Measured

indicating a removal efficiency of 85% (Figure 4). This corresponds to initial and final liquid concentrations of 30.4 and 2.4 umol/ℓ (Figure 7). The initial liquid concentration used in the linearization procedure was 21.0 umol/ℓ. The calculated rate constant for DCM was .056/hr with a correlation coefficient of -0.981 (Figure 9).

The initial total mass of DCA in the reactor, as displayed in Figure 5, was 33.3 umoles with 14.1 umoles remaining after 48 hours. The removal efficiency was 58% after 48 hours. Initial and final liquid concentrations were 37.3 and 13.1 umol/ℓ (Figure 7) with an initial concentration of 31.8 umol/ℓ for determining the rate constant. The degradation rate constant for DCA was calculated to be 0.023/hr with a corresponding correlation coefficient of -0.986 (Figure 9).

The total mass of DCE in the second batch test was 36.9 umoles with approximately 20.0 umoles remaining after 48 hours and 11.5 umoles remaining after approximately 129 hours. These data indicate a removal efficiency of 46% after 48 hours and 69% removal for DCE after 129.5 hours. The initial liquid concentration was 58.1 umol/ℓ with remaining concentrations of 13.5 umol/ℓ after 48 hours and 10.4 umol/ℓ after approximately 129 hours (Figure 8). The calculated rate constant for DCE, with 29.2 umol/ℓ as the initial concentration, was 0.008 with a correlation coefficient of -0.983 (Figure 10).

No volatile metabolites were detected in the batch degradation of DCM, DCA, or DCE.

Model Predictions

Model predictions based on the degradation rate constants obtained in the batch experiments are presented in Figures 11 through 16. Predicted metabolite concentrations are shown for metabolites with assumed degradation rate constants 2X, 5X, 10X, 50X, and 100X as fast as those of the parent compound. Predicted metabolite concentrations are expressed as a fraction of the original parent compound concentration. Batch model concentrations are presented as a function of time and continuous flow as a function of hydraulic retention time.

The models for DCM and DCA, (Figures 11 through 14) indicated that the maximum concentration of metabolite for the given set of rate constants would accumulate in a system operating in batch mode. However, these batch concentrations appear only transiently. The continuous flow models indicated that for a given retention time, the predicted concentration would appear consistently. The predicted optimum retention time for accumulation of DCM metabolites was between 5 and 15 hours and occurs for the case where $k_m$ is twice that of $k_p$. At these conditions, a concentration of up to 17% of the parent compound's initial concentration is predicted (Figure 12). For DCA, a 10 to 30 hour retention time would lead to a maximum accumulation of 17% of the parent compound's concentration (Figure 14). For biodegradation of DCE in a batch reactor, the maximum metabolite concentration should occur after 60 hours (Figure 15).

No metabolites were detected in the batch DCE removal

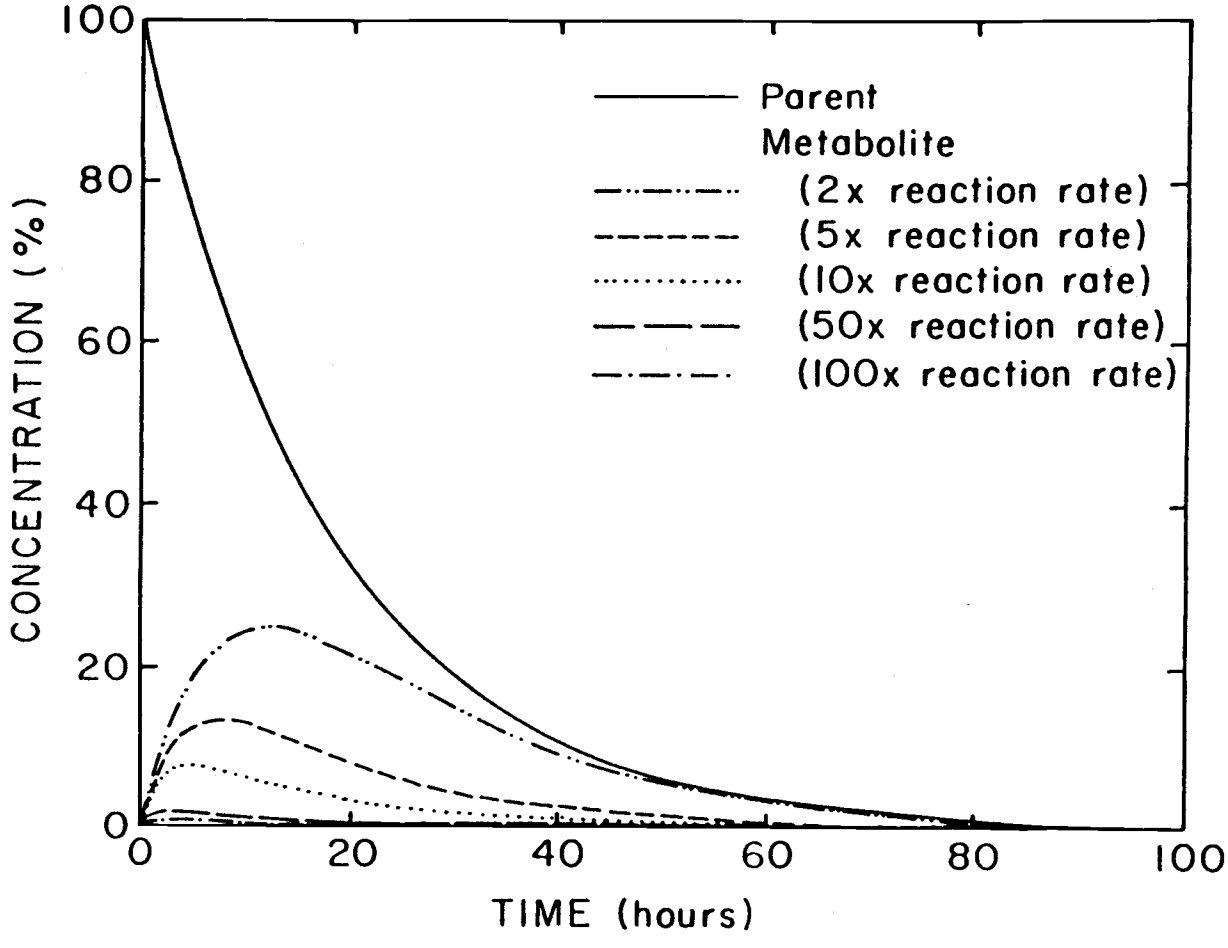


Figure 11. Batch model predicting metabolite concentrations in dichloromethane degradation as a function of time.

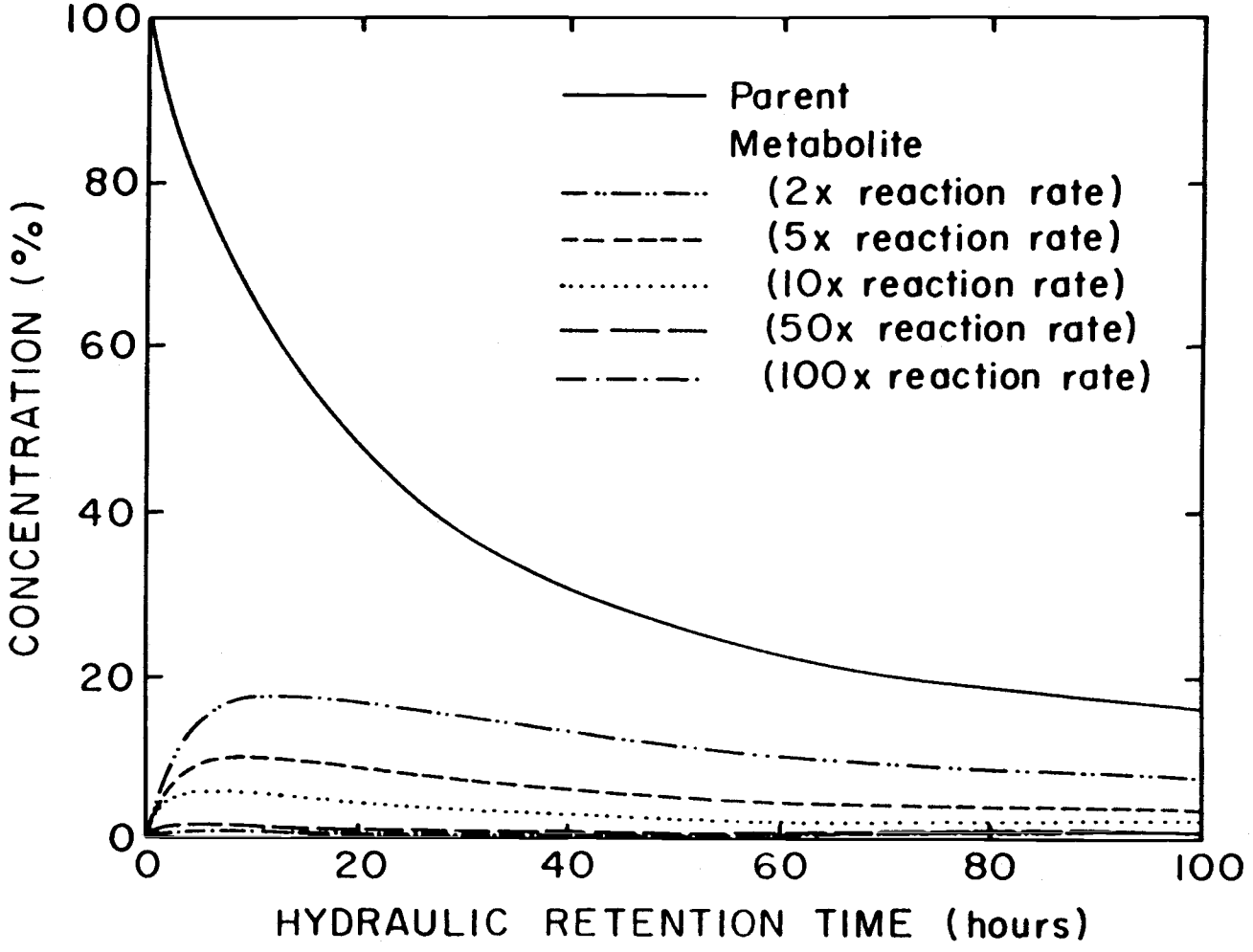


Figure 12. Continuous flow model predicting metabolite concentrations in dichloromethane degradation as a function of time.

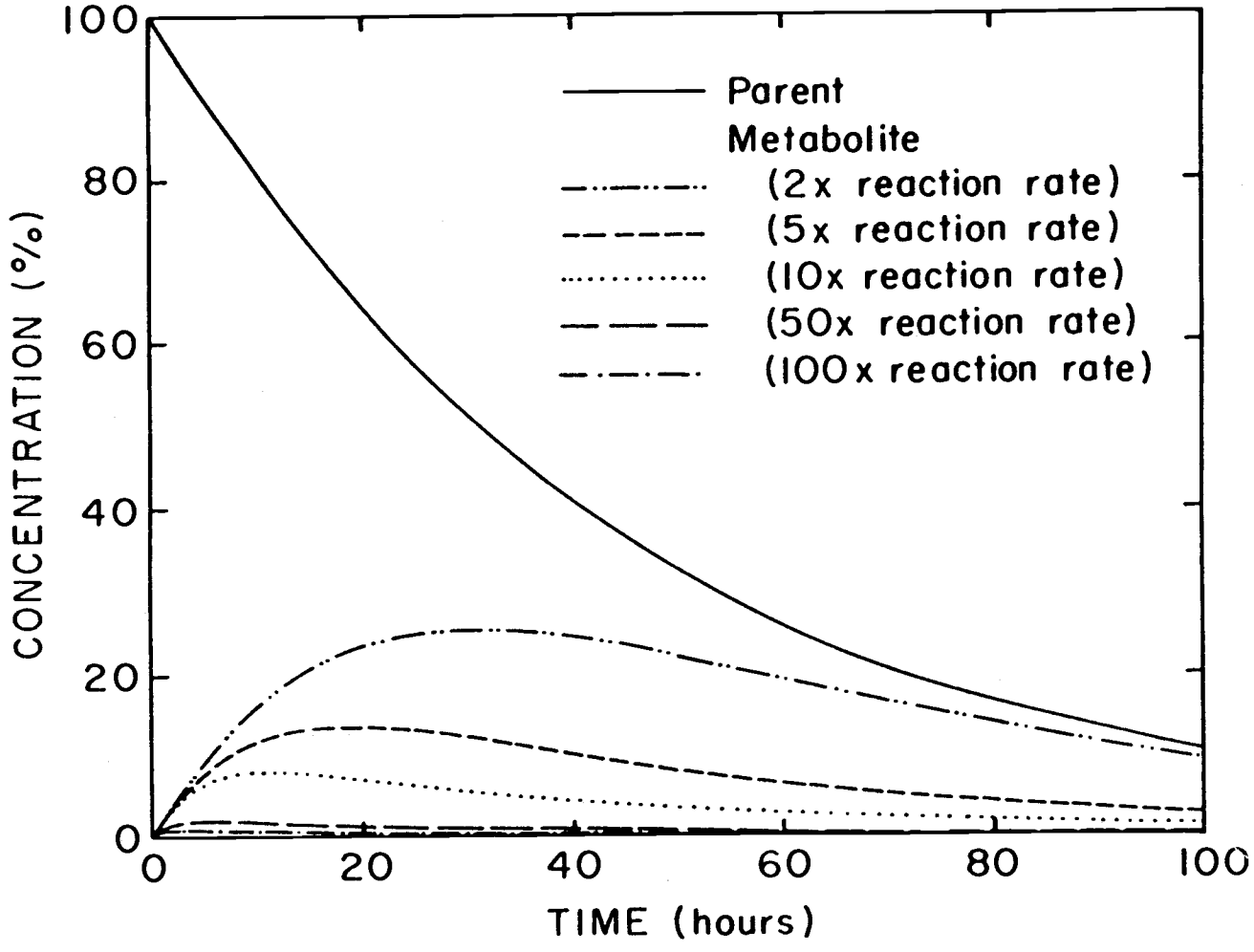


Figure 13. Batch model predicting metabolite concentrations in 1,2-dichloroethane degradation as a function of time.

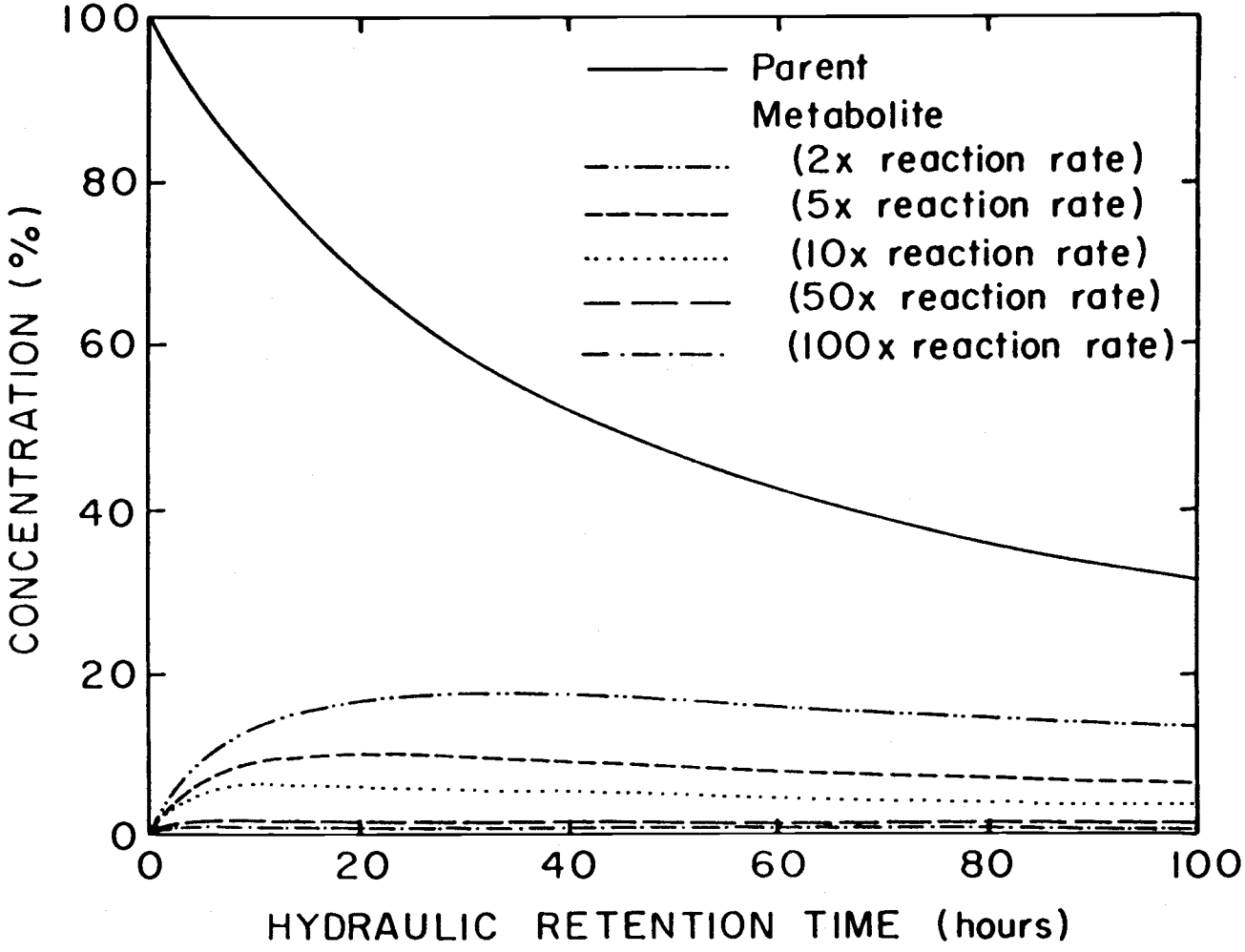


Figure 14. Continuous flow model predicting metabolite concentrations in 1,2-dichloroethane degradation as a function of time.

