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## DISCLAIMER

# Kinetics of In situ Bioremediation of Hanford Groundwater

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## ABSTRACT

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the 40+ years of operations at the U.S. Department of Energy's (DOE) Hanford Site. Some of these wastes were discharged to the soil column and many of the waste components, including nitrate, carbon tetrachloride ($CCl_4$), and several radionuclides, have been detected in the Hanford groundwater. Current DOE policy prohibits the disposal of contaminated liquids directly to the environment, and may require the remediation of existing contaminated groundwaters. In situ bioremediation is one technology currently being developed at Hanford to meet the need for cost effective technologies to clean groundwater contaminated with $CCl_4$, nitrate, and other organic and inorganic contaminants. This paper focuses on the latest results of an on-going effort to quantify the biological and chemical reactions that would occur during in situ bioremediation

## INTRODUCTION

The Hanford Site, located in southeastern Washington State, is an area of approximately 600 square miles that was selected in 1943 for producing nuclear materials in support of the United States' effort in World War II. Hanford's operations over the last 40+ years have been dedicated to nuclear materials, electrical generation, diverse types of research, and waste management. Some of these operations have produced aqueous and organic wastes that were subsequently discharged to the soil column. In the 200 west area of the Hanford Site, plutonium recovery processes discharged carbon tetrachloride ($CCl_4$) bearing solutions to three liquid waste disposal facilities: a trench, tile field, and crib. A minimum of 637-t of $CCl_4$ was disposed to the subsurface, primarily between 1955 and 1973, along with co-contaminants such as tributyl phosphate; lard oil; cadmium; nitrates; hydroxides; fluorides; sulfates; chloroform; and various radionuclides, including plutonium (1). Near the disposal site, $CCl_4$ vapors have been encountered in the vadose zone during well drilling operations, and groundwater contamination from $CCl_4$ is extensive, covering 5-$km^2$. Concentrations up to 1000 times the Environmental Protection Agency's (EPA) drinking water standard of 5-ppb have been measured in the groundwater. In addition, nitrate concentrations up to 10 times the EPA drinking water standard of 44-ppm have been measured in the same area of the site. Bioremediation is one technology currently being developed at Hanford to meet the need for cost- effective technologies to clean groundwater.

The Department of Energy (DOE) and its operating contractor at Hanford, Westinghouse Hanford Company, are currently pursuing an Expedited Response Action (ERA) to remove volatile $CCl_4$ from the vadose zone using soil vapor extraction (2). In collaboration with the ERA, DOE's Office of Technology Development has selected the 200 West Area $CCl_4$ site as the host for the VOC-Arid Integrated Demonstration. The objective of the Integrated Demonstration is to develop, demonstrate, evaluate, and

transfer for deployment new technologies for all phases of cleanup of volatile organic and associated contaminants in the subsurface. This program brings forward new technologies that provide more effective, less expensive, and safer methods for cleanup. Bioremediation is one technology being developed as part of the VOC-Arid Integrated Demonstration.

The current understanding of microbial degradation of $CCl_4$ is limited. However, $CCl_4$ biodegradation has been demonstrated with a number of different bacteria. The conditions that favor biodegradation of $CCl_4$ are predominantly anaerobic. For example, Bouwer and McCarty observed that cultures of sewage treatment bacteria biodegraded $CCl_4$ to $CO_2$ and other metabolites under methanogenic (3) and denitrifying (4) conditions. Sulfate-reducing microorganisms have also demonstrated the ability to destroy $CCl_4$ (5,6). In addition, Semprini et al. (7) speculated that sulfate reducing bacteria were responsible for the $CCl_4$ degradation they observed during a field test of in situ bioremediation. Biodegradation of $CCl_4$ under denitrification conditions is of particular interest at Hanford because of the occurrence of both $CCl_4$ and nitrates in the unconfined aquifer. Both Hansen (8) and Criddle et al. (9) identified Pseudomonas species capable of degrading $CCl_4$ with acetate as the electron donor and nitrate and the terminal electron acceptor.

