COLUMN STUDIES ON THE BIOTRANSFORMATION OF PESTICIDES IN GROUNDWATER UNDER VARIOUS ELECTRON ACCEPTOR CONDITIONS

By

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OF PESTICIDES IN GROUNDWATER

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CHAPTER I

INTRODUCTION

The pollution of aquifers with organic chemicals is a problem that continues to raise questions regarding the quantity and quality of world water resources. Ground water supplies about one-half of United States drinking water needs, and withdrawal rates are increasing by about 25% per decade (Beeman and Suflita 1987). Since about one-half the U.S. population relies on ground water as a source of potable water, concern about contamination of this resource has grown considerably in the last 20-25 years (Beeman and Suflita 1987).

Pesticides in Ground Water

Reports of the presence of pesticides in ground water supplies have heightened the increasing concern about contamination of water resources in recent years. A number of water quality surveys, both nationwide and specific to the Midwest, have reported finding many of the most commonly used pesticides in both raw ground waters and in finished drinking waters (Cohen *et al.* 1986; Hallberg 1987; Ritter 1990). Furthermore, evidence exists that the percentage of wells with detectable amounts of pesticides is increasing (Hallberg *et al.* 1987). Four of the more commonly used pesticides are the triazine herbicide atrazine , the acetanilide herbicides alachlor and propachlor, and the heterocyclic herbicide bromacil.

Bromacil is generally used on noncropland areas for control of a wide range of annual and perennial grasses, broadleaf weeds, and certain woody species (Pease 1966).

Atrazine, alachlor, and propachlor are used as pre- and post-emergence herbicides for the control of broadleaf and grassy weeds in numerous crops including corn and wheat. Atrazine is also used extensively for control of roadside weeds (CPP 1991). Nationally, alachlor and atrazine account for 25 percent of total pesticide use by weight (Wilber and Parkin 1991). These pesticides are also two of the most frequently detected pesticides in Midwestern ground water supplies.

Drinking Water Standards for Pesticides

It is known that the ingestion of drinking water contaminated with pesticides can be dangerous to human health (NAS 1977). The EPA has issued Maximum Contaminant Levels (MCL) for alachlor and atrazine of 2 and 3 μg/L, respectively (Pontius 1992). Propachlor is listed as a contaminant to be monitored, and bromacil is on the Priority List as a contaminant due to be regulated in June, 1993 (Pontius 1992).

Biorestoration of Contaminated Ground Water

The discovery of sites with ground water contaminated by these pesticides and concern about their effects on health has led researchers to seek new methods to use in remediating these sites. *In-situ* bioremediation is a promising technique currently under investigation. Biotransformation can be a significant process affecting the fate of organic contaminants in the subsurface.

Considerable research has been performed in recent years addressing various aspects of the fate and transport of pesticides following their application. It has been demonstrated that many pesticides are biodegraded in the subsurface under a variety of conditions. It is also well documented that the phenomena of sorption and desorption are major factors in the movement of pesticides in ground water (Sabatini and Austin 1990). However, numerous questions remain regarding specific fate processes and the effect of

various environmental factors, such as electron acceptor conditions, the presence of exogenous carbon sources, and the degree to which sorption phenomena make the pesticides unavailable to the microbial community for further transformation.

Research Objectives

With these facts in mind, a research project was initiated to investigate the fate of atrazine, alachlor, propachlor, and bromacil under conditions similar to those found in ground water and under the following electron acceptor conditions: nitrate reduction, methanogenesis, and aerobic respiration. The primary objectives of this research were the following:

1. To investigate the effect of electron acceptor condition on the biotransformation of atrazine, alachlor, bromacil, and propachlor.
2. To describe the effect of sorption on these systems and evaluate its impact on the availability of pesticides for biotransformation.
3. To investigate the effect of acetate as an added carbon source on such systems.
4. To determine the abiotic effect of sulfide on these pesticides.