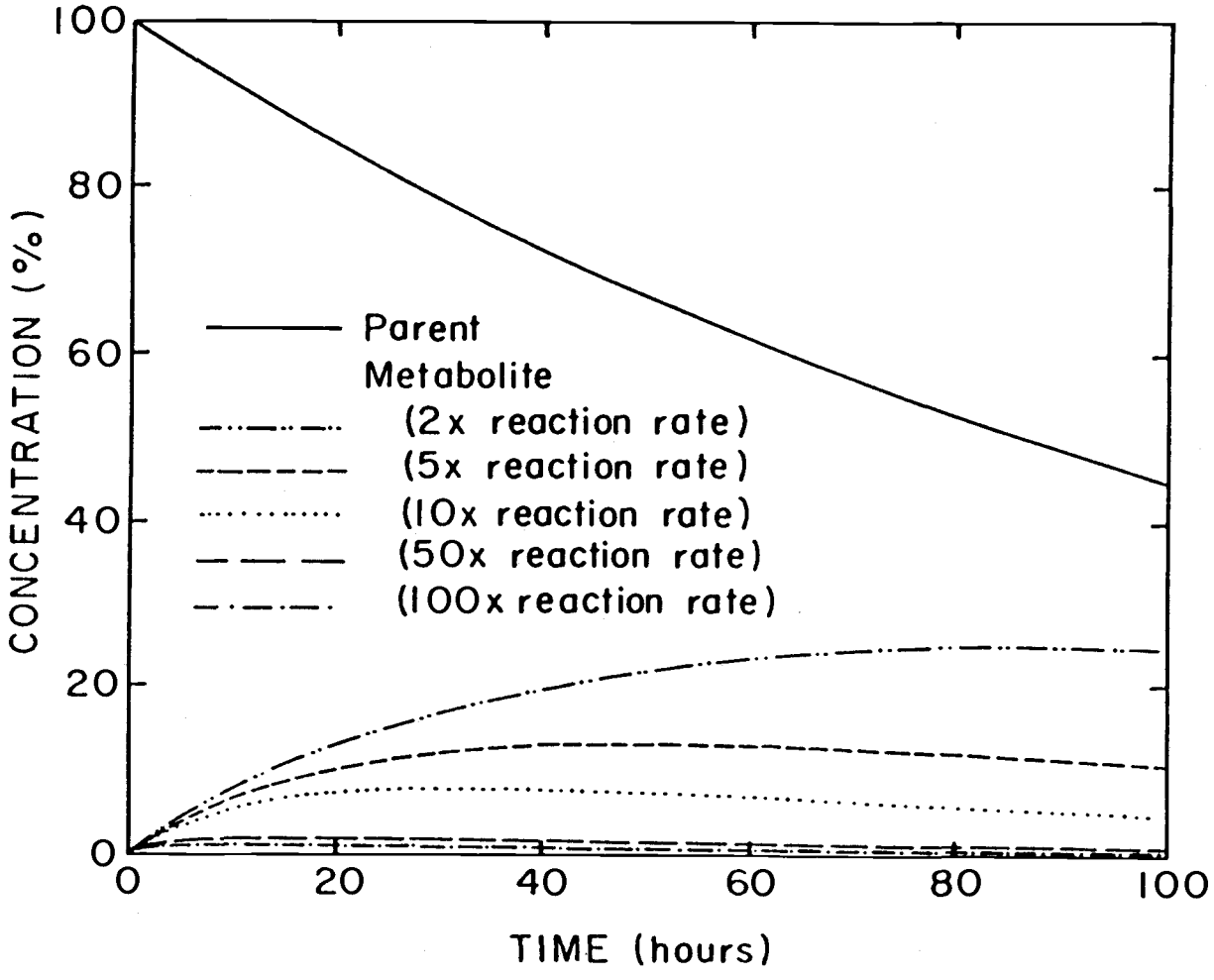


Figure 15. Batch model predicting metabolite concentrations in cis 1,2-dichloroethene degradation as a function of time.

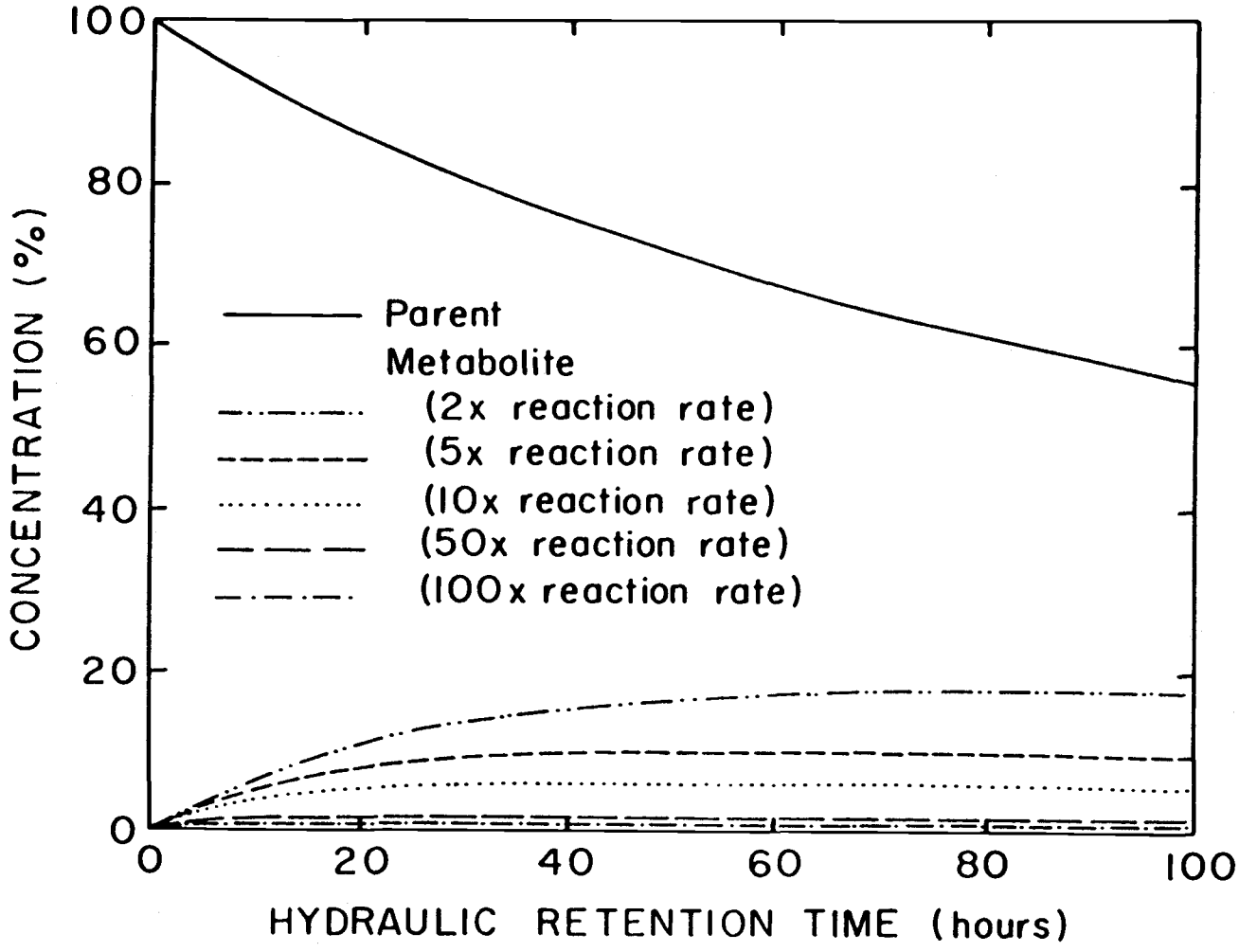


Figure 16. Continuous flow model predicting metabolite concentrations in cis 1,2-dichloroethene degradation as a function of time.

experiment, so it was decided to forego continous flow degradation of DCE. The predicted steady-state concentrations for continuous flow (Figure 16) were lower than those which were undetected in batch. Since, optimum concentrations of metabolite were predicted for such a long time period during batch degradation, it was unlikely that the optimum concentration was "missed" in sampling. It is more likely that the predicted concentration was not detectable and the lower predicted continuous flow concentrations would be less likely to be detected.

A retention time of 20 hours was chosen for continuous flow operation to attempt to accumulate metabolites of DCM and DCA. It was believed that this retention time would be the most likely to yield degradation products of both parent compounds.

Continuous Flow Experiment

Mass balances for DCM and DCA during continuous flow operation are shown in Figures 17 and 18. Concentrations of chlorinated compounds were compared to a theoretical concentration of the chlorinated compounds assuming no degradation. This tracer curve predicted the concentration of organic compound in the liquid phase (effluent) as a function of time for an inert compound in a completely mixed flow reactor (CMFR). The theoretical curve is not ideal because the measured influent concentrations varied from 32 to 45 umol/$\ell$ over the 7 day experimental period. Concentrations of the inert tracer in the reactor were estimated by a finite difference model (Appendix A).

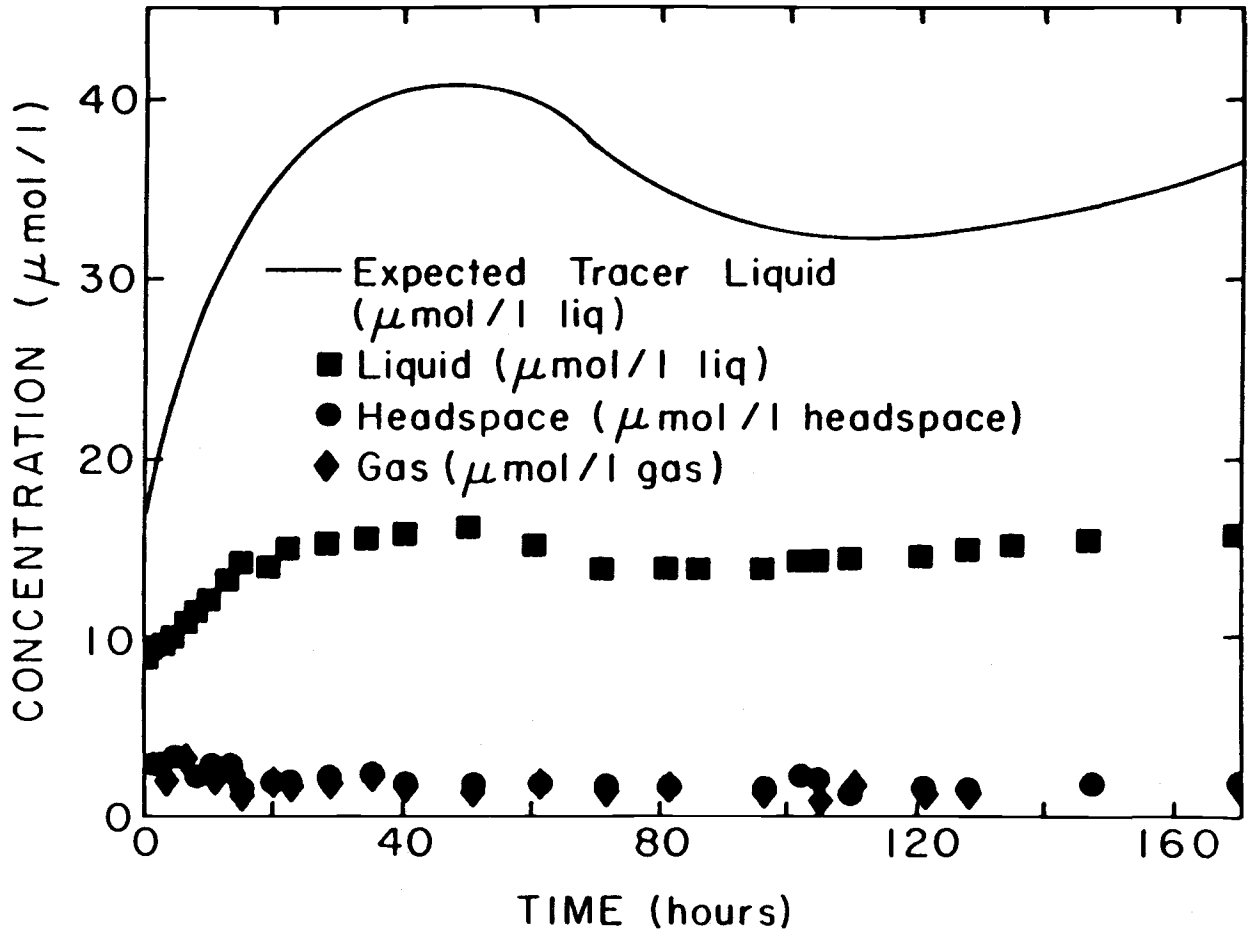


Figure 17. Dichloromethane concentrations during continuous flow degradation.

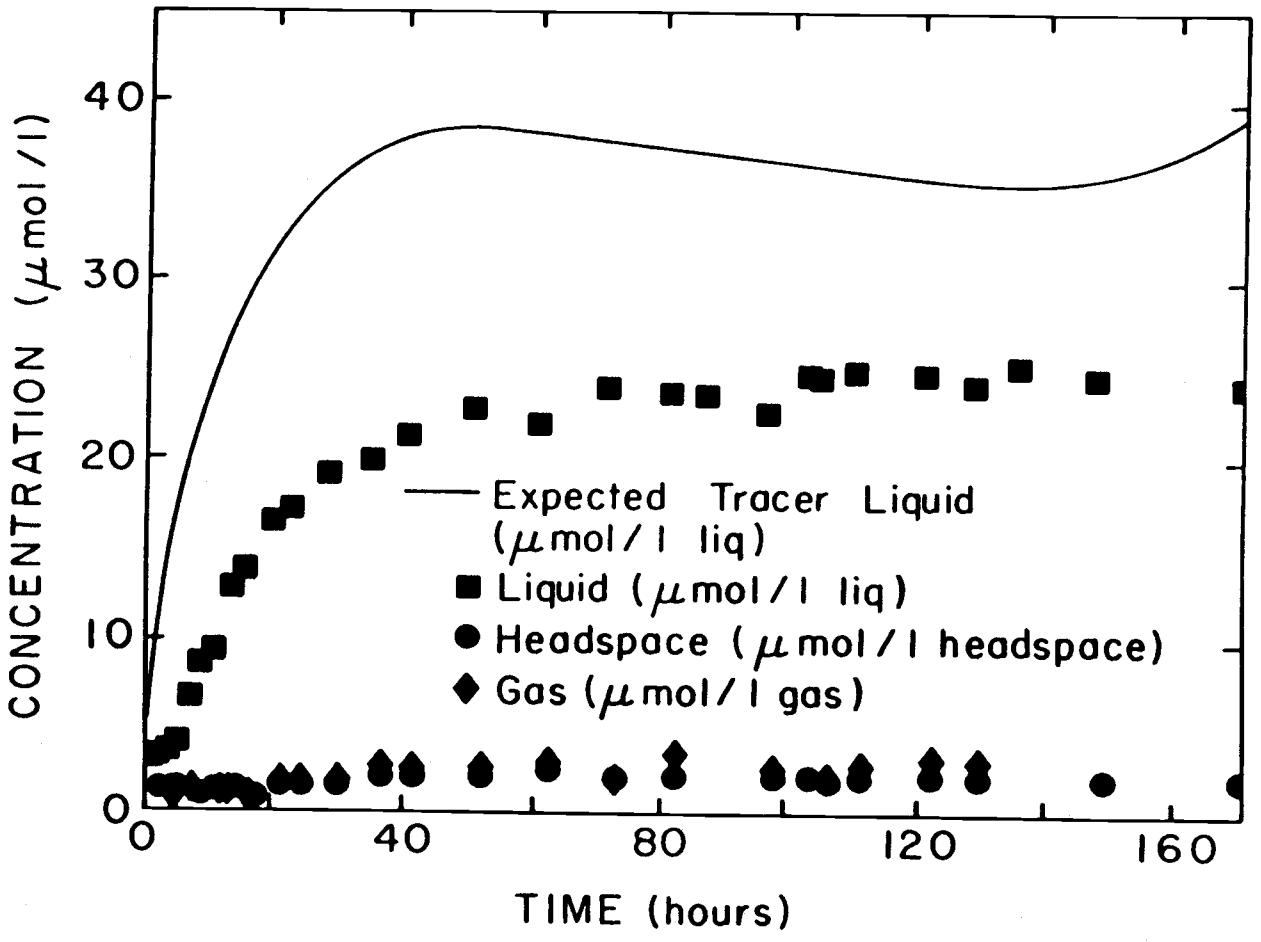


Figure 18. 1,2-dichloroethane concentrations during continuous flow degradation.