The potential of stimulating microorganisms indigenous to the Hanford site to degrade both nitrate and $CCl_4$ has been demonstrated at the laboratory-, bench-, and pilot-scales. (10,11). For example, a pilot-scale, agitated slurry reactor processing a simulated groundwater feed which contained 400-ppm nitrate, 200-ppb $CCl_4$, and acetate as the primary carbon source, demonstrated greater than 99% and 93% destruction of nitrate and $CCl_4$, respectively. Analysis of all product streams indicated that the concentration of nitrate and $CCl_4$ were reduced to levels below the drinking water standards. These promising results with indigenous Hanford microorganisms has lead to the speculation that it may be possible to introduce the appropriate nutrients to the subsurface and induce the native bacteria to biodegrade both the nitrate and $CCl_4$ contamination in situ. This paper outlines the latest results in an effort to design and implement an in situ bioremediation test site. At present, work is proceeding to measure hydrological and chemical properties of the bioremediation test site and to rigorously study the kinetics of contaminant degradation and the growth of the indigenous microorganisms (12). This information is being incorporated into 1- and 3-dimensional simulations of in situ bioremediation to help design proper remediation conditions. In this paper new insights on the microbial degradation of $CCl_4$ are presented and discussed in relation to the design of an in situ bioremediation system.

## MATERIALS AND METHODS

Batch experiments were performed to obtain reliable data that will allow credible prediction of the results of the application of in situ bioremediation technologies. These experiments provide data that can be used to verify mathematical expressions that describe the biological reactions involved in the degradation of $CCl_4$ and nitrate and the simultaneous production of biomass. They are designed to allow the investigation of the effects of various experimental conditions upon the proposed expressions. The experimental parameters that are being considered are the pH at which the reaction is conducted and the concentration of $CCl_4$, acetate, nitrate, nitrite and biomass that is present initially in the reactor. The data that is being collected as a function of time includes the biomass, $CCl_4$, acetate, nitrate, and nitrite concentrations; and the pH and redox potential of the media. The experimental apparatus is shown schematically on Figure 1.

A soil sample was obtained in a sterile fashion from a test well on the Hanford site (12). A bacterial consortia from this soil sample was isolated and used as the reactor inoculum. Simulated groundwater (SG) amended with acetate was used for cultivating cells. The SG was formulated to ion concentrations that approximate the major ion concentrations of the Hanford groundwater. The reactor was sterilized in an autoclave, charged with sterile SG, and purged with nitrogen for 16-hours to deoxygenate the system. Once deoxygenated, the sodium nitrate and acetic acid were added to the reactor

to adjust the nitrate and acetate concentrations to 2400- and 2000-ppm, respectively. Finally, the pH of the reactor was adjusted to 7.2 and the system was charged with 500-ppb $CCl_4$ and enough biomass to achieve a dry weight in the reactor of 25-mg/l.

Once charged, the reactor was placed in a constant temperature water bath maintained at 17°C, and mixed with a stir bar. Periodically, the reactor pressure, temperature, pH and oxidation/reduction potential (ORP) were recorded. When necessary, a sterile 10% $H_2SO_4$ solution was added to return the pH to the original value. To measure $CCl_4$ levels, 2.5-ml aqueous samples were removed from the reactor using a sterile syringe containing 0.5-ml of hexane. This mixture was then dispensed directly into approximately 2-ml of hexane in a graduated cylinder that was kept in ice. The cylinder was closed with a teflon lined cap, and the hexane volume and sample volume were recorded. The $CCl_4$ was extracted into the hexane by shaking the cylinder for 1-min to mix the phases. After mixing, the phases were allowed to separate and the hexane layer was extracted and placed in a GC vial for later analysis.

Anions and biomass were measured periodically by withdrawing a 10-ml aqueous sample from the reactor. Biomass levels were determined by either of two methods: dry weight per unit volume, i.e. Total Suspended Solids (TSS), or optical absorbance. The absorbance method was used if the biomass concentration was estimated to be below 100-mg/L. In this case, approximately 4-ml of the 10-ml sample was sonicated for 30-sec and its absorbance was then measured at 450-nm. From this absorbance reading, the TSS of the biomass was determined from a standard curve. If the biomass concentration was estimated to be above 100-mg/L, then the TSS was directly measured from the weight of a filtered sample that was dried at 106 °C for a minimum of 1-hr. In either case, the sample was filtered through a 0.45-$\mu$m membrane to remove biomass. The filtrate was collected and analyzed on an ion chromatograph to determine acetate, nitrate, nitrite, chloride and sulfate concentrations.

