

COMPARATIVE EFFICIENCY OF MICROBIAL SYSTEMS
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INATION IN HANFORD GROUND WATER

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COMPARATIVE EFFICIENCY OF MICROBIAL SYSTEMS FOR DESTROYING CARBON TETRACHLORIDE CONTAMINATION IN HANFORD GROUNDWATER

M. J. Truex, R. S. Skeen, S. M. Caley, and D.J. Workman

ABSTRACT

Past waste disposal practices at the U.S. Department of Energy's (DOE) Hanford site have resulted in carbon tetrachloride and nitrate contamination in the groundwater. In-situ bioremediation is currently being investigated as a cost effective means to destroy these groundwater contaminants. A key factor in the cost effectiveness of the process is the nutrient amendments required to stimulate microbial destruction of the contaminants. Current and previous research has focused on determining the reaction kinetics and microbial processes for carbon tetrachloride destruction using acetate as the electron donor and the indigenous microbes. In this study, kinetic experiments were conducted and a first-order model was used to compare the rate of carbon tetrachloride destruction and biomass production stimulated using acetate, ethanol, glycerol, and methanol as substrates under denitrification conditions. All of the substrates stimulated comparable carbon tetrachloride destruction and biomass production. Glycerol treatments exhibited a unique biphasic pattern of carbon tetrachloride destruction. In addition, it was determined that the rate of carbon tetrachloride destruction was first order with respect to carbon tetrachloride concentration.

INTRODUCTION

Past waste disposal practices at the U.S. Department of Energy's (DOE) Hanford site have resulted in carbon tetrachloride (CCl_4) and nitrate contamination in the groundwater. *In situ* bioremediation is currently being investigated as a cost effective means to destroy these groundwater contaminants. The cost effectiveness of bioremediation is significantly influenced by the nutrient amendments required to sustain the contaminant destruction reactions (Skeen et al. 1992, Truex et al. 1992). This is particularly important for bioremediation of CCl_4 because its biodestruction is the result of a cometabolic process. Nutrient amendments are also important in controlling the growth characteristics of the bacteria to prevent biofouling. Current and previous research has focused on determining the reaction kinetics and microbial processes for CCl_4 destruction using acetate as the electron donor for indigenous microbes (Brouns et al. 1990, Amos 1992, Semprini et al. 1991). This study was conducted to determine if electron donors other than acetate may be more cost effective, or may provide a better means of process control, for *in situ* bioremediation of CCl_4 contamination. Three alternative electron donors, glycerol, methanol, and ethanol, were screened for their ability to stimulate CCl_4 destruction. Detailed reaction kinetic experiments using an indigenous microbial consortium with these substrates and with acetate were conducted to determine the efficiency of each in destroying CCl_4 .

MATERIALS AND METHODS

A bacterial consortium isolated from aquifer sediments at the DOE's Hanford site was stored in 50% glycerol at -70°C as a stock cell culture. The growth medium used for all cell culturing and kinetic experiments was a simulated groundwater (SGW) (Amos 1992) amended with the appropriate electron donor and acceptor for the specific experiment. Cell culture and inoculation protocol were as described by Amos (1992). Kinetic experiments (duplicated under

similar conditions) were conducted using acetate, methanol, ethanol, and glycerol as electron donors at an initial concentration of 0.1 mole of carbon/L. Nitrate was provided at an initial concentration that was not limiting for the duration of the experiments. Abiotic controls were performed to assay for loss of CCl_4 due to the experimental procedure.