The liquid concentration data represents the measured effluent concentration data. Initially, volatilization of the compounds was significant as the compounds equilibrated between the liquid and gas phases. After approximately 3.5 retention times, the gas and liquid phases reached equilibrium and volatilization was minimized as shown by the constant concentrations in the headspace and gas compartments (Figures 17 and 18). The reactor was assumed to be operating at steady-state. Sorption of the compounds to the biofilm and the reactor also occurred immediately after addition of the chemicals. Degradation is shown after the initial equilibration period since the effluent concentration is significantly less than the predicted tracer curve. Average degradation constants with a 20 hour retention time determined from the liquid concentration versus time curves after 3.5 retention times were 0.059 $hr^{-1}$ and 0.022 $hr^{-1}$ for DCM and DCA respectively (Table 1). Removals of DCM and DCA were 56% and 23% respectively.

No metabolites were detected in continuous flow degradation of DCA or DCM with a 20 hour retention time.

Degradation of 2-chloroethanol

The batch removal of 2-chloroethanol (CEO) from the reactor liquid is shown in Figure 19. The chromatogram area of CEO in the liquid was normalized to the initial area as a function of time. With the initial normalized area equal to 1, the area after 9.15 hours is approximately 0.18 representing an 82% removal. Linearization of these data (Figure 21) yielded a degradation rate

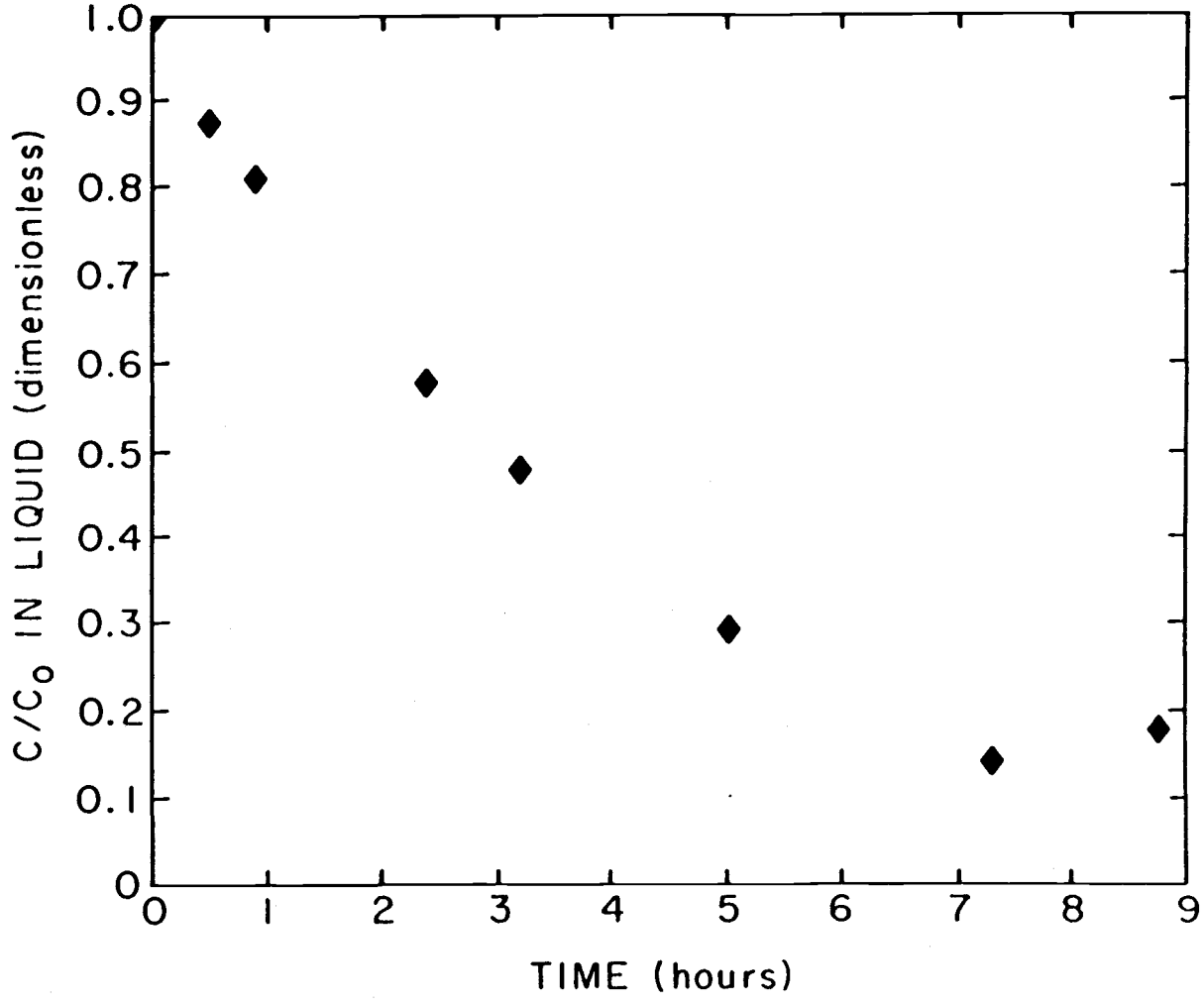


Figure 19. Normalized liquid concentrations of 2-chloroethanol during batch degradation of 2-chloroethanol.

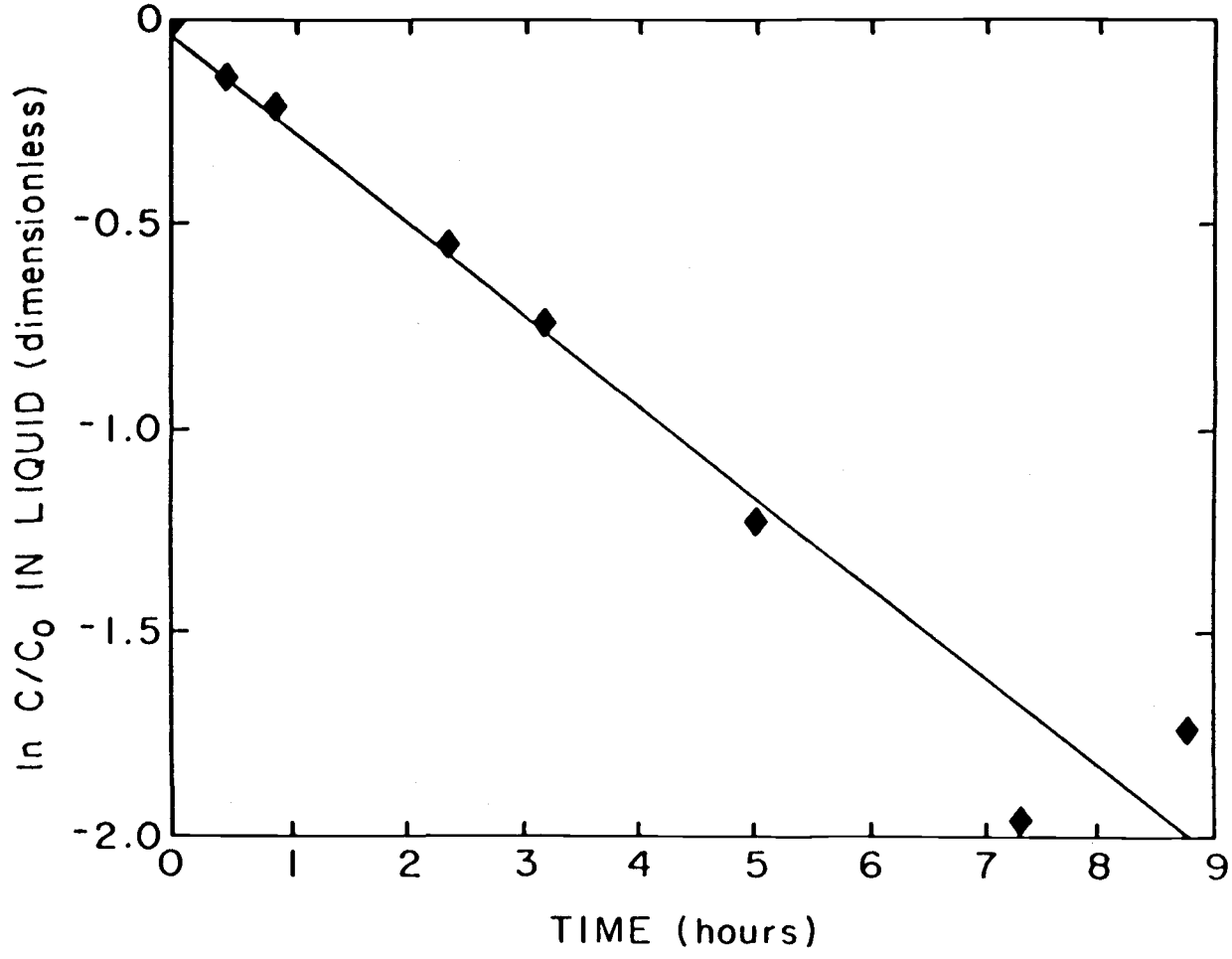


Figure 20. Linearized liquid concentration of 2-chloroethanol during batch degradation.
Slope = $-k_p$ $k_p = 0.224\ hr^{-1}$

constant of 0.224 $hr^{-1}$ with a correlation coefficient of -0.997. No CEO was detected in the headspace or gas compartments throughout the experiment.

Control Experiments

The total mass of chlorinated compounds as a function of time during the control experiments is shown in Figure 21. These were performed in two separate experiments but are represented in one figure for ease of comparison. Except for an initial immediate decrease, the mass of the chlorinated compounds did not decrease significantly throughout the experiment. The calculated means of the total mass of DCM, DCA, and DCE were 32.61±2.41, 34.60±0.85, and 21.93±1.54 umoles, repectively. These masses were 93, 98, and 91 percent of the initial total masses, respectively.

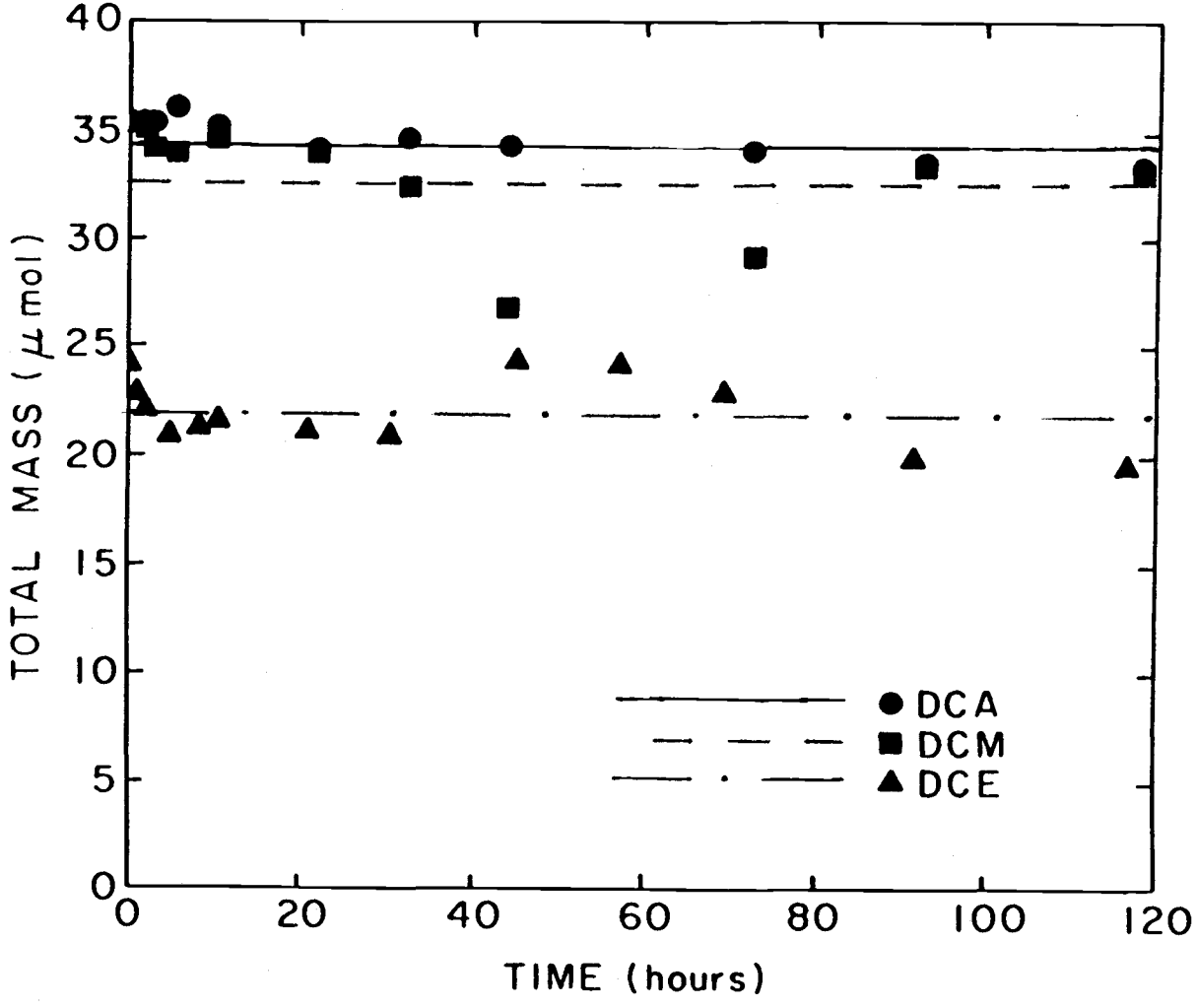


Figure 21. Total mass (headspace and liquid) of dichloromethane, 1,2-dichlorethane, and cis 1,2 dichloroethene during control experiments.

# DISCUSSION

## Degradation of DCM, DCA, and DCE

Biodegradation of chlorinated methanes, ethanes, and ethenes by the methylotrophic GPMS biofilm appears to be similar to that obtained in other methylotrophic degradation studies. Rates for batch degradation for the GPMS system were measured as 0.056, 0.023, and 0.008 $hr^{-1}$ or a ratio of 7.0 : 2.9 : 1.0 for DCM, DCA, and DCE, respectively. A similar ratio of degradation rates of methane, ethane, and ethene (3.8 : 2.5 : 1.0) was reported by Patel et al. (1979, 1980) in their work with MMO isolated from methylotrophs. Yokoto et al. (1986) calculated degradation rates of DCM and DCA by measuring chloride production in methylotrophic chemostats. The ratio of degradation of DCM : DCA (1.7 : 1.0) corresponds to that obtained with the GPMS system (2.4 : 1.0). Hensen et al. (unpublished) compared rates of removal of chlorinated 1- and 2- carbon compounds by natural-gas stimulated organisms in soil columns. They reported that their observations were probably due to cometabolism of chlorinated compounds by methylotrophic bacteria. They observed degradation rates of 0.91, 0.60, and >1.2 $hr^{-1}$ and removals of 94%, 85%, and >98% for DCM, DCA, and DCE, respectively. The ratio of DCM : DCA removal (1.1 : 1.0) compares to that of the GPMS system where 85% and 58% (1.5 : 1.0) of DCM and DCA were removed after 48 hours batch degradation, as does the ratio of rates of degradation for DCM and DCA.

However, the much higher removal and degradation rate of DCE reported by Hensen et al. is in contrast to that obtained by the GPMS system.