A review of literature pertinent to the study will be presented in Chapter II. The materials used and the experimental methods employed will be reviewed in Chapter III. Chapter IV will present a discussion of the results obtained in the study. In Chapter V, the findings of the research will be summarized. Also in Chapter V, limitations of the study will be discussed, and theoretical and practical suggestions for advancement of this study will be offered.

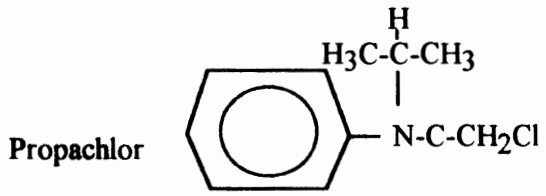
CHAPTER II

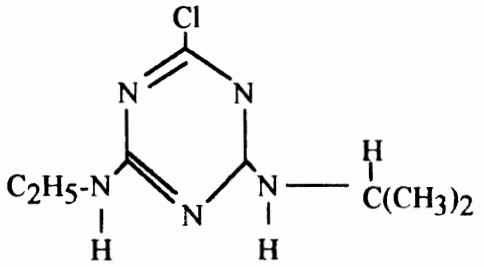
LITERATURE REVIEW

Pesticides in Ground Water

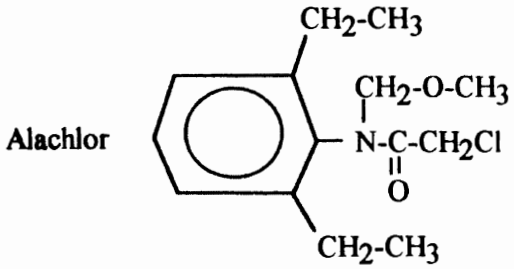
As mentioned in Chapter I, many commonly used pesticides have been found in ground waters. Bromacil, atrazine, alachlor, and propachlor are four such pesticides. Figure 1 illustrates the chemical structures of these pesticides. Hebb and Wheeler (1978) found bromacil (5-bromo-3-sec-butyl-6-methyluracil) in Florida ground water at concentrations of 1.25 mg/L within four months after land application. Eight months later, concentrations had decreased to 100 μg/L. Hallberg (1987) reported typical bromacil concentrations of 300 μg/L in several ground water monitoring studies. The EPA (1988) reports that bromacil has been detected in ground water in at least 2 states.

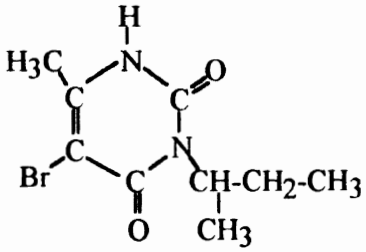
Atrazine (2-chloro-4-[ethylamino]-6-[isopropylamino]-1,3,5-triazine), alachlor (2-chloro-2',6'-diethyl-N-methoxymethyl acetanilide), and propachlor (2-chloro-N-isopropyl acetanilide) have also been detected in ground water. Hallberg (1985) reported alachlor concentrations as high as 16.6 μg/L in ground water in northeast Iowa. In Wisconsin, alachlor was detected in 47 of 377 samples with 21 samples exceeding 2.0 μg/L (Holden 1986). The EPA (1988) reports that alachlor has been detected in ground water in 12 states, and atrazine has been found in 13. Atrazine probably has been detected more widely in ground water than any other herbicide (Ritter 1990). Junk *et al.* (1980) measured atrazine concentrations as high as 88 μg/L in ground water samples in Nebraska.