The gas chromatograph used in this research to determine the $CCl_4$, $CHCl_3$, and $CH_2Cl_2$ concentrations was an HP model 5890 Series II equipped with an electron capture detector. Nitrogen was used as the carrier gas at 6.6-ml/min. This gas chromatograph was equipped with a 30-m DB-624 column (J&W Scientific) having a 0.53-mm ID.

The ion chromatograph used was a Dionex 4000i which was equipped with a conductivity detector and a cation suppression unit which exchanges hydrogen for the cations contained in solution. This cation exchange unit is used to remove the effect of the cations upon the conductivity of the exit stream. The eluent was 40-mmolar $Na_2SO_4$ and 5% methanol which was fed at 1-ml/min. The column used was a Dionex PAX 100 anion exchange column.

The pH and ORP were determined using sealed probes and an Orion 720A meter. Pressure was determined using a Omega DP-2000 pressure transducer.

## RESULTS AND DISCUSSION

When the batch reactor was operated as described above, the results shown in Figures 4-6 were obtained. From Figure 2b, it is observed that the biomass concentration follows a classic growth curve. The biomass first experiences an approximate 25-hr period of little or no growth as the bacteria adapt to the new, nutrient rich environment. For the next 18-hrs the microbes exhibit rapid growth, followed by a 24-hr period where growth stops, and subsequently the levels of biomass declines. Throughout the growth cycle, nitrate and acetate were consumed in the course of the biological denitrification reactions. This can be seen on Figure 2a. Nitrite, on the other hand, was first produced as the nitrate was consumed, and then was itself consumed by the biomass. This is in agreement with well established denitrification pathways, which can be summarized as follows (13):

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

From Figure 3, it is observed that the total pressure in the reactor increases with time, despite the fact that samples are being withdrawn from this sealed vessel. Sample removal would tend to cause the pressure to decrease if gaseous components were not being produced in the reactor. Interestingly, it is not until the nitrite starts to be consumed that the reactor pressure begins to increase. This is consistent with the denitrification pathway shown above since the only gaseous species that are predicted to be produced are $CO_2$, from cell respiration; and NO, $N_2O$, and $N_2$ from nitrite degradation. In addition, at the pH of the reaction, a large portion of the $CO_2$ that was generated by the consumption of the acetate would partition into the aqueous phase. Thus, it is not surprising that the pressure begins to rise rapidly while the nitrite is consumed.

Finally, Figure 4 shows the $CCl_4$ concentration as a function of time. Here, it is observed that when the cells are not growing, no $CCl_4$ degradation occurs. However, a dramatic decrease in the $CCl_4$ concentration occurred as the microbes pass from the phase of no growth to the active growth phase. In the very short time span of 6 hours, the aqueous $CCl_4$ concentration dropped from 387 to 327-ppb, accounting for more than 60% of the ultimate $CCl_4$ degradation. This behavior is consistent with the results of Hansen (8) who observed in a similar batch experiment that approximately 60% of the total microbial degradation of $CCl_4$ occurred during the first day of growth. However, he did not sample biomass at a sufficiently small interval to demonstrate the relationship between $CCl_4$ degradation and the growth phase. Past work with these organisms suggest that the microbes used in Hansen's work would have a growth curve similar to that shown in Figure 2b (8). Hence, the degradation obtained in his experiments most likely took place when cells were metabolizing the substrate but not rapidly growing. A situation similar to the experiments reported here.

If indeed $CCl_4$ is only degraded by microbes when they are exhibiting active metabolism but not active growth, then the implementation of in situ bioremediation will be impacted. Nutrient feeding strategies must be devised to maintain the bacteria in the phase of metabolism where $CCl_4$ degradation occurs. Most likely, a pulse addition of nutrients will need to be used. Such feeding schemes would have the added advantage of extending well life by reducing clogging caused by biomass growth near the injection well.

The fact that a pulsing feeding strategy can repetitively stimulate microbes to degraded $CCl_4$ has been demonstrated by Hansen (8). In his work, he demonstrated $CCl_4$ destruction in cultures grown to the stationary phase. He then demonstrated that by adding additional nutrients, further $CCl_4$ degradation occurred. The amount of $CCl_4$ degraded after the second nutrient addition was similar to that obtained after the first addition.