Continuous flow operation with a 20-hour retention time resulted in significant removal of DCM and DCA from the reactor, producing degradation rates similar to those obtained in the batch experiments. This suggests that the same removal process is occurring in continuous flow and in batch mode. Possible mechanisms of removal of the chlorinated compounds include degradation, sorption, and volatilization. In continuous flow at steady-state, the compounds have equilibrated between the liquid, gas, and solid phases. Thus, further losses by sorption and volatilization are insignificant. Degradation is the only significant removal process, and the calculated removal rates are due to degradation processes only. Since the rate constants for continuous flow are almost identical to those obtained in the batch experiments (0.059 and 0.022 $hr^{-1}$ for DCM and DCA in continuous flow and 0.056 and 0.023 $hr^{-1}$ in batch), the data suggest that sorption and volatilization must also be insignificant in the batch experiments, and that biodegradation is the significant removal mechanism in the batch experiments, also. In addition, the correlation coefficients obtained in linearizing the batch liquid concentration data (-0.981, -0.986, and -0.983), support the model's first-order biodegradation assumption.

No volatile metabolites were detected in either batch or continuous flow degradation of DCM and DCA, or in batch

degradation of DCE. No reports of volatile metabolites of DCM degradation were found in the literature. However, Yokota et al. (1986) suggested that dehalogenation of substituted n-alkanes by oxygenase enzymes occurs according to the following reaction sequence:

$$R\text{-}CH_2Cl \xrightarrow{\text{oxygenase}} R\text{-}CHClOH \xrightarrow{\text{spontaneous}} R\text{-}CHO \xrightarrow{\text{dehydrogenase}} R\text{-}COOH$$

Based on this scheme, DCM and DCA would be degraded to alcohols which would undergo further spontaneous degradation. Colby et al. (1977) similarly suggested that metabolites were not detected due to the instability of the 1-substituted methanol derivatives. Vogel and Criddle (1987) also reported that products of aerobic degradation of chlorinated methanes are unstable.

Two metabolites have been identified in the methylotrophic degradation of DCA. Janssen et al. (1985) reported the production of 2-chloroethanol for degradation with crude extract of methylotrophic cells, while Yokoto et al. (1986) found production of 2-chloroacetic acid in the degradation of DCA by resting methylotrophic cells. Yokoto noted that the enzyme responsible for the degradation of DCA was an oxygenase with biochemical properties similar to that of the MMO enzyme identified in rats. Based on the detection of several other enzymes (mainly dehalogenases and dehydrogenases) a complete pathway for the degradation of DCA has been proposed by Janssen et al.(1985):

$CH_2Cl$-$CH_2Cl$------>$CH_2Cl$-$CH_2OH$------>$CH_2Cl$-CHO--->

dichloroethane 2-chloroethanol chloroacetaldehyde

--->$CH_2Cl$-COOH------>$CH_2OH$-COOH

chloroacetic acid 2-hydroxyacetic acid

with the hydroxylated carboxylic acids rapidly metabolized by various enzymes.

The production of 2-chloroacetate in the degradation of 2-chloroethanol (Stucki et al., 1983), supports this proposed pathway. This pathway is also in accordance with the biodegradative process proposed by Vogel et al. (1987) based on the chemical properties of chlorinated one- and two- carbon compounds.

Fogel et al. (1986) report the production of unidentified volatile chlorinated compounds by methylotrophic DCE degradation.

Model Predictions

The model predictions presented in Figures 11 through 16 indicate that maximum metabolite concentrations amount to only a small fraction of the parent compound's initial concentration if it is assumed that the metabolite degrades at least twice as fast as the parent compound (i.e. $k_m > 2k_p$). This assumption is based on the information regarding proposed pathways of chlorinated aliphatic compound degradation by the MMO enzyme and the biochemical properties of the proposed metabolites. Smaller metabolite concentrations result for the higher metabolite

degradation rates. The relative degradation rates of metabolite to parent compound (2X, 5X, 10X, 50X, and 100X) were chosen to allow for a broad range of potential metabolites.

If a metabolite detection limit of 5% of the parent compound's initial concentration is assumed, the models show that metabolite detection will occur only if the metabolite degrades at a rate of less than 10 times that of the parent compound. For the relative rates examined, the maximum predicted accumulation is 25% of the parent compound's initial concentration. This occurs for a relative degradation rate of 2 : 1 metabolite to parent compound in a batch reactor.

The faster the parent compound degrades, the smaller the time interval during which detectable concentrations of metabolite are present. For example, Figure 11 shows that detectable concentrations of DCM metabolites are present for only several hours in batch degradation. Detectable concentrations of products of DCE, which degrades much slower than DCM, are present for many hours as indicated by Figure 15.

Continuous flow models for each compound predict lower steady-state concentrations of metabolites than those predicted for batch operation. Maximum predicted accumulation for the case where the metabolite degrades twice as fast as the parent compound amounts to approximately 17% of the parent compound's concentration.

Monitoring of the liquid composition throughout the time period indicated as favorable for accumulation of metabolites in batch degradation for DCM, DCA, and DCE did not result in the

detection of metabolites. However, it is more likely to detect metabolites in a continuous flow system operating at steady-state than in a batch reactor where the metabolite's presence is transient. Any small change in the degradative rate of the biofilm could produce a maximum metabolite concentration at a different time than predicted. When steady-state continous flow operation is achieved, concentrations remain relatively constant and the possibility of "missing" transient concentrations is eliminated.

The models for DCE, however, indicated no advantage to operating in continuous flow. For metabolites with degradation rates less than or equal to ten times that of DCE, batch degradation should have produced detectable levels of metabolites anytime after 15 hours after the initial injection. Since no metabolites were detected in any samples throughout the 129-hour testing period, it was unlikely that there was ever a detectable concentration of metabolite present.

Though no volatile metabolites were detected at these steady-state conditions, it is possible to determine the relative degradation rates of the metabolic products and their corresponding parent compounds. The models suggest that the concentration of degradation products which would be present in the reactor at a given retention time highly depends on the relative degradation rates of metabolite and parent compound. Since no metabolites were detected, it is determined that the degradation rates of the products must be greater than approximately ten times

that of the parent compound.

Degradation of 2-Chloroethanol

The degradation rate of CEO was measured as 0.224 $hr^{-1}$. This is approximately 10 times as fast as the degradation rate of DCA. The detection limit of CEO, based on the analytical procedure employed in this study, is approximately 3 umol/$\ell$. This corresponds to approximately 6% of the original concentration of DCA. The model predicts that, at steady state, there should be a concentration of metabolite of approximately 5% of the original DCA concentration (2.31 umol/$\ell$). Since this is only slightly less than the detection limit, it is difficult to conclude whether or not CEO could be a metabolic product.

CEO degradation is approximately 30 times that of DCE. Based on the DCE models, (Figures 15 and 16,) the detection limit would have to be approximately 1% of the parent compound's concentration in order for CEO to be detected in DCE degradation studies. The actual detection limit of 2-chloroethanol was appoximately 5%.

Arguments Supporting Biodegradation

An issue of significant concern during this study was the minimization of unaccounted losses of the volatile compounds. Though no nondegradable tracer was analyzed in this particular study, the results presented here can be compared to those obtained in a nearly identical reactor by Ely (1986). In these earlier experiments, the total mass of carbon tetrachloride (CT) in the system remained relatively constant enabling its use as a tracer.

The lower vapor pressures and chemical concentrations in this current research suggest an even smaller chance of undetected volatilization.

It is also necessary to consider the extent of sorption of the compounds onto the reactor materials, membrane, or biofilm material. Since there is probably no way to completely avoid sorption, an attempt was made to consider relative extents of adsorption of the compounds based on relative octanol/water coefficients ($K_{OW}$). Ely (1986) observed only slight immediate losses due to sorption based on the CT tracer data. Table 2 shows the ($K_{OW}$) for all compounds examined in these experiments. Since the octanol/water coefficient for CT is higher than for any other compound investigated, the effects of sorption would be greatest for the CT. Losses due to sorption in the current research would be expected to also occur immediately and to be less significant than those observed by Ely.

Photolytic degradation of the chlorinated compounds is assumed negligible since the reactor is maintained in a dark enclosure.

Therefore, the mechanisms which could be responsible for degradation in the liquid phase are chemical and biological destruction of the compounds. The hydrolytic half-lives of the DCM, DCA, and DCE are each greater than 50 years (EPA, 1979) indicating that chemical degradation would be quite slow. The control study data supports this hypothesis. Thus, the significant removal mechanism in operation in this experiment is concluded to be biodegradation.

TABLE 2

Summary of Octanol/Water Coefficients ($K_{ow}$)

| Compound | log $K_{ow}$ |
|---|---|
| Dichloromethane | 1.25[a] |
| 1,2-Dichloroethane | 1.48[b] |
| cis 1,2-Dichloroethene | 1.48[c] |
| 2-chloroethanol | 0.03[d] |
| Carbon Tetrachloride | 2.83[d] |
| Chloroform | 1.97[a]<br>1.97[d] |

[a] Hansch et al., 1975
[b] Radding et al., 1977
[c] estimated by Tute method, 1971
[d] Leo et al., 1971

# SUMMARY AND CONCLUSIONS

## Summary

This research has demonstrated the use of a methylotrophic GPMS biofilm to degrade dichloromethane, dichloroethane, and dichloroethene. The degradation is similar to previously reported results achieved with suspended cultures of methylotrophs or with isolated MMO. This suggests that methylotrophic GPMS biofilms could be effective in treating the wide variety of recalcitrant compounds known to be amenable to degradation by the MMO enzyme. The GPMS system provides degradation in either batch or continuous flow configurations The advantages of the GPMS biofilm is its effectiveness in oxidizing low concentrations of recalcitrant compounds and its biological stability. No toxic metabolites are accumulated from the GPMS methylotrophic degradation of dichloromethane, dichloroethane, and dichloroethene.

## Conclusions

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

1. The GPMS methylotrophic biofilm can degrade low concentrations of dichloromethane, 1,2- dichloroethane, and cis 1,2-dichloroethene in aqueous samples. Degradation rates decrease in the order of chlorinated methane, ethane, and ethene.

2. The GPMS biofilm system can be used in batch or continuous flow treatment processes.

3. Control studies performed in the absence of the biofilm indicate that the compounds are not significantly degraded by chemical processes.

4. No metabolites are detectable from the methylotrophic degradation of dichloromethane, 1,2-dichloroethane, or cis 1,2 dichloroethene in the GPMS system.

5. The rate of degradation of 2-chloroethanol in the GPMS system is approximately ten times that of DCA and 30 times that of DCE.

6. A model describing metabolite concentrations as a function of time and retention time can be used to determine relative degradation rates of parent compounds and metabolic products.

7. The relative degradation rate of any metabolic product of 1,2-dichloroethane or of dichloromethane is probably at least ten times greater than the degradation rate of the parent compound.

# RECOMMENDATIONS FOR FURTHER RESEARCH

This research has demonstrated that the methylotrophic biofilm is effective in degrading one and two carbon compounds. Though no volatile metabolites were accumulated in the degradation, it is possible that highly reactive metabolic intermediates were produced and degraded simulataneously. The predictive model thus indicated a minimum biodegradation rate for the intermediates.

Further research should be directed towards determining whether the biofilm can degrade other groundwater pollutants such as chlorinated aromatic compounds. It seems reasonable that the metabolic products of these more complex molecules would be less susceptible to degradation (than chlorinated alcohols, for example) and would thus be more easily accumulated. The degradation then could be accomplished at different retention times in continuous flow mode accumulating the corresponding steady-state concentrations of metabolites. Application of the model then could determine the relative degradation rate of parent compound to metabolite ($k_p$ to $k_m$).

Another possibility for further research is to determine the effect of varying the $O_2$ : $CH_4$ ratio in the gaseous substrate. Methane is known to supress the oxidation rate of trichloroethylene (Strand, 1988). Decreasing the $CH_4$ concentration may produce higher degradation rates of the trace organic compounds. There may be a lower limit of $CH_4$ that must be available to sustain the degradation capabilities of the biofilm.

Finally, refinements need to be made in the GPMS system to gather more quantitative data. The biofilm used for these experiments varied spatially in thickness and density. A reactor with controlled and uniform mixing would eliminate these difficulties.

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# APPENDICES

# APPENDIX A

## Finite Difference Method

CALCULATION OF TRACER CURVE

The tracer curve is calculated using a finite difference method. A mass balance is performed on the compound in the continuous flow reactor. The concentration is represented as a function of time C(t). Calculations are made for small time changes ( t).

Influent concentration was measured periodically.

Retention Time is calculated from measured liquid flow rate (Q) and volume (V).

Mass balance on the compound in reactor

$V\Delta c/\Delta t = Q\,Co - Q\,C$ Co = initial concentration, C = concentration at time t

$\Delta c/\Delta t = (1/T)\,(Co-C)$ T = Retention time = V/Q

Calculation for change in concentration for time change

C(t) = C(t-1) + (change in concentration during t)

$C = Co + (\Delta c/\Delta t)\,t$ $\Delta t$ = time since last liquid concentration measurement

| ##########SAMPLE INFO.########## | | | | INFLUENT | INFLUENT | | | | TRACER | TRACER |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | time | DCM | DCA | Retention | dc/dt | dc/dt | CONC | CONC |
| date | hour | minute | elapsed | CONC | CONC | Time | DCM | DCA | DCM | DCA |
| 6 | 10 | 37 | 0.85 | 33.57 | 34.54 | 20.3 | 1.38 | 1.42 | 17.50 | 6.20 |
| 6 | 12 | 18 | 2.53 | 44.06 | 45.12 | 16.1 | 1.65 | 2.42 | 20.28 | 10.27 |
| 6 | 14 | 0 | 4.23 | 43.84 | 43.88 | 20.5 | 1.15 | 1.64 | 22.23 | 13.06 |
| 6 | 19 | 29 | 9.72 | 42.80 | 44.29 | 16.7 | 1.23 | 1.87 | 28.98 | 23.31 |
| 6 | 22 | 21 | 12.58 | 42.90 | 42.79 | 14.5 | 0.96 | 1.34 | 31.73 | 27.16 |
| 7 | 0 | 15 | 14.48 | 42.56 | 43.22 | 15.8 | 0.69 | 1.02 | 33.04 | 29.09 |
| 7 | 5 | 27 | 19.68 | 42.28 | 43.43 | 21.5 | 0.43 | 0.67 | 35.27 | 32.56 |
| 7 | 9 | 24 | 23.63 | 45.13 | 41.55 | 20.9 | 0.47 | 0.43 | 37.14 | 34.26 |
| 7 | 14 | 16 | 28.50 | 43.55 | 40.34 | 17.3 | 0.37 | 0.35 | 38.94 | 35.97 |
| 7 | 20 | 45 | 34.98 | 42.42 | 40.02 | 16.7 | 0.21 | 0.24 | 40.29 | 37.54 |
| 8 | 3 | 5 | 41.32 | 40.74 | 36.98 | 18.5 | 0.02 | -0.03 | 40.45 | 37.35 |
| 8 | 12 | 26 | 50.67 | 41.62 | 38.67 | 20.1 | 0.06 | 0.07 | 40.99 | 37.96 |
| 8 | 22 | 39 | 60.88 | 40.40 | 38.89 | 18.1 | -0.03 | 0.05 | 40.66 | 38.49 |
| 8 | 23 | 45 | 61.98 | 39.15 | 35.92 | 18.1 | -0.08 | -0.14 | 40.56 | 38.33 |
| 9 | 9 | 5 | 71.32 | 32.10 | 36.41 | 18.1 | -0.47 | -0.11 | 36.20 | 37.34 |
| 9 | 10 | 19 | 72.55 | 33.99 | 37.73 | 18.1 | -0.12 | 0.02 | 36.05 | 37.37 |
| 9 | 19 | 20 | 81.57 | 33.04 | 36.66 | 14.7 | -0.20 | -0.05 | 34.20 | 36.93 |
| 10 | 10 | 27 | 96.68 | 32.33 | 36.92 | 22.1 | -0.08 | -0.00 | 32.92 | 36.92 |
| 10 | 15 | 0 | 101.23 | 33.38 | 37.57 | 22 | 0.02 | 0.03 | 33.02 | 37.06 |
| 10 | 16 | 57 | 103.18 | 33.28 | 36.46 | 23.1 | 0.01 | -0.03 | 33.04 | 37.01 |
| 10 | 23 | 47 | 110.02 | 31.14 | 34.17 | 22.1 | -0.09 | -0.13 | 32.45 | 36.13 |
| 11 | 10 | 17 | 120.52 | 33.59 | 35.00 | 22.9 | 0.05 | -0.05 | 32.97 | 35.61 |
| 11 | 18 | 16 | 128.50 | 34.67 | 34.79 | 22 | 0.08 | -0.04 | 33.59 | 35.32 |
| 12 | 0 | 47 | 135.02 | 33.26 | 33.54 | 20.8 | -0.02 | -0.09 | 33.49 | 34.76 |
| 12 | 12 | 31 | 146.75 | 33.35 | 35.60 | 20.8 | -0.01 | 0.04 | 33.41 | 35.24 |
| 12 | 14 | 5 | 148.32 | 39.48 | 39.37 | 20.8 | 0.29 | 0.20 | 33.87 | 35.55 |
| 13 | 11 | 5 | 169.32 | 37.44 | 39.53 | 24.6 | 0.15 | 0.16 | 36.92 | 38.95 |