Atrazine





Bromacil

Figure 1. Chemical Structures of Propachlor, Alachlor, Atrazine, and Bromacil

Biorestoration of Contaminated Ground Water

It is known that biotransformation can be a significant process affecting the fate of organic contaminants in the subsurface. Diverse and metabolically active microorganisms have been found in both shallow and deep aquifers, and they have been observed to transform many commonly detected contaminants (Ghiorse and Wilson 1988; Thomas and Ward 1989). Laboratory investigations have demonstrated that a potential exists for enhanced biotransformation of pesticides under a variety of electron acceptor conditions. These studies suggest that *in-situ* biorestoration with a selected native bacterial population stimulated by the addition of a primary substrate and nutrients is possible (Lanzarone and McCarty 1990). This process has potential advantages, especially if developed for aquifers containing organic contaminants that are difficult to degrade, significantly sorbed to aquifer solids, and/or present at low concentrations (Lanzarone and McCarty 1990).

Effects of Electron Acceptor Conditions on Biotransformations

An important environmental factor influencing biotransformations is the electron acceptor utilized by microorganisms for deriving energy from an electron donor. Microorganisms preferentially utilize electron acceptors that provide maximum free energy during respiration (Stumm and Morgan 1981). Of the common electron acceptors used by microorganisms, oxygen typically provides the most free energy to microorganisms during electron transfer (Cobb and Bouwer 1991). It is known that the addition of exogenous carbon substrates to an aquifer may result in rapid depletion of dissolved oxygen, resulting in anaerobic electron acceptor conditions (Cobb and Bouwer 1991). The electron acceptor condition will then be determined by that electron acceptor present in the media which is most thermodynamically favored and thus utilized by the microbial population for deriving energy from an electron donor (Stumm and Morgan 1981). Use of nitrate, sulfate, and carbon dioxide typically yields decreasing amounts of free energy during

electron transfer according to the order listed (Cobb and Bouwer 1991). Furthermore, it is known that the electron acceptor condition under which bioremediation is performed can effect the success and rate of the biodegradation of xenobiotic compounds, including pesticides (Berry *et al.* 1987; Bouwer and McCarty 1985; Kuhn and Suflita 1989).

Biotransformation Studies of Alachlor and Atrazine

A number of studies have investigated the biotransformation of atrazine and alachlor. For example, Pothuluri *et al.* (1990) studied the effects of various redox conditions and reported that alachlor was more readily degraded under aerobic than anaerobic conditions in soil. The addition of extraneous carbon sources to soil samples was found to enhance the rate of alachlor biotransformation.

Novick and Alexander (1985) found the degradation of alachlor to be a cometabolic process. In mineralization studies using soil-water suspensions and lake water, they found only very small percentages of ^{14}C ring-labeled alachlor were recoverable as $^{14}CO_2$ after 30 days incubation. Four transformation products were separated by thin layer chromatography, but were not identified (Novick and Alexander 1985). Further studies found that propachlor was much more readily degraded than alachlor in these systems, though no organism capable of mineralizing either compound could be isolated. Furthermore, none of the pesticide carbon was found to be assimilated by the biomass, leading to the conclusion that the degradative processes were cometabolic (Novick and Alexander 1985).

Microbial metabolism of atrazine has also been studied previously. The fate of atrazine in the environment has been most recently reviewed by Erickson and Lee (1989). They state that the ethyl and isopropyl side chains of atrazine are the only parts of the atrazine molecule capable of providing energy to microorganisms through oxidative phosphorylation, and thus, the "bioenergetic incentive for microbial biodegradation

associated with [atrazine] is found in the alkyl side chains." Despite this relatively small energetic incentive, there have been reports of microbial communities using atrazine as a growth substrate. Erickson and Lee (1989) report that dealkylation appears to be the first step in the microbial degradation of chlorinated s-triazines. Behki and Kahn (1986) observed dealkylations of both ethyl and isopropyl groups by a *Pseudomonas* species, and reported that these reactions occur much faster than the hydrolytic dechlorination. They also reported that growth of the culture was directly linked to the atrazine utilization. More recently, Nair and Schnoor (1992) compared atrazine mineralization under aerobic and nitrate-reducing conditions in soil. They report much more rapid mineralization when oxygen was present that when nitrate served as the terminal electron acceptor. Wilber and Parkin (1991) report the biotransformation of alachlor and, to a lesser degree, atrazine, in continuous flow, acetate-fed biofilm reactors, under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions. Under all four conditions, alachlor was degraded more rapidly than atrazine.