The concept that microbes only metabolize $CCl_4$ during early stages of growth can also be used to explain the results obtained by Semprini et al. (7) during a field demonstration of in situ $CCl_4$ destruction. In that study, Semprini et al. found that $CCl_4$ degradation occurred only at a significant distance from the injection well. Near the injection point, nutrient concentrations were high and microbes exhibited vigorous growth as indicated by the clogging of the system after 70 days. However, in the region where $CCl_4$ was degraded, nutrient concentrations were low. In addition, a pulse feeding strategy was used in their work, which would cause microbes in the region where $CCl_4$ was degraded to be exposed to nutrients levels which cycled from low to nonexsistant levels. Exposed to such a nutrient profile, the microbes would tend to remain in a metabolically active, but not rapidly growing, state. This state would be similar to that experienced by cells which are in the transition between the lag and exponential growth phase. This notion provides an alternative explanation to the suggestion by Semprini et al. that two biomass populations were responsible for the observed $CCl_4$ degradation.

## CONCLUSIONS

The results presented in this paper suggest that $CCl_4$ degradation occurs via a metabolic pathway that is not associated with vigorous growth. This result would have significant impact on the formulation of a

feeding strategy for in situ bioremediation. A successful feeding strategy would need to maintain the microbes in an metabolically active state, but not actively growing. This would maximizing $CCl_4$ degradation while minimizing cell growth which would cause well plugging.

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## FIGURE LEGENDS

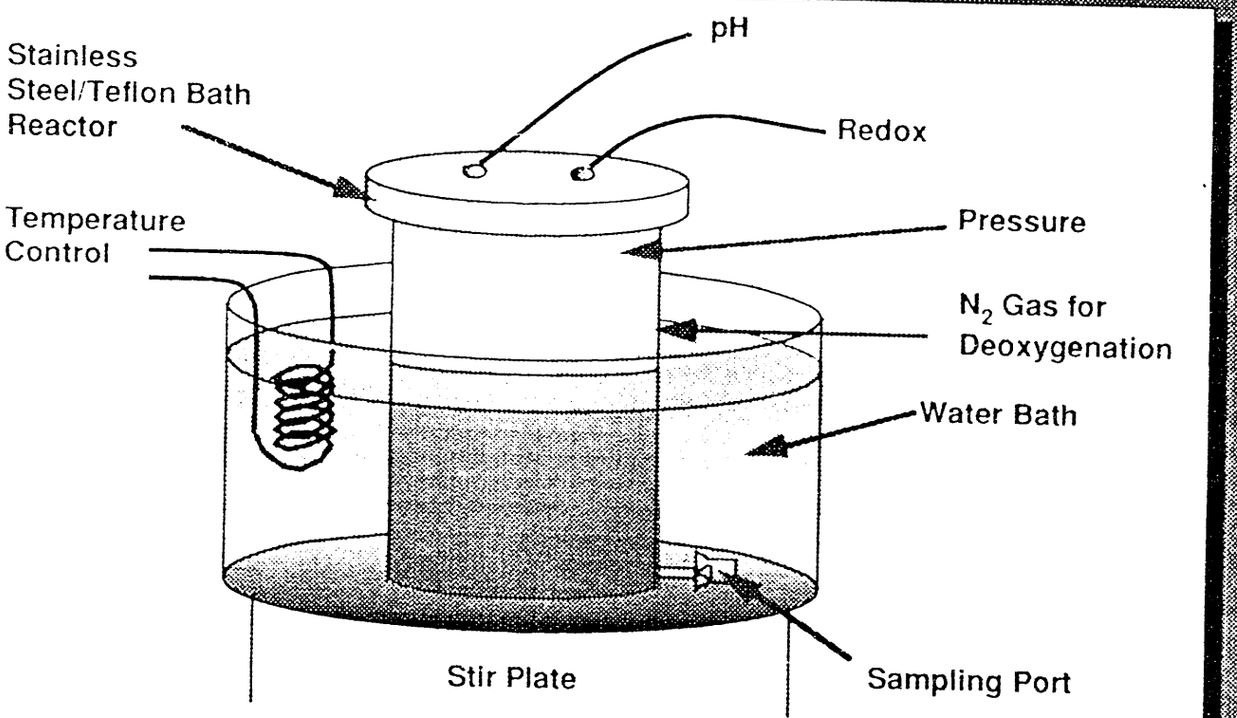
Figure 1. Schematic of the equipment used in the batch $CCl_4$ degradation experiments.