Filename: APNDXA

# APPENDIX B

## Batch Degradation Data

BATCH Experiment (DCA & DCM)

LIQUID DATA

| date | hour | minute | time | elapsed | DCM area | DCA area | DCM conc | DCA conc | DCM mass | DCA mass |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 11 | 52 | 7.71E+05 | 0.00 | 6594 | 23063 | | | 30.84 | 35.22 |
| 30 | 12 | 16 | 7.71E+05 | 0.40 | 4993 | 19590 | 30.41 | 37.27 | 23.17 | 29.40 |
| 30 | 13 | 32 | 7.71E+05 | 1.67 | 3410 | 15858 | 20.97 | 31.81 | 15.98 | 24.24 |
| 30 | 14 | 24 | 7.71E+05 | 2.53 | 2948 | 15034 | 18.20 | 30.16 | 13.87 | 22.98 |
| 30 | 15 | 13 | 7.71E+05 | 3.35 | 2756 | 14952 | 17.06 | 30.00 | 13.00 | 22.86 |
| 30 | 16 | 2 | 7.71E+05 | 4.17 | 2574 | 14331 | 15.96 | 28.75 | 12.16 | 21.91 |
| 30 | 16 | 51 | 7.71E+05 | 4.98 | 2418 | 14175 | 15.02 | 28.44 | 11.45 | 21.67 |
| 30 | 18 | 14 | 7.71E+05 | 6.37 | 2205 | 13690 | 13.74 | 27.47 | 10.47 | 20.93 |
| 30 | 20 | 9 | 7.71E+05 | 8.28 | 1996 | 13210 | 12.49 | 26.51 | 9.52 | 20.20 |
| 30 | 22 | 22 | 7.71E+05 | 10.50 | 1763 | 12798 | 11.09 | 25.69 | 8.45 | 19.57 |
| 1 | 1 | 14 | 7.71E+05 | 13.37 | 1479 | 11817 | 9.39 | 23.73 | 7.15 | 18.08 |
| 1 | 5 | 30 | 7.71E+05 | 17.63 | 1156 | 11037 | 7.45 | 22.17 | 5.68 | 16.89 |
| 1 | 9 | 19 | 7.71E+05 | 21.45 | 957 | 10429 | 6.41 | 20.75 | 4.89 | 15.81 |
| 1 | 13 | 48 | 7.71E+05 | 25.93 | 723 | 9295 | 5.01 | 18.48 | 3.82 | 14.08 |
| 1 | 17 | 36 | 7.71E+05 | 29.73 | 601 | 8393 | 4.28 | 16.68 | 3.26 | 12.71 |
| 1 | 22 | 25 | 7.71E+05 | 34.55 | 479 | 7375 | 3.54 | 14.64 | 2.70 | 11.16 |
| 2 | 12 | 35 | 7.71E+05 | 48.72 | 295 | 6618 | 2.44 | 13.13 | 1.86 | 10.00 |

HEADSPACE DATA

| date | hour | minute | time | elapsed | DCM area | DCA area | DCM conc | DCA conc | DCM mass | DCA mass |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 12 | 42 | 7.71E+05 | 0.83 | 1751 | 3109 | 0.78 | 1.16 | 2.67 | 3.95 |
| 30 | 14 | 0 | 7.71E+05 | 2.13 | 2926 | 4626 | 1.23 | 1.51 | 4.17 | 5.14 |
| 30 | 15 | 37 | 7.71E+05 | 3.75 | 3187 | 5205 | 1.32 | 1.64 | 4.50 | 5.59 |
| 30 | 17 | 50 | 7.71E+05 | 5.97 | 2222 | 3806 | 0.96 | 1.32 | 3.27 | 4.50 |
| 30 | 19 | 44 | 7.71E+05 | 7.87 | 1879 | 3363 | 0.83 | 1.22 | 2.83 | 4.15 |
| 30 | 21 | 59 | 7.71E+05 | 10.12 | 2172 | 4788 | 0.94 | 1.55 | 3.20 | 5.26 |
| 1 | 0 | 50 | 7.71E+05 | 12.97 | 2662 | 5851 | 1.13 | 1.79 | 3.83 | 6.10 |
| 1 | 5 | 6 | 7.71E+05 | 17.23 | 1395 | 3173 | 0.65 | 1.18 | 2.21 | 4.00 |
| 1 | 8 | 54 | 7.71E+05 | 21.03 | 3522 | 1189 | 1.28 | 0.39 | 4.34 | 1.32 |
| 1 | 12 | 14 | 7.71E+05 | 24.37 | 2223 | 5136 | 0.78 | 1.33 | 2.66 | 4.52 |
| 1 | 17 | 12 | 7.71E+05 | 29.33 | 1696 | 4636 | 0.58 | 1.21 | 1.97 | 4.11 |
| 1 | 22 | 1 | 7.71E+05 | 34.15 | 1821 | 4564 | 0.63 | 1.19 | 2.13 | 4.05 |
| 2 | 11 | 47 | 7.71E+05 | 47.92 | 1582 | 3742 | 0.54 | 1.00 | 1.82 | 3.39 |

GAS DATA

| date | hour | minute | time | elapsed | DCM area | DCA area | DCM conc | DCA conc | DCM mass | DCA mass |
|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 13 | 5 | 7.71E+05 | 1.22 | 755 | 4132 | 0.41 | 1.86 | 0.20 | 0.93 |
| 30 | 14 | 49 | 7.71E+05 | 2.95 | 1144 | 7450 | 0.56 | 2.16 | 0.28 | 1.08 |
| 30 | 16 | 27 | 7.71E+05 | 4.58 | 901 | 5363 | 0.46 | 1.68 | 0.23 | 0.84 |
| 30 | 18 | 44 | 7.71E+05 | 6.87 | 1355 | 8848 | 0.64 | 2.48 | 0.32 | 1.24 |
| 30 | 20 | 35 | 7.71E+05 | 8.72 | 813 | 5172 | 0.43 | 1.64 | 0.22 | 0.82 |
| 30 | 22 | 46 | 7.71E+05 | 10.90 | 716 | 4539 | 0.40 | 1.49 | 0.20 | 0.75 |
| 1 | 1 | 38 | 7.71E+05 | 13.77 | 366 | 3644 | 0.26 | 1.26 | 0.13 | 0.64 |
| 1 | 5 | 55 | 7.71E+05 | 18.05 | 43 | 2525 | 0.14 | 1.03 | 0.07 | 0.51 |
| 1 | 9 | 43 | 7.71E+05 | 21.85 | 1633 | 3034 | 0.56 | 0.83 | 0.28 | 0.41 |
| 1 | 13 | 24 | 7.71E+05 | 25.53 | 697 | 2598 | 0.20 | 0.72 | 0.10 | 0.36 |
| 1 | 17 | 59 | 7.71E+05 | 30.12 | 1250 | 1984 | 0.41 | 0.58 | 0.20 | 0.29 |
| 1 | 22 | 50 | 7.71E+05 | 34.97 | 848 | 1145 | 0.26 | 0.38 | 0.13 | 0.19 |
| 2 | 12 | 12 | 7.71E+05 | 48.33 | 873 | 5807 | 0.26 | 1.49 | 0.13 | 0.74 |

| | DCM | DCA |
|---|---|---|
| avg time | ****total | mass**** |
| 0.82 | 26.05 | 33.28 |
| 2.54 | 18.32 | 29.20 |
| 4.17 | 16.90 | 28.34 |
| 6.40 | 14.06 | 26.67 |
| 8.29 | 12.56 | 25.17 |
| 10.51 | 11.85 | 25.58 |
| 13.37 | 11.12 | 24.82 |
| 17.64 | 7.96 | 21.41 |
| 21.44 | 9.51 | 17.55 |
| 25.28 | 6.57 | 18.96 |
| 29.73 | 5.43 | 17.11 |
| 34.56 | 4.96 | 15.40 |
| 48.32 | 3.82 | 14.14 |

| ####ln(C/Co)#### DCM | DCA | Time elapsed |
|---|---|---|
| ########################## | | |
| 0.00 | 0.00 | |
| 0.00 | 0.00 | 0.00 |
| -0.37 | -0.16 | 1.27 |
| -0.51 | -0.21 | 2.13 |
| -0.58 | -0.22 | 2.95 |
| -0.64 | -0.26 | 3.77 |
| -0.71 | -0.27 | 4.58 |
| -0.79 | -0.30 | 5.97 |
| -0.89 | -0.34 | 7.88 |
| -1.01 | -0.37 | 10.10 |
| -1.18 | -0.45 | 12.97 |
| -1.41 | -0.52 | 17.23 |
| -1.56 | -0.59 | 21.05 |
| -1.80 | -0.70 | 25.53 |
| -1.96 | -0.80 | 29.33 |
| -2.15 | -0.93 | 34.15 |
| -2.52 | -1.04 | 48.32 |

Linear Regression DCA

| | | | |
|---|---|---|---|
| Sx | 178.9166 | A = | -0.13433 (intercept) |
| Sy | -6.13197 | | |
| Sxy | -112.318 | B = | -0.02301 (slope) (kp) |
| Sxsq | 3836.653 | | |
| Sysq | 3.433749 | r = | -0.98615 |
| SxSx | 32011.17 | | |
| SySy | 37.60117 | | |
| n | 15 | | |

| x | y | x*y | x^2 | y^2 | x | calc.Y |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | -0.13433 |
| 1.266666 | -0.15851 | -0.20079 | 1.604444 | 0.025128 | 1.266666 | -0.16347 |
| 2.133333 | -0.21171 | -0.45166 | 4.551111 | 0.044825 | 2.133333 | -0.18342 |
| 2.95 | -0.21717 | -0.64065 | 8.702499 | 0.047163 | 2.95 | -0.20221 |
| 3.766666 | -0.25945 | -0.97729 | 14.18777 | 0.067318 | 3.766666 | -0.22100 |
| 4.583333 | -0.27036 | -1.23918 | 21.00694 | 0.073098 | 4.583333 | -0.23979 |
| 5.966666 | -0.30506 | -1.82022 | 35.60111 | 0.093064 | 5.966666 | -0.27162 |
| 7.883333 | -0.34063 | -2.68533 | 62.14694 | 0.116031 | 7.883333 | -0.31573 |
| 10.1 | -0.37220 | -3.75928 | 102.0099 | 0.138537 | 10.1 | -0.36674 |
| 12.96666 | -0.45165 | -5.85646 | 168.1344 | 0.203992 | 12.96666 | -0.43270 |
| 17.23333 | -0.51966 | -8.95555 | 296.9877 | 0.270051 | 17.23333 | -0.53088 |
| 21.05 | -0.58567 | -12.3284 | 443.1025 | 0.343014 | 21.05 | -0.61870 |
| 25.53333 | -0.70141 | -17.9095 | 651.9511 | 0.491986 | 25.53333 | -0.72187 |
| 29.33333 | -0.80411 | -23.5874 | 860.4444 | 0.646606 | 29.33333 | -0.80931 |
| 34.15 | -0.93430 | -31.9065 | 1166.222 | 0.872929 | 34.15 | -0.92014 |

Linear Regression DCM

| | | | |
|---|---|---|---|
| Sx | 178.9166 | A = | -0.37497 (intercept) |
| Sy | -15.5596 | | |
| Sxy | -260.135 | B = | -0.05552 (slope) (kp) |
| Sxsq | 3836.653 | | |
| Sysq | 21.59549 | r = | -0.98100 |
| SxSx | 32011.17 | | |
| SySy | 242.1040 | | |
| n | 15 | | |

| x | y | x*y | x^2 | y^2 | x | calc.Y |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | -0.37497 |
| 1.266666 | -0.37155 | -0.47063 | 1.604444 | 0.138052 | 1.266666 | -0.44530 |
| 2.133333 | -0.51330 | -1.09505 | 4.551111 | 0.263482 | 2.133333 | -0.49343 |
| 2.95 | -0.57798 | -1.70505 | 8.702499 | 0.334064 | 2.95 | -0.53878 |
| 3.766666 | -0.64487 | -2.42903 | 14.18777 | 0.415866 | 3.766666 | -0.58413 |
| 4.583333 | -0.70532 | -3.23272 | 21.00694 | 0.497478 | 4.583333 | -0.62948 |
| 5.966666 | -0.79423 | -4.73893 | 35.60111 | 0.630809 | 5.966666 | -0.70629 |
| 7.883333 | -0.88990 | -7.01544 | 62.14694 | 0.791937 | 7.883333 | -0.81272 |
| 10.1 | -1.00861 | -10.1869 | 102.0099 | 1.017300 | 10.1 | -0.93581 |
| 12.96666 | -1.17540 | -15.2410 | 168.1344 | 1.381576 | 12.96666 | -1.09500 |
| 17.23333 | -1.40662 | -24.2408 | 296.9877 | 1.978588 | 17.23333 | -1.33192 |
| 21.05 | -1.55666 | -32.7678 | 443.1025 | 2.423209 | 21.05 | -1.54386 |
| 25.53333 | -1.80380 | -46.0570 | 651.9511 | 3.253696 | 25.53333 | -1.79282 |
| 29.33333 | -1.96181 | -57.5466 | 860.4444 | 3.848734 | 29.33333 | -2.00383 |
| 34.15 | -2.14958 | -73.4081 | 1166.222 | 4.620699 | 34.15 | -2.27129 |

Filename: BATCH1

BATCH Experiment (DCE)

LIQUID

| date | hour | minute | time elapsed | | area | conc | mass |
|---|---|---|---|---|---|---|---|
| 14 | 12 | 24 | 7.72E+05 | -0.87 | 0 | 0.21 | 0.14 |
| 14 | 13 | 16 | 7.72E+05 | 0.00 | 11579 | 58.11 | 40.03 |
| 14 | 13 | 41 | 7.72E+05 | 0.42 | 9674 | 48.58 | 33.47 |
| 14 | 14 | 49 | 7.72E+05 | 1.55 | 6923 | 34.83 | 23.99 |
| 14 | 15 | 51 | 7.72E+05 | 2.58 | 5803 | 29.22 | 20.14 |
| 14 | 16 | 50 | 7.72E+05 | 3.57 | 5490 | 27.66 | 19.06 |
| 14 | 18 | 43 | 7.72E+05 | 5.45 | 5124 | 25.83 | 17.80 |
| 14 | 20 | 56 | 7.72E+05 | 7.67 | 4959 | 25.00 | 17.23 |
| 14 | 22 | 59 | 7.72E+05 | 9.72 | 4772 | 24.07 | 16.58 |
| 15 | 1 | 17 | 7.72E+05 | 12.02 | 4745 | 23.93 | 16.49 |
| 15 | 5 | 27 | 7.72E+05 | 16.18 | 4330 | 21.86 | 15.06 |
| 15 | 9 | 46 | 7.72E+05 | 20.50 | 4360 | 22.01 | 15.16 |
| 15 | 13 | 27 | 7.72E+05 | 24.18 | 4085 | 20.63 | 14.22 |
| 15 | 17 | 30 | 7.72E+05 | 28.23 | 3867 | 19.54 | 13.47 |
| 16 | 1 | 22 | 7.72E+05 | 36.10 | 3739 | 18.90 | 13.03 |
| 16 | 9 | 57 | 7.72E+05 | 44.68 | 3397 | 17.19 | 11.85 |
| 16 | 17 | 45 | 7.72E+05 | 52.48 | 3221 | 16.31 | 11.24 |
| 17 | 5 | 47 | 7.72E+05 | 64.52 | 3058 | 15.50 | 10.68 |
| 17 | 17 | 30 | 7.72E+05 | 76.23 | 2732 | 13.87 | 9.56 |
| 17 | 21 | 46 | 7.72E+05 | 80.50 | 2573 | 13.07 | 9.01 |
| 18 | 9 | 38 | 7.72E+05 | 92.37 | 2442 | 12.42 | 8.56 |
| 18 | 21 | 13 | 7.72E+05 | 103.95 | 1967 | 10.04 | 6.92 |
| 19 | 9 | 11 | 7.72E+05 | 115.92 | 2109 | 10.75 | 7.41 |
| 19 | 21 | 30 | 7.72E+05 | 128.23 | 2044 | 10.43 | 7.19 |

HEADSPACE

| date | hour | minute | time | elapsed | area | conc | mass |
|---|---|---|---|---|---|---|---|
| 14 | 12 | 49 | 7.72E+05 | -0.45 | 0 | -0.05 | -0.17 |
| 14 | | | | | | -0.05 | |
| 14 | 14 | 6 | 7.72E+05 | 0.83 | 3317 | 0.77 | 2.89 |
| 14 | 15 | 15 | 7.72E+05 | 1.98 | 6077 | 1.45 | 5.44 |
| 14 | 16 | 17 | 7.72E+05 | 3.02 | 6702 | 1.61 | 6.02 |
| 14 | 17 | 14 | 7.72E+05 | 3.97 | 8170 | 1.97 | 7.38 |
| 14 | 19 | 8 | 7.72E+05 | 5.87 | 8859 | 2.14 | 8.01 |
| 14 | 21 | 20 | 7.72E+05 | 8.07 | 6940 | 1.67 | 6.24 |
| 14 | 23 | 26 | 7.72E+05 | 10.17 | 9458 | 2.29 | 8.56 |
| 15 | 1 | 41 | 7.72E+05 | 12.42 | 9638 | 2.33 | 8.73 |
| 15 | 5 | 50 | 7.72E+05 | 16.57 | 10457 | 2.54 | 9.49 |
| 15 | 10 | 10 | 7.72E+05 | 20.90 | 9520 | 2.31 | 8.62 |
| 15 | 13 | 50 | 7.72E+05 | 24.57 | 9635 | 2.33 | 8.73 |
| 15 | 17 | 54 | 7.72E+05 | 28.63 | 10474 | 2.54 | 9.50 |
| 16 | 1 | 46 | 7.72E+05 | 36.50 | 8613 | 2.08 | 7.78 |
| 16 | 10 | 20 | 7.72E+05 | 45.07 | 8078 | 1.95 | 7.29 |
| 16 | 18 | 43 | 7.72E+05 | 53.45 | 7516 | 1.81 | 6.77 |
| 17 | 6 | 11 | 7.72E+05 | 64.92 | 8302 | 2.00 | 7.50 |
| 17 | 17 | 53 | 7.72E+05 | 76.62 | 7281 | 1.75 | 6.55 |
| 17 | 22 | 10 | 7.72E+05 | 80.90 | 6972 | 1.68 | 6.27 |
| 18 | 10 | 3 | 7.72E+05 | 92.78 | 6686 | 1.61 | 6.00 |
| 18 | 21 | 33 | 7.72E+05 | 104.28 | 5566 | 1.33 | 4.97 |
| 19 | 9 | 34 | 7.72E+05 | 116.30 | 5404 | 1.29 | 4.82 |
| 19 | 21 | 53 | 7.72E+05 | 128.62 | 4013 | 0.94 | 3.53 |

GAS

| date | hour | minute | time | elapsed | area | conc | mass |
|---|---|---|---|---|---|---|---|
| 14 | 11 | 59 | 7.72E+05 | -1.28 | 0 | -0.05 | -0.02 |
| 14 | | | | | | -0.05 | |
| 14 | 14 | 25 | 7.72E+05 | 1.15 | 4624 | 1.10 | 0.55 |
| 14 | 15 | 33 | 7.72E+05 | 2.28 | 5778 | 1.38 | 0.69 |
| 14 | 16 | 34 | 7.72E+05 | 3.30 | 7242 | 1.74 | 0.87 |
| 14 | 17 | 32 | 7.72E+05 | 4.27 | 8744 | 2.11 | 1.06 |
| 14 | 19 | 27 | 7.72E+05 | 6.18 | 10669 | 2.59 | 1.29 |
| 14 | 21 | 40 | 7.72E+05 | 8.40 | 11271 | 2.74 | 1.37 |
| 14 | 23 | 50 | 7.72E+05 | 10.57 | 9636 | 2.33 | 1.17 |
| 15 | 1 | 57 | 7.72E+05 | 12.68 | 6883 | 1.65 | 0.83 |
| 15 | 6 | 6 | 7.72E+05 | 16.83 | 10001 | 2.42 | 1.21 |
| 15 | 10 | 26 | 7.72E+05 | 21.17 | 11464 | 2.79 | 1.39 |
| 15 | 14 | 6 | 7.72E+05 | 24.83 | 11805 | 2.87 | 1.43 |
| 15 | 18 | 5 | 7.72E+05 | 28.82 | 11199 | 2.72 | 1.36 |
| 16 | 2 | 1 | 7.72E+05 | 36.75 | 10920 | 2.65 | 1.33 |
| 16 | 10 | 40 | 7.72E+05 | 45.40 | 10671 | 2.59 | 1.29 |
| 16 | 18 | 27 | 7.72E+05 | 53.18 | 10185 | 2.47 | 1.23 |
| 17 | 6 | 25 | 7.72E+05 | 65.15 | 10043 | 2.43 | 1.22 |
| 17 | 18 | 11 | 7.72E+05 | 76.92 | 9708 | 2.35 | 1.18 |
| 17 | 22 | 26 | 7.72E+05 | 81.17 | 9318 | 2.26 | 1.13 |
| 18 | 10 | 24 | 7.72E+05 | 93.13 | 8108 | 1.96 | 0.98 |
| 18 | 21 | 44 | 7.72E+05 | 104.47 | 7610 | 1.83 | 0.92 |
| 19 | 9 | 45 | 7.72E+05 | 116.48 | 7043 | 1.69 | 0.85 |
| 19 | 22 | 13 | 7.72E+05 | 128.95 | 6502 | 1.56 | 0.78 |

| AVG TIME | TOTAL DCE MASS |
|---|---|
| -0.87 | -0.05 |
| 0.00 | 40.03 |
| 0.80 | 36.91 |
| 1.94 | 30.13 |
| 2.97 | 27.03 |
| 3.93 | 27.49 |
| 5.83 | 27.10 |
| 8.04 | 24.84 |
| 10.15 | 26.32 |
| 12.37 | 26.05 |
| 16.53 | 25.76 |
| 20.86 | 25.18 |
| 24.53 | 24.38 |
| 28.56 | 24.33 |
| 36.45 | 22.14 |
| 45.05 | 20.43 |
| 53.04 | 19.25 |
| 64.86 | 19.39 |
| 76.59 | 17.29 |
| 80.86 | 16.40 |
| 92.76 | 15.54 |
| 104.23 | 12.81 |
| 116.23 | 13.08 |
| 128.60 | 11.50 |

| ln(C/Co) DCE | Time elapsed |
|---|---|
| 0.00 | 0 |
| -0.05 | 0.98 |
| -0.12 | 2.87 |
| -0.16 | 5.08 |
| -0.19 | 7.13 |
| -0.20 | 9.43 |
| -0.29 | 13.60 |
| -0.28 | 17.92 |
| -0.35 | 21.60 |
| -0.40 | 25.65 |
| -0.44 | 33.52 |
| -0.53 | 42.10 |
| -0.58 | 49.90 |
| -0.63 | 61.93 |
| -0.75 | 73.65 |
| -0.80 | 77.92 |
| -0.86 | 89.78 |
| -1.07 | 101.37 |
| -1.00 | 113.33 |
| -1.03 | 125.65 |

Linear Regression DCE

| | | | |
|---|---|---|---|
| Sx | 873.4166 | A = | -0.12929 intercept |
| Sy | -9.73885 | | |
| Sxy | -682.030 | B = | -0.00818 slope (k |
| Sxsq | 69490.81 | | |
| Sysq | 6.916965 | r = | -0.98325 |
| SxSx | 762856.6 | | |
| SySy | 94.84527 | | |
| n | 20 | | |

| x | y | x*y | x^2 | y^2 | calc.Y |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | -0.12929 |
| 0.983333 | -0.05503 | -0.05411 | 0.966944 | 0.003029 | -0.13734 |
| 2.866666 | -0.12348 | -0.35399 | 8.217777 | 0.015249 | -0.15277 |
| 5.083333 | -0.15594 | -0.79273 | 25.84027 | 0.024319 | -0.17092 |
| 7.133333 | -0.19405 | -1.38428 | 50.88444 | 0.037658 | -0.18771 |
| 9.433333 | -0.19968 | -1.88367 | 88.98777 | 0.039873 | -0.20655 |
| 13.6 | -0.29036 | -3.94897 | 184.96 | 0.084312 | -0.24067 |
| 17.91666 | -0.28352 | -5.07986 | 321.0069 | 0.080387 | -0.27602 |
| 21.6 | -0.34803 | -7.51757 | 466.56 | 0.121128 | -0.30619 |
| 25.65 | -0.40230 | -10.3191 | 657.9225 | 0.161849 | -0.33935 |
| 33.51666 | -0.43559 | -14.5997 | 1123.366 | 0.189745 | -0.40378 |
| 42.1 | -0.53040 | -22.3300 | 1772.41 | 0.281330 | -0.47407 |
| 49.9 | -0.58293 | -29.0886 | 2490.01 | 0.339818 | -0.53795 |
| 61.93333 | -0.63418 | -39.2771 | 3835.737 | 0.402189 | -0.63650 |
| 73.65 | -0.74529 | -54.8910 | 5424.322 | 0.555466 | -0.73245 |
| 77.91666 | -0.80432 | -62.6701 | 6071.006 | 0.646934 | -0.76740 |
| 89.78333 | -0.85571 | -76.8290 | 8061.046 | 0.732250 | -0.86458 |
| 101.3666 | -1.06794 | -108.254 | 10275.20 | 1.140516 | -0.95944 |
| 113.3333 | -0.99965 | -113.294 | 12844.44 | 0.999307 | -1.05744 |
| 125.65 | -1.03033 | -129.461 | 15787.92 | 1.061596 | -1.15831 |

Filename: BATCH2

# APPENDIX C

## Continuous Flow Degradation Data

Continuous Flow Experiment
DCM & DCA

LIQUID DATA

| date | hour | minute | time number | time elapsed | area | area | DCM CONC | DCA CONC | DCM MASS | DCA MASS |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 9 | 46 | 7.71E+05 | 0.00 | 845 | 1434 | 9.11 | 3.26 | 6.94 | 2.49 |
| 6 | 11 | 1 | 7.71E+05 | 1.25 | 910 | 1420 | 9.70 | 3.23 | 7.39 | 2.46 |
| 6 | 12 | 42 | 7.71E+05 | 2.93 | 918 | 1642 | 9.77 | 3.68 | 7.44 | 2.80 |
| 6 | 14 | 25 | 7.71E+05 | 4.65 | 966 | 1779 | 10.20 | 3.95 | 7.77 | 3.01 |
| 6 | 16 | 2 | 7.71E+05 | 6.27 | 1052 | 3075 | 10.97 | 6.54 | 8.36 | 4.99 |
| 6 | 17 | 42 | 7.71E+05 | 7.93 | 1119 | 3991 | 11.58 | 8.38 | 8.82 | 6.38 |
| 6 | 19 | 54 | 7.71E+05 | 10.13 | 1189 | 4423 | 12.21 | 9.24 | 9.30 | 7.04 |
| 6 | 22 | 45 | 7.71E+05 | 12.98 | 1328 | 6201 | 13.46 | 12.80 | 10.25 | 9.75 |
| 7 | 0 | 47 | 7.71E+05 | 15.02 | 1403 | 6637 | 14.13 | 13.67 | 10.77 | 10.41 |
| 7 | 5 | 3 | 7.71E+05 | 19.28 | 1416 | 8070 | 14.25 | 16.53 | 10.86 | 12.60 |
| 7 | 8 | 8 | 7.71E+05 | 22.37 | 1443 | 8622 | 15.17 | 17.06 | 11.56 | 13.00 |
| 7 | 13 | 53 | 7.71E+05 | 28.12 | 1465 | 9617 | 15.39 | 19.05 | 11.73 | 14.52 |
| 7 | 20 | 21 | 7.71E+05 | 34.58 | 1490 | 10048 | 15.64 | 19.91 | 11.92 | 15.17 |
| 8 | 1 | 56 | 7.71E+05 | 40.17 | 1519 | 10736 | 15.93 | 21.29 | 12.14 | 16.22 |
| 8 | 12 | 2 | 7.71E+05 | 50.27 | 1507 | 11269 | 16.25 | 22.85 | 12.38 | 17.41 |
| 8 | 22 | 16 | 7.71E+05 | 60.50 | 1417 | 10829 | 15.35 | 21.97 | 11.69 | 16.74 |
| 9 | 8 | 32 | 7.71E+05 | 70.77 | 1447 | 11582 | 14.07 | 24.14 | 10.72 | 18.39 |
| 9 | 18 | 27 | 7.71E+05 | 80.68 | 1436 | 11434 | 13.98 | 23.84 | 10.65 | 18.17 |
| 9 | 23 | 45 | 7.71E+05 | 85.98 | 1443 | 11355 | 14.03 | 23.68 | 10.69 | 18.05 |
| 10 | 9 | 29 | 7.71E+05 | 95.72 | 1436 | 11115 | 13.99 | 22.77 | 10.66 | 17.35 |
| 10 | 15 | 26 | 7.71E+05 | 101.67 | 1489 | 12162 | 14.41 | 24.86 | 10.98 | 18.95 |
| 10 | 17 | 30 | 7.71E+05 | 103.73 | 1487 | 12110 | 14.39 | 24.76 | 10.97 | 18.87 |
| 10 | 23 | 7 | 7.71E+05 | 109.35 | 1503 | 12269 | 14.52 | 25.08 | 11.07 | 19.11 |
| 11 | 9 | 52 | 7.71E+05 | 120.10 | 1489 | 12460 | 14.71 | 24.89 | 11.21 | 18.97 |
| 11 | 17 | 14 | 7.71E+05 | 127.47 | 1539 | 12104 | 15.16 | 24.18 | 11.55 | 18.42 |
| 12 | 0 | 22 | 7.71E+05 | 134.60 | 1546 | 12646 | 15.22 | 25.26 | 11.60 | 19.25 |
| 12 | 12 | 6 | 7.71E+05 | 146.33 | 1515 | 11954 | 15.51 | 24.70 | 11.82 | 18.82 |
| 13 | 10 | 41 | 7.71E+05 | 168.92 | 1546 | 11776 | 15.79 | 24.35 | 12.03 | 18.55 |

GAS DATA

| date | hour | minute | number time | time elapsed | area | area | DCM CONC | DCA CONC | DCM MASS | DCA MASS |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 10 | 10 | 7.71E+05 | 0.40 | 6079 | 3922 | 2.20 | 0.86 | 1.10 | 0.43 |
| 6 | 11 | 54 | 7.71E+05 | 2.13 | 5610 | 3543 | 2.00 | 0.76 | 1.00 | 0.38 |
| 6 | 13 | 30 | 7.71E+05 | 3.73 | 8584 | 5926 | 3.26 | 1.33 | 1.63 | 0.67 |
| | | | | | | | -0.37 | -0.08 | -0.19 | -0.04 |
| 6 | 16 | 26 | 7.71E+05 | 6.67 | 5693 | 5605 | 2.03 | 1.26 | 1.02 | 0.63 |
| 6 | 18 | 19 | 7.71E+05 | 8.55 | 6690 | 6384 | 2.46 | 1.44 | 1.23 | 0.72 |
| 6 | 20 | 42 | 7.71E+05 | 10.93 | 3472 | 5057 | 1.09 | 1.13 | 0.55 | 0.56 |
| 6 | 23 | 36 | 7.71E+05 | 13.83 | 5547 | 7590 | 1.97 | 1.73 | 0.99 | 0.86 |
| 7 | 1 | 35 | 7.71E+05 | 15.82 | 4543 | 7340 | 1.55 | 1.67 | 0.77 | 0.83 |
| 7 | 6 | 6 | 7.71E+05 | 20.33 | 4407 | 8736 | 1.49 | 2.00 | 0.74 | 1.00 |
| 7 | 8 | 56 | 7.71E+05 | 23.17 | 5604 | 10371 | 2.42 | 2.66 | 1.21 | 1.33 |
| 7 | 15 | 9 | 7.71E+05 | 29.38 | 3550 | 9617 | 1.50 | 2.47 | 0.75 | 1.23 |
| 7 | 21 | 34 | 7.71E+05 | 35.80 | 2887 | 9522 | 1.20 | 2.44 | 0.60 | 1.22 |
| 8 | 2 | 42 | 7.71E+05 | 40.93 | 4040 | 10974 | 1.71 | 2.82 | 0.86 | 1.41 |
| 8 | 13 | 9 | 7.71E+05 | 51.38 | 3167 | 8133 | 1.48 | 2.19 | 0.74 | 1.09 |
| 8 | 23 | 31 | 7.71E+05 | 61.75 | 2432 | 7144 | 1.16 | 1.95 | 0.58 | 0.97 |
| | | | | | | | -0.09 | -0.04 | -0.05 | -0.02 |
| 9 | 9 | 48 | 7.71E+05 | 72.03 | 3594 | 12724 | 1.52 | 3.25 | 0.76 | 1.63 |
| | | | | | | | 0.02 | -0.00 | 0.01 | -0.00 |
| 9 | 19 | 6 | 7.71E+05 | 81.33 | 3165 | 9369 | 1.34 | 2.39 | 0.67 | 1.20 |
| | | | | | | | 0.02 | -0.00 | 0.01 | -0.00 |
| 10 | 10 | 8 | 7.71E+05 | 96.37 | 2524 | 7758 | 1.08 | 1.98 | 0.54 | 0.99 |
| 10 | 16 | 20 | 7.71E+05 | 102.57 | 3963 | 11083 | 1.64 | 2.81 | 0.82 | 1.40 |
| 10 | 18 | 45 | 7.71E+05 | 104.98 | 3789 | 12108 | 1.57 | 3.06 | 0.79 | 1.53 |
| 11 | 0 | 13 | 7.71E+05 | 110.45 | 3031 | 11525 | 1.28 | 2.92 | 0.64 | 1.46 |
| 11 | 11 | 0 | 7.71E+05 | 121.23 | 3318 | 12855 | 1.58 | 3.63 | 0.79 | 1.82 |
| 11 | 17 | 57 | 7.71E+05 | 128.18 | 3077 | 12813 | 1.45 | 3.62 | 0.73 | 1.81 |
| 12 | 13 | 8 | 7.71E+05 | 147.37 | 3441 | 12813 | 1.49 | 3.09 | 0.75 | 1.54 |
| 13 | 11 | 44 | 7.71E+05 | 169.97 | 2203 | 8979 | 1.01 | 2.21 | 0.50 | 1.11 |

HEADSPACE
DATA

| date | hour | minute | time | elapsed | area | area | DCM CONC | DCA CONC | DCM MASS | DCA MASS |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 9 | 21 | 7.71E+05 | -0.42 | 7433 | 4406 | 2.77 | 0.97 | 9.42 | 3.30 |
| 6 | 11 | 25 | 7.71E+05 | 1.65 | 8136 | 5427 | 3.07 | 1.21 | 10.43 | 4.13 |
| 6 | 13 | 5 | 7.71E+05 | 3.32 | 7905 | 5503 | 2.97 | 1.23 | 10.10 | 4.19 |
| 6 | 14 | 48 | 7.71E+05 | 5.03 | 8616 | 6482 | 3.27 | 1.46 | 11.12 | 4.98 |
| 6 | 18 | 5 | 7.71E+05 | 8.32 | 5969 | 4423 | 2.15 | 0.97 | 7.31 | 3.31 |
| 6 | 20 | 17 | 7.71E+05 | 10.52 | 7437 | 6305 | 2.77 | 1.42 | 9.42 | 4.84 |
| 6 | 23 | 4 | 7.71E+05 | 13.30 | 7505 | 6714 | 2.80 | 1.52 | 9.52 | 5.17 |
| 7 | 1 | 12 | 7.71E+05 | 15.43 | 4698 | 4257 | 1.61 | 0.94 | 5.48 | 3.18 |
| 7 | 5 | 50 | 7.71E+05 | 20.07 | 5273 | 5879 | 1.86 | 1.32 | 6.31 | 4.49 |
| 7 | 8 | 32 | 7.71E+05 | 22.77 | 4411 | 5307 | 1.88 | 1.34 | 6.40 | 4.56 |
| 7 | 14 | 40 | 7.71E+05 | 28.90 | 4877 | 7074 | 2.09 | 1.80 | 7.11 | 6.13 |
| 7 | 21 | 10 | 7.71E+05 | 35.40 | 5386 | 7361 | 2.32 | 1.89 | 7.88 | 6.39 |
| 8 | 2 | 29 | 7.71E+05 | 40.72 | 4357 | 7177 | 1.86 | 1.83 | 6.31 | 6.22 |
| 8 | 12 | 54 | 7.71E+05 | 51.13 | 3768 | 6908 | 1.74 | 1.89 | 5.91 | 6.42 |
| 8 | 23 | 11 | 7.71E+05 | 61.42 | 4036 | 7781 | 1.85 | 2.10 | 6.30 | 7.14 |
| | | | | | | | -0.09 | -0.04 | -0.32 | -0.15 |
| 9 | 9 | 28 | 7.71E+05 | 71.70 | 4068 | 7760 | 1.71 | 1.98 | 5.82 | 6.74 |
| | | | | | | | 0.02 | -0.00 | 0.08 | -0.02 |
| 9 | 18 | 51 | 7.71E+05 | 81.08 | 4082 | 7948 | 1.72 | 2.03 | 5.84 | 6.90 |
| | | | | | | | 0.02 | -0.00 | 0.08 | -0.02 |
| 10 | 9 | 54 | 7.71E+05 | 96.13 | 3790 | 6957 | 1.57 | 1.78 | 5.35 | 6.05 |
| 10 | 15 | 50 | 7.71E+05 | 102.07 | 5813 | 8218 | 2.37 | 2.09 | 8.04 | 7.11 |
| 10 | 18 | 20 | 7.71E+05 | 104.57 | 5412 | 7503 | 2.21 | 1.91 | 7.51 | 6.51 |
| 10 | 23 | 32 | 7.71E+05 | 109.77 | 3275 | 7250 | 1.37 | 1.85 | 4.67 | 6.29 |
| 11 | 10 | 41 | 7.71E+05 | 120.92 | 3436 | 7847 | 1.65 | 2.19 | 5.60 | 7.43 |
| 11 | 17 | 37 | 7.71E+05 | 127.85 | 3249 | 7502 | 1.55 | 2.09 | 5.26 | 7.10 |
| 12 | 12 | 55 | 7.71E+05 | 147.15 | 4041 | 7549 | 1.73 | 1.89 | 5.87 | 6.42 |
| 13 | 11 | 28 | 7.71E+05 | 169.70 | 3777 | 7220 | 1.62 | 1.81 | 5.52 | 6.16 |

INFLUENT DATA

| date | hour | minute | time number | time elapsed | area | area | DCM CONC | DCA CONC | DCM MASS | DCA MASS |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | | | 7.71E+05 | | | | 1.51 | 0.39 | 1.15 | 0.30 |
| 6 | 10 | 37 | 7.71E+05 | 0.85 | 3563 | 17074 | 33.57 | 34.54 | 25.59 | 26.32 |
| 6 | 12 | 18 | 7.71E+05 | 2.53 | 4728 | 22361 | 44.06 | 45.12 | 33.57 | 34.38 |
| 6 | 14 | 0 | 7.71E+05 | 4.23 | 4704 | 21742 | 43.84 | 43.88 | 33.41 | 33.43 |
| 6 | 19 | 29 | 7.71E+05 | 9.72 | 4588 | 21950 | 42.80 | 44.29 | 32.61 | 33.75 |
| 6 | 22 | 21 | 7.71E+05 | 12.58 | 4599 | 21196 | 42.90 | 42.79 | 32.69 | 32.60 |
| 7 | 0 | 15 | 7.71E+05 | 14.46 | 4562 | 21415 | 42.56 | 43.22 | 32.43 | 32.94 |
| 7 | 5 | 27 | 7.71E+05 | 19.68 | 4531 | 21519 | 42.28 | 43.43 | 32.22 | 33.10 |
| 7 | 9 | 24 | 7.71E+05 | 23.63 | 4439 | 20867 | 45.13 | 41.55 | 34.39 | 31.66 |
| 7 | 14 | 16 | 7.71E+05 | 28.50 | 4281 | 20260 | 43.55 | 40.34 | 33.19 | 30.74 |
| 7 | 20 | 45 | 7.71E+05 | 34.98 | 4168 | 20101 | 42.42 | 40.02 | 32.32 | 30.50 |
| 8 | 3 | 5 | 7.71E+05 | 41.32 | 4000 | 18579 | 40.74 | 36.98 | 31.04 | 28.18 |
| 8 | 12 | 26 | 7.71E+05 | 50.67 | 4044 | 19178 | 41.62 | 38.67 | 31.71 | 29.47 |
| 8 | 22 | 39 | 7.71E+05 | 60.88 | 3922 | 19289 | 40.40 | 38.89 | 30.78 | 29.64 |
| 8 | 23 | 45 | 7.71E+05 | 61.98 | 3797 | 17800 | 39.15 | 35.92 | 29.83 | 27.37 |
| 9 | 9 | 5 | 7.71E+05 | 71.32 | 3701 | 17717 | 32.10 | 36.41 | 24.46 | 27.74 |
| 9 | 10 | 19 | 7.71E+05 | 72.55 | 3938 | 18379 | 33.99 | 37.73 | 25.90 | 28.75 |
| 9 | 19 | 20 | 7.71E+05 | 81.57 | 3819 | 17843 | 33.04 | 36.66 | 25.18 | 27.93 |
| 10 | 10 | 27 | 7.71E+05 | 96.68 | 3729 | 18189 | 32.33 | 36.92 | 24.64 | 28.13 |
| 10 | 15 | 0 | 7.71E+05 | 101.23 | 3860 | 18516 | 33.38 | 37.57 | 25.43 | 28.63 |
| 10 | 16 | 57 | 7.71E+05 | 103.18 | 3848 | 17960 | 33.28 | 36.46 | 25.36 | 27.78 |
| 10 | 23 | 47 | 7.71E+05 | 110.02 | 3580 | 16815 | 31.14 | 34.17 | 23.73 | 26.04 |
| 11 | 10 | 17 | 7.71E+05 | 120.52 | 3587 | 17517 | 33.59 | 35.00 | 25.60 | 26.67 |
| 11 | 18 | 16 | 7.71E+05 | 128.50 | 3707 | 17411 | 34.67 | 34.79 | 26.42 | 26.51 |
| 12 | 0 | 47 | 7.71E+05 | 135.02 | 3550 | 16787 | 33.26 | 33.54 | 25.34 | 25.56 |
| 12 | 12 | 31 | 7.71E+05 | 146.75 | 3497 | 17404 | 33.35 | 35.60 | 25.41 | 27.13 |
| 12 | 14 | 5 | 7.71E+05 | 148.32 | 4241 | 19285 | 39.48 | 39.37 | 30.08 | 30.00 |
| 13 | 11 | 5 | 7.71E+05 | 169.32 | 4015 | 19369 | 37.44 | 39.53 | 28.53 | 30.12 |

Filename: FLOWDATA

Reactor Data For Continuous Flow Experiment

12/6/87 - 12/13/87

| Date | Time Hour | Time Min | | Time Elapsed | Head P | Liquid level | Liquid volume | liquid collected | Flow rate | Retention Time |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 10 | 38 | 770818.6 | 0 | 0 | 6.9 | 560 | | | |
| 6 | 11 | 2 | 770819.0 | 0.4 | 0.2 | 6.8 | 551 | 19 | 27.2 | 20.3 |
| 6 | 11 | 25 | 770819.4 | 0.8 | 0.2 | 6.7 | 543 | 22 | 36.2 | 15.0 |
| 6 | 12 | 13 | 770820.2 | 1.6 | 0.2 | 6.7 | 543 | 27 | 33.7 | 16.1 |
| 6 | 13 | 7 | 770821.1 | 2.5 | 0.2 | 6.7 | 543 | | | |
| 6 | 13 | 32 | 770821.5 | 2.9 | 0.2 | 6.7 | 543 | 34 | 25.8 | 21.0 |
| 6 | 14 | 27 | 770822.4 | 3.8 | 0.2 | 6.7 | 543 | 25 | 27.3 | 19.9 |
| 6 | 14 | 50 | 770822.8 | 4.2 | 0.1 | 6.7 | 543 | | | |
| 6 | 15 | 14 | 770823.2 | 4.6 | 0 | 6.7 | 543 | 16 | 20.4 | 26.6 |
| 6 | 16 | 5 | 770824.0 | 5.4 | 0 | 6.7 | 543 | 29 | 34.1 | 15.9 |
| 6 | 17 | 3 | 770825.0 | 6.4 | 0 | 6.75 | 547 | 29 | 34.2 | 16.0 |
| 6 | 17 | 45 | 770825.7 | 7.1 | 0 | 6.9 | 560 | 21 | 47.4 | 11.8 |
| 6 | 19 | 36 | 770827.6 | 9.0 | 0 | 6.9 | 560 | 62 | 33.5 | 16.7 |
| 6 | 20 | 46 | 770828.7 | 10.1 | 0 | 7 | 568 | 35 | 36.9 | 15.4 |
| 6 | 22 | 51 | 770830.8 | 12.2 | 0 | 7.3 | 592 | 61 | 41.0 | 14.5 |
| 6 | 23 | 52 | 770831.8 | 13.2 | 0 | 7.3 | 592 | 38 | 37.4 | 15.8 |
| 7 | 1 | 5 | 770833.0 | 14.4 | 0 | 7.15 | 580 | 55 | 35.2 | 16.5 |
| 7 | 5 | 3 | 770837.0 | 18.4 | 0 | 7 | 568 | 117 | 26.4 | 21.5 |
| 7 | 8 | 10 | 770840.1 | 21.5 | -0.3 | 7.2 | 584 | 71 | 28.0 | 20.9 |
| 7 | 9 | 0 | 770841 | 22.4 | 0 | 7.2 | 584 | | | |
| 7 | 14 | 0 | 770846 | 27.4 | 0.1 | 6.9 | 560 | 213 | 32.3 | 17.3 |
| 7 | 16 | 5 | 770848.0 | 29.4 | 0 | 7 | 568 | 50 | 27.9 | 20.3 |
| 7 | 17 | 27 | 770849.4 | 30.8 | 0 | 7 | 568 | 52 | 38.0 | 14.9 |
| 7 | 20 | 3 | 770852.0 | 33.4 | 0 | 6.9 | 560 | 95 | 33.4 | 16.7 |
| 8 | 1 | 48 | 770857.8 | 39.2 | 0.2 | 6.5 | 527 | 196 | 28.4 | 18.5 |
| 8 | 12 | 0 | 770868 | 49.4 | 0 | 7 | 568 | 248 | 28.3 | 20.1 |
| 8 | 21 | 34 | 770877.5 | 58.9 | 0.2 | 6.6 | 535 | 316 | 29.6 | 18.1 |
| 9 | 8 | 7 | 770888.1 | 69.5 | 0.3 | 6.2 | 503 | 325 | 27.7 | 18.1 |
| 9 | 14 | 0 | 770894 | 75.4 | 0.3 | 6.3 | 511 | 146 | 26.2 | 19.5 |
| 9 | 17 | 7 | 770897.1 | 78.5 | 0.2 | 6.5 | 527 | 65 | 26.1 | 20.2 |
| 9 | 18 | 39 | 770898.6 | 80.0 | 0.1 | 6.9 | 560 | 26 | 38.1 | 14.7 |
| 9 | 23 | 59 | 770903.9 | 85.3 | -0.2 | 7.4 | 600 | 96 | 25.6 | 23.4 |
| 10 | 9 | 12 | 770913.2 | 94.6 | 0 | 7.4 | 600 | 250 | 27.1 | 22.1 |
| 10 | 14 | 0 | 770918 | 99.4 | -0.2 | 7.6 | 616 | 118 | 28.0 | 22.0 |
| 10 | 17 | 27 | 770921.4 | 102.8 | -0.3 | 7.6 | 616 | 92 | 26.7 | 23.1 |
| 10 | 22 | 0 | 770926 | 107.4 | 0 | 7.3 | 592 | 146 | 26.7 | 22.1 |
| 11 | 9 | 50 | 770937.8 | 119.2 | 0 | 7.5 | 608 | 298 | 26.6 | 22.9 |
| 11 | 17 | 15 | 770945.2 | 126.6 | 0 | 7.3 | 592 | | | |
| 12 | 0 | 20 | 770952.3 | 133.7 | 0 | 7.2 | 584 | 207 | 28.1 | 20.8 |
| 12 | 10 | 55 | 770962.9 | 144.3 | 0 | 6.9 | 560 | 309 | 26.9 | 20.8 |
| 13 | 10 | 40 | 770986.6 | 168.0 | 0 | 7.1 | 576 | 540 | 23.4 | 24.6 |

Filename: REACDATA

TO CALCULATE K

| time elapsed | ###EFFLUENT### conc DCM | DONC DCA | ###INFLUENT#### CONC DCM | DONC DCA | RETENTION TIME | K DCM | K DCA | % removal DCM | % removal DCA |
|---|---|---|---|---|---|---|---|---|---|
| ######### | ########## | | | | | | | | |
| 60.50 | 15.35 | 21.97 | 40.40 | 38.89 | 18.1 | -0.090 | -0.042 | 0.62 | 0.43 |
| 70.77 | 14.07 | 24.14 | 32.10 | 36.41 | 18.1 | -0.071 | -0.028 | 0.56 | 0.34 |
| 80.68 | 13.98 | 23.84 | 33.04 | 36.66 | 19.05 | -0.072 | -0.028 | 0.58 | 0.35 |
| 95.72 | 13.99 | 22.77 | 32.33 | 36.92 | 22.1 | -0.059 | -0.028 | 0.57 | 0.38 |
| 101.67 | 14.41 | 24.86 | 33.38 | 37.57 | 22 | -0.060 | -0.023 | 0.57 | 0.34 |
| 103.73 | 14.39 | 24.76 | 33.28 | 36.46 | 23.1 | -0.057 | -0.020 | 0.57 | 0.32 |
| 109.35 | 14.52 | 25.08 | 31.14 | 34.17 | 22.1 | -0.052 | -0.016 | 0.53 | 0.27 |
| 120.10 | 14.71 | 24.89 | 33.59 | 35.00 | 22.9 | -0.056 | -0.018 | 0.56 | 0.29 |
| 127.47 | 15.16 | 24.18 | 34.67 | 34.79 | 21.85 | -0.059 | -0.020 | 0.56 | 0.30 |
| 134.60 | 15.22 | 25.26 | 33.26 | 33.54 | 20.8 | -0.057 | -0.016 | 0.54 | 0.25 |
| 146.33 | 15.51 | 24.70 | 33.35 | 35.60 | 20.8 | -0.055 | -0.021 | 0.53 | 0.31 |
| 168.92 | 15.79 | 24.35 | 37.44 | 39.53 | 24.6 | -0.056 | -0.025 | 0.58 | 0.38 |
| | | | | after 3 RT | | -0.062 | -0.024 | 0.56 | 0.33 |
| | | | | after 3.5 RT | | -0.059 | -0.022 | 0.56 | 0.32 |
| | | | | | | avg | avg | avg | avg |

Filename: FLOWDATA

Calculation of Tracer Curve

| ###INFLUENT### | | | | | ###Tracer Curve### | |
|---|---|---|---|---|---|---|
| DCM CONC | DCA CONC | Retention Time | dc/dt DCM | dc/dt DCA | C DCM | C DCA |
| 33.57 | 34.54 | 20.3 | 1.38 | 1.42 | 17.50 | 6.20 |
| 44.06 | 45.12 | 16.1 | 1.65 | 2.42 | 20.28 | 10.27 |
| 43.84 | 43.88 | 20.5 | 1.15 | 1.64 | 22.23 | 13.06 |
| 42.80 | 44.29 | 16.7 | 1.23 | 1.87 | 28.98 | 23.31 |
| 42.90 | 42.79 | 14.5 | 0.96 | 1.34 | 31.73 | 27.16 |
| 42.56 | 43.22 | 15.8 | 0.69 | 1.02 | 33.04 | 29.09 |
| 42.28 | 43.43 | 21.5 | 0.43 | 0.67 | 35.27 | 32.56 |
| 45.13 | 41.55 | 20.9 | 0.47 | 0.43 | 37.14 | 34.26 |
| 43.55 | 40.34 | 17.3 | 0.37 | 0.35 | 38.94 | 35.97 |
| 42.42 | 40.02 | 16.7 | 0.21 | 0.24 | 40.29 | 37.54 |
| 40.74 | 36.98 | 18.5 | 0.02 | -0.03 | 40.45 | 37.35 |
| 41.62 | 38.67 | 20.1 | 0.06 | 0.07 | 40.99 | 37.96 |
| 40.40 | 38.89 | 18.1 | -0.03 | 0.05 | 40.66 | 38.49 |
| 39.15 | 35.92 | 18.1 | -0.08 | -0.14 | 40.56 | 38.33 |
| 32.10 | 36.41 | 18.1 | -0.47 | -0.11 | 36.20 | 37.34 |
| 33.99 | 37.73 | 18.1 | -0.12 | 0.02 | 36.05 | 37.37 |
| 33.04 | 36.66 | 14.7 | -0.20 | -0.05 | 34.20 | 36.93 |
| 32.33 | 36.92 | 22.1 | -0.08 | -0.00 | 32.92 | 36.92 |
| 33.38 | 37.57 | 22 | 0.02 | 0.03 | 33.02 | 37.06 |
| 33.28 | 36.46 | 23.1 | 0.01 | -0.03 | 33.04 | 37.01 |
| 31.14 | 34.17 | 22.1 | -0.09 | -0.13 | 32.45 | 36.13 |
| 33.59 | 35.00 | 22.9 | 0.05 | -0.05 | 32.97 | 35.61 |
| 34.67 | 34.79 | 22 | 0.08 | -0.04 | 33.59 | 35.32 |
| 33.26 | 33.54 | 20.8 | -0.02 | -0.09 | 33.49 | 34.76 |
| 33.35 | 35.60 | 20.8 | -0.01 | 0.04 | 33.41 | 35.24 |
| 39.48 | 39.37 | 20.8 | 0.29 | 0.20 | 33.87 | 35.55 |
| 37.44 | 39.53 | 24.6 | 0.15 | 0.16 | 36.92 | 38.95 |

Filename: APNDXA

# APPENDIX D

## Degradation of 2-chloroethanol Data

CHLOROETHANOL (CEO) DEGRADATION

LIQUID

| date | hour | minute | time elapsed | | area A/Ao | |
|---|---|---|---|---|---|---|
| 10 | 13 | 9 | 7.72E+05 | 0.00 | 0 | |
| 10 | 14 | 26 | 7.72E+05 | 0.00 | 99727 | |
| 10 | 14 | 51 | 7.72E+05 | 0.00 | 22094 | 1.00 |
| 10 | 15 | 20 | 7.72E+05 | 0.48 | 19313 | 0.87 |
| 10 | 15 | 45 | 7.72E+05 | 0.90 | 17880 | 0.81 |
| 10 | 17 | 14 | 7.72E+05 | 2.38 | 12763 | 0.58 |
| 10 | 18 | 3 | 7.72E+05 | 3.20 | 10565 | 0.48 |
| 10 | 19 | 52 | 7.72E+05 | 5.02 | 6523 | 0.29 |
| 10 | 22 | 9 | 7.72E+05 | 7.30 | 3123 | 0.14 |
| 10 | 23 | 37 | 7.72E+05 | 8.77 | 3895 | 0.18 |

| CEO | Time elapsed |
|---|---|
| 0.00 | 0.00 |
| 0.00 | 0.00 |
| -0.13 | 0.48 |
| -0.21 | 0.90 |
| -0.55 | 2.38 |
| -0.74 | 3.20 |
| -1.22 | 5.02 |
| -1.96 | 7.30 |
| -1.74 | 8.77 |

Linear Regression CEO

| | | | |
|---|---|---|---|
| Sx | 28.05 | A = | -0.03100 intercept |
| Sy | -6.54476 | | |
| Sxy | -39.5424 | B = | -0.22448 slope |
| Sxsq | 172.2752 | | |
| Sysq | 9.236924 | r = | -0.97951 |
| SxSx | 786.8025 | | |
| SySy | 42.83391 | | |
| n | 8 | | |

| x | y | x*y | x^2 | y^2 | calc.Y |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | -0.03100 |
| 0.483333 | -0.13452 | -0.06502 | 0.233611 | 0.018097 | -0.13950 |
| 0.9 | -0.21162 | -0.19046 | 0.81 | 0.044784 | -0.23303 |
| 2.383333 | -0.54875 | -1.30786 | 5.680277 | 0.301132 | -0.56601 |
| 3.200000 | -0.73775 | -2.36083 | 10.24000 | 0.544288 | -0.74934 |
| 5.016666 | -1.21997 | -6.12019 | 25.16694 | 1.488330 | -1.15715 |
| 7.3 | -1.95651 | -14.2825 | 53.29000 | 3.827939 | -1.66972 |
| 8.766666 | -1.73561 | -15.2155 | 76.85444 | 3.012350 | -1.99896 |

Filename: CEO

# APPENDIX E

## Control Experiments Data

HYDROLYSIS DCM&DCA

LIQUID DATA

| date | hour | minute | time | elapsed | DCM area | DCA area | DCM conc | DCA conc | DCM mass | DCA mass |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 12 | 20 | 7.69E+05 | 0.00 | 7753 | 27556 | 56.24 | 54.77 | 32.90 | 32.04 |
| 10 | 13 | 26 | 7.69E+05 | 1.10 | 7260 | 26763 | 52.79 | 53.18 | 30.88 | 31.11 |
| 10 | 14 | 53 | 7.69E+05 | 2.55 | 7095 | 26286 | 51.63 | 52.23 | 30.21 | 30.55 |
| 10 | 17 | 18 | 7.69E+05 | 4.97 | 7240 | 26937 | 52.65 | 53.53 | 30.80 | 31.31 |
| 10 | 22 | 12 | 7.69E+05 | 9.87 | 7068 | 26167 | 51.44 | 51.99 | 30.10 | 30.41 |
| 11 | 10 | 7 | 7.69E+05 | 21.78 | 6755 | 25198 | 49.25 | 50.05 | 28.81 | 29.28 |
| 11 | 20 | 37 | 7.69E+05 | 32.28 | 5367 | 25747 | 39.54 | 51.15 | 23.13 | 29.92 |
| 12 | 8 | 22 | 7.69E+05 | 44.03 | 5974 | 25244 | 43.79 | 50.14 | 25.62 | 29.33 |
| 13 | 12 | 36 | 7.69E+05 | 72.27 | 6768 | 24851 | 49.34 | 49.36 | 28.87 | 28.87 |
| 14 | 8 | 47 | 7.69E+05 | 92.45 | 6830 | 24997 | 49.78 | 49.65 | 29.12 | 29.04 |
| 15 | 10 | 21 | 7.69E+05 | 118.02 | 6765 | 24564 | 49.46 | 48.78 | 28.94 | 28.54 |

GAS DATA

| date | hour | minute | time | elapsed | DCM area | DCA area | DCM conc | DCA conc | DCM mass | DCA mass |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 12 | 44 | 7.69E+05 | 0.40 | 11311 | 23088 | 4.37 | 6.33 | 2.32 | 3.36 |
| 10 | 13 | 50 | 7.69E+05 | 1.50 | 16405 | 29467 | 6.31 | 8.08 | 3.35 | 4.28 |
| 10 | 15 | 19 | 7.69E+05 | 2.98 | 18373 | 32521 | 7.06 | 8.91 | 3.74 | 4.72 |
| 10 | 17 | 53 | 7.69E+05 | 5.55 | 18627 | 32232 | 7.16 | 8.83 | 3.79 | 4.68 |
| 10 | 22 | 36 | 7.69E+05 | 10.27 | 18828 | 32572 | 7.24 | 8.92 | 3.83 | 4.73 |
| 11 | 10 | 31 | 7.69E+05 | 22.18 | 16797 | 33410 | 6.46 | 9.15 | 3.42 | 4.85 |
| 11 | 21 | 1 | 7.69E+05 | 32.68 | 17746 | 31214 | 6.82 | 8.55 | 3.62 | 4.53 |
| 12 | 8 | 37 | 7.69E+05 | 44.28 | 17293 | 33406 | 6.65 | 9.15 | 3.52 | 4.85 |
| 13 | 13 | 21 | 7.69E+05 | 73.02 | 20984 | 35303 | 8.06 | 9.67 | 4.27 | 5.12 |
| 14 | 9 | 12 | 7.69E+05 | 92.87 | 18004 | 30417 | 6.92 | 8.34 | 3.67 | 4.42 |
| 15 | 10 | 45 | 7.69E+05 | 118.42 | 18452 | 32020 | 7.09 | 8.77 | 3.76 | 4.65 |

| AVG TIME | ****TOTAL MASS**** DCM | DCA |
|---|---|---|
| 0.20 | 35.22 | 35.40 |
| 1.30 | 34.23 | 35.39 |
| 2.77 | 33.95 | 35.27 |
| 5.26 | 34.59 | 35.99 |
| 10.07 | 33.93 | 35.14 |
| 21.98 | 32.24 | 34.13 |
| 32.48 | 26.75 | 34.45 |
| 44.16 | 29.14 | 34.18 |
| 72.64 | 33.14 | 34.00 |
| 92.66 | 32.79 | 33.46 |
| 118.22 | 32.70 | 33.19 |

Filename: HYDRO

HYDROLYSIS EXPERIMENT
DCE

LIQUID

| date | hour | minute | time | elapsed | area | conc | mass |
|---|---|---|---|---|---|---|---|
| 31 | 12 | 5 | 7.72E+05 | 0.00 | 0 | 0.21 | 0.14 |
| 31 | 12 | 55 | 7.72E+05 | 0.00 | 6563 | 33.02 | 22.79 |
| 31 | 13 | 52 | 7.72E+05 | 0.95 | 5816 | 29.29 | 20.21 |
| 31 | 14 | 41 | 7.72E+05 | 1.77 | 5449 | 27.45 | 18.94 |
| 31 | 17 | 40 | 7.72E+05 | 4.75 | 5075 | 25.58 | 17.65 |
| 31 | 20 | 56 | 7.72E+05 | 8.02 | 5172 | 26.07 | 17.99 |
| 31 | 23 | 10 | 7.72E+05 | 10.25 | 5245 | 26.43 | 18.24 |
| 1 | 9 | 44 | 7.72E+05 | 20.82 | 5055 | 25.48 | 17.58 |
| 1 | 19 | 18 | 7.72E+05 | 30.38 | 5057 | 25.49 | 17.59 |
| 2 | 10 | 15 | 7.72E+05 | 45.33 | 5023 | 30.36 | 20.95 |
| 2 | 22 | 5 | 7.72E+05 | 57.17 | 4989 | 30.16 | 20.81 |
| 3 | 10 | 20 | 7.72E+05 | 69.42 | 4718 | 28.53 | 19.69 |
| 4 | 8 | 37 | 7.72E+05 | 91.70 | 4849 | 24.36 | 16.81 |
| 5 | 9 | 47 | 7.72E+05 | 116.87 | 4729 | 23.76 | 16.40 |

HEADSPACE

| date | hour | minute | time | elapsed | area | conc | mass |
|---|---|---|---|---|---|---|---|
| 31 | 12 | 28 | 7.72E+05 | 0.38 | 0 | -0.05 | -0.02 |
| 31 | 12 | 55 | 7.72E+05 | 0.00 | 12451 | 3.03 | 1.36 |
| 31 | 13 | 32 | 7.72E+05 | 0.62 | 24089 | 5.90 | 2.66 |
| 31 | 14 | 27 | 7.72E+05 | 1.53 | 28475 | 6.99 | 3.14 |
| 31 | 17 | 27 | 7.72E+05 | 4.53 | 29384 | 7.21 | 3.24 |
| 31 | 20 | 44 | 7.72E+05 | 7.82 | 30054 | 7.38 | 3.32 |
| 31 | 22 | 56 | 7.72E+05 | 10.02 | 30110 | 7.39 | 3.33 |
| 1 | 9 | 31 | 7.72E+05 | 20.60 | 30817 | 7.57 | 3.40 |
| 1 | 18 | 48 | 7.72E+05 | 29.88 | 29381 | 7.21 | 3.24 |
| 2 | 9 | 43 | 7.72E+05 | 44.80 | 28929 | 7.33 | 3.30 |
| 2 | 21 | 41 | 7.72E+05 | 56.77 | 28655 | 7.26 | 3.27 |
| 3 | 10 | 1 | 7.72E+05 | 69.10 | 27423 | 6.95 | 3.13 |
| 4 | 8 | 12 | 7.72E+05 | 91.28 | 26703 | 6.73 | 3.03 |
| 5 | 9 | 17 | 7.72E+05 | 116.37 | 26676 | 6.73 | 3.03 |

| AVG TIME | DCE MASS |
|---|---|
| ********** | ********** |
| 0.19 | 0.12 |
| 0.00 | 24.15 |
| 0.78 | 22.87 |
| 1.65 | 22.09 |
| 4.64 | 20.90 |
| 7.92 | 21.31 |
| 10.13 | 21.57 |
| 20.71 | 20.99 |
| 30.13 | 20.84 |
| 45.07 | 24.25 |
| 56.97 | 24.08 |
| 69.26 | 22.82 |
| 91.49 | 19.84 |
| 116.62 | 19.42 |
| avg | 21.93 |
| s = | 1.54 |

| | ****TOTAL MASS**** | |
|---|---|---|
| AVG TIME | DCM | DCA |
| 0.20 | 35.22 | 35.40 |
| 1.30 | 34.23 | 35.39 |
| 2.77 | 33.95 | 35.27 |
| 5.26 | 34.59 | 35.99 |
| 10.07 | 33.93 | 35.14 |
| 21.98 | 32.24 | 34.13 |
| 32.48 | 26.75 | 34.45 |
| 44.16 | 29.14 | 34.18 |
| 72.64 | 33.14 | 34.00 |
| 92.66 | 32.79 | 33.46 |
| 118.22 | 32.70 | 33.19 |
| avg | 32.61 | 34.60 |
| s = | 2.41 | 0.85 |

Filename: HYDRO2