Sorption of these pesticides has also been studied previously. Peter and Weber (1985) ran a series of soil columns and found between 10 and 20 percent of the alachlor remained bound. The amounts adsorbed were most highly correlated with the fraction of organic matter in each of the soils tested. Using field lysimeters with Plainfield sand, Bowman (1990) reported that alachlor moved no further than 30 cm from the surface to which it was applied and was less mobile than atrazine. Column studies performed by Alhajjar *et al.* (1990) using Plainfield sand and Plano silt loam, gave the opposite results, with alachlor being considerably more mobile than atrazine.

Biotransformation Studies of Bromacil

Bromacil is considered to be moderately to highly mobile and relatively less biodegradable than many other herbicides (Rhodes *et al.* 1970). It can persist in soil for at

least 2 years in effective phytotoxic amounts (Bovey *et al.* 1967) and has been shown to leach rapidly through soil toward ground water, particularly during periods of heavy rainfall immediately following its field application (Hebb and Wheeler 1978). Chaudhry and Cortez (1988) isolated from soil a *Pseudomonas* sp. capable of aerobically using bromacil as its sole carbon and energy source. In studies with soil reactors inoculated with the bacterium, approximately 75% of the bromacil was degraded within 48 hours, "whereas no loss of bromacil was noticed in uninoculated samples over the same period." The addition of glucose as a second carbon source "almost completely stopped utilization of the pesticide by the microorganism." More recently, Adrian and Suflita (1990) studied the fate of bromacil in anoxic aquifer slurries. They report minimal transformation occurring in nitrate- and sulfate-reducing conditions. However, under methanogenic conditions, bromacil was shown to be reductively debrominated, producing 3-*sec*-butyl-6-methyluracil. Evidence of an abiotic transformation was also found, but was not investigated further.

Biotransformation Studies of Propachlor

Novick and Alexander (1985) studied the fate of propachlor in sewage and lake water and found it was extensively transformed. They report that more than 70% of the propachlor was transformed in 21 days in lake water, whereas 35% was transformed in 45 hours in sewage samples. Further, when glucose was added to the sewage samples, transformation rates improved to 90%. The same study also examined the fate of alachlor under identical conditions and found it to be much less susceptible to transformation than propachlor. Although propachlor and alachlor have similar chemical structures (both are acetanilide herbicides), it is possible that propachlor is more susceptible to transformation because it is less substituted (Novick and Alexander 1985). Steen and Collette (1989) studied the microbial degradation of seven amides, including propachlor, and found a wide

range of transformation patterns. They developed a second-order transformation rate constant for each and found propachlor to be transformed at rates up to 5 orders-of-magnitude greater than others.

Effects of Sulfide on Transformation of Pesticides

When confirming loss of pesticides by biotransformation, it is important to consider the potential abiotic reaction of the pesticides with constituents of the aqueous media. In particular, reaction with bisulfide is important since it is produced under sulfate-reducing conditions. Barbash and Reinhard (1989) report that the reaction of bisulfide with chlorinated aliphatic compounds is significant under conditions considered environmentally relevant. While the reactivity of alachlor and atrazine with bisulfide has been examined previously (Wilber and Parkin 1991), no data were found which reported on the reaction of bisulfide with bromacil and propachlor.

Column Methods

In order to evaluate the potential for field enhancement of biotransformation, laboratory-scale procedures are desirable for determining the biotransformation potential of native bacteria and for evaluating the effect of nutrient and substrate additions on transformation rates. Column methodologies described by Siegrist and McCarty (1987) and Lanzarone and McCarty (1990) have been used effectively in evaluating both the potential for *in-situ* biotransformation of halogenated compounds and the effect of operating variables, such as nutrient and substrate additions, on the degree of transformation that might be achieved.