Experiments were conducted in a gastight, 1-L reaction vessel designed to allow samples to be removed from the reactor with negligible losses of CCl_4 . Experimental procedures were identical to those used by Amos (1992). Briefly, the vessel was sterilized in an autoclave; pressure tested to 10 psi to ensure there were no gas leaks; and then charged with SGW, the specific electron donor, and CCl_4 . Carbon tetrachloride was added in a saturated water solution to produce a nominal aqueous concentration between 300 $\mu\text{g/L}$ and 4,500 $\mu\text{g/L}$. Based on preliminary experiments, a 3- to 4-hour lag time before injection of the bacterial inoculum was used to allow the CCl_4 to reach equilibrium partitioning between the gas and aqueous phases. Bacteria were inoculated at a nominal biomass concentration of 25 mg-dry weight/L as measured by total suspended solids. The starting aqueous volume in the reactor was 750 mL, and the pH of the reactor was buffered at 7.0. The reactor was operated as a mixed batch reactor in a constant temperature bath (17°C) under denitrification conditions.

Periodic samples were withdrawn to determine the aqueous-phase concentration of the solution anions, biomass, and CCl_4 . Carbon tetrachloride was extracted into hexane and measured using gas chromatography. Solution anion concentrations were measured from filtered aqueous samples using ion chromatography. Biomass was measured spectrophotometrically and correlated to total suspended solids (mg-dry weight/L) using a standard curve. Values for kinetic parameters that could not be estimated directly from experimental data were numerically determined using the software Simusolv (Dow Chemical Company, Midland, Michigan).

RESULTS AND DISCUSSION

Denitrification rates were not significantly different for any of the treatments. In addition, each treatment demonstrated greater destruction of CCl_4 than the losses measured in abiotic controls. For comparison purposes, the biomass production and CCl_4 destruction rates for each treatment were quantified by numerically fitting the experimental data to the rate expressions described by equations 1 and 2. In these equations, X is the biomass concentration (mg-dry weight/L), CT is the CCl_4 concentration ($\mu\text{g/L}$), and K_{CT} (hr^{-1} (mg-dry weight/L) $^{-1}$) and K_X (hr^{-1}) are rate constants.

$$\frac{d(X)/dt}{X} = K_X(X) \quad (1)$$

$$\frac{d(CT)/dt}{CT} = -K_{CT}(X)(CT) \quad (2)$$

During the fitting procedure, the amount of CCl_4 that partitioned into the headspace of the reactor was calculated using a Henry's law constant of $0.856 \text{ (mg/L gas phase)}/(\text{mg/L aqueous phase})$ (Gossett 1987) to account for changes in the headspace volume due to sampling and assuming instantaneous equilibrium.

Cell growth rate was assumed to be first order in cell numbers (biomass). For the CCl_4 destruction reaction, the rate of CCl_4 destruction was estimated to be first order with respect to CCl_4 concentration based on an initial rate analysis of the two experiments using acetate as the electron donor, and two additional experiments using acetate performed at different initial concentrations of CCl_4 . These experiments provided CCl_4 data over a range from $300 \mu\text{g/L}$ to $4,500 \mu\text{g/L}$. In this analysis, the natural logarithm of the initial rate of change in CCl_4 concentration, normalized by the initial biomass concentration, is plotted versus the natural logarithm of the initial CCl_4 concentration. As shown in equation 3, the slope of the resulting line

indicates the order of the CCl_4 destruction rate.

$$\ln[-d(\text{CT})/dt(1/X)] = \ln(K_{\text{CT}}) + n[\ln(\text{CT})] \quad (3)$$

Equation 3 was obtained by evaluating equation 2 at a specific instant in time and by assuming that the reaction depends on CCl_4 concentration to the n^{th} power. This method for determining the power dependency can be applied with the assumption that the biomass and CCl_4 concentration are constant over the time period used to estimate the initial rate. The initial CCl_4 destruction rate was estimated by linear regression of the data for the first day of incubation. During this time period, the biomass and CCl_4 concentrations changed an average of 35% and 13%, respectively. The small variation in CCl_4 concentration was acceptable for the assumption of constant CCl_4 concentration. Although the assumption of constant biomass does not appear to be entirely valid, the initial rate analysis results were relatively insensitive to changes in biomass concentration over the observed range. This is indicated by the similar power dependencies calculated when the minimum ($n=1.07$), average ($n=1.18$), and maximum ($n=1.25$) values of the first-day biomass concentrations were used in the initial rate analysis. The r^2 values for the linear regression of data for equation 3 used to generate these n values were 0.86, 0.87, and 0.92, respectively. From these three estimates of the power dependency, the assumption that the rate of CCl_4 destruction is first order with respect to CCl_4 concentration is justified.

Reaction constants for CCl_4 destruction were comparable for the acetate, methanol, and ethanol treatments (Table 1). The biomass production constant, which is a measure of the exponential growth rate, was also similar for acetate, ethanol, and methanol treatments. The time-phase pattern of contaminant destruction for the duration of the experiment was similar for all treatments except for glycerol. For this reason, equations 1 and 2 did not accurately describe the experimental data for glycerol and the results are not shown in Table 1. Two additional

experiments were performed with glycerol as the substrate and similar results were obtained. Three of the four glycerol treatments exhibited a biphasic pattern where greater than 80% of the CCl_4 destruction occurred in the first 30 to 40 hours of the experiment (Figure 1A). Denitrification and biomass production, however, exhibited the opposite trend (Figure 1B). In treatments with the other substrates, the time-phase pattern of CCl_4 destruction followed a first-order decrease and the lag phase before exponential microbial growth was much shorter than the lag phase for glycerol treatments.

Because glycerol can be used as a substrate for fermentation, the initial CCl_4 destruction may have occurred during fermentation reactions that took place before vigorous denitrification began. Potentially fumarate, a TCA cycle intermediate, may have been the electron acceptor supporting the fermentation of glycerol. Significant CCl_4 destruction previously has been demonstrated in batch experiments amended with glycerol and fumarate, while treatments conducted with glucose fermentation (glycolysis) did not promote significant CCl_4 destruction (J.K. Fredrickson, unpublished data). Other mechanisms may have been responsible for the biphasic pattern of CCl_4 destruction in the glycerol treatments. However, these mechanisms have not been fully investigated.

The impact of these results for *in situ* bioremediation of CCl_4 may be important relative to cost effectiveness, optimization, and control of the process. Each of these compounds may be potentially useful for stimulating *in situ* bioremediation of CCl_4 because each induced similar rates of CCl_4 destruction and microbial growth. Additional information on regulatory constraints, relative cost per unit mass of CCl_4 destroyed, and the microbial growth response in a porous medium will determine which substrate will be most cost effective for application at the Hanford site. Experiments to evaluate these additional cost and application issues are currently under way. Glycerol, in particular, may offer significant advantages because nitrate may not be required to stimulate CCl_4 destruction. Use of a substrate such as glycerol, where both denitrification and

fermentation reactions can support CCl_4 destruction, would greatly reduce the amount of nitrate required for contaminant remediation at the Hanford site, and consequently, may result in an improved cost effectiveness over systems using substrates that require denitrification conditions. Optimization of nutrient conditions may result in relatively high CCl_4 destruction rates and low denitrification rates using glycerol as an electron donor.

ACKNOWLEDGMENT

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LIST OF FIGURE AND TABLE CAPTIONS

TABLE 1. Reaction constants for a first-order model of CCl_4 destruction (K_{CT}) and biomass production (K_X) in kinetic experiments.

FIGURE 1. Typical time-phase pattern of CCl_4 destruction (A), denitrification, and biomass production (B) in batch kinetic experiments using glycerol as the electron donor.

TABLE 1.

<u>Treatment</u>	<u>$K_{CT} (x10^{-4})$</u>	<u>%VE</u>	<u>$K_x (x10^{-3})$</u>	<u>%VE</u>
Acetate	1.2 +/- 0.4	57, 90	9.6 +/- 6.1	89, 99
Methanol	1.3 +/- 0.8	79, 45	7.4 +/- 3.1	96, 71
Ethanol	1.0 +/- 0.7	66, 66	10 +/- 11	76, 98

%VE = the percent of data variation explained by the first-order model (first experiment, second experiment)

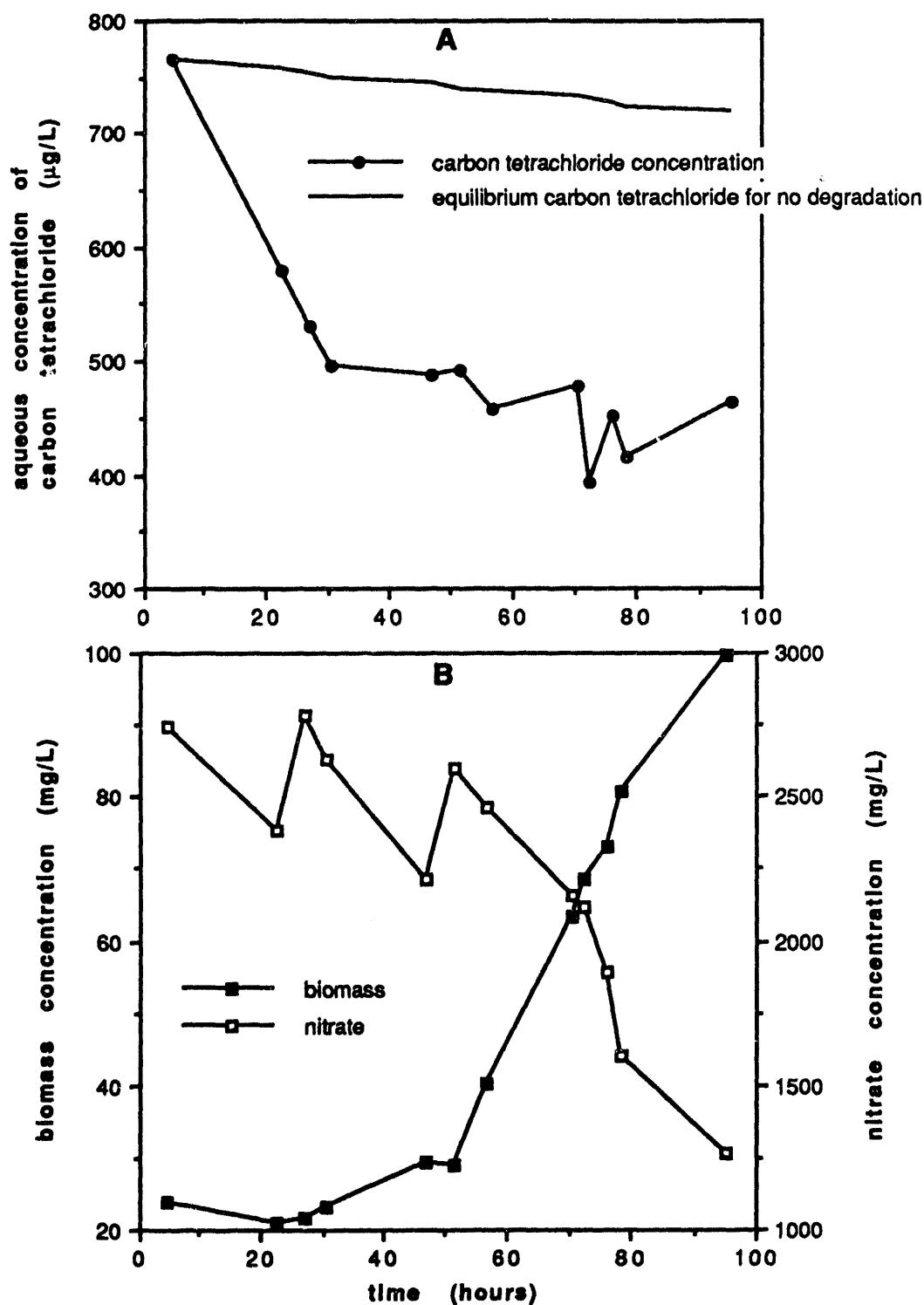


FIGURE 1.

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