Figure 2. Results from the batch microbial kinetic experiment. (a) Acetate, Nitrate, and Nitrite concentrations as a function of reaction time. (b) Biomass concentration as a function of time.

Figure 3. Pressure in the batch reactor during the microbial kinetic experiment represented in Figure 2.

Figure 4. Carbon tetrachloride concentrations during the microbial kinetic experiment represented in Figure 2.

# Batch Kinetics Reactor




RD203158 2.7

FIGURE 1

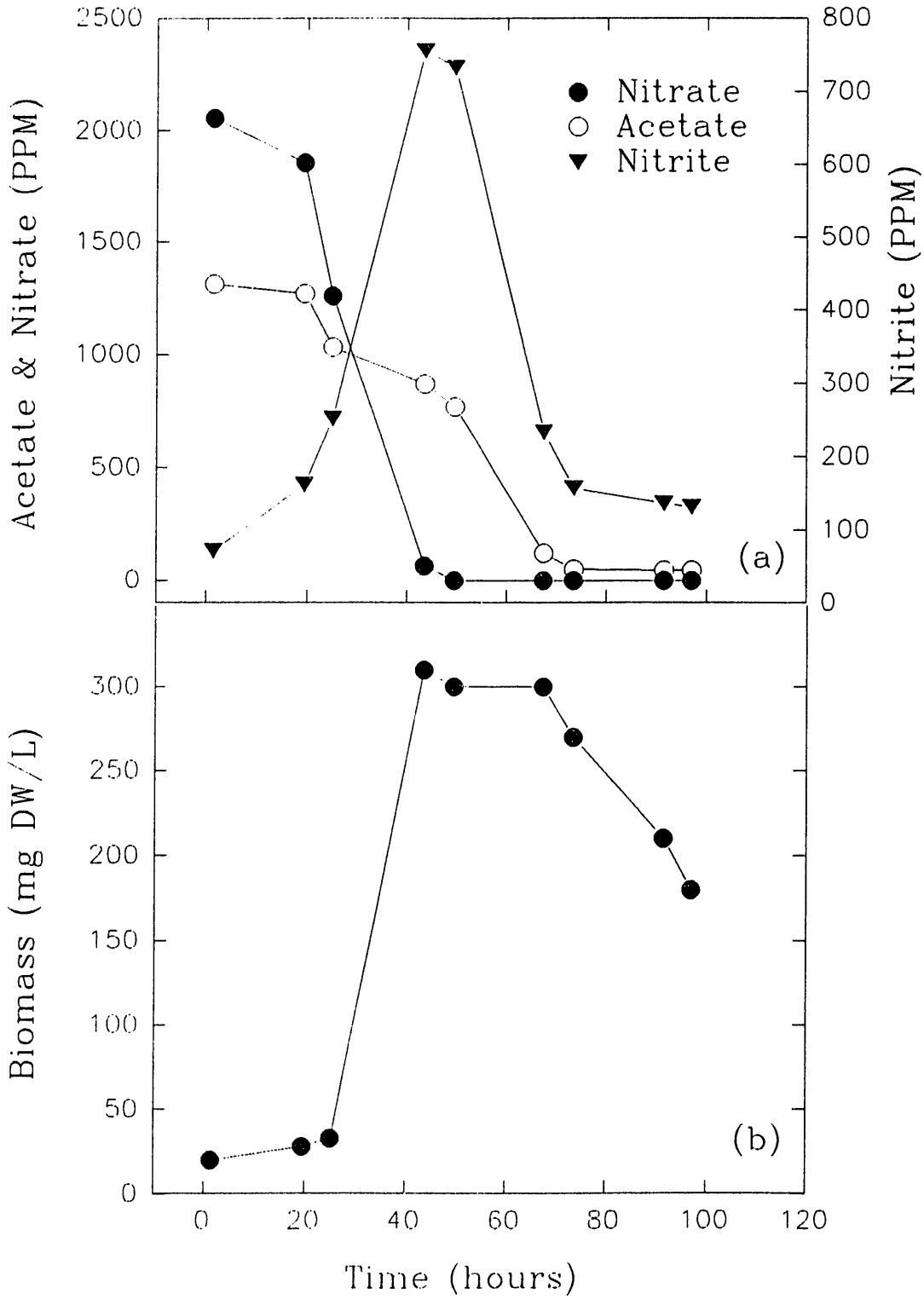

Acetate & Nitrate (PPM)
Nitrite (PPM)
Nitrate
Acetate
Nitrite
(a)
(b)
Biomass (mg DW/L)
Time (hours)
2500
2000
1500
1000
500
0
800
700
600
500
400
300
200
100
0
300
250
200
150
100
50
0
0
20
40
60
80
100
120

FIGURE 2

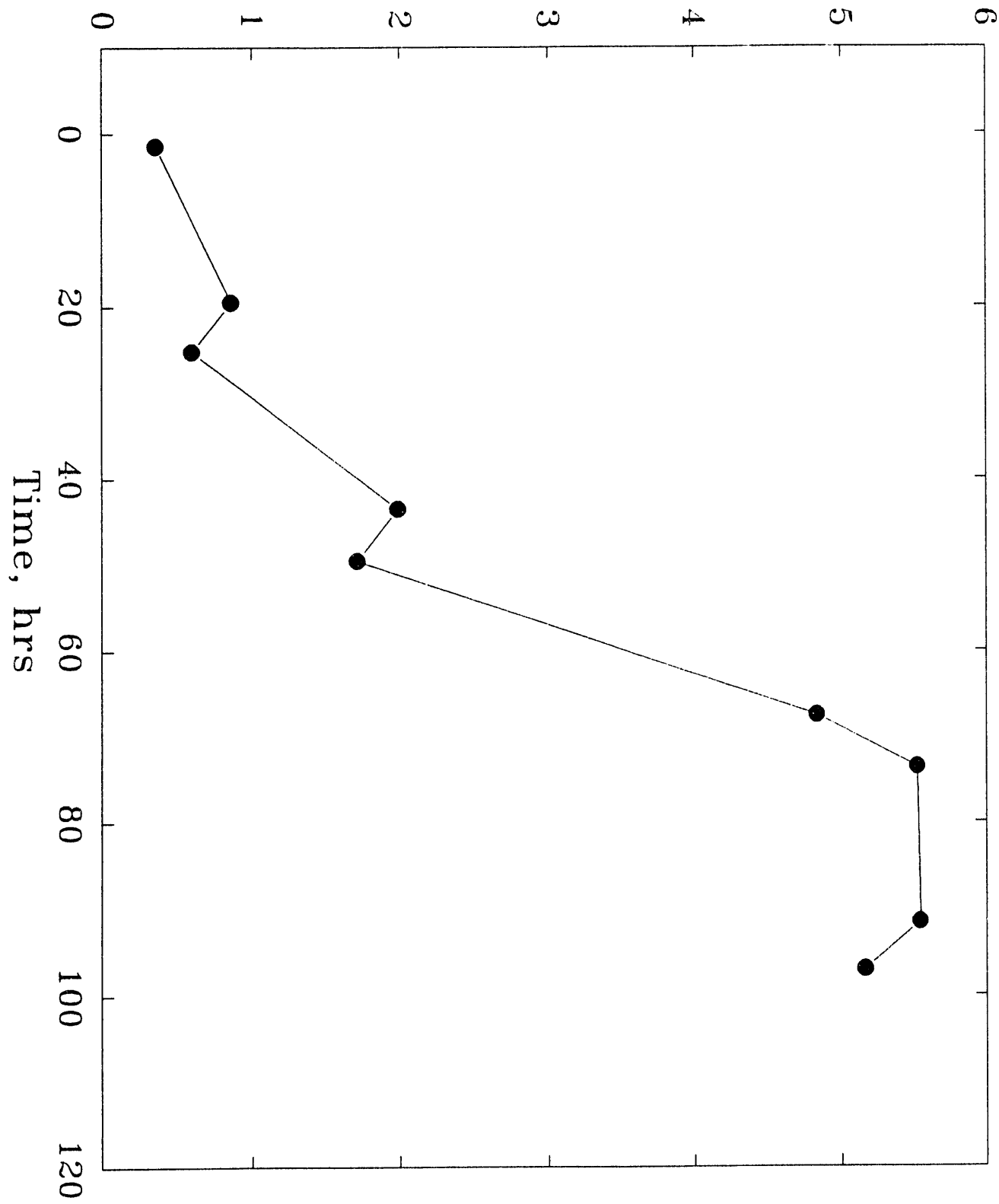



FIGURE 3

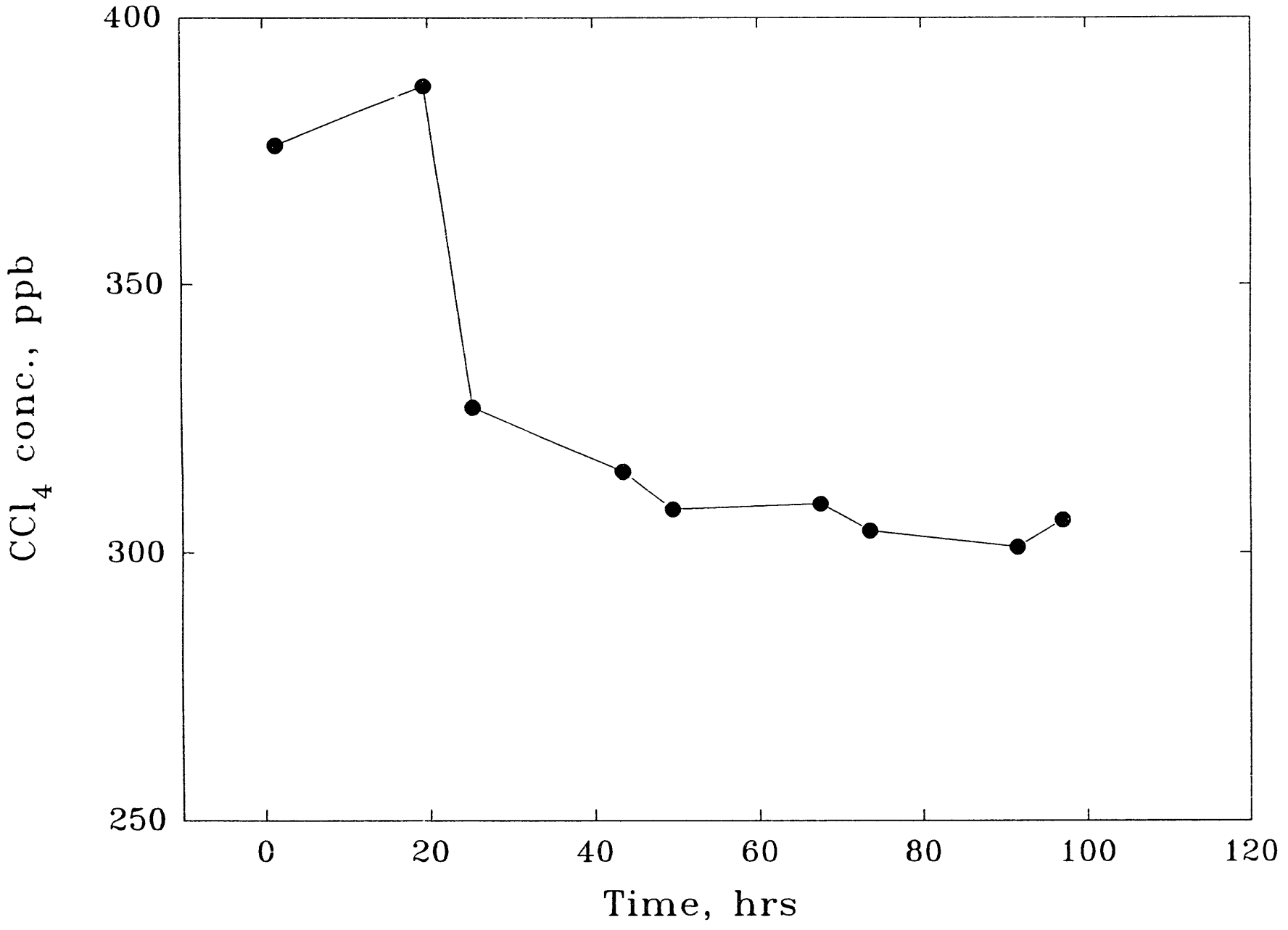



FIGURE 4

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