Summary

Many studies have been conducted to examine the biotransformation of alachlor and atrazine. Less information is available regarding the biotransformation of bromacil and propachlor. Few of these studies, however, specifically address the interaction of soil adsorption with biotransformation, or the effect of added carbon sources. This research will result in a better understanding of the interactions among soil, pesticides, and the native bacteria populations under various electron acceptor conditions. It will provide additional information about the usefulness of acetate as an added carbon and energy source to these microorganisms, and its effect on their metabolism of pesticides. Further, investigations of abiotic reactions of pesticides with aqueous media constituents such as bisulfide will be useful in determining their effect on transformation of pesticides. These results should be helpful in assessing the potential for *in-situ* bioremediation as a technique for treating soils and ground water contaminated with these pesticides.

CHAPTER III

METHODS AND MATERIALS

This chapter will present a review of chemicals used and experimental methods employed in this research.

Chemicals

All chemicals used in this study were commercially available and were used without further purification. Methanol and ethyl acetate were HPLC grade solvents or better. Aqueous stock solutions of each pesticide were prepared from pure, analytical grade chemicals obtained from Supelco (atrazine), Monsanto (alachlor and propachlor), and DuPont (bromacil). All other compounds used for the feed solution were analytical grade or better.

Solid-Phase Extraction Procedures

Pesticide concentrations in the aqueous phase were measured by the solid-phase extraction (SPE) method described by Thurman *et al.* (1990). PrepSep C_{18} cartridges (FisherScientific, Fair Lawn, NJ) containing 360 mg of 40 μm C_{18} bonded silica were used. The C_{18} cartridges were prepared by washing with 3 mL of methanol, 3 mL of ethyl acetate, 3 mL of methanol, and 2 mL of distilled water. The column effluent sample (100 mL) was passed through the PrepSep cartridge using a PrepTorr Vacuum Box (Fisher Scientific, Fair Lawn, NJ). The cartridge was dried first with air to remove residual water and then eluted with 2.0 mL of ethyl acetate.

Pesticide Analysis

The ethyl acetate eluates were analyzed by gas chromatography (GC). The extracts were stored in the dark at 4°C until analysis. The extracts were injected (3 μL) on a DB-5 fused silica capillary column (film thickness, 0.25 μm; inner diameter, 0.25 mm; length, 30 m; J & W Scientific, Folsom, CA) in a model 5890 Hewlett-Packard Series II GC equipped with an electron capture detector (ECD). Quantification was achieved by injecting standards, treated like samples, and comparing relative areas under each separated peak recorded by a model 3396 Hewlett Packard Series II integrator. The minimum detectable concentration for propachlor, alachlor, and bromacil was 5 μg/L, while atrazine was detectable consistently to 25 μg/L. Injections were made in the split mode (ratio 1:45) at an injector temperature of 200°C and a column temperature of 175° C. Helium was the carrier gas, with a flow rate of 45 mL/min and a head pressure of 25 psi. A 95% argon/5% methane mixture was used as the ECD make-up gas. The column temperature was held at 175°C for 1 minute and then increased at a rate of 5°C/min to a final temperature of 185°C.

Acetate Analysis

The acetate samples were spiked with four drops of 88% formic acid and stored in the dark at 4°C until analysis. The samples were analyzed by gas chromatography (GC). Aqueous samples were injected (1 μL) on a glass column (60/80 Carbopak C/0.3% Carbowax 20M/0.1% H_3PO_4; inner diameter, 2 mm; length, 24 in, Supelco, Bellefonte, PA) in a model 5890 Hewlett-Packard Series II GC equipped with a flame ionization detector (FID). Quantification was achieved in the same manner as described for pesticide analysis. The minimum detectable concentration was 2.5 mg/L.

Semi-Continