AN EVALUATION OF MICROBIAL TRANSFORMATION

PROPERTIES FOR SELECTED PESTICIDES:

LINDANE AND 2,4-DICHLOROPHENOXY-

ACETIC ACID

By

JOSE ATILANO PEREIRA
II

Bachelor of Science

in Civil Engineering

Oklahoma State University

Stillwater, Oklahoma

1985

AN EVALUATION OF MICROBIAL TRANSFORMATION

PROPERTIES FOR SELECTED PESTICIDES:

LINDANE AND 2,4-DICHLOROPHENOXY-

ACETIC ACID

Thesis Approved:

_____
Thesis Adviser

_____

_____

_____
Dean of the Graduate College

i

ACKNOWLEDGEMENTS

I would like to express special thanks and appreciation to my adviser, Dr. W. F. McTernan, for his encouragement, support and guidance throughout my graduate program and thesis preparation. Appreciation is also expressed to other members of my committee: Dr. W. W. Clarkson and Dr. D. R. Snethen for their suggestions and directions in my studies.

I am thankful for the friend ship of Mrs. T. V. Garner, Mr. S. P. Ho and Mr. R. G. Petricek, my colleagues in the graduate program for their moral support.

Most of all, I would like to express sincerest thanks and gratitude to my wife, Patricia D. Pereira, my in-laws, Ross and Reba F. Vann, and my parents, Jose A. and Gladys M. Pereira for their encouragement and support throughout this course of study.

This investigation was made possible in part through the financial support of the OSU University Center for Water Research and Oklahoma Water Resources Research Institute.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Modern agricultural methods often include the extensive
use of a wide range of different herbicides and insecticides
(1). In Oklahoma, a total of 3,808,416 pounds of herbicides
and insecticides were applied in 1981 (2). In addition to
positive features associated with the control of designated
pests, these chemicals may persist in the soil and in under-
laying groundwater. Environmental contamination due to the
excessive use of pesticides has become a great concern to
the public and to regulatory officials. This concern is
linked to the pesticides potential toxicity to nontarget or-
ganisms and to their possible adverse effects on man (3).

The persistence of pesticides in groundwater beyond
their points of maximum utility in the environment can be
attributed to various physical, chemical and biological
factors (4). These include the lack of chemical and phy-
sical adsorption onto soil clays and organics as well as
diminished microbial decay in subsurface horizons. If a
pesticide is neither retained in the soil nor decayed by
native microflora, it possesses a potential risk to shallow
water table aquifers. Among these factors, concentration of
the compound may affect its susceptibility to microbial

1

attack. Organic chemicals may persist in some environments as a result of low prevailing concentration or low solubility in water (5).

Many of the chemical compounds present in the natural environments at low concentrations are mineralized, but there is evidence that bacteria are not able to grow solely at the expense of organic compounds which are present at low concentrations (6,7). However, the mineralization of synthetic organic compounds at very low concentrations may be carried out by microorganisms whose growth is supported by organic compounds that are present at higher concentrations (8).

Two general types of dual-substrate models can be distinguished. The first class of models describes circumstances in which two compounds contribute substantially to the growth rate of the population (9,10). Models of the second general type are for circumstances in which the substrate of interest is present at very low concentration and therefore is not important in determining the growth rate of the active organisms. Growth of the active organisms in such cases would be governed almost entirely by the concentration of one or more alternative substrates (8).

Biological breakdown of 2,4-Dichlorophenoxyacetic acid (2,4-D) in soils has been reported by several investigators, while various species of bacteria capable of catalyzing this decomposition have been isolated (11,12,13,14). Similarly,

the breakdown of 2,4-D in lake waters has been observed
(4,5). Suggested metabolic mechanisms for these decomposi-
tions include utilizing the pesticide as the sole source of
carbon (primary substrate) or by utilizing it in conjunction
with another carbon source (secondary substrate) (5). It
has been postulated (15,16,17) that bacteria can consume a
xenobiotic compound at low concentrations by secondary util-
ization, if the population is supported through energy ob-
tained from another substrate that is present in higher
concentrations above a minimum substrate (Smin) level. The
compound at higher concentration is termed the primary
substrate, and that at low concentration is termed the
secondary substrate (15,16,17). A mechanism available to
describe the biological removal of contaminant substrates at
concentrations less than a thermodynamic minimum has been
described by Bouwer and McCarty as secondary utilization
(18). It has been shown that in the presence of a primary
carbon and energy source, trace levels of organic contami-
nants can be biologically metabolized. Kincannon, et al.
have demonstrated similar results with priority pollutant
removal in dispersed growth reactors (19). One apparent
explanation for these observations is that large organic
molecules cannot pass the semipermeable membranes of typi-
cal bacteria or fungi. Exocellular enzymes excreted by the
cells cleave these large molecules into useable sizes for
transport across the membranes. Pesticides and other

organic contaminants as well as traditional substrates can be initially broken into smaller sized units by enzymes found within the heterogeneous population comprising the population. Once this occurs the shorter units originating from the contaminants are indistinguishable by transport enzymes from similar materials originally located in primary substrates, and they are subsequentially metabolized by the cells. The mineralization of low levels of a contaminant substrate may be enhanced by the addition of an easily metabolized compound (6,8,17).

The objective of this study was to evaluate that part of the pesticide transport problem which could be attributed to biological decomposition. Sodium acetate was used as primary carbon source, and Lindane and 2,4-Dichlorophenoxyacetic acid (2,4-D) were used in sequential studies as secondary carbon sources or substrates to determine acclimation times as well as decomposition rates. A range of pesticide concentrations was selected to approximate a variety of field conditions as well as placing additional stress in terms of toxicity on the bacteria population for single application of an individual pesticide. These pesticides are among those most commonly used in Oklahoma (2) and are quite soluble in water, making them potential candidates for groundwater pollution. The solubility of these pesticides in water is presented in Table 1.

This study employed mixed cultures taken from a

TABLE I

SOLUBILITY OF SELECTED PESTICIDES
IN WATER (20)

Pesticide Compound	Solubility in Water (mg/L)	Temperature C
Lindane	10	20
2,4-D	620	25

wastewater treatment plant (primary clarifier effluent) and grown in a continuous flow reactor using sodium acetate as carbon source until the population reached steady state conditions. Acclimation of these bacteria to the pesticide was not attempted in this phase of the effort. The continuous flow reactor was employed to obtain a source of mixed bacteria of relatively constant genetic traits for subsequent experiments.

After steady state conditions were obtained, and a genetically constant bacterial population was assumed to exist, samples were taken from the continuous flow reactor and inoculated in batch enrichment units that contained sodium acetate, nutrients, buffers and the pesticide of concern at concentrations of 50, 500 and 5000 micrograms per liter to evaluate biological breakdown.

Data generated from these efforts included chemical oxygen demand (COD) as a measure of primary substrate presence, bacterial numbers, pH, dissolved oxygen, and the pesticide concentration of concern.

CHAPTER II

MATERIALS AND METHODS

Continuous Flow Reactor

A continuous flow culture reactor was employed to provide a source of unacclimated bacteria for subsequent batch testing. This reactor is shown in Figure 1 and was used to obtain a constant genetic source of bacteria for these experiments and for later researchers. The basal medium used in this reactor is listed in Table 2.

Sodium acetate, present at a total concentration of 5000 mg/liter, was the growth-limiting substrate in the medium. This was selected because it is an easily metabolized substrate and provides a low energy single carbon source. The relatively low levels of energy available from the acetate selected prototrophic organisms capable of living in relatively stressed environments. These organisms were intended to approximate the activities of soil-based oligotrophs.

Continuous feeding of fresh medium was effected through the use of a C-flex tubing. The medium was pumped through this tubing at a rate of 350 ml/Hr. The mean hydraulic retention time was equal to 7.14 hours, enough time

7
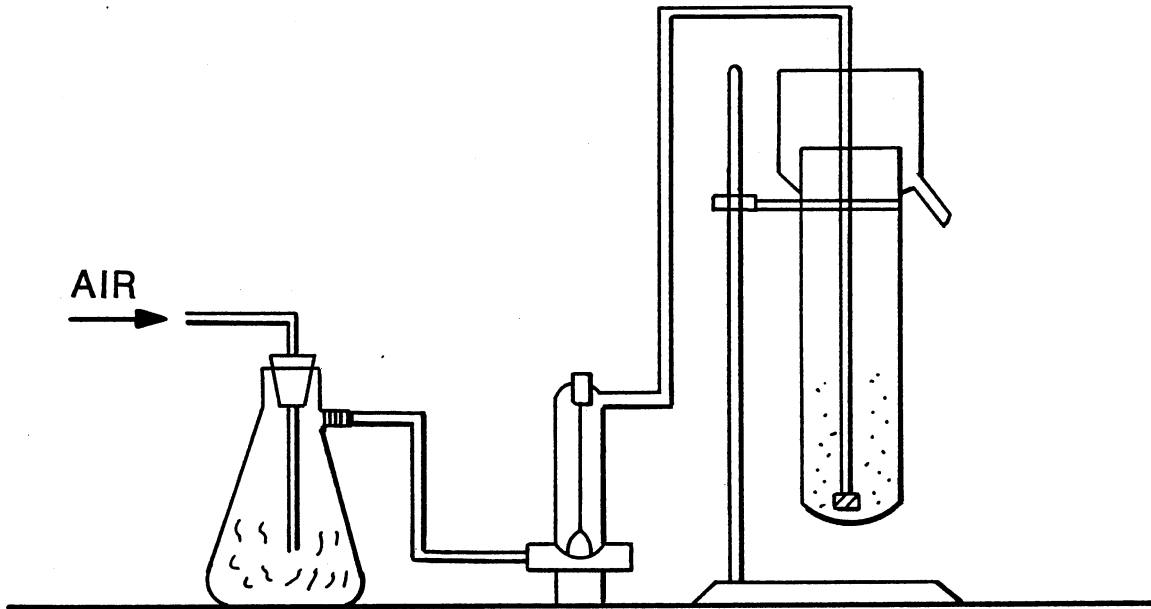
Figure 1. Continuous Flow Reactor

TABLE II

BASAL MEDIUM CONCENTRATION. A VOLUME OF 1.00 TO 2.00 ML (PER LITER OF DISTILLED WATER) OF THESE NUTRIENTS WAS USED (21)

Constituents	Concentration (g/L)
Sodium Acetate	5.00
Buffer:	
KH PO	8.50
K HPO	21.75
Na HPO *7H O	33.40
NH Cl	1.70
MgSO *7H O	22.50
CaCl	27.50
FeCl *6H O	0.25

for the cells to reproduce before washout from the reactor occurred. A constant volume was maintained by an overflow weir leading to a collection reservoir. Aeration and agitation were effected by an air line admitted to the bottom of the reactor at an approximate rate of 2.2 L/Hr. A Bendix Environmental Science Division air flow meter was employed to control air flow rate. A stone cylinder with a 1 x 7/8 inch diameter was used as a diffuser, and a cotton filter was placed in the air line to clean the compressed air (Figure 1).

Influent and effluent samples from the continuous flow reactor were taken and analysed for COD (21,22). When the effluent concentrations remained constant, a steady state condition was considered to be established. The reactor was then used to provide bacteria for subsequent experiments.

Batch units were inoculated with unacclimated bacteria from this reactor to evaluate the biological breakdown of selected pesticides in the presence of varying concentrations of primary substrates.

Sterilization of the media was accomplished by autoclaving the feed solution (20 liters) for approximately 2 hours at a temperature of $248\,^{\circ}F$ and a pressure of 15 psi. The tubing connecting the feed reservoir with the reactor was also sterilized and changed periodically to minimize contamination of influent.

Batch Reactors

Batch experiments were carried out in 300 ml BOD glass bottles. The basal media used in these units contained 1.5 ml/L of the nutrients concentrations described in Table 2, and 100 mg/L of sodium acetate. Lindane at concentrations tions of 50 and 500 parts per billion or 2,4-dichloropheno-xyacetic acid (2,4-D) at concentrations of 50, 500, and 5000 parts per billion were added, as individual pesticides. The solution was saturated with oxygen and the pH was adjusted to between 6 - 8 standard units with sodium hydroxide. Bacterial cells were extracted from the contin-uous flow reactor and centrifuged at a force of 1250 G for approximately 15 minutes to separate them from the culture fluid. The supernatant was decanted and the pellet was re-suspended in BOD dilution water to original volume to further wash residual culture fluid from inoculum. To each of these batch units containing 150 ml of medium, 1 ml of bacteria cells was added to complete a final volume of 151 milliliters per bottle. The BOD bottles were then stoppered and incubated in the dark at 21 $^{\circ}$C. The size of the incuba-tion vessels was chosen to have a large surface area of liquid exposed to the air to ensure aerobic conditions (23). Dissolved oxygen and pH were determined on a daily basis by measuring them directly in the batch units with an oxygen electrode probe (Model 97-08; Orion Research, Inc.) con-nected to an Orion Ionilyzer (model 301). The electrode

was calibrated with water-saturated air before each set
of readings. These determinations were completed while
other samples were proceeded for primary substrate utiliza-
tion, bacterial growth, pH and pesticide removal. Dupli-
cates of each reactor were sampled daily. No reactor was
sampled more than once to minimize contamination.

Reagents, Pesticide and Other
Laboratory Protocols

The organic chemicals used for these experiments were
of +99% purity. Organic solvents used for extraction were
pesticide grade.

All glassware was soaked in dichromate-sulfuric acid
for 2 to 3 hours, rinsed several times with tap water and
distilled water, then rinsed with methanol, methylene chlo-
ride, dried with nitrogen gas and stored in the oven at
248 F for several hours prior to use.

Analytical Methods

Duplicate samples from parallel batch units were ini-
tially taken daily, and filtered through 0.45 micro meter
acrodisc disposable filter units (Gelman Sciences, Inc., Ann
Arbor, MI) for the COD analyses and subsequent pesticide
extractions. The pesticide analyses were performed with a
Perkin-Elmer 2000 model gas chromatograph equipped with a Ni
(63) electron capture detector. A combination of 95% argon
and 5% methane was used as the carrier gas at a flow rate of

40 ml/min. Operating temperatures for the inlet port and detector were 250 $^{\circ}$C for both. The column was 2 meters by 2 millimeters (inside diameter) and packed with 3% SP-2100 on 100 to 120-mesh (Supelco Co.).

The output from the detector was connected to a re-cording integrator Perkin-Elmer LCI-100 model. 2,4-D and Lindane were calculated on the basis of peak area measure-ments by comparison with an external EPA standard prepared in 2,2,4-Trimethylpentane (Isooctane).

Pesticide Extraction

Extraction of water samples containing Lindane was com-pleted by using a microextraction procedure (24). This approach was developed for use in the U.S. Environmental Agency verification sampling and analysis program, and was chosen because it uses small sample volumes (10 ml) and has a reported detection limit of nano-grams (ng).

All samples were extracted with hexane and shaken by hand in the inverted position. After agitation, the phases were allowed to separate and the solvent containing the in-secticide was pipeted into a 2 ml vial, capped with a teflon lid and stored in the freezer at a temperature below 4 $^{\circ}$C to avoid volatilization.

Extraction of water samples containing 2,4-D, was completed by an available method for the analysis of phenoxy herbicides (25). This method has been found to be

effective for the analysis of acidic herbicides (25). The extraction of acidic herbicides from water samples is a process involving acidification of the sample with mineral acid, saturation with sodium chloride and extraction with ether. Simultaneous extraction of both the acid and ester, if present, will be effected with this extraction procedure. Cleanup of water samples containing 2,4-D consists of removal of coextracted material from the herbicide and preparing to for derivation (25). The first step is accomplished by adding a volume (40 ml) of ether to remove the unwanted compounds with dilute aqueous sodium bicarbonate solution. Much of the coextracted material is left in a highly colored layer while the herbicide is extracted into the bicarbonate layer. After removal of the organic layer, the bicarbonate solution is slowly acidified and slowly saturated with sodium chloride to control carbon dioxide evolution. The acidic herbicide is then reextracted into ether, which will then be evaporated to a smaller volume (0.5 ml) for derivization. This technique often removes many interferences from substrate to be analyzed that could affect the integrated area of the peak. Averages of quintuplet trials of percent recoveries of selected pesticides added to control samples are presented in Table 3. These control samples were prepared by the same extraction and derivization methods employed in the experimental conditions in this research.

TABLE III

MEAN PERCENT RECOVERY OF
SELECTED PESTICIDES

Pesticide Compound	Number of Injections	Mean % Recovery
Lindane	5	98
2,4-D	5	81

Microbial Population Determinations

The growth of bacteria was measured daily during incubation period by the pour plate technique (21). Duplicates of 1 milliliter and 0.1 milliliter of 100-fold dilutions were plated with a sterile medium containing (per liter of deionized water) 17 grams of plate count agar (PCA) (Difco Laboratories). Colony counts were made after 72 hours of incubation at 21 °C. From each of the dilution bottles, sample volumes of 1 ml and 0.1 ml were pipeted into the sterilized petri dishes. The melted medium was then poured into the petri dish and mixed with the test portion. This was done by gently lifting the cover of the petri dish to avoid extra contamination, then allowing the plates to solidify on a level surface. After the medium solidified, the plates were inverted to minimize condensation and placed in the incubator.

Sterility Controls

The sterility of the medium, dilution water blanks and plates was checked by pouring control plates for each series of samples. This was done in duplicate at each time that pour plate determinations were made.

Chemical Oxygen Demand

COD was used to determine the amount of primary

substrate (sodium acetate) utilized by the bacteria in the
batch units. Duplicate samples were taken and analyzed
daily from the respective batch reactors, until the residual
substrate levels fell below the detection limit, which was
approximately 1 mg/L. These experiments were done when
sodium acetate was used in combination with either pesticide
as a single application, and when used in control units
without pesticide addition.

Control experiments containing mineral salts, and
sodium acetate at the same concentrations as described, were
used to monitor system performance when no pesticide was
added. Sodium acetate served as the only source of carbon
to the controls. Duplicate samples were taken daily for COD
and bacterial counts. Also control experiments containing
similar concentrations of mineral salts, sodium acetate, and
pesticides in the absence of bacterial inoculum were set up
to determine if any volatilization or non-bacterial decom-
position of the pesticide occurred during the period of in-
cubation.

Modeling

Several models were available describing the bacterial
decomposition kinetics of primary and secondary substrates.
Two of these are included in Table 4. Others available from
similar sources were reviewed for possible use in this
study. The two presented were selected for their ease of

TABLE IV

TWO MODELS FOR THE KINETICS OF
DEGRADATION OF PRIMARY AND
SECONDARY SUBSTRATES

Model and Characteristics	Equation and Inequalities	Source
I.- Zero Order		(26)
Differential form	$-dS/dt = K1$	
Integral form	$S = So - K1*t$	
Necessary conditions	$Xo \gg So$ and $So \ll Ks$	
II.- First Order		(26)
Differential form	$-dS/dt = K3*S$	
Integral form	$S = So \ exp(-k3t)$	
Necessary conditions	$Xo \gg So$ and $So \ll Ks$	

Where:

    S = Substrate concentration
    t = Time
    So = Substrate concentration at time zero
    Xo = Half-saturation constant
    K1 = Slope of the line
    K3 = Slope of the line

use and general overall applicability to the proposed effort.

These models were compared to the collected data for the acetate and pesticide removal. Those best fitting the data were considered to be the most appropriate.

The zero order model assumes that the reaction rate is not affected by the substrate concentration, while the concentration becomes the driving force of the system with a first order model.

CHAPTER III

RESULTS

To test the ability of these organisms to simultan-
eously utilize a single pesticide and sodium acetate as sub-
strates, a series of batch experiments were performed. The
biological decomposition of Lindane and 2,4-D was tested in-
dividually at various concentrations while possible toxicity
effects were observed from the decay coefficients.

Lindane

Lindane at concentrations of 50 and 500 ppb was ino-
culated into batch culture enrichments units as described
previously. The data collected from these experiments are
presented in Table 5. This table presents the averages of
duplicate trials of pesticide concentration remaining,
dissolved oxygen, pH, and COD. Data are also given from a
set of control experiments where pesticide and sodium
acetate were present in the absence of bacterial inoculum to
determine volatilization or other non-bacterial pesticide
removal. Similarly, controls without pesticide addition
were established to better define the effect of the respec-
tive pesticide on overall system performance.

Figures 2 and 3 present the data collected for Lindane

20

TABLE V

AVERAGES OF DUPLICATE DATA VALUES
COLLECTED FOR LINDANE EXPERIMENTS

TIME	Pesticide Conc. Remaining (ppb)		D.O (mg/L)		pH		COD (mg/L)		Controls When No Pesticide Was Present.	Controls (no bacteria) Pesticide Concentration	
(Days)	50 (ppb)	500 (ppb)	50 (ppb)	500 (ppb)	50 (ppb)	500 (ppb)	50 (ppb)	500 (ppb)	(mg/L)	50 (ppb)	500 (ppb)
0	49.98	490.00	8.2	8.1	6.8	7.0	47	47	47	49.9	498.0
1	40.74	476.72	7.7	7.6	6.1	6.3	32	35	25	49.9	498.0
2	30.46	457.10	7.6	7.4	6.1	6.2	25	25	20	49.9	498.0
3	36.40	417.10	7.4	7.4	6.1	6.1	15	20	10	49.9	497.5
4	36.50	398.57	7.0	7.2	6.1	6.0	8	10	5	49.9	498.0
5	30.20	298.33	6.6	6.2	6.1	6.0	3	5	2	49.9	498.0
6	26.48	280.10	6.6	6.5	6.1	6.1	1	3	1	49.5	497.8
7	27.80	246.80	6.4	6.3	6.1	6.0	BDL	BDL	BDL	49.5	498.0
8	39.68	330.20	6.4	6.3	6.1	6.0	BDL	BDL	BDL	49.5	498.0
9	33.50	330.45	6.3	6.2	6.0	6.1	BDL	BDL	BDL	49.5	497.8
10	36.40	315.65	6.3	6.2	6.1	6.1	BDL	BDL	BDL	49.5	498.0
11	39.50	298.90	6.1	6.0	6.0	6.1				49.5	498.0
12	34.80	292.60	6.1	6.0	6.0	6.1				49.5	498.0
13	34.03	290.34	6.0	5.8	6.0	6.1				49.5	497.8
14	32.20	298.20	5.8	5.7	6.0	6.0				49.7	497.5
15	27.20	290.40	5.8	5.7	6.0	6.1				49.7	498.0
16	27.10	287.70	5.6	5.5	6.1	6.1				49.5	497.8
17	27.95	265.36	5.5	5.2	6.0	6.0				49.5	497.8
18	27.50	210.40	5.0	5.0	6.0	6.1				49.5	498.0
19	27.40	237.30	4.8	5.0	6.0	6.1				49.5	498.0
20	26.40	223.00	4.7	4.6	6.0	6.1				49.5	497.8
21	26.24	219.47	4.8	4.4	6.0	6.0				49.5	498.0
22	26.05	214.78	4.6	4.3	6.0	6.0				49.5	497.8
23	25.33	211.80	4.6	4.3	6.0	6.0				49.5	497.8
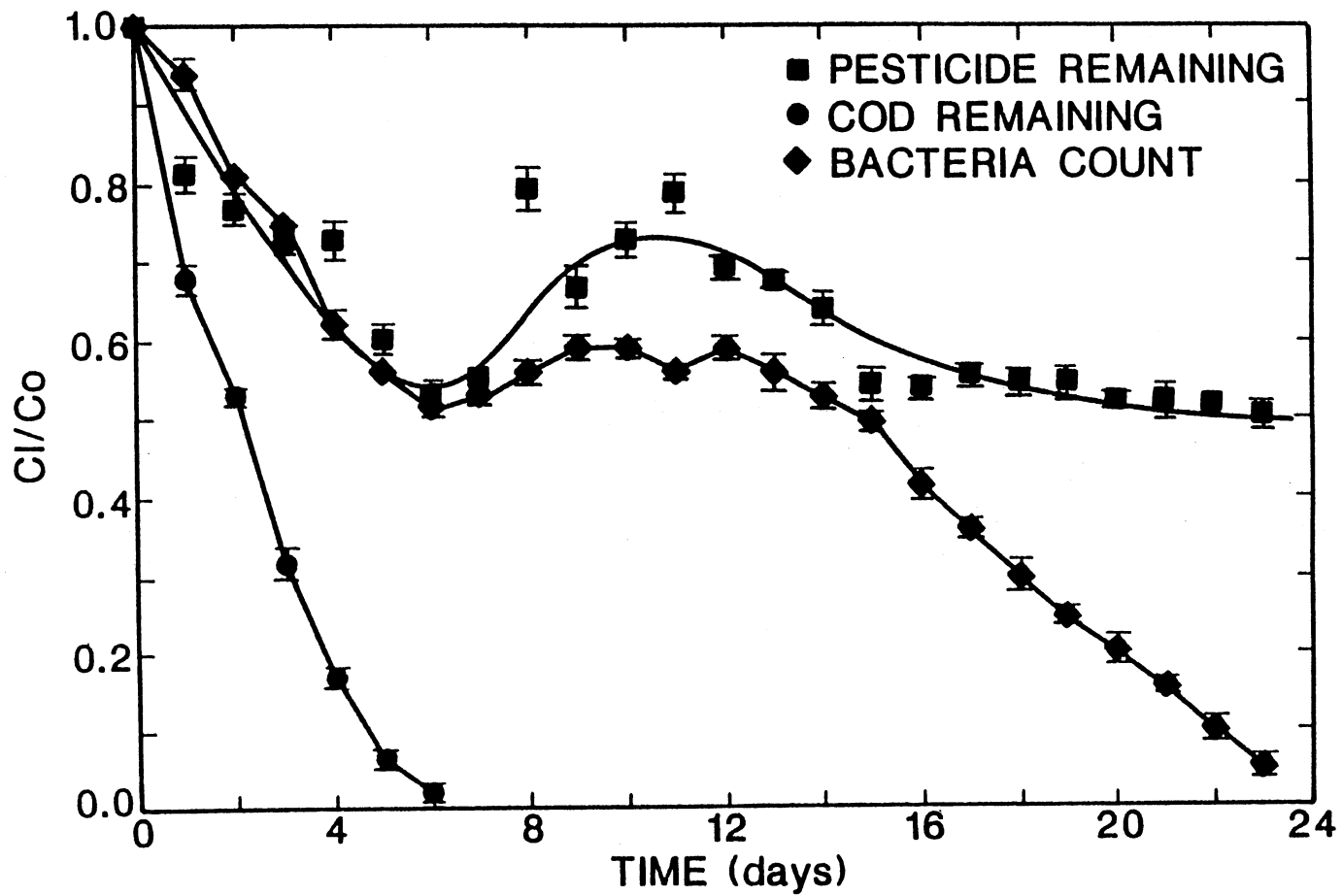
Figure 2. Experimental Results of Bacterial Growth and Sodium Acetate and Lindane Decay (50 ppb) in Batch Culture Tests.
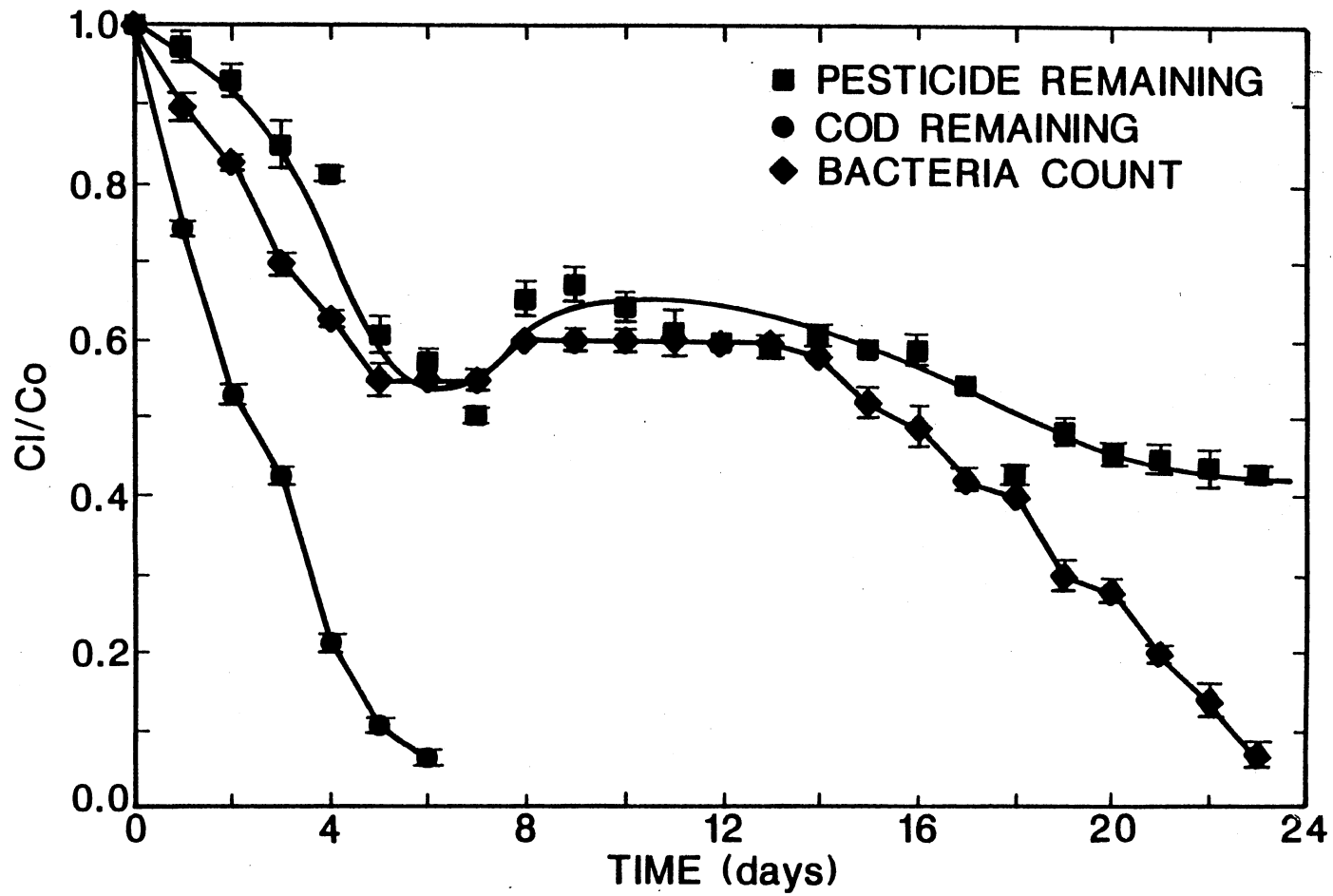
Figure 3. Experimental Results of Bacterial Growth and Sodium
Acetate and Lindane Decay (500 ppb) in Batch Culture
Tests.

at 50 and 500 ppb respectively. Insecticide and COD re-
movals are plotted over time as well as the daily bacterial
count for the period of experimentation. These figures show
that the primary substrate as measured by COD was disappear-
ing while the bacteria was acclimating to the new system,
which consisted of a lower primary substrate than was used
in the original culture vessel as well as containing pesti-
cide. Mean values as well as the ranges of duplicate deter-
minations are shown on these and subsequent figures.

The removal of the insecticide was observed to become
predominant at approximately day 12 and after 14 or 15
days of incubation for 50 and 500 ppb respectively. The
concentration remaining in the samples (Table 5), showed
that 50% (50 ppb) and 57% (500 ppb) of the insecticide was
removed in the 23 days of these experiments. It also can be
observed that pesticide concentration decreased and then
subsequently increased. This seems due to a possible ad-
sorption of the pesticide onto the bacteria cells in the
reactor vessel, followed by a desorption phase. A final
decrease was then observed and is attributed to a removal or
decay phase. The adsorption of the insecticide onto bac-
teria cells was attributed to the relatively lower solubil-
ity of the insecticide (lindane) compared to other
pesticides (such as 2,4-D) in water. To determine the in-
secticide adsorption onto bacteria cells, samples for pesti-
cide extraction were filtered. The bacterial solids

remaining on the 0.45 um filter paper were physically re-
moved, resuspended in hexane, and kept in the freezer for
later injection. Table 6 presents the pesticide concentra-
tion adsorbed onto the bacteria cells. This table shows the
averages of duplicate trials or replicates completed. Ad-
sorption onto bacterial cells was shown to be a significant
mechanism for pesticide removal, particularly in the early
stages of incubation.

Figure 4, in conjunction with Table 5, presents the
data collected for the control experiments where sodium ace-
tate and pesticide of concern (lindane) were present without
bacterial inoculum. This figure shows that both concentra-
tions remained constant and that neither volatilization of
the pesticide nor bacterial contamination of the experiments
were thought to have occurred during the period of incuba-
tion.

Figure 5 presents the data collected for the control
experiments where no pesticide was present. This figure
illustrates the change in COD and bacterial count with time
for the period of experimentation. This figure shows that
that the substrate was disappearing while the bacteria were
acclimating to the new system as with the systems where the
pesticide was also introduced into the reactors. After the
substrate concentration fell below the level of detection,
the growth of bacteria started to decrease due to a lack of
available food or energy. Endogenous respiration is assumed

TABLE VI

AVERAGES OF DUPLICATE TRIALS OF
ADSORPTION OF LINDANE
ONTO BACTERIA CELLS

Days Incubation	Initial Pesticide Concentration (ppb)	Avg. of Duplicate Amount of Pest. Adsorbed (ppb)
2	50	3
3	50	4
4	50	5
4	500	93
6	500	61
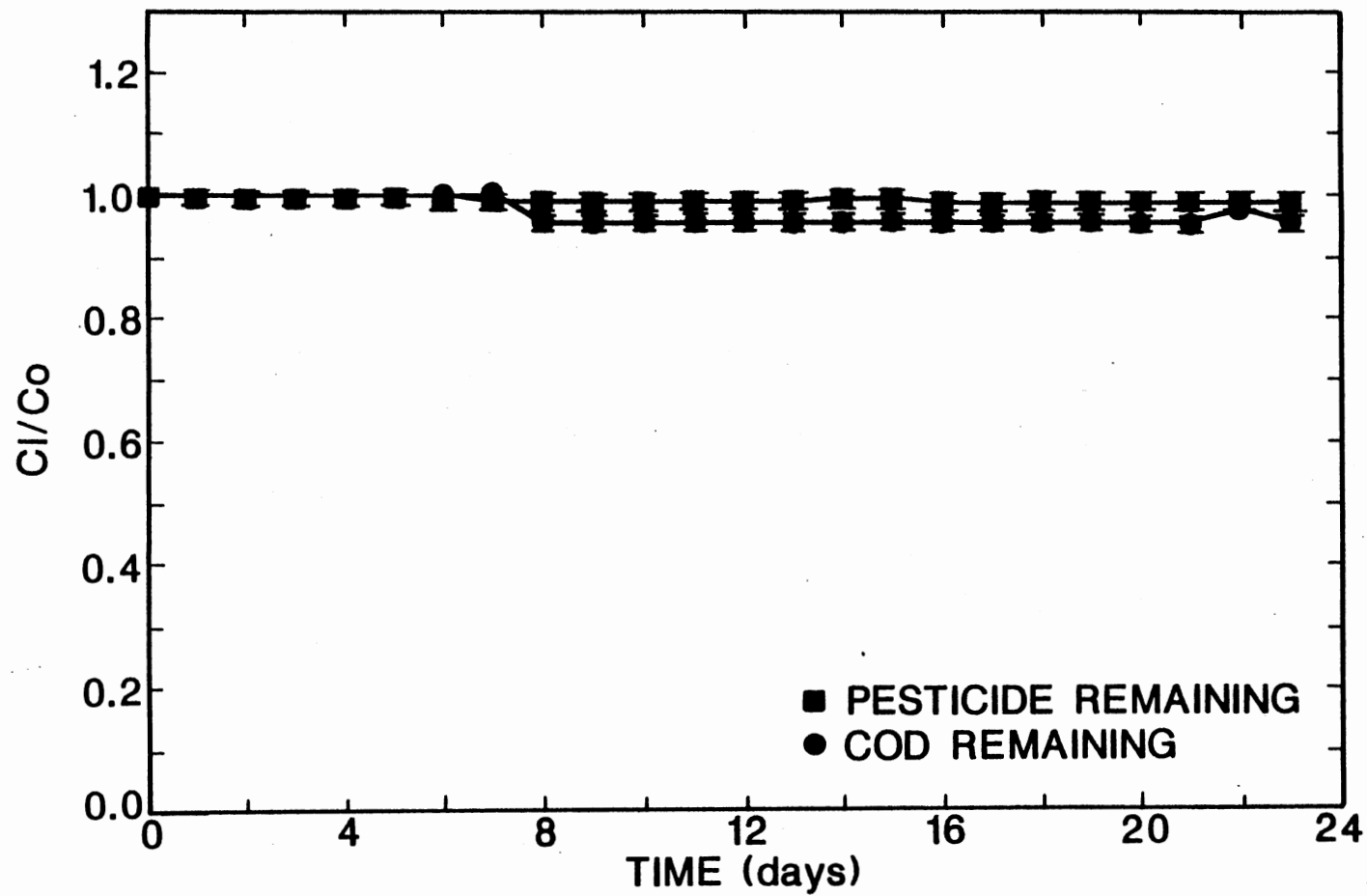
Figure 4. Volatilization Control for COD and Lindane at 50, and 500 ppb.
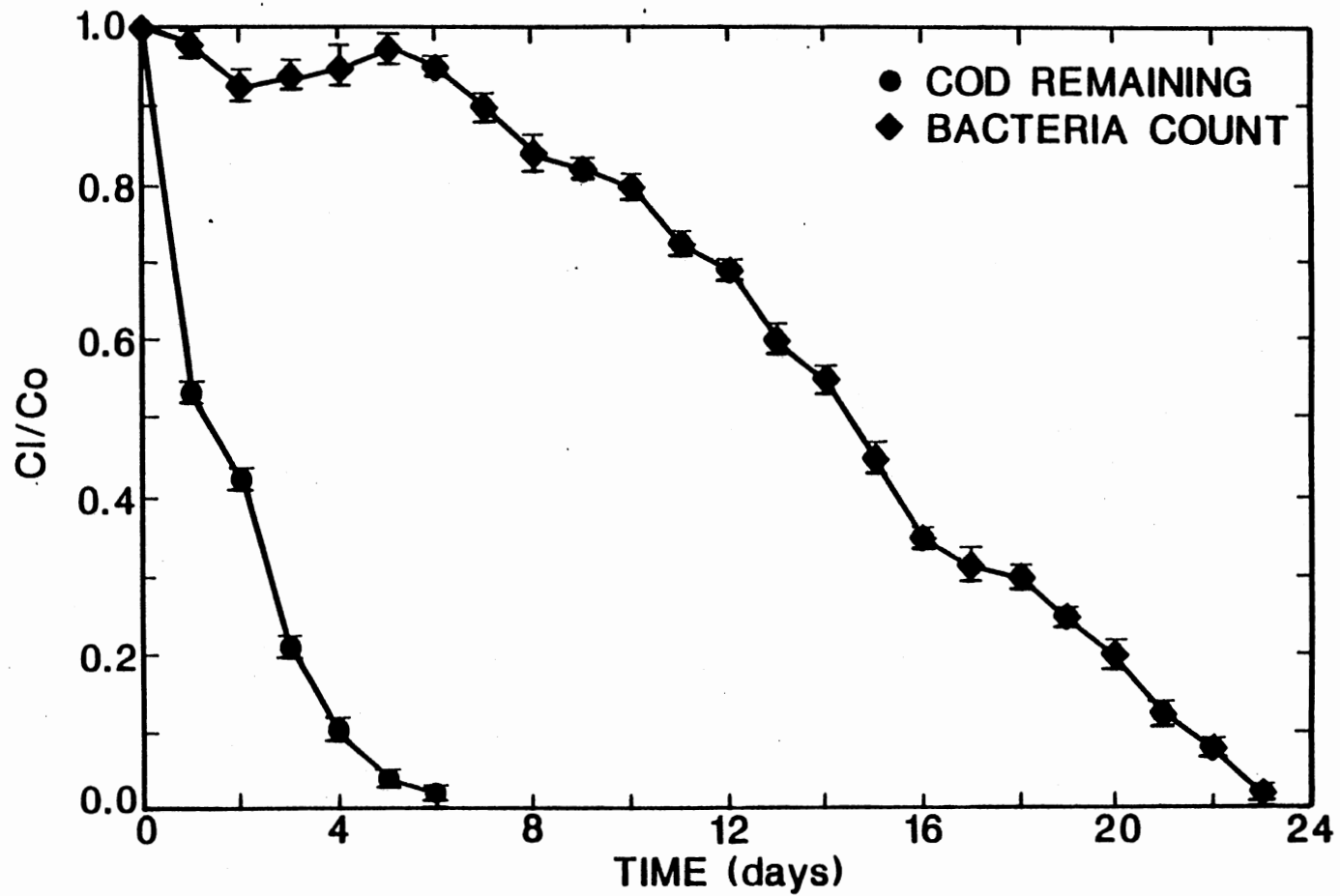
Figure 5. Lindane Control Units for Growth and Substrate Removal
Data.

to become increasingly prevalent. A substrate removal con-
stant of 0.6424 mg/L day and microbial growth and death rate
of 0.019 per day and 0.1700 per day were obtained for this
set of experiments. These primary substrate removal rate
constants and microbial growth and death rates will be com-
pared to those determined when pesticide was present in sub-
sequent figures.

Figure 6 and 7 present the primary substrate and
secondary substrate removal rate constants, determined for
the set of experiments where 50 and 500 ppb of lindane was
included in the inoculation. These were determined by using
linear regression of the natural log (ln) of the substrate
concentrations with time. These figures summarize the
various overall kinetic coefficients calculated from the
collected data. This approach generates first order removal
kinetics for comparison purposes. Other functional forms
will be compared later in this thesis. These removal con-
stant values are presented in Table 7. At a higher pesti-
cide concentration the removal rate of the primary substrate
was lower than that obtained at the lower pesticide concen-
tration or when no pesticide was present in the system, but
the pesticide decay rate was lower in the 50 ppb reactors
than in the 500 ppb vessels.

Table 8, in conjunction with Figures 2 and 3, presents
the measurement of the bacterial population, monitored
through the total incubation period for both concentrations
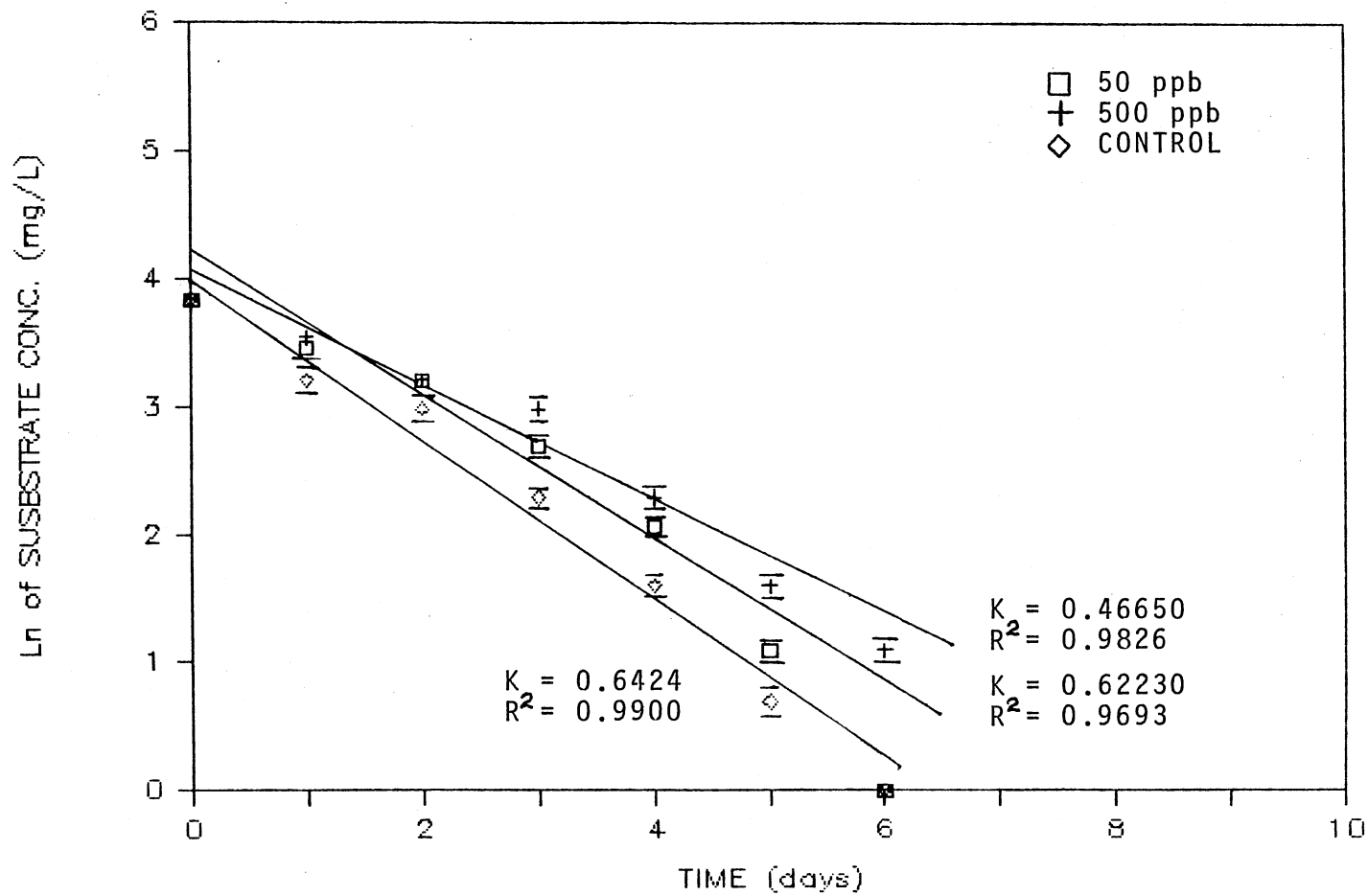
Figure 6. Removal Rate Constants (K) and Correlation
Coefficients for Sodium Acetate without Pesticide
and with Lindane at 50 and 500 ppb.
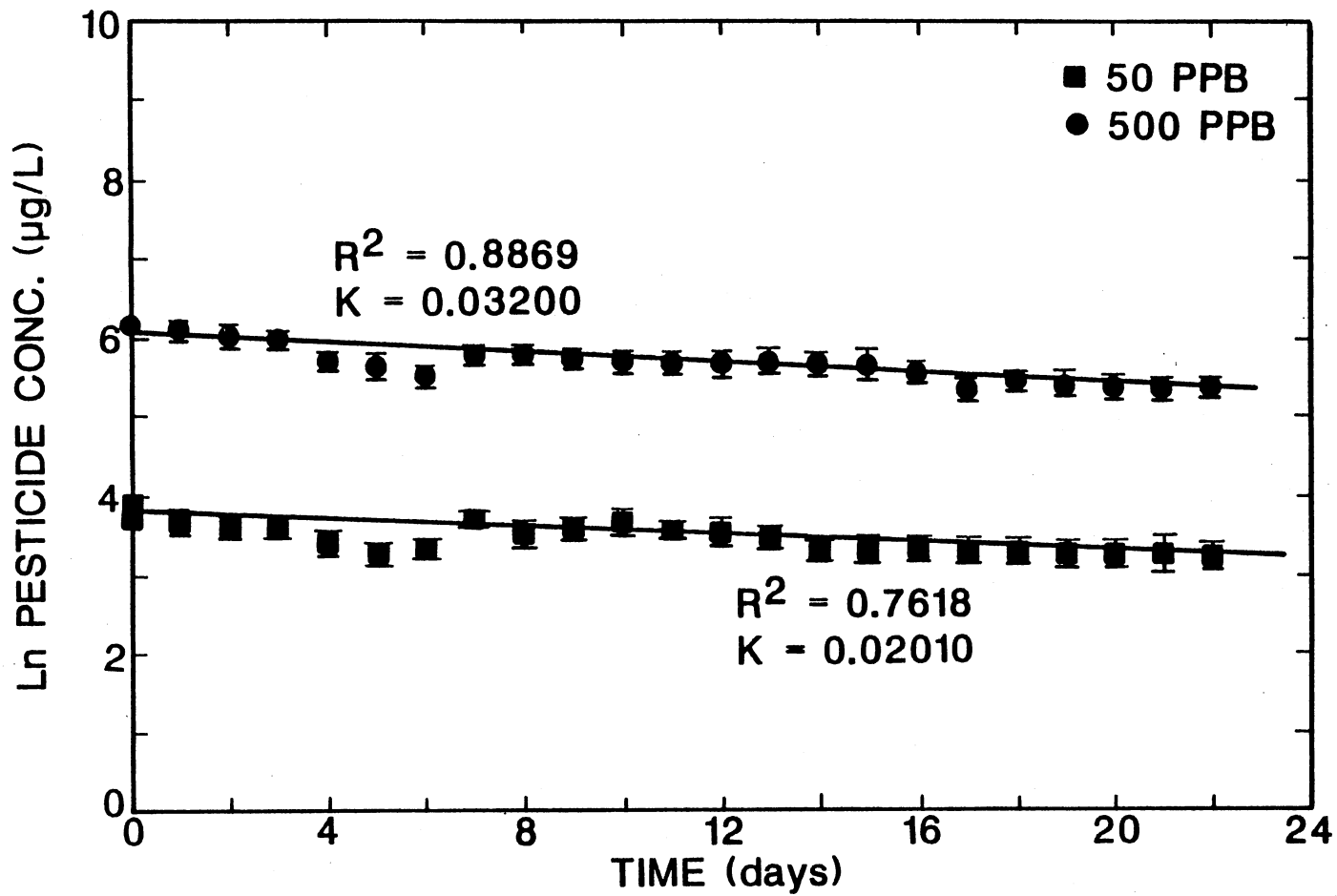
Figure 7. Removal Rate Constants (K) and Correlation Coefficients for Lindane at 50 and 500 ppb.

TABLE VII

REMOVAL RATE (K) VALUES FOR SODIUM
ACETATE AND LINDANE AS WELL
AS CONTROL UNITS

Pesticide of interest	Pesticide Concentration (ppb)	Sodium Acetate K (mg/L/day)	Pesticide K (µg/L/day)
Lindane	50	0.62230	0.02010
Lindane	500	0.46650	0.03200
Lindane	Control	0.64240	N/A

TABLE VIII

AVERAGES OF DUPLICATE TRIALS OF BACTERIA COUNT (CELLS/ML) FOR LINDANE EXPERIMENTS, AND CONTROL UNITS WHERE NO PESTICIDE WAS PRESENT

Time	Bacteria Count x (10E6 Cells/ml)				Controls Where No Pest. Was Present	
	50 ppb	Ci/Co	500 ppb	Ci/Co	Cells x 10E6	Ci/Co
0	16.00	1.00	10.00	1.00	20.00	1.00
1	15.00	0.94	9.00	0.90	19.60	0.98
2	13.00	0.81	8.30	0.83	18.50	0.93
3	12.00	0.75	7.00	0.70	18.80	0.94
4	10.00	0.63	6.30	0.63	19.00	0.95
5	9.00	0.56	5.50	0.55	19.50	0.98
6	8.30	0.52	5.50	0.55	19.00	0.95
7	8.50	0.53	5.50	0.55	18.00	0.90
8	9.00	0.56	6.00	0.60	16.80	0.84
9	9.50	0.59	6.00	0.60	16.50	0.83
10	9.50	0.59	6.00	0.60	16.00	0.80
11	9.00	0.56	6.00	0.60	14.50	0.73
12	9.50	0.59	6.00	0.60	13.80	0.69
13	9.00	0.56	6.00	0.60	12.00	0.60
14	8.50	0.53	5.80	0.58	11.00	0.55
15	8.00	0.50	5.20	0.52	9.00	0.45
16	6.70	0.42	4.90	0.49	7.00	0.35
17	5.80	0.36	4.20	0.42	6.30	0.32
18	4.80	0.30	4.00	0.40	6.00	0.30
19	4.00	0.25	3.00	0.30	5.00	0.25
20	3.30	0.21	2.80	0.28	4.00	0.20
21	2.50	0.16	2.00	0.20	2.50	0.13
22	1.60	0.10	1.40	0.14	1.60	0.08
23	0.85	0.05	0.70	0.07	0.40	0.02

of lindane. Population size initially decreased as bacteria were exposed to the lower concentration of acetate in the media relative to the larger incubation reactor designed to provide seed. A probable toxicity of the insecticide to the microorganisms was subsequently noticed as the pesticide concentration was increased. The population went from 16x10E6 cells/ml (50 ppb) to 10x10E6 cells/ml (500 ppb). This was later partially confirmed with growth and death rate coefficients. This toxicity is related to removal rates of the primary substrate obtained as pesticide concentration was increased.

Table 9 presents the microbial growth rates determined for the points when the bacterial response reaches a new level appropriate to the decrease in primary substrate encountered in the batch reactors. These were determined by plotting the log of the bacteria count with time.

Table 10 presents the microbial death phase rates obtained for this set of experiments. These were determined by plotting the natural log (ln) of the bacteria count versus time. These values were calculated from the point where the bacterial population was decreasing in total number to the end of the experiment.

Comparing the microbial growth and death rates between the concentrations used in this set of experiments and to controls where no pesticide was present, note that after the bacteria became acclimated to the new system or media, the

TABLE IX

MICROBIAL GROWTH RATES FOR BACTERIA
INOCULATED IN LINDANE SOLUTIONS
OF 50 AND 500 PPB, AS WELL AS
CONTROLS WHERE NO PESTICIDE
WAS PRESENT

Pesticide of interest	Initial Pesticide Concentration (ppb)	Growth Rates per day
Lindane	50	0.0202
Lindane	500	0.0190
Control	N/A	0.0190

TABLE X

MICROBIAL DEATH PHASE RATES FOR BACTERIA
INOCULATED IN LINDANE SOLUTIONS
OF 50 AND 500 PPB, AS WELL AS
CONTROLS WHERE NO PESTICIDE
WAS PRESENT

Pesticide of interest	Initial Pesticide Concentration (ppb)	Death Rates per day
Lindane	50	0.0938
Lindane	500	0.0819
Control	N/A	0.1700

growth rate values at both pesticide concentrations were
approximately equal to each other and that determined for
the control system.

When comparing the death rates values, there was a
decrease as the pesticide concentration was increased,
possibly showing that the pesticide had become a substrate
available to the acclimated microorganisms, thereby slowing
the effects of the death phase. Further, when compared to
the control system, the microbial death rates were sequen-
tially lower as the pesticide concentration was increased.

2,4-Dichlorophenoxyacetic Acid

2,4-D at concentrations of 50, 500 and 5000 ppb was in-
cubated in the same type of enrichment vessels described
previously. The data collected for these experiments are
presented in Table 11. This table presents the averages of
duplicate trials of pesticide concentration remaining,
dissolved oxygen, pH, and COD. Data are also given from a
set of control experiments where pesticide at various con-
centrations, and sodium acetate were present in the absence
of bacterial inoculum to provide volatilization and non-
bacterial removal checks. Also presented are the results
from experiments where the pesticide was not added to the
reaction vessels to determine unaffected rate functions and
responses.

Figures 8, 9 and 10 present the data collected for

TABLE XI

AVERAGES OF DUPLICATE DATA VALUES
COLLECTED FOR 2,4-D EXPERIMENTS

| TIME	Pesticide Conc. Remaining (ppb)			D.O (mg/L)			pH			COD (mg/L)			Controls When No Pesticide Was Present.	Controls (no bacteria) Pesticide Concentration		
(Days)	50 (ppb)	500 (ppb)	5000 (ppb)	50 (ppb)	500 (ppb)	5000 (ppb)	50 (ppb)	500 (ppb)	5000 (ppb)	50 (ppb)	500 (ppb)	5000 (ppb)	(mg/L)	50 (ppb)	500 (ppb)	5000 (ppb)
0	49.9	491	4987.7	8.2	8.7	8.9	7.0	7.2	7.2	47	47	47	47	49.9	493	4990.0
1	48.0	474.1	4869.6	7.6	8.3	7.4	6.6	6.6	6.8	32	40	45	25	49.8	492.8	4989.5
2	46.1	434.3	4734.6	7.3	7.8	7.5	6.4	6.2	6.1	25	35	40	20	49.5	493.0	4990.0
3	39.8	409.0	4737.7	7.1	7.5	6.4	6.2	5.9	5.9	20	29	35	10	49.8	493.0	4990.0
4	30.2	325.5	4500.3	6.8	6.8	6.0	5.9	5.9	6.0	15	20	25	5	49.8	492.5	4990.0
5	27.2	214.6	4191.7	6.2	6.7	5.8	5.9	5.9	5.8	5	10	12	2	49.8	492.6	4989.0
6	24.5	191.5	4633.1	6.0	6.1	5.8	6.0	6.1	5.8	2	8	8	1	49.5	492.8	4989.3
7	20.3	182.0	4239.6	5.9	5.9	5.5	6.0	5.9	5.8	1	2	3	BDL	49.5	493.0	4989.4
8	17.3	163.5	4000.0	5.7	5.8	5.5	5.9	5.9	5.9	BDL	BDL	BDL	BDL	49.5	493.0	4990.0
9	12.5	150.4	3865.7	5.5	5.8	5.2	6.0	5.9	6.0	BDL	BDL	BDL	BDL	49.5	493.0	4989.6
10	10.5	141.4	3493.0	5.5	5.5	5.0	5.9	6.0	6.1	BDL	BDL	BDL	BDL	49.5	493.0	4990.0
11	9.8	114.2	3973.3	5.2	5.2	5.8	5.9	6.0	6.1					49.5	492.8	4990.0
12	5.1	110.0	3524.2	5.0	5.0	4.8	6.0	6.0	6.0					49.5	492.5	4990.0
13	4.9	102.7	3338.4	4.8	4.9	4.6	6.0	6.0	6.0					49.5	492.8	4990.0
14	2.0	92.4	3289.9	4.6	4.6	4.5	6.0	6.0	6.0					49.5	492.8	4990.0
15	1.7	82.0	3200.2	4.5	4.6	4.3	6.0	6.0	6.0					49.5	492.8	4990.0
16	1.7	82.7	3195.7	4.5	4.5	4.3	6.0	6.0	6.0					49.5	492.8	4990.0
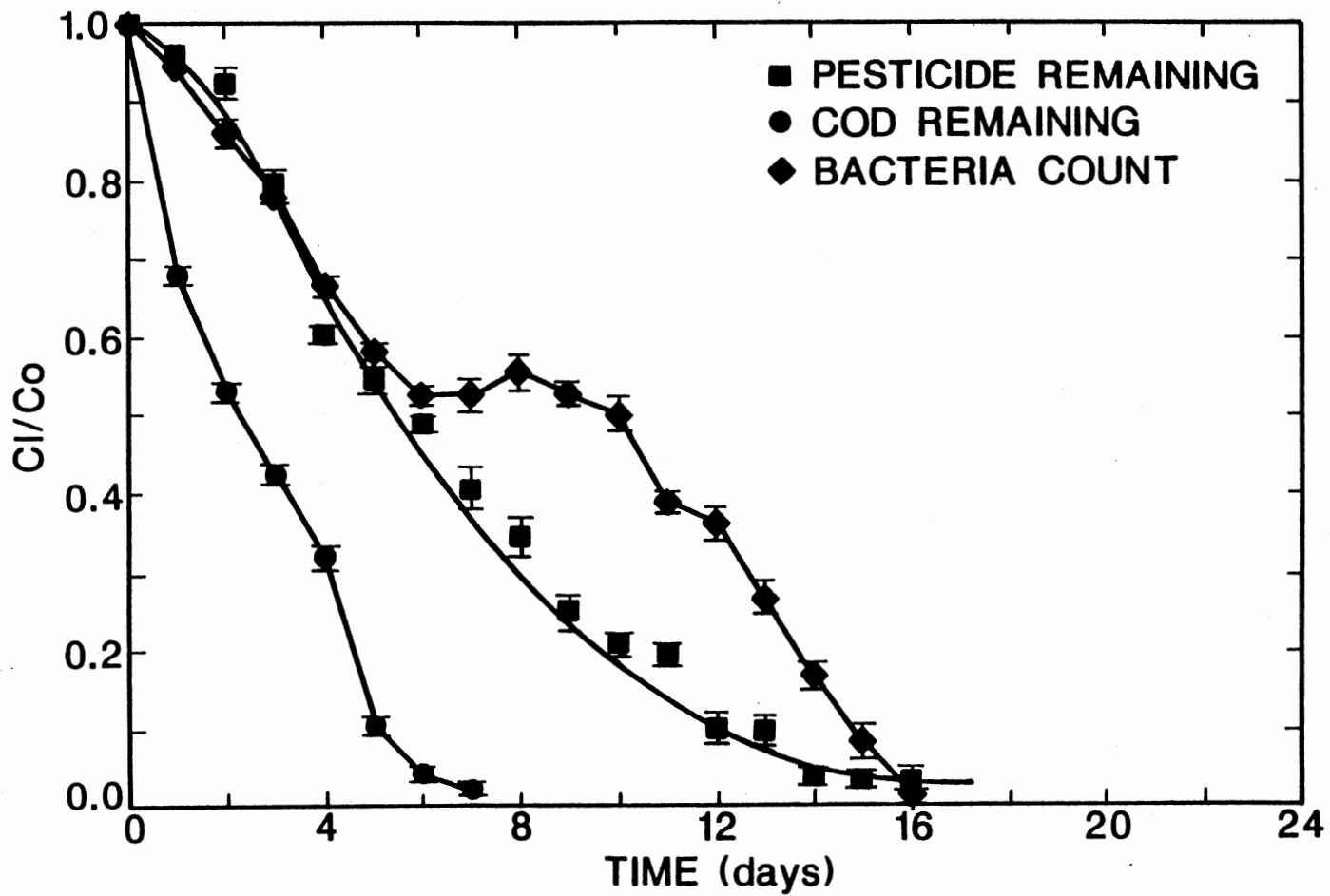
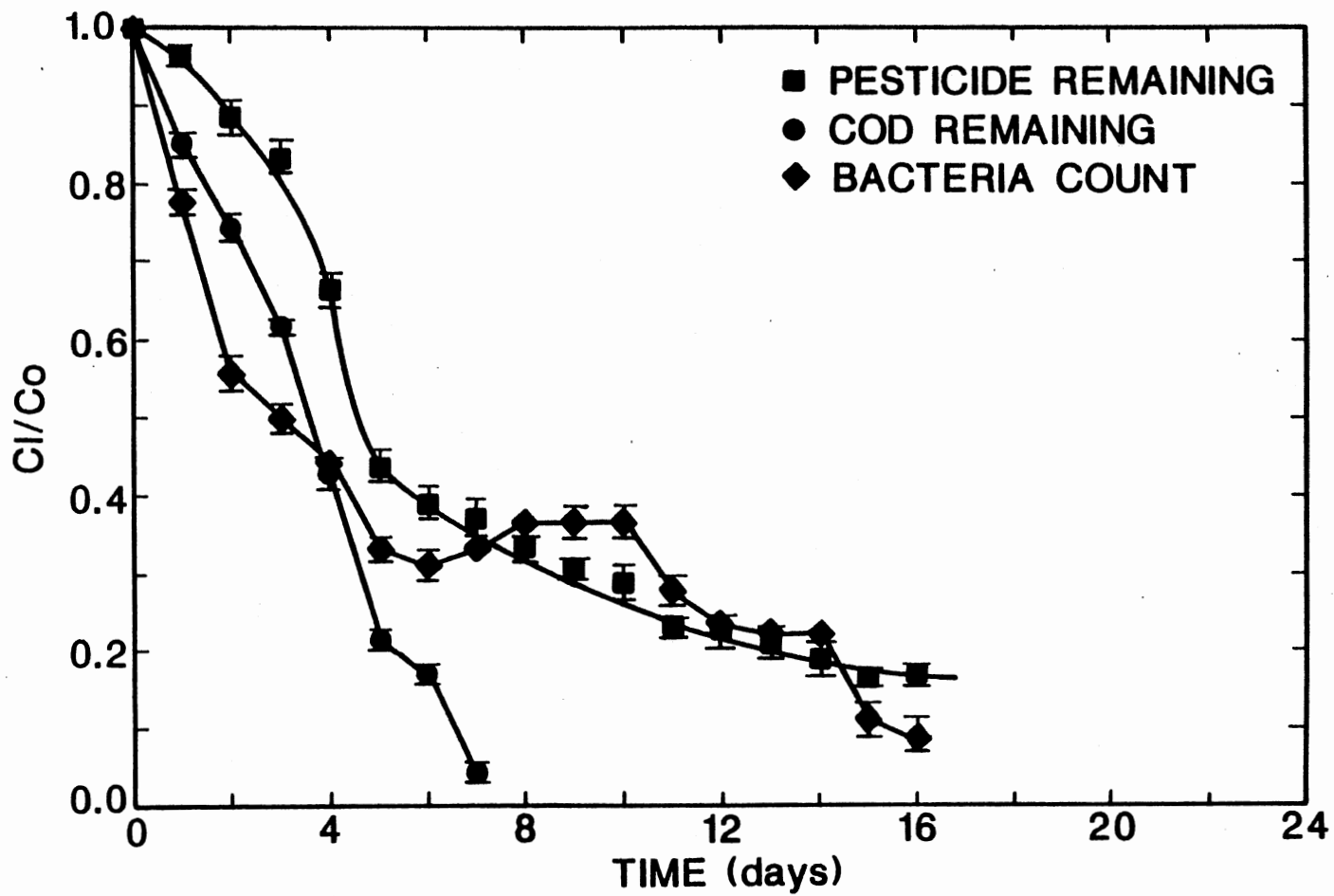Figure 8. Experimental Data for Microbial Growth and Sodium Acetate and Pesticide Removal (2,4-D = 50 ppb)

Figure 9. Experimental Data for Microbial Growth and Sodium
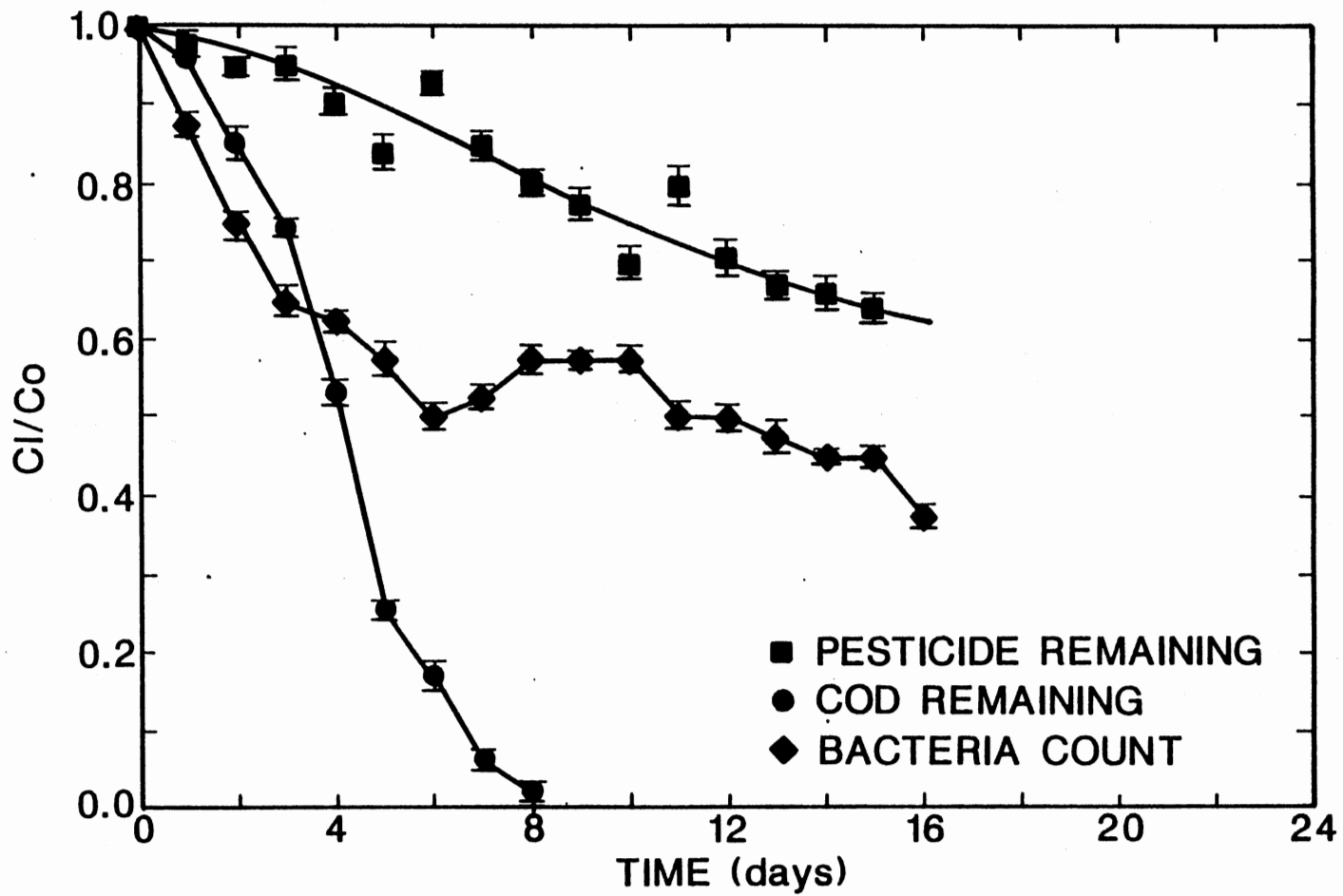Acetate and Pesticide Removal (2,4-D = 500 ppb).

Figure 10.   Experimental Data for Microbial Growth and Sodium
Acetate and Pesticide Removal (2,4-D = 5000 ppb)

2,4-D experiments at 50, 500, and 5000 ppb respectively. These figures plot the herbicide and COD removal, and bacterial count for the period of experimentation. These figures show that the primary substrate as measured by COD was disappearing while the bacteria was acclimating to the new system, which consisted of a lower concentration of primary substrate (100 mg/L) than was used in the original culture vessel (5000 mg/L) as well as containing pesticide. Mean values as well as the ranges of duplicate determinations are shown on these and subsequent figures. Degradation of the herbicide started approximately 1 to 2 days after incubation, the rate becoming faster after this initial period. Concentrations remaining in the samples after sixteen days showed that at 96% (50 ppb), 84% (500 ppb) and 35.93% (5000 ppb) of the herbicide was removed.

Figure 11, in conjunction with Table 11, presents the data collected for the control experiments where sodium acetate and 2,4-D were present without bacterial inoculum. This figure shows that both concentrations remained constant and that neither volatilization of the pesticide nor bacterial contamination of the experiments were thought to have occurred during the period of incubation.

Figure 12 presents the data collected for the control experiments where no pesticide was present, indicating the COD and bacterial count with time for the period of experimentation. It is shown that the substrate was disappearing
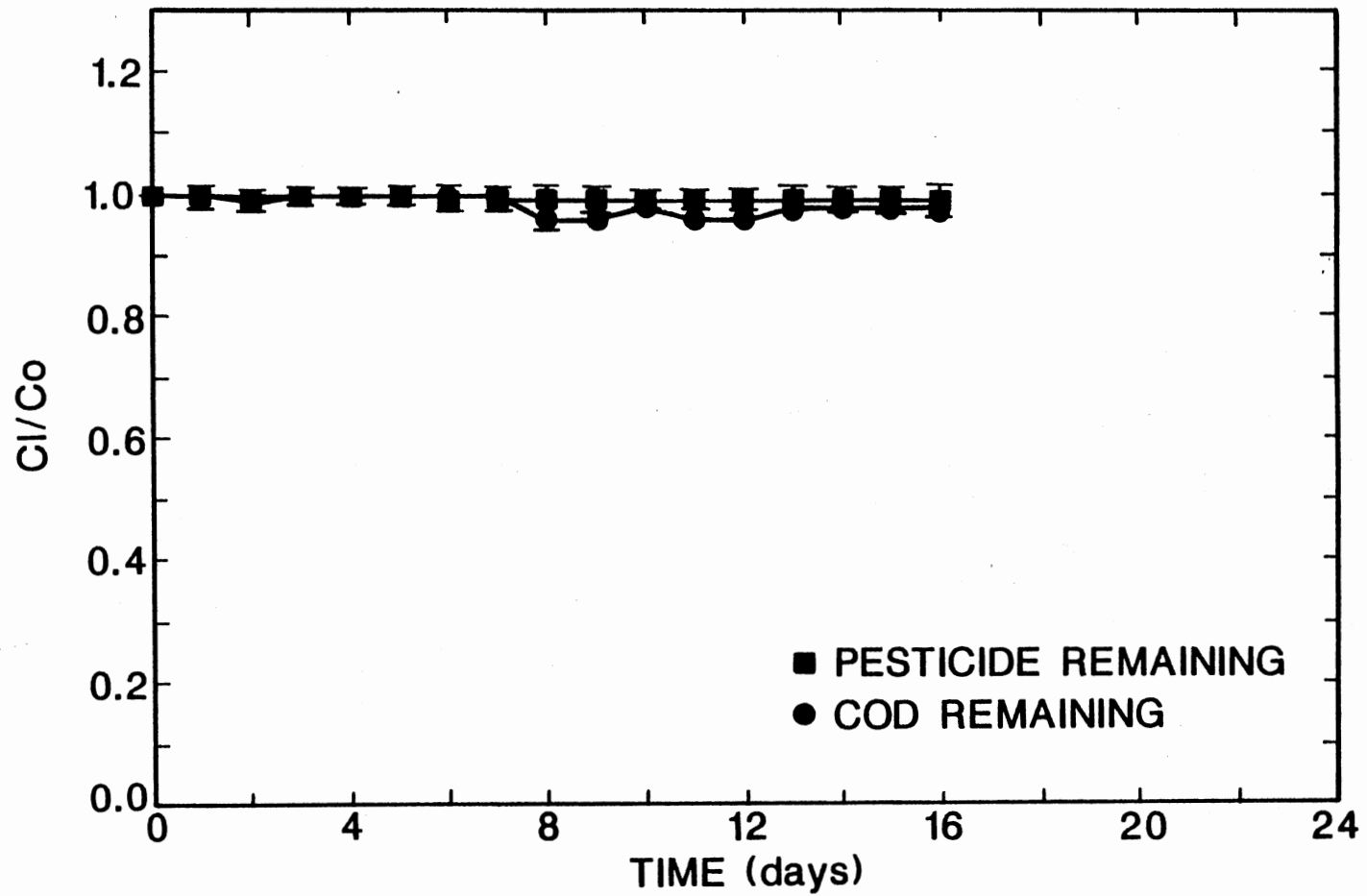
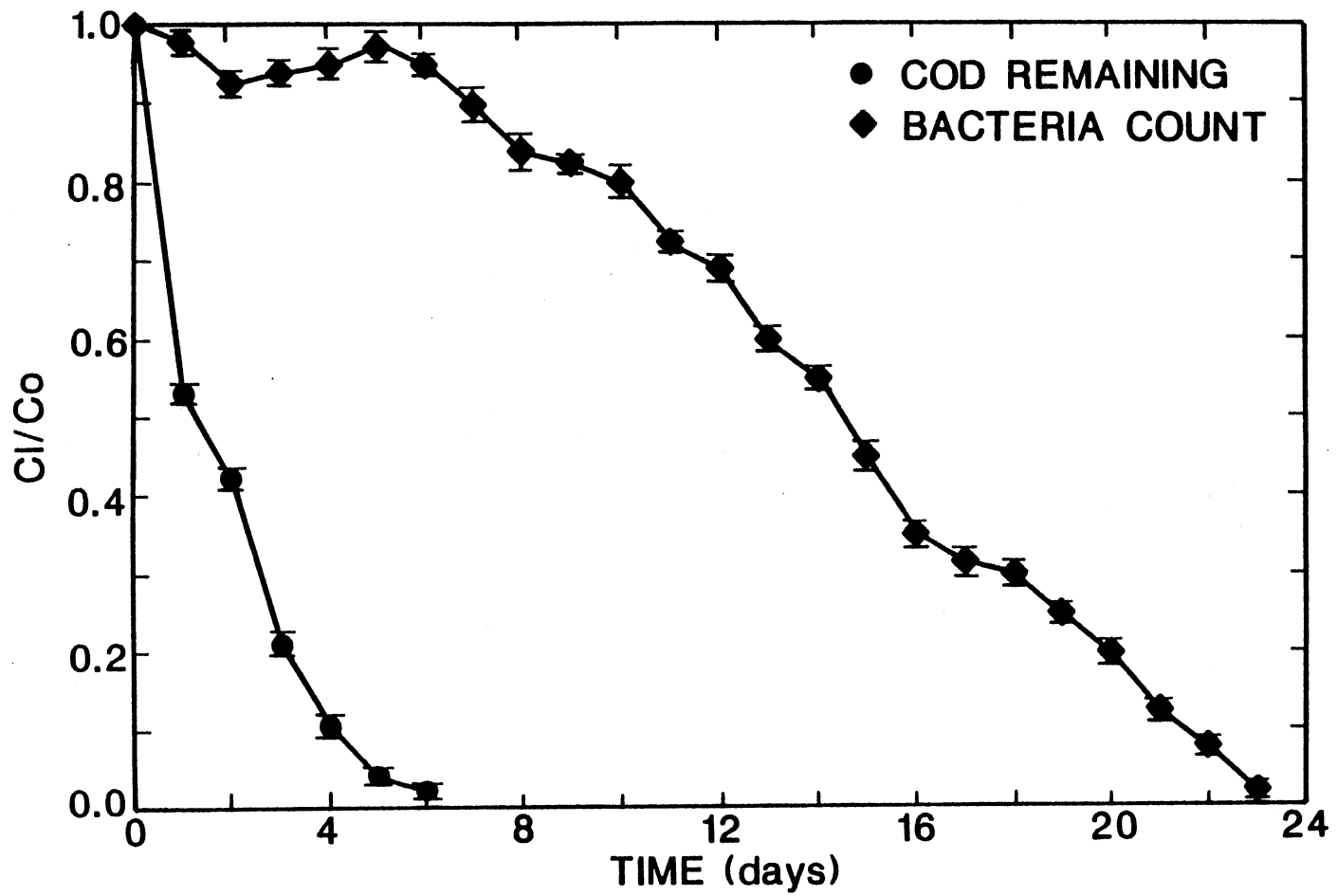Figure 11. Volatilization Control for COD and 2,4-D at 50, 500
and 5000 ppb.

Figure 12.   2,4-D Control Units for Growth and Substrate Removal
Data.

while the bacteria was acclimating to the new system as with the systems where the pesticide was also introduced into the reactors. After the substrate concentration fell below the level of detection, the growth of bacteria started to decrease due to a lack of available food or energy. Endogenous respiration is assumed to become increasingly prevalent. A substrate removal constant of 0.6424 mg/L day and microbial growth and death rates of 0.019 per day and 0.1700 per day were determined from this set of experiments. Subsequent figures will compare the primary substrate removal rate constant and microbial growth and death rates to those determined where pesticide was present at various concentrations. This figure is the same as presented for lindane experiments.

Figures 13 and 14 present the primary substrate and secondary substrate removal rate values determined for this set of experiments. These were determined by plotting the natural log (ln) of the substrates concentrations versus time. These figures summarize the various overall kinetic coefficients calculated from the collected data. This approach generates first order removal kinetics for comparison purposes. Other functional forms will be compared later in this thesis. Linear regression techniques were then used to fit these data to a "best" model. These removal rate (k) values are presented in Table 12.

This table shows that at a higher pesticide concentra-
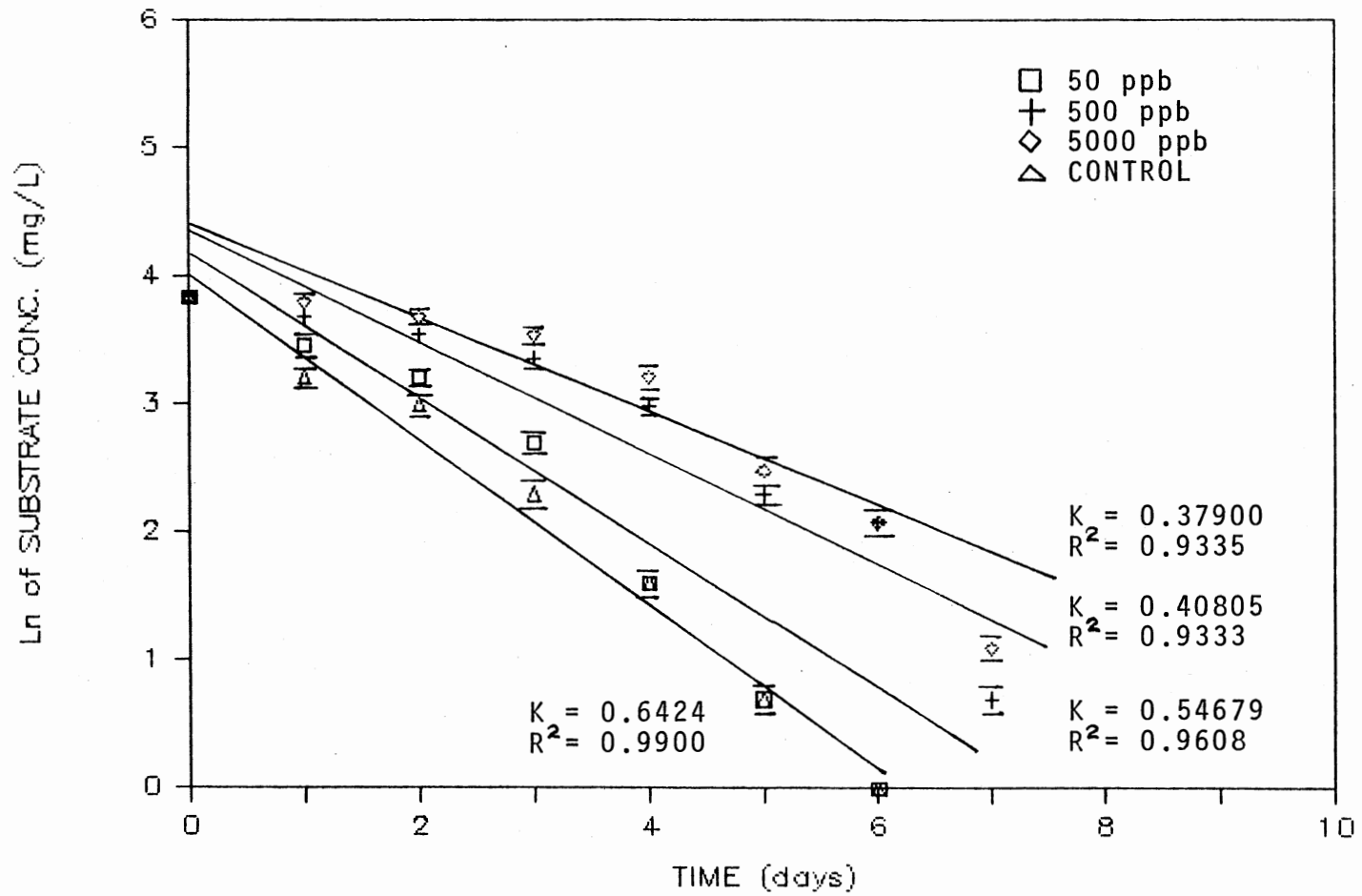
Figure 13.   Removal Rate Constants (K) and Correlation
            Coefficients for Sodium Acetate without Pesticide
            and with 2,4-D at 50, 500, and 5000 ppb.
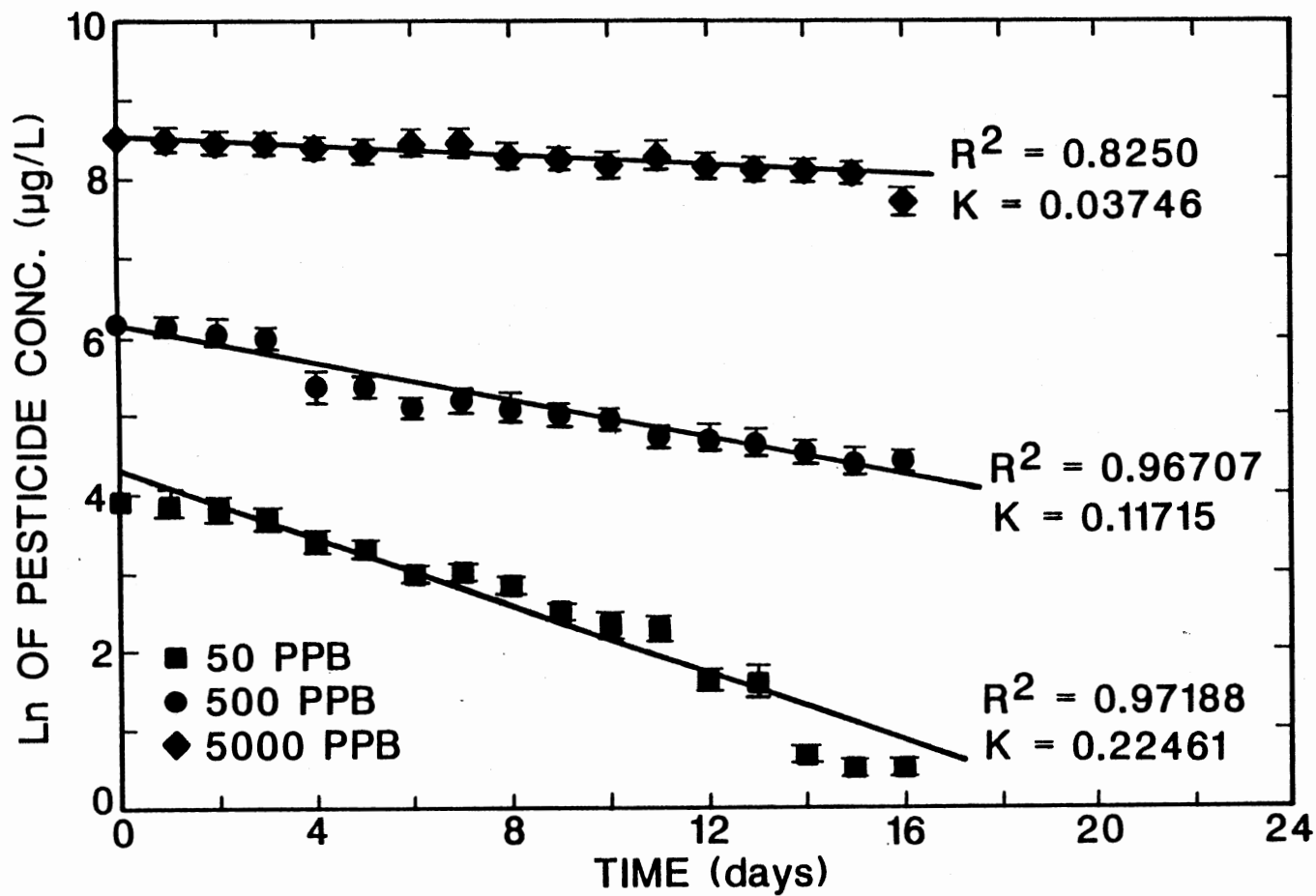
Figure 14.   Removal Rate Constants (K) and Correlation
Coefficients for 2,4-D at 50, 500, and 5000 ppb.

TABLE XII

REMOVAL RATE (K) VALUES FOR SODIUM
ACETATE AND 2,4-D AS WELL
AS CONTROL UNITS

Pesticide of interest	Pesticide Concentration (ppb)	Sodium Acetate K (mg/L/day)	Pesticide K (µg/L/day)
2,4-D	50	0.54679	0.22570
2,4-D	500	0.40805	0.12195
2,4-D	5000	0.37900	0.02998
Control	0	0.64240	N/A

tion the removal rate of the primary substrate was lower than that obtained at the lower pesticide concentration or when no pesticide was present in the system.

Table 13, in conjunction with figures 8, 9 and 10, represents the measurement of bacterial population, monitored through the incubation period for all the concentrations. These data show that as with lindane, the population size decreased as bacteria were exposed to the lower concentration of acetate in the media. Toxicity of the herbicide to the microorganisms was noticed as pesticide concentration was increased. The presence of increased herbicide concentrations lowers the primary substrate removal rates (Table 12).

Table 14 presents the microbial growth rates for the points when the bacteria appeared acclimated to the new incubation systems. These were determined by taking the slopes of a natural logarithm plot of the bacteria count over time.

This table shows that the microbial growth rate increases with increasing pesticide but exhibits a subsequent decrease at the highest 2,4-D concentration used. This indicates that increasing pesticide concentration offers a higher energy level to the acclimated bacteria, but that apparent toxic responses associated with the highest pesticide concentration soon diminish the rate of growth.

Table 15 presents the microbial death phase rates

TABLE XIII

AVERAGES OF DUPLICATE TRIALS OF BACTERIA
COUNT (CELLS/ML) FOR 2,4-D EXPERIMENTS,
AND CONTROL UNITS WHERE NO
PESTICIDE WAS PRESENT

Time	Bacteria Count x (10E6 Cells/ml)						Controls Where No Pest. Was Present	
	50 ppb	Ci/Co	500 ppb	Ci/Co	5000 ppb	Ci/Co	cells x 10E6	Ci/Co
0	18.00	1.00	9.00	1.00	4.00	1.00	20.00	1.00
1	17.00	0.94	7.00	0.78	3.50	0.88	19.60	0.98
2	15.50	0.86	5.00	0.56	3.00	0.75	18.50	0.93
3	14.00	0.78	4.50	0.50	2.60	0.65	18.80	0.94
4	12.00	0.67	4.00	0.44	2.50	0.63	19.00	0.95
5	10.50	0.58	3.00	0.33	2.30	0.58	19.50	0.98
6	9.50	0.53	2.80	0.31	2.00	0.50	19.00	0.95
7	9.50	0.53	3.00	0.33	2.10	0.53	18.00	0.90
8	10.00	0.56	3.30	0.37	2.30	0.58	16.80	0.84
9	9.50	0.53	3.30	0.37	2.30	0.58	16.50	0.83
10	9.00	0.50	3.30	0.37	2.30	0.58	16.00	0.80
11	7.00	0.39	2.50	0.28	2.00	0.50	14.50	0.73
12	6.50	0.36	2.10	0.23	2.00	0.50	13.80	0.69
13	4.80	0.27	2.00	0.22	1.90	0.48	12.00	0.60
14	3.00	0.17	2.00	0.22	1.80	0.45	11.00	0.55
15	1.50	0.08	1.00	0.11	1.80	0.45	9.00	0.45
16	0.20	0.01	0.80	0.09	1.50	0.38	7.00	0.35

TABLE XIV

MICROBIAL GROWTH RATES FOR BACTERIA
INOCULATED IN 2,4-D SOLUTIONS
OF 50, 500 AND 5000 PPB,
AS WELL AS CONTROLS
WHERE NO PESTICIDE
WAS PRESENT

Pesticide of interest	Initial Pesticide Concentration (ppb)	Growth Rates per day
2,4-D	50	0.0110
2,4-D	500	0.0360
2,4-D	5000	0.0283
Control	N/A	0.0190

TABLE XV

MICROBIAL DEATH PHASE RATES FOR BACTERIA
INOCULATED IN 2,4-D SOLUTIONS
OF 50, 500 and 5000 PPB,
AS WELL AS CONTROLS
WHERE NO PESTICIDE
WAS PRESENT

Pesticide of interest	Initial Pesticide Concentration (ppb)	Death Rates per day
2,4-D	50	0.2000
2,4-D	500	0.0952
2,4-D	5000	0.0249
Control	N/A	0.1700

obtained for this set of experiments. These were determined by plotting the natural log (ln) of the bacteria count over time. These values were calculated at the point where the bacteria population was decreasing in total number and were continued until the end of the experimental data.

This table shows that as pesticide concentration increases the microbial death rates decreases. This is explained best by assuming that the acclimated bacteria could use the higher pesticide concentration as a growth substrate. During the time periods covered by these calculations, the available substrates assumed to exist are these pesticides and endogenous sources.

In comparing the microbial growth and death phase rates among the concentrations used for this set of experiments and to those where no pesticide was present, note that as the pesticide concentration was increased from 50 ppb to 5000 ppb, the growth rates increased as well, but the death rates decreased. The growth rate of the control experiments was higher than that obtained at 50 ppb and lower than that obtained at 500 and 5000 ppb. The death rates were lower at the two highest pesticide concentrations than were obtained when no pesticide was present. This is explained best by assuming that the acclimated bacteria could use the higher pesticide concentration as a substrate. The energy available to the acclimated culture from the pesticide far exceeded that from similar concentration of acetate or endogenous source.

Comparison of 2,4-D Data with Lindane Data

Table 16 presents the comparison of 2,4-D versus
Lindane, concerning their behavior in the presence of these
unacclimated microorganisms.

The data presented on this table showed some simi-
larities in terms of pesticide and acetate removal and
bacterial growth, but also exhibited some differences.

When comparing the primary substrate removal from both
pesticides to control units, these were slowed as the pesti-
cide concentration increased over time. This retardance was
related to the possible toxicity of each pesticide as the
concentration was increased making the unacclimated mixed
culture of heterotrophic bacteria more sensitive to the
toxic substance.

When comparing the microbial growth and death rates be-
tween pesticides as well as to controls, note that with
lindane, as the pesticide concentration was increased, the
growth and death rates were less affected by increased
pesticide concentration. A slight decrease in both rates
with increased pesticide concentration suggests alternative
mechanisms involved in microbial response to the pesticide.
As 2,4-D concentration increased, the growth rate also ini-
tially increased but exhibited a subsequent decrease at the
highest concentration used. The death rates were decreased
as the pesticide concentration increased.

The pesticide percent removal when compared between

TABLE XVI

COMPARISON OF 2,4-D VERSUS LINDANE
FOR THE CONDITIONS OF EXPERIMENT

Pesticide of Concern	Initial Conc. (ppb)	Pesticide Removal rate (ug/L.day)	Pesticide Removal (%)	Microbial Death rate (days -1)	Microbial Growth rate (days -1)	Sodium Acetate Removal (mg/L.day)
LINDANE	50	0.02010	50	0.0938	0.0202	0.6223
LINDANE	500	0.03200	57	0.0819	0.0190	0.4665
2,4-D	50	0.22461	96	0.2000	0.0110	0.5468
2,4-D	500	0.11715	84	0.0952	0.0360	0.4081
2,4-D	5000	0.03746	36	0.0249	0.0283	0.3790
CONTROL	NA	NA	NA	0.1700	0.0190	0.6424

these two pesticides, showed that 2,4-D had a higher percent removal than lindane. This was attributed to sodium acetate being possibly somewhat analogous to 2,4-D in chemical structure. This suggests that enzymes capable of metabolizing the 2,4-D were already present in the unacclimated population. This lowered acclimation times caused a more rapid decay of the 2,4-D relative to the lindane. Table 17 summarizes the various overall kinetic coefficients calculated from the collected data. The removal coefficients for sodium acetate as well as for the 50 and the 5000 ppb 2,4-D experiments were determined from day 1 to the end of the experiments, while the 500 ppb 2,4-D data were analyzed as two separate events. The lindane determinations were done from the time of bacterial acclimation.

TABLE XVII

KINETIC COEFFICIENTS CALCULATED FOR LINDANE AND 2,4-D EXPERIMENTS

Pesticide of Concern	Pesticide Conc. (ppb)	Simulation Time Period (days)		Overall Removal Kinetic (base e)	
		Sodium Acetate Removal	Pesticide Removal	Sodium Acetate	Pesticide
LINDANE	50	0 - 6	12 - 23	0.6223	0.02620
LINDANE	500	0 - 6	9 - 23	0.4665	0.03381
2,4-D	50	0 - 7	1 - 16	0.5468	0.22570
2,4-D	500	0 - 7	1 - 5	0.4081	0.15220
2,4-D	500	NA	5 - 16	NA	0.09212
2,4-D	5000	0 - 7	1 - 16	0.3790	0.02998
CONTROL	NA	0 - 6	NA	0.6424	NA

CHAPTER IV

ADDITIONAL ANALYSIS: MODELING

The models presented in Table 4 were evaluated for the application to these data sets. A determination of the best fit of each was considered to be a preliminary indication of the utility of each of these approaches.

To determine the model of best fit for each substrate disappearance curve, the values of the slopes either from natural log plots of remaining concentrations for the first order approximation or from similar plots of the unaltered original data for the zero order simultion, as well as, the initial substrate concentration, and introducing them as the kinetic coefficients into the integrated forms of the models presented in Table 4. The removal K's specified for the zero and first order models were taken to be equal to the specific reaction rate constants for each of the consti-tuents presented in Table 17. The concentrations used in these determinations corresponded to that remaining in solu-tion at the beginning of the simulation time period.

Lindane Modeling

Sodium acetate disappearance in conjunction with each insecticide concentration as single pesticide application

was the first condition to be modeled. These models were developed by taking the values of the slopes either from natural log plots of the remaining concentrations plots for the first order approximations or from similar plots of the unaltered original data for the zero order simulation, and introducing them as the kinetic coefficients into the integrated forms of the zero and first order models.

Figures 15, 16 and 17 present a comparison of the actual data collected during this effort with that simulated by the zero order and first order models for primary substrate removal in the presence of lindane as well as for the control trials. These figures showed that the zero order model under predicted the actual data while the first order predicted greater removals than observed in the experimental trials. Also the first order model had a better fit at the beginning and ending of the simulation time period than did the zero order approach. Therefore, the primary substrate removal was considered to be better described by the first order model than the zero order.

The mineralization of lindane at 50 and 500 ppb was also modeled. The data used in this activity were only those points collected form a post-acclimation phase. Simulation times for lindane mineralization at concentrations of 50 and 500 ppb were from day 12 to day 23, and from day 9 to day 23 respectively.

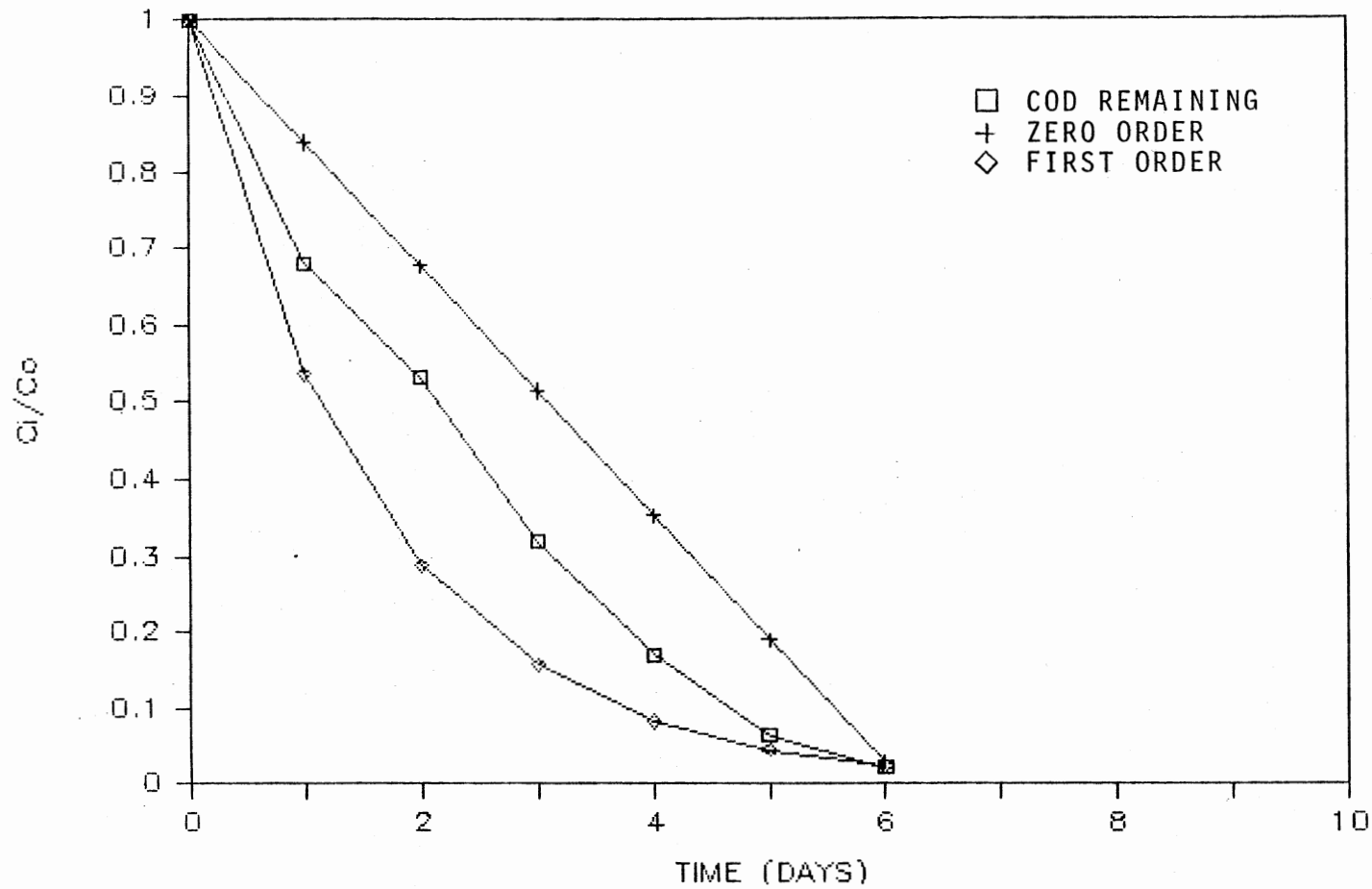Figures 18 and 19 present the model approach for the

Figure 15.   A Comparison of Model Results for Sodium Acetate
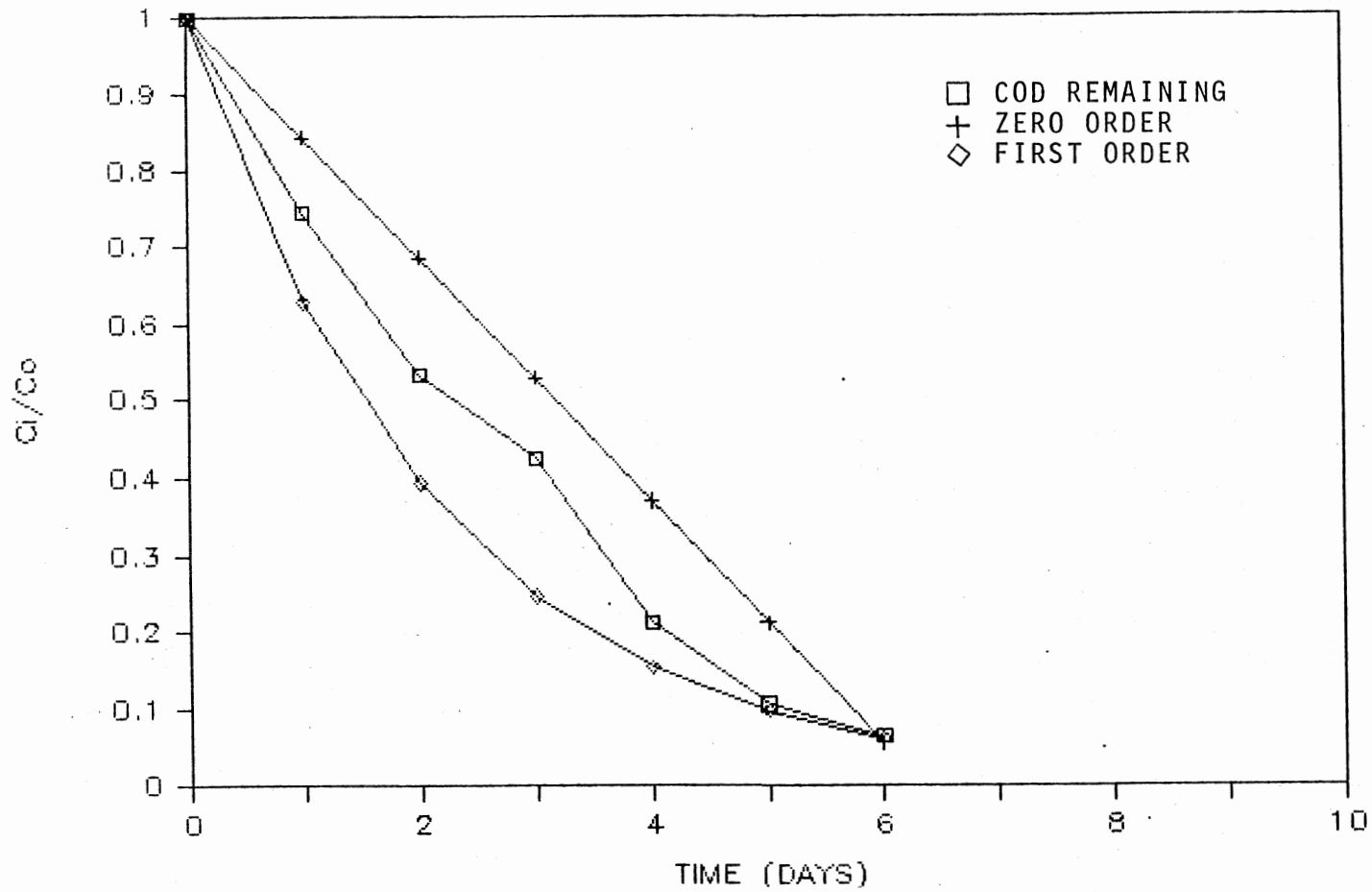Utilization with Lindane = 50 ppb.

Figure 16.   A Comparison of Model Results for Sodium Acetate
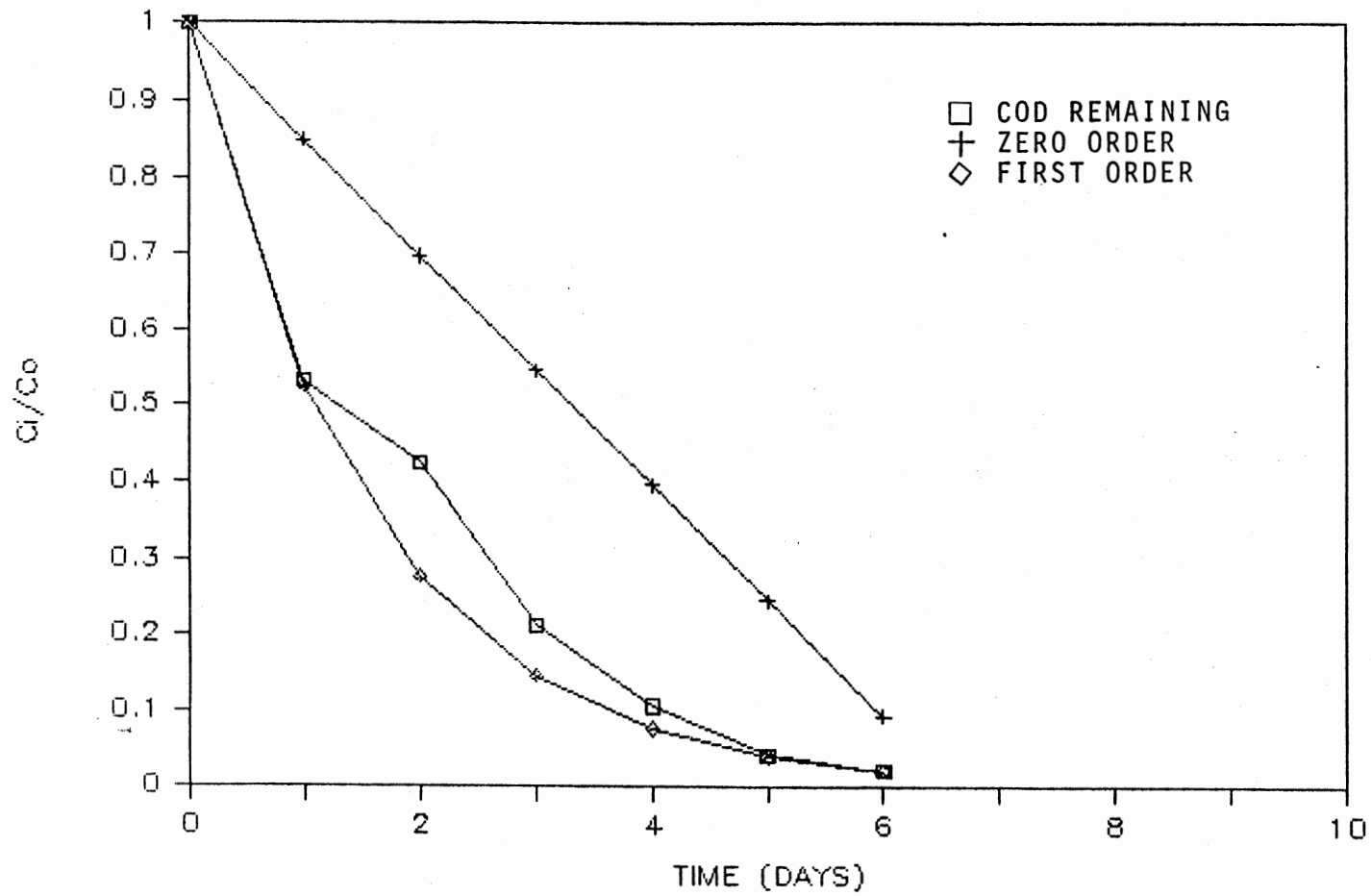Utilization with Lindane = 500 ppb.

Figure 17. A Comparison of Model Results for Sodium Acetate
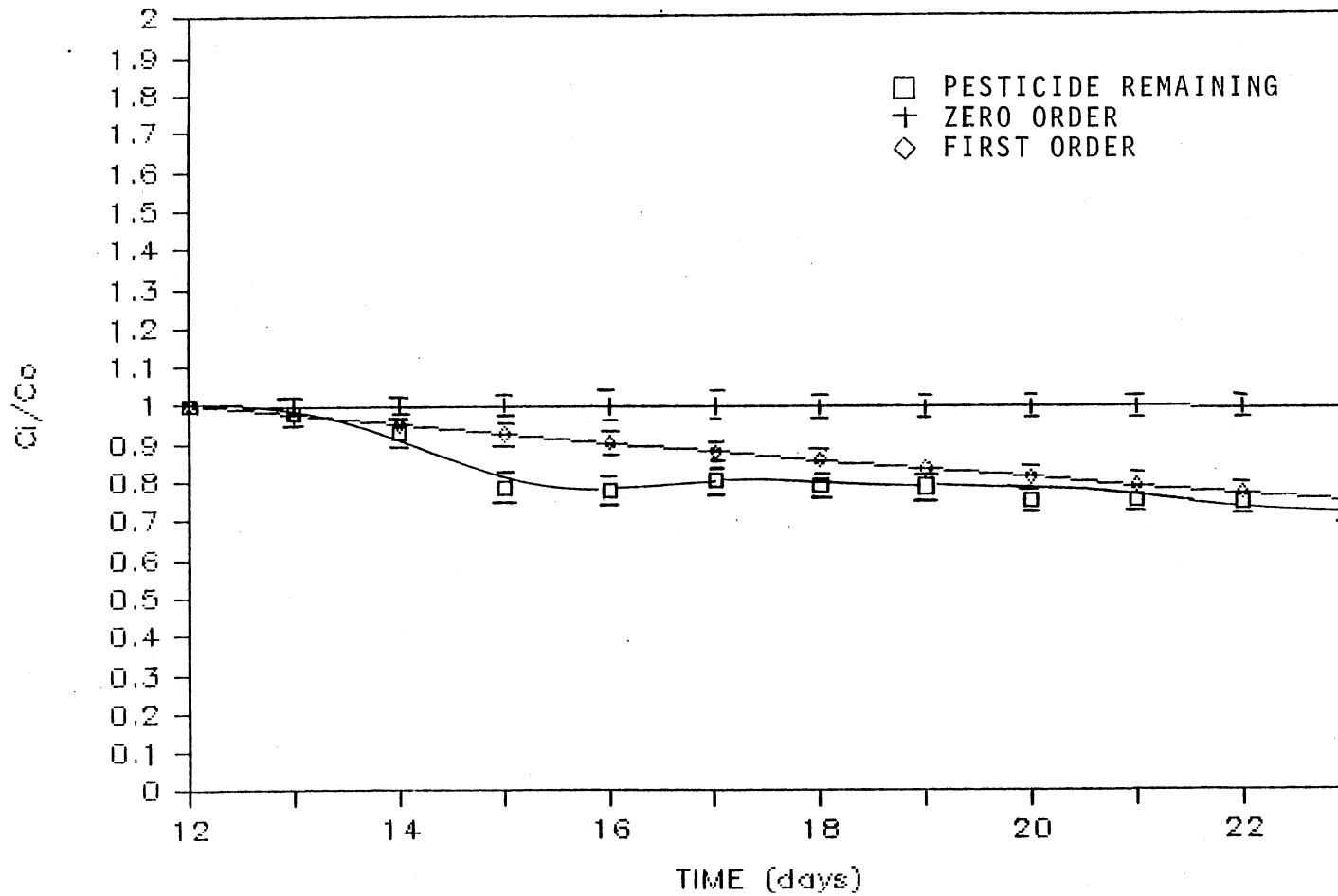Utilization (Control)

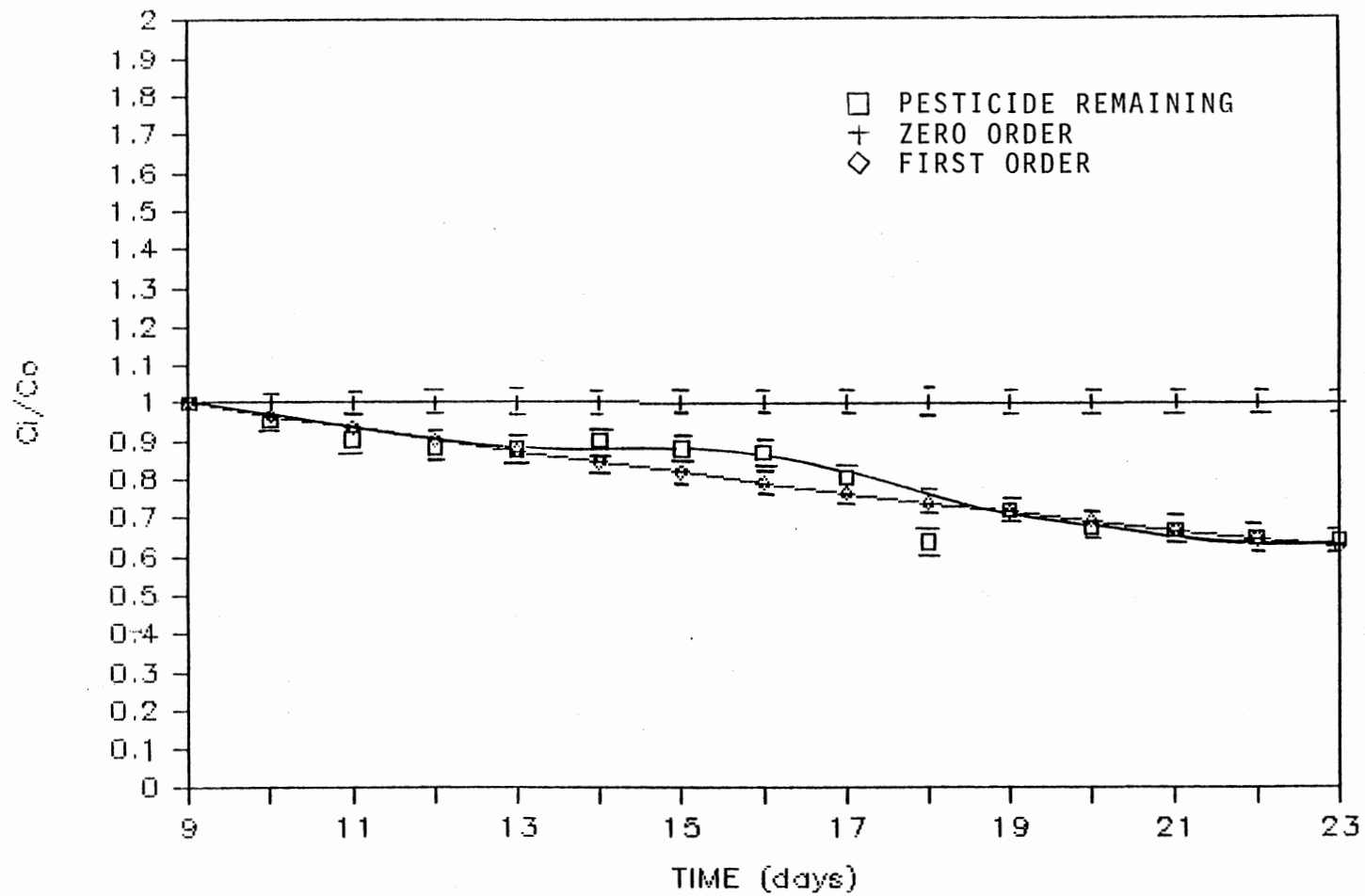Figure 18. A Comparison of Model Results for Lindane Removal at 50 ppb.

Figure 19. A Comparison of Model Results for Lindane Removal
at 500 ppb.

best fit of the pesticide removed from the point where the bacteria were acclimated to the new system. These figures showed that the first order model had a better fit to the actual data than did the zero order approach. These figures show that the concentration appears to be the driving force of the system.

2,4-D Modeling

Sodium acetate mineralization data for 50, 500 and 5000 ppb of 2,4-D and for the control were utilized in the same manner as described previously.

Figures 20, 21, 22, and 23 present a comparison of the actual data collected during this effort with that simulated by the first and zero order model types. These figures showed that primary substrate removal in the presence of 2,4-D at 500 and 5000 ppb concentrations was best fit by the zero order model when compared to the first order model. The primary substrate removal when 2,4-D equaled 50 ppb as well as in the control trials where no pesticide was present showed that the zero order model under predicted the actual data while the first order predicted greater removals than was observed in the experimental trials. Also the first order approach had a better fit to the actual data curve at the beginning and ending of the simulation time periods. Therefore, the primary substrate removal at 2,4-D equal to 50 ppb as well as in the control units was considered to be

Figure 20.   A Comparison of Model Results for Sodium Acetate
Utilization with 2,4-D = 50 ppb.

Figure 21.   A Comparison of Model Results for Sodium Acetate
             Utilization with 2,4-D = 500 ppb.
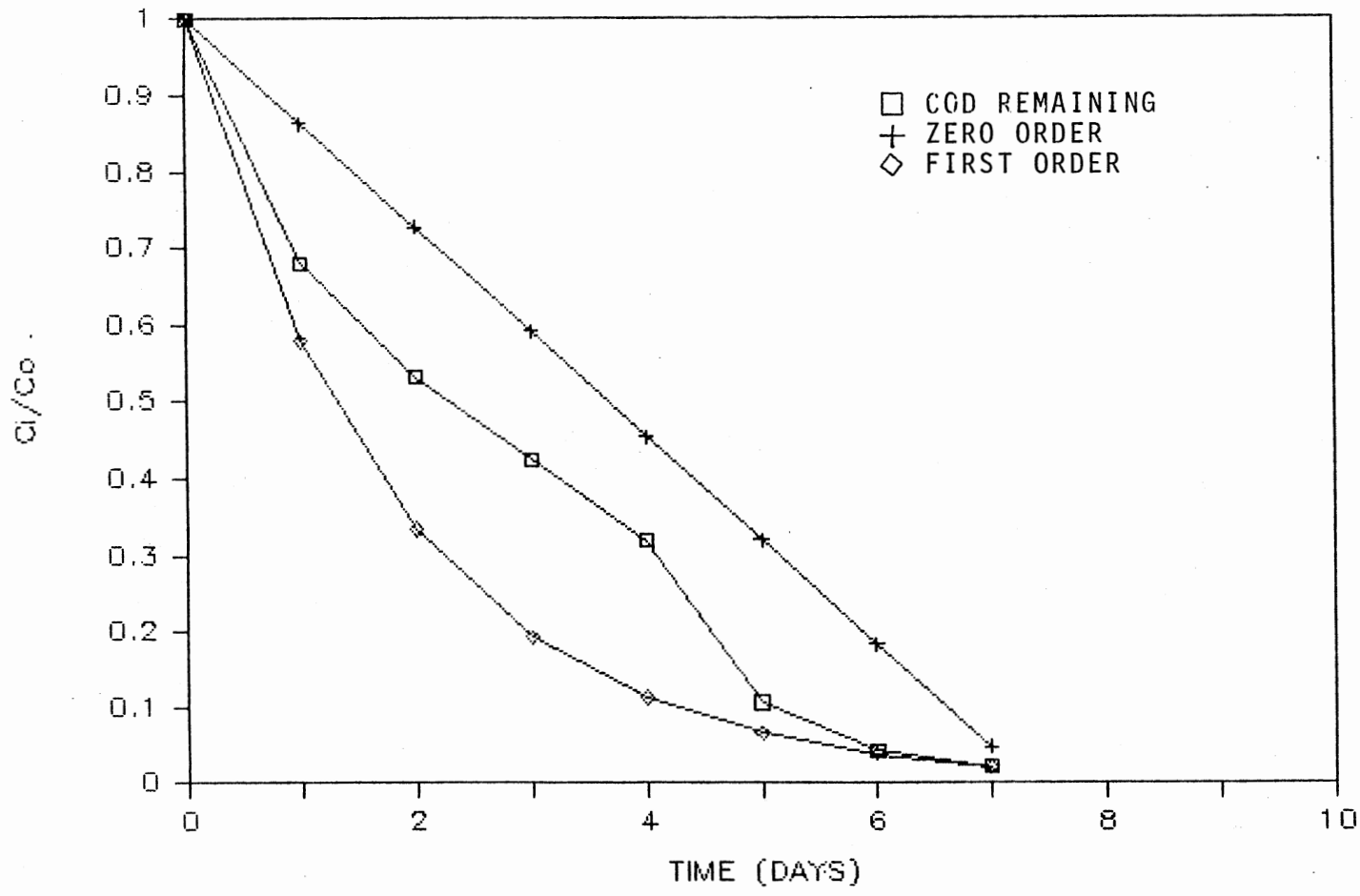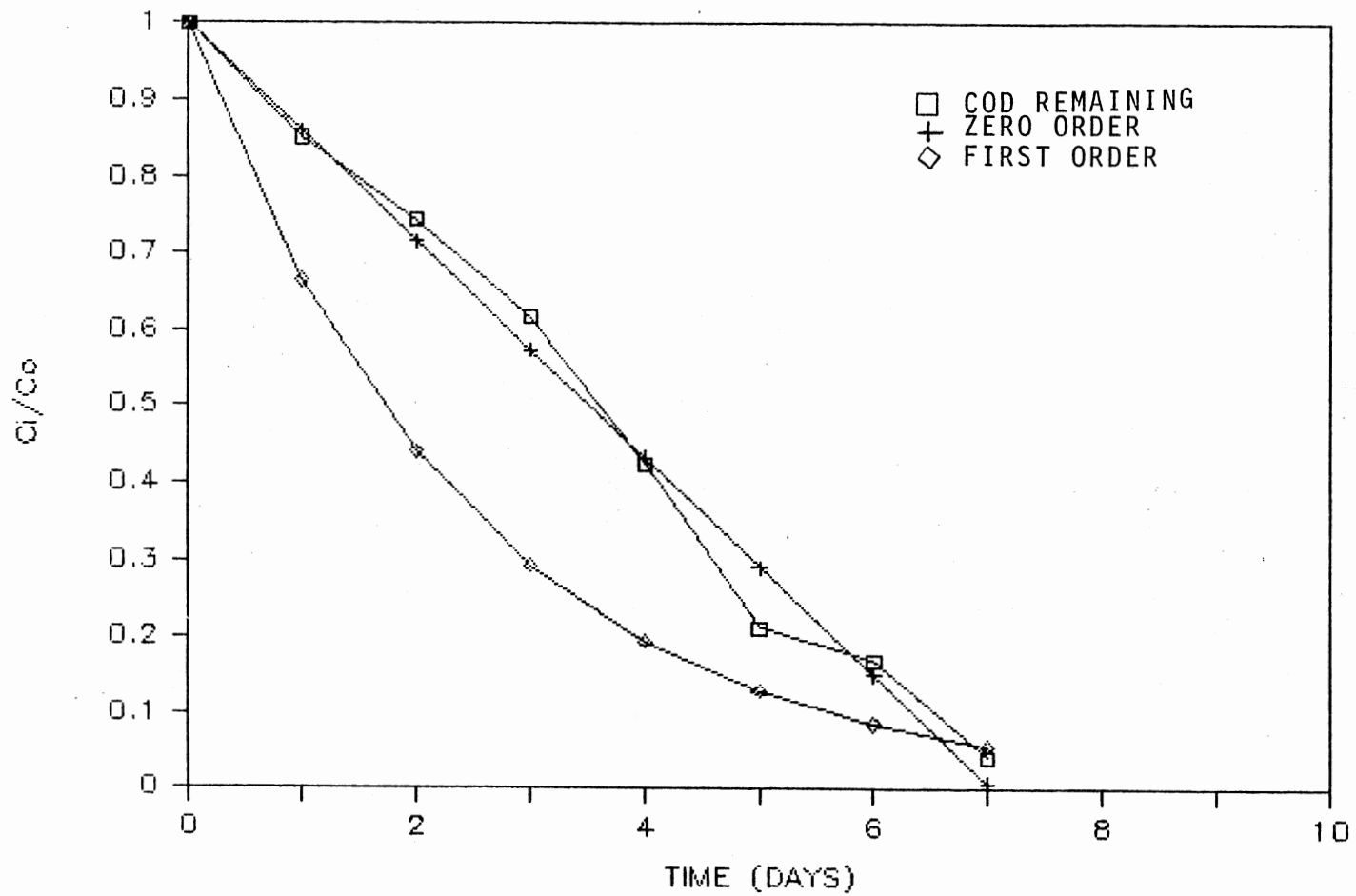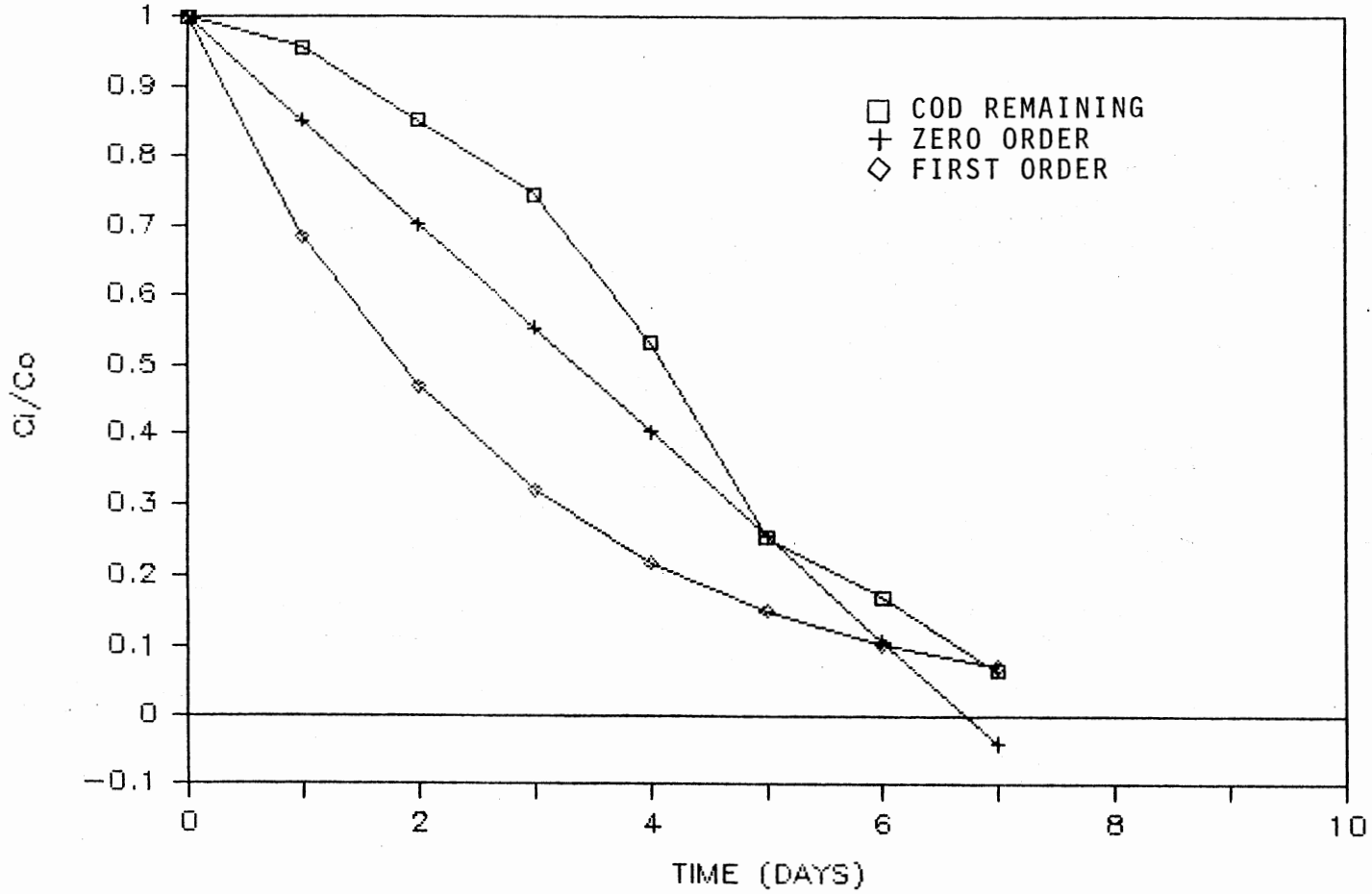
Figure 22. A Comparison of Model Results for Sodium Acetate Utilization with 2,4-D = 5000 ppb.
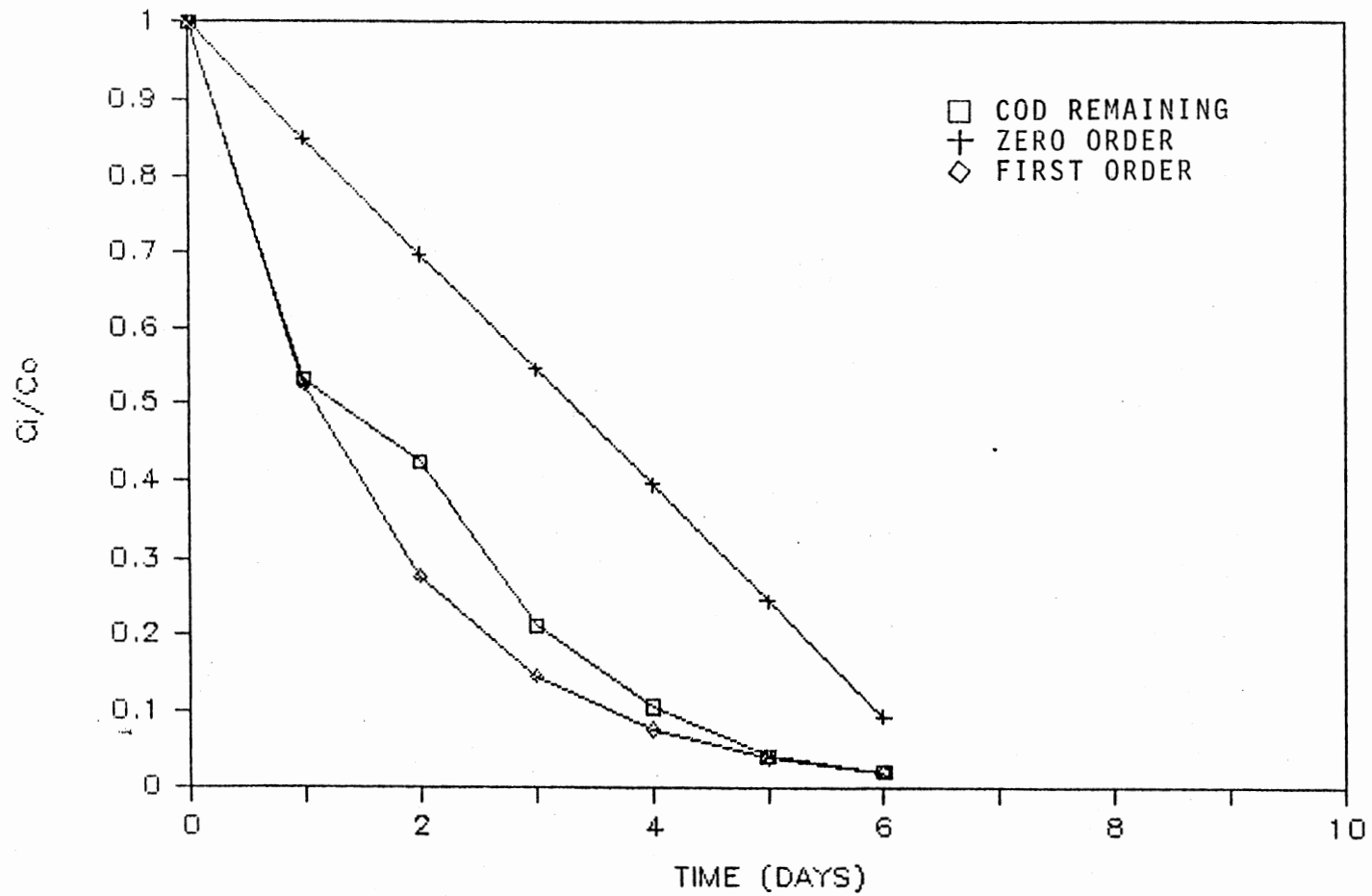
Figure 23. A Comparison of Model Results for Sodium Acetate Utilization (Control)

better described by the first order model than the zero order approach.

The mineralization of the herbicide at 50, 500 and 5000 ppb was also modeled. These data for the secondary substrate disappearance were also best fitted by the first order model for all three concentrations when compared to the zero order model. Mineralization of 2,4-D at 500 ppb was divided into two simulation time periods to best analyze the actual mineralization curve. The first simulation time consisted from day 0 to day 5, and the second from day 5 to end of incubation time (day 16). Both simulation times were better approached by the first order model when compared to the zero order model.

Figures 24, 25, 26, and 27, present the simulation results for the pesticide removed at the various concentrations. These figures showed that the first order model had a better fit to the actual data curve than did the zero order approach. Simulation time periods at concentrations of 50, 500, and 5000 ppb were from day 0 to day 16; day 0 to day 5 and day 5 to day 16; and day 0 to day 16, respectively. This exercise was approached in the same manner as described previously.

Table 18 provides a comparison of the modeled results to the actual COD and pesticide removal data collected during this effort. A comparison of the two models presented in Table 4 was included in this analysis.
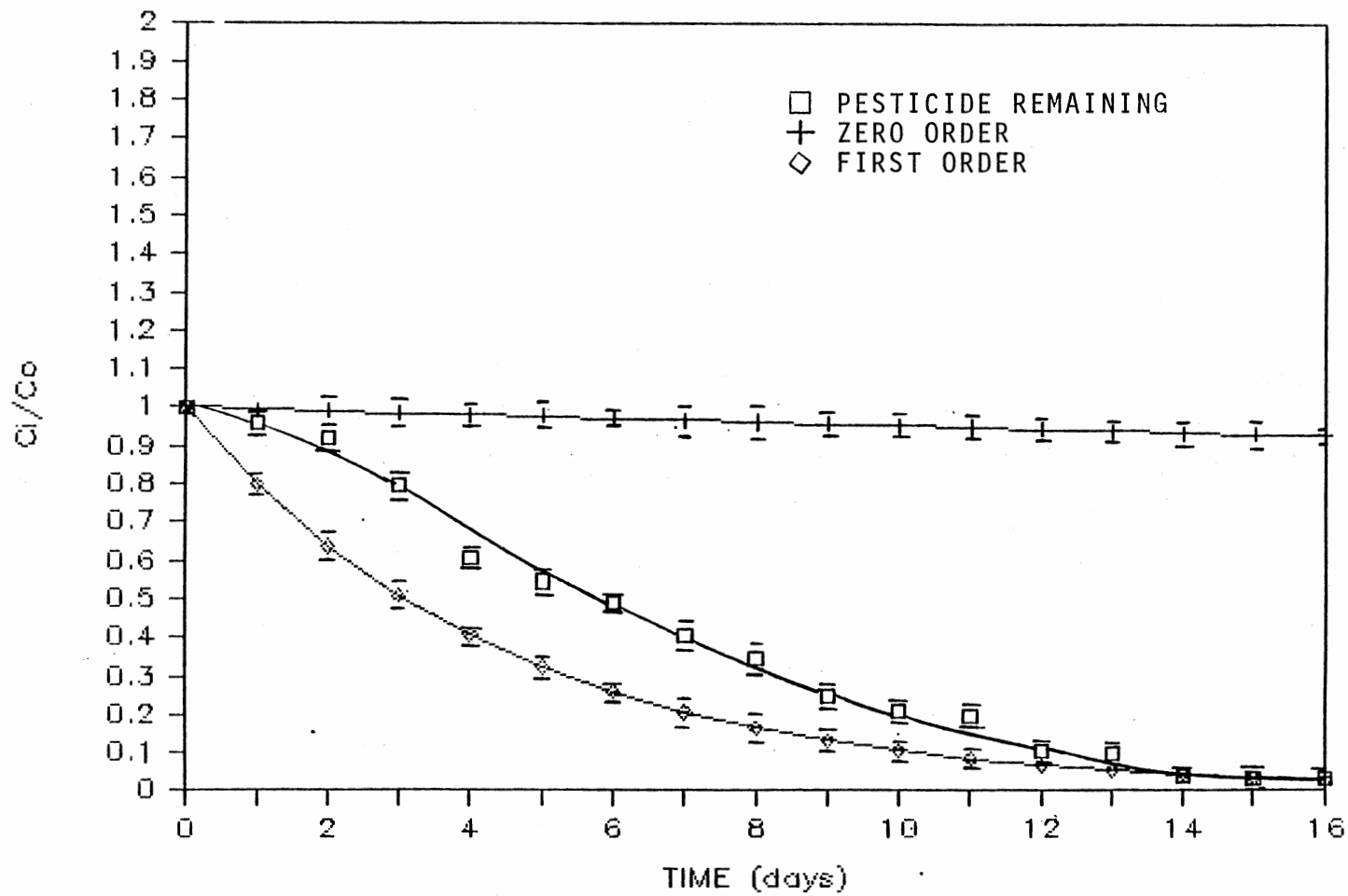
Figure 24. A Comparison of Model Results for 2,4-D Removal
at 50 ppb.
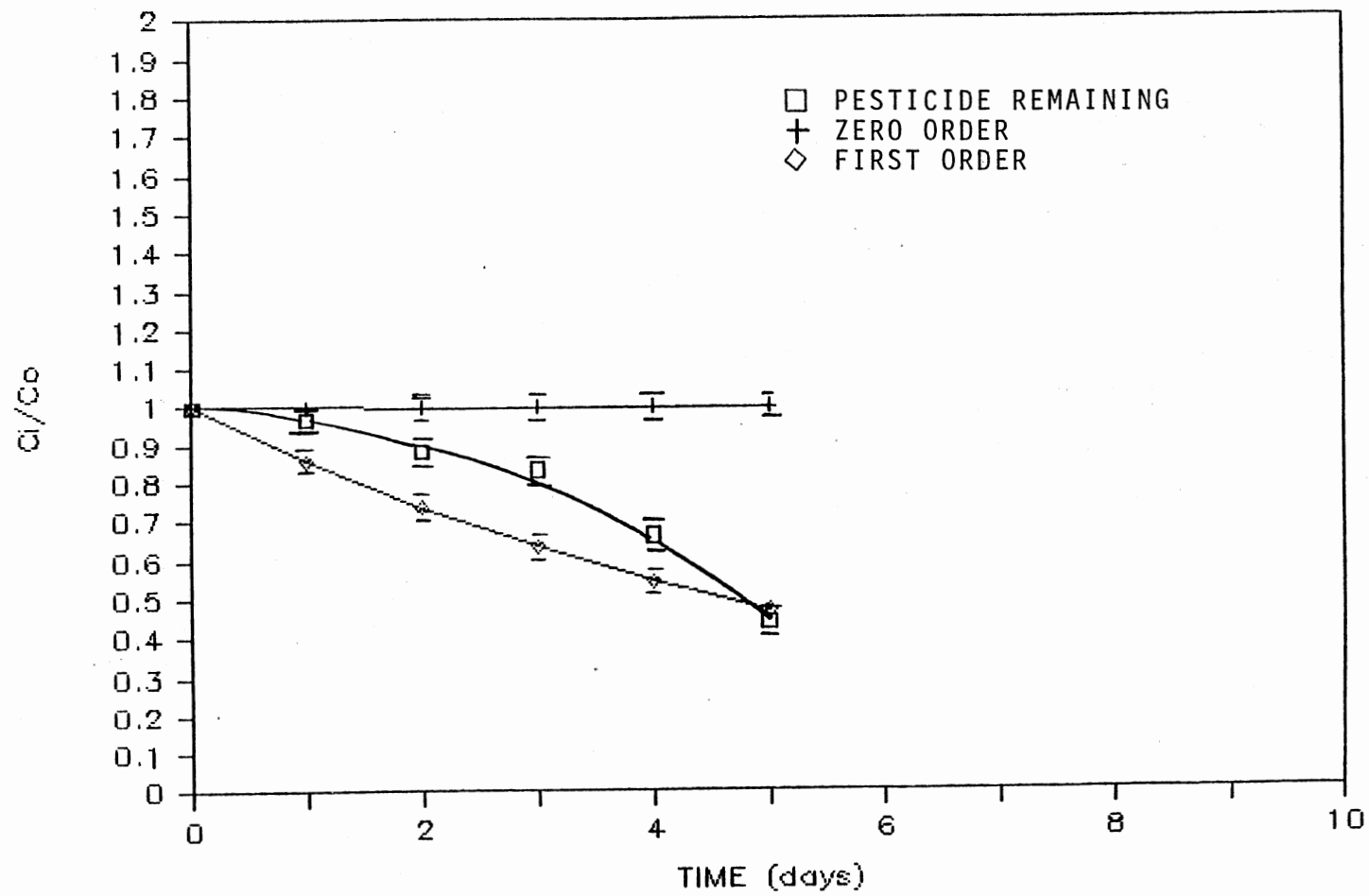
Figure 25. A Comparison of Model Results for 2,4-D removal
at 500 ppb.
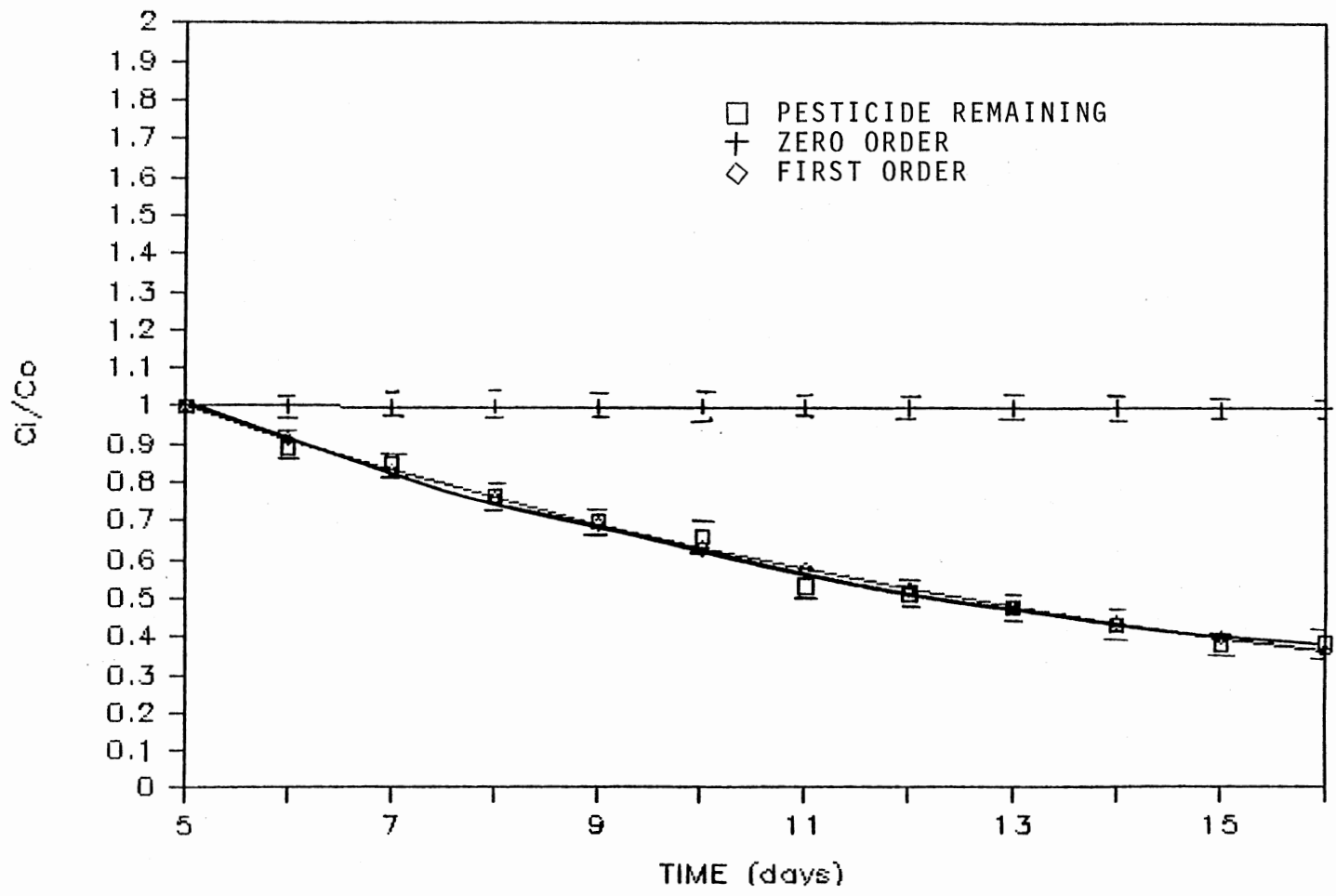
Figure 26. A Comparison of Model Results for 2,4-D Removal at 500 ppb
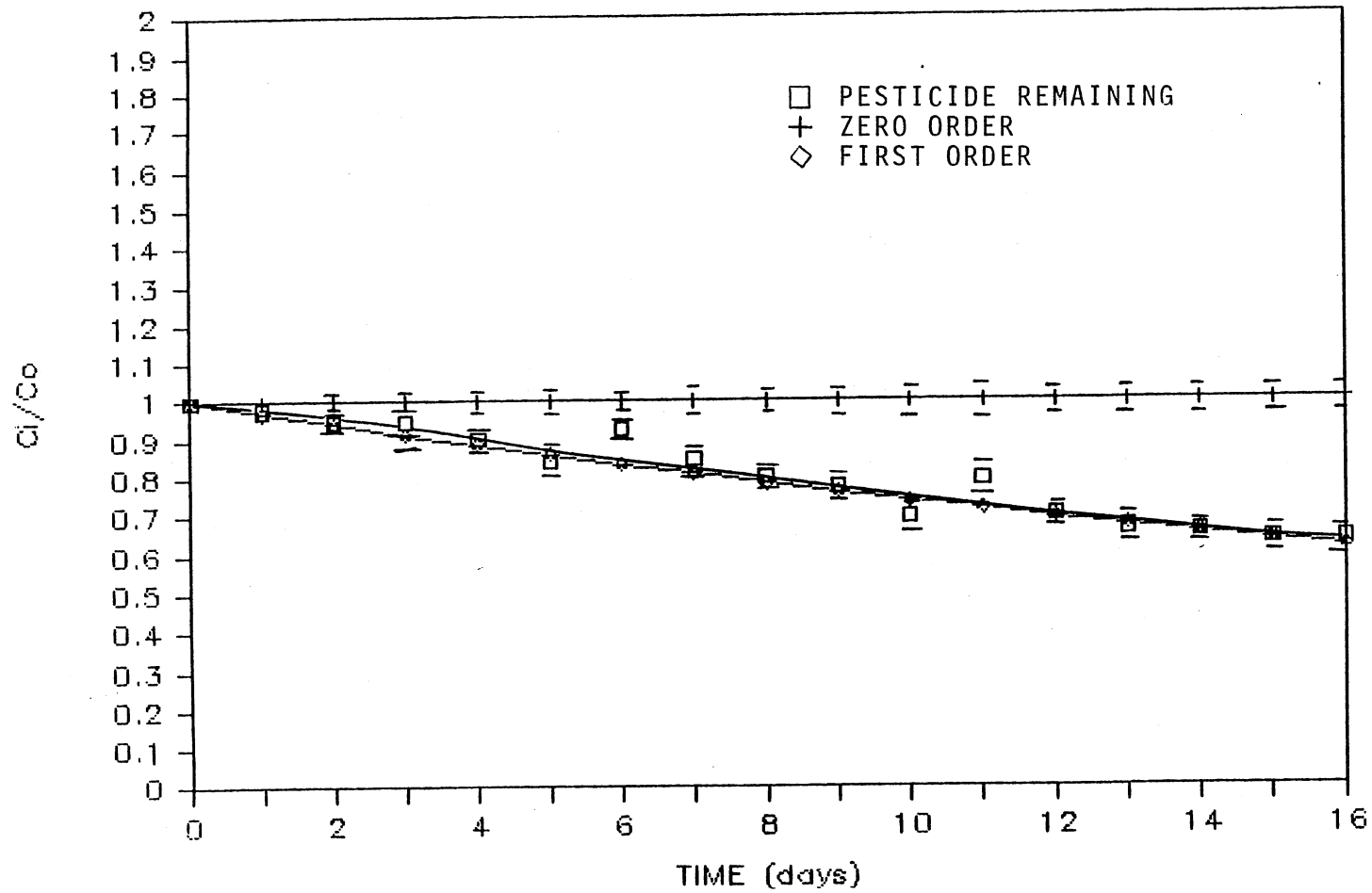
Figure 27. A Comparison of Model Results for 2,4-D Removal
at 5000 ppb.

TABLE XVIII

MODELS OF BEST FIT FOR SODIUM ACETATE
DISAPPEARANCE IN THE PRESENCE OF LINDANE
OR 2,4-D AS WELL AS PESTICIDE OF CONCERN
AND CONTROLS

Pesticide of Concern	Pesticide Conc. (ppb)	Simulation of Sodium Acetate	Simulation of Pesticide	Model of Best Approach
LINDANE	50	YES	NO	First Order
		NO	YES	First Order
LINDANE	500	YES	NO	First Order
		NO	YES	First Order
2,4-D	50	YES	NO	First Order
		NO	YES	First Order
2,4-D	500	YES	NO	Zero Order
		NO	YES	First Order
2,4-D	5000	YES	NO	Zero Order
		NO	YES	First Order
CONTROL	NA	YES	NA	First Order

The purpose of using these models to best fit the actual data obtained in this research, is to intially evaluate the ability of simplified expressions to model the critical components of primary and secondary substrate removal. It was found that mineralization of primary substrates as well as secondary substrates were best approached by either the first or zero order model, whichever showed a closer fit to the actual data curve at beginning and ending simulation time periods.

CHAPTER V

DISCUSSION

This research was structured to evaluate the interactions between a low energy primary substrate and increasing concentrations of higher energy, but potentially toxic secondary substrates when inoculated into a mixed culture of heterotrophic bacteria previously unacclimated to the toxic substrates. The pesticides used as secondary substrates in this effort were lindane and 2,4-D. They were initially inoculated into a culture broth of sodium acetate, buffers, nutrients, and the bacterial population. The results showed some similarities in terms of pesticide and acetate removal and bacterial growth but also exhibited some differences.

Lindane

The mineralization of lindane by the unacclimated mixed culture of heterotrophic bacteria was divided into two phases. Phase one consisted of an adsorption and a desorption stage. In this phase the pesticide was being adsorbed to the bacteria cells due to the affinity of the insecticide to adsorb to any suspended solid present in an aquatic system. This affinity was possibly attributed to

77

the relatively lower solubility of the insecticide compared to other pesticides. Subsequently the insecticide was being desorbed as these bacteria were acclimating to a new source of food or energy. This adsorption onto bacteria cells was identified as a possible factor responsible for the removal of the insecticide during the first days of incubation. Using average values from duplicate samples up to 93 ppb or over 18% of the total pesticide load was adsorbed during the first four days of incubation.

The second phase described the removal or decay of the secondary substrate (lindane), by these microorganisms once they were either acclimated to the new system or were unable to extract sufficient energy from the orginal primary substrate, sodium acetate, causing a shift to alternative carbon and energy sources, lindane or endogenous materials.

The initial primary substrate removal of sodium acetate as measured by COD was slowed as the pesticide concentration was increased when compared to controls when no pesticide was present. The sodium acetate removal rates at 50 and 500 ppb were slowed by approximately 3.13% and 27.38% respectively when compared to the controls. This retardance of the sodium acetate utilization or disappearance as measured by COD was either due to the toxicity of the pesticides to the unacclimated bacteria or was a function of acclimation times needed to provide the enzymes capable of metabolizing the lindane molecule. After COD remaining was lowered to

levels below 1 part per million, lindane metabolism was seen to increase. A lag phase of approximately 9 to 11 days was observed for lindane metabolism at both test concentrations employed. This was identified as a possible time needed for these microorganisms to produce the enzymes capable of metabolizing lindane. It was assumed that lindane had become a sequential primary substrate to the acclimated mixed culture of heterotrophic bacteria.

Pesticide removal at the higher concentration (500 ppb) was observed to be slightly higher than at 50 ppb. This was accomplished after the bacterial population had become acclimated to the new system, and were apparently using lindane as a primary substrate. The higher concentrations of lindane produced better growth and slowed the death phase apparently because more substrate and energy was available in those systems with the 500 ppb pesticide solutions.

Comparing the bacterial growth rate results obtained in this effort among the various pesticide concentrations and when no pesticide was present, it was often difficult to detect differences. Therefore, lindane was probably not toxic to the bacterial population at the time of bacterial growth, that is after acclimation. Also when comparing results as pesticide concentration was increased and when no pesticide was present, higher death rate values were ob-tained at the lowest pesticide concentration as well as when no pesticide was present. This was attributed to the

capability of these microorganisms to use the pesticide as a sequential primary substrate source much better after sodium acetate had disappeared. Therefore, it was assumed that time was needed for acclimation of this bacterial population or that the secondary substrate was becoming a sequential becoming a sequential primary substrate after the true primary substrate (sodium acetate) had disappeared.

A hypothesis of lindane degradation was then developed from the data plots of Figures 2 and 3, as well as from the adsorption data presented in Table 5. As described previously, lindane was initially adsorbed onto the bacterial cells causing the pesticide concentration to decrease during the first six days of incubation. Subsequently, the pesticide concentration increased due to a desorption phase. Finally a decrease was again observed due to apparent biological decay. Parallel studies performed on the adsorption of lindane and 2,4-D on dried bacteria cells demonstrated that lindane had a higher adsorption capacity than did 2,4-D (27). Since almost all lindane decay appeared to occur after most of the sodium acetate had disappeared, lindane was identified as a possible sequential primary substrate rather than a true secondary substrate. However, after comparing the pesticide removal and growth and death rate values between the various pesticides concentrations and when no pesticide was present, an alternative hypothesis is possible. That is, acclimation time was needed for these

microorganisms to develop the enzymes capable of metabol-
izing the lindane molecule, and that lindane did not show
inhibitory properties after the bacteria had acclimated to
the new system as the pesticide concentration was increased.

From the set of controls monitoring the growth of
bacteria where no pesticide was present (Figure 5), note
that the substrate was disappearing while the bacteria was
being acclimated to the new system. Even though the sub-
strate was disappearing after approximately 5 to 6 days of
incubation, this substrate disappearance was attributed to
the fact that when these bacteria were extracted from the
continuous flow reactor previously described, they were re-
moved from an environment containing sodium acetate at 5000
mg/L and immediately placed in the batch units containing
sodium acetate at 100 mg/L. Also, control experiments where
sodium acetate and pesticide of concern were present without
bacterial inoculum had shown that both the pesticide and the
sodium acetate concentrations remained constant and that
little volatilization of the pesticide nor other non-biolog-
ical pesticide and sodium acetate loss occurred during the
period of incubation.

The disappearance or mineralization of lindane for both
concentrations was best modeled by the first order model, at
simulation times where the microorganisms were possibly ac-
climated to the new media. This model gave a closer fit to
the actual data than did the zero order model. These models

were utilized by taking the slope values of the linearized plots of natural log (ln) of the pesticide concentration versus time of the actual data, and using them in the integrated forms of the models compared. A comparison of the simulated curve with the actual data was made.

The disapperance of the sodium acetate when present with the various pesticide concentrations as well as for the control trials, was also best simulated by the first order model. This model gave a closer fit to the actual data than did the other model evaluated (zero order).

The selection of this model shows that as the pesticide concentration increases the fitting of this model to the COD removed data becomes less appropriate when compared to the actual data curve. This seems to result from either toxicity of the pesticide to the unacclimated bacteria or from a divergence from the acclimation times needed for these microorganisms to produce the enzymes capable of metabolizing the lindane molecule.

2,4-Dichlorophenoxyacetic Acid

In these experiments, 2,4-D was metabolized by the inoculated microorganisms at the same time as the sodium acetate was removed. This indicated that little acclimation time was needed to prepare the bacteria for this oxidation. This was attributed to the possible presence of enzymes produced by this bacteria grown on sodium acetate which were

capable of metabolizing a common acetic based compound present in the sodium acetate and 2,4-D. This led to belief that 2,4-D was probably a true secondary substrate and not a primary substrate that required acclimation.

Adsorption of the herbicide was not observed during the decomposition of 2,4-D. Neither volatilization nor other abiotic removal mechanisms were noted in the control experiments. Therefore, the dominant mechanism reponsible for the disappearance of 2,4-D was considered to be biological decay. This finding was supported by several other studies which reported that 2,4-D was easily degraded by mixed heterotrophic bacteria in the presence of an easily utilizable substrate (1,28).

Toxicity of the pesticide to the unacclimated bacteria was observed, as the primary substrate removal as measured by COD was lowered by the increased pesticide concentration, and when compared to controls when no pesticide was present. The primary substrate removal rates of 50, 500, and 5000 ppb were slowed by approximately 14.9%, 36.5%, and 41.0% respectively when compared to the control. The retardance of primary substrate utilization or disappearance as measured by COD was possibly related to the toxicity of the pesticide as its concentration increased. This was later confirmed by the results obtained for the total pesticide removal rates over the period of incubation, which in this case decreased as pesticide concentration was increased.

Comparing the bacterial growth and death rates obtained in this effort among the various concentrations and when no pesticide was present, it was observed that the growth rate values increased from 50 to 500 ppb but later decreased at the highest pesticide concentration (5000 ppb) showing inhibitory conditions to these microorganisms.

Observations from the death rate values obtained in this effort showed that some of the pesticide was being utilized as concentration was increased. This was confirmed when death rate values were compared to controls and among the various pesticide concentrations, which showed decreases in death rate as the pesticide concentration was increased.

A hypothesis for 2,4-D degradation developed from these results is as follows. 2,4-D metabolisms consisted of a decay phase where no significant adsorption of the herbicide was observed. Since decay of 2,4-D was noted at the same time the primary substrate (sodium acetate) was disappearing, 2,4-D was recognized as a probable secondary substrate rather than an alternative primary substrate. Also, 2,4-D showed toxicity to the unacclimated bacteria as well as post-acclimated mixed culture at the highest pesticide concentration at times of bacterial growth. This indicates that even though the 2,4-D molecule can be used for cell maintenance, not all of the cells are capable of using the pesticide without a measure of toxic response.

From the set of controls monitoring the growth of bacteria where no pesticide was present (Figure 12), the same results were obtained as those found in the lindane study. That is, the control experiments where sodium acetate and the pesticide were present without bacterial inoculum had shown that both the pesticide and the sodium acetate concentrations remained constant and that neither volatilization of the pesticide nor little non-biological pesticide and sodium acetate loss occurred during the period of incubation.

The disappearance or mineralization of 2,4-D for all three concentrations was best approached by a first order model. The incubation times used for these simulations of pesticide mineralization were different for the various concentrations. At 50 and 5000 ppb the simulation modeling times were from day 1 to the end of the experiment (day 16), while at 500 ppb the simulation time was divided into two phases: phase one, from day 1 to day 5, and phase two, from day 5 to day 16. This was done to best approximate the actual data curve, which showed a different slope path for these data at these times.

This modeling effort shows that as the pesticide concentration increases the comparison of the actual data with the simulated curves diverges. This suggests that toxic effects were present.

The disappearance of the sodium acetate when present

with 2,4-D at 50 ppb as well as for the control trials was best approximated by the first order model, while when present at concentrations of 500 and 5000 ppb the primary substrate removal was best modeled by the zero order approach. The selection of the model to best fit the actual data was based on how well these models approximated the experimental data curves.

When comparing the degradation of both pesticides, 2,4-D showed a faster degradation rate in a shorter incubation time (16 days) when compared to the lindane experiments conducted at similar concentrations. This was possibly related to the chemical structure or composition of the 2,4-D that was somewhat analogous to the sodium acetate used as the principal growth substrate. This assumes that the enzymes required for the breakdown of the molecule were present in the previously unacclimated culture.

Toxicity or acclimation time needed for these microorganisms to produce the enzymes capable of metabolizing the pesticides was noted for each of the pesticides when compared between the different concentrations used as well as when compared to controls. 2,4-D acclimation times were not actually required for these microorganisms, since almost immediate breakdown of the 2,4-D molecule was observed. Therefore, only toxicity was the possible factor responsible for the decrease in primary substrate (sodium acetate) removal as the pesticide concentrations was increased. This

was later confirmed at the highest concentration used in this research (5000 ppb), where inhibitory conditions to the mixed culture of heterotrophic bacteria was observed. This demonstrates that even though 2,4-D was a more easily degraded compound, lindane was likely to be used at higher concentration by these acclimated microorganisms without apparent toxic responses.

The disappearance of lindane during the first days of incubation was attributed to the adsorption of the pesticide onto bacteria cells, possibly due to its relatively lower solubility in water when compared to other pesticides.

CHAPTER VI

CONCLUSIONS

The objectives of this research were to evaluate criti-
cal components or elements of the microbial decomposition of
selected pesticides. These fundamental elements were consi-
dered critical in the transport and transformation of pesti-
cides in saturated and unsaturated groundwater systems.
Systems were constructed and operated utilizing an unaccli-
mated mixed culture of heterotrophic bacteria grown in the
presence of an easily utilizable low energy substrate
(sodium acetate). The relatively low levels of energy
available from the acetate apparently selected prototrophic
organisms capable of survival in low energy environments.
Parallel additions of two pesticides, lindane and 2,4-Di-
chlorophenoxyacetic acid were made in each batch reactor.
Controls to monitor volatilization and other non-biological
pesticide removal mechanisms were established, as were
systems without the pesticide additions. These last con-
trols were intended to monitor system performance for
acetate removal as well as microbial growth and death with-
out pesticide presence.

Chemical oxygen demand and plate count techniques used
in this research were selected and conducted in compliance

with Standard Methods for the Examination of Water and Wastewater (14th edition) (21). Other techniques such as DO and pH readings were routinely updated with current standards according to their procedures. Pesticide presence was determined by gas chromatography.

Degradation of lindane was obtained for an incubation period of 23 days, where more than half of its concentration was removed. Lindane decomposition appeared to be divided into two phases: an adsorption/desorption phase where little decay was noted and a microbial decomposition phase where pesticide disappearance was documented. This lead to a hypothesis being formed which postulated that lindane was not metabolized until the primary substrate was removed at low levels. The lindane was then metabolized as a subsequent primary substrate. In this way, the removal of lindane approximated diauxic growth, with a toxic initial response to the subsequent primary substrate.

Toxicity of the insecticide was observed with one of the experimental indicators, the overall substrate removal coefficient, but was not seen in either the microbial death or growth rates. This is interpreted that the lindane, once acclimation had occurred was stimulatory, rather than inhibitory to the culture population. Modeling prediction of sodium acetate and lindane removal at 50 and 500 ppb was felt to be best approached by a first order model for both substrates.

Degradation of 2,4-D was obtained in a shorter incubation period than was lindane. The majority of the pesticide at the lowest concentration (50 ppb) was removed or decayed by the unacclimated mixed culture of heterotrophic bacteria in 16 days. More importantly, 2,4-D decay started almost immediately with culture initiation unlike lindane where a noticable lag of approximately 10 days was observed. A hypothesis was developed for these decomposition systems, where the 2,4-D serves as a true secondary substrate or cometabolite and provides energy and substrate to cells over the length of incubation period. Increasing toxicity of this secondary substrate was noted, however with increasing pesticide concentration. This initial acclimation to 2,4-D was partially attributed to structural analogies between the original food and energy source, the sodium acetate and the 2,4-D. In the case the enzymes or similar enzymes responsible for the breakdown of 2,4-D molecule were apparently already present, making it easier for its initial decomposition relative to the parallel lindane based systems which required acclimation time to generate the appropriate enzymes.

Control units for the volatilization of both pesticides and possible contamination of the units, as presented in Figures 4 and 11, showed that little non-biological pesticide loss occurred during the period of incubation. These also demonstrated how accurate and consistent the

recovery of both pesticides as well as sodium acetate was obtained during the period of incubation.

The second set of controls intended to monitor the performance of the systems where no pesticide was present (Figures 5 and 12) showed that the sodium acetate (primary substrate) disappeared faster than in those systems where pesticide was present at the various concentrations.

From the results obtained in this effort as well as in previous research conducted by other investigators, it is apparent that the biological removal of pesticides is potentially significant but that different mechanisms combining physical and chemical as well as microbial properties may be involved in the final transformation/transport system. Similarly, the manner in which the resident microbial populations acclimate to the pesticide strongly affects transport. The utilization of the pesticide as a cometabolite or as a sequential primary substrate will also significantly affect the transport of these materials in groundwater systems.

SELECTED REFERENCES

1. Lapping, H. M., Greaves, M. P., and Slater, J. H., "Degradation of the Herbicide Mecoprop [2-(2-Methyl-4-Chlorophenoxy)Propionic Acid] by a Synergistic Microbial Community", Applied Environmental Microbiology, 49, (1985), pp.429-433.

2. Daniels, B. T., "An Evaluation of Methods for Defining the Variability in Pesticide Contamination of Groundwater in Oklahoma, Oklahoma State University, Master Thesis, May, 1988.

3. Ou, Li. Tl, and Sikaka, H. C., "Extensive Degradation of Silvex bySynergistic Action of Aquatic Microorganisms", Journal of Agricultural Food Chemistry, 25, (1977), pp. 1336-1339.

4. Chakrabarty, A. M., Biodegradation and Detoxification of Environmental Pollutant, CRC Press (1982).

5. Boethling, R. S., and Alexander, M., "Effect of Organic Chemical on their Biodegradation by Natural Communties", Applied and Environmental Microbiology, 37, (1979), pp. 1211-1216.

6. Law, A. T., and Button, D. K., J. M., "Multiple-Carbon Limited Growth Kinetics of a Marine Coryne Bacterium", Journal of Bacteriology, 129, (1977), pp. 115-123.

7. Shetata, T. E., and Marr, A. G., "Effect of Nutrient Concentration on the Growth of Escherichia Coli", Journal of Bacteriology, 107, (1971), pp. 210-216.

8. Schmidt, S. K., Simkins, S., and Alexander, M., "Model for the Kinetics of Biodegradation of Organic Compounds not Supporting Growth", Applied Environmental Microbiology, August, 1985, pp. 323-331.

9. Smouse, P. E., "Mathematical Models for Continuous Culture GrowthDynamics of Mixed Population Subsisting on a Heterogeneous Resource Base". I. Simple Competition, Theor. Popul. Biol., 17, (1980), pp. 16-36.

10. Yoon, H., Klinzing, G., and Blanch, H. W., "Competi-
     tion for Mixed Substrates by Microbial
     Populations", Biotechnology Bioengineer, 19, (1977)
     pp. 1193-1210.

11. Roy, D., "Biodegradation of Chlorophenxyacetic Acid
     Herbicides", Directions in Hazardous Waste
     Research, October (1986).

12. Audus, L. J., "Nature", 166, (1950), p. 356.

13. Steenson, T. I., Walker, N., ournal of General Micro-
     biology 18, (1958), p. 692.

14. Steenson, T. I., Walker, N., Plant Soil, 8 (1956), p.
     17.

15. McCarty, P.L., Rittmann, B. E., and Reinhard, M.,
     "Trace Organics in Groundwater", nvironmental
     Science Technology 15, (1981) pp. 40-51.

16. Rittmann, B. E., McCarty, P. L., and Roberts, P. V.,
     "Trace Organics Biodegradation in Aquifer Recharge"
     Groundwater, 19 (1980) pp.236-243.

17. Laura, T., McCarty, P. L., and Zehnder, A. J. B.,
     "Secondary Substrate Utilization of Methylene
     Chloride by an Isolated Strain of Pseudomonas
     sp", Applied Environmental Microbiology, April
     (1984), pp. 825-830.

18. Bouwer, E. J. , "Secondary Utilization of Trace
     Halogenated Organic Compounds in Biofilms",
     Environmental Progress, Vol. 4, No. 1, (1985)
     pp. 43-46.

19. Kincannon, D. F., Weinert, A., Padorr, R., and Stover,
     E. L., "Predicting the Treatability of Multiple
     Organic Priority Pollutants from Single Pollutant
     Treatability Studies", Proceedings 37th Purdue
     Industrial Waste Conference. Purdue University,
     West Lafayette, Indiana, 1982.

20. Quellete, R. P., and King, J. A., "Chemical Week Pesti-
     cides", Technical data on Pesticides, 1977.

21. Greenberg, A. E., Trussell, R. R., Clesceri, L. S.,
     Standard Method for the Examination of Water and
     WasteWater., 14th Edition, American Public Health
     Association, 1975.

22. Chemical Oxygen Demand procedure by the Hach Method,
     Hach Company, Loveland Colorado, 1986.

23. Subba-Rao, R. V., Rubin, H. E., and Alexander, M.,
     Kinetics and Extent of Mineralization of Organic
     Chemical at Trace Levels in Freshwater and Sewage",
     Applied and Environmental Microbiology, 43, (1982),
     pp. 1139-1150.

24. Keith, L. H., "Advances in the Identification and
     Analysis of Organic Pollutants in Water", Vol. 1,
     Ann Arbor Science Publishers, Inc., Ann Arbor, MI,
     pp. 235-266 (1981).

25. Jensen, D., Analysis of Residues of Acidic Herbicides,
     John Riley and Sons, 1981.

26. Simkins, S., and Alexander, M., "Models for Mineraliz-
     ation Kinetics with the Variables of Substrate Con-
     centration and Population Density", Applied and
     Environmental Microbiology, June (1984) pp. 1299-
     1306.

27. Polycarp, H., "Adsorption of Lindane and 2,4-D on Dried
     Bacteria Cells", Master of Science Thesis, Oklahoma
     State University, 1988.

28. Walker, R. L., and Newman, A. S., "Microbial Decompos-
     ition of 2,4-Dichlorophenoxyacetic Acid", Applied
     Microbiology, Vol. 4, No. 4, (1956), pp. 201-206.

VITA -2

Jose Atilano Pereira

Candidate for the Degree of

Master of Science

Thesis: AN EVALUATION OF MICROBIAL TRANSFORMATION
           PROPERTIES FOR SELECTED PESTICIDES: LINDANE
           AND 2,4-Dichlorophenoxyacetic Acid

Major Field: Environmental Engineering

Biographical:

  Personal Data: Born in Caucagua, Miranda, Venezuela,
      October 19, 1961, the son of Jose A. and Gladys M.
      Pereira

  Education: Graduated from the Miltary High School of
      Gral. J. A. Anzoategui, Pto. Piritu, Anzoategui,
      Venezuela, in 1978; received the Bachelor of
      Engineering Degree in Civil Engineering at
      Oklahoma State University, in December, 1985; com-
      pleted requirements for Master of Science Degree
      at Oklahoma State University, in July, 1988.

  Professional Experience: Research Assistant,
      Department of Civil Engineering, Oklahoma State
      University, January, 1987 to June, 1988; Military
      service at a Military High School in Venezuela,
      July, 1973 to July, 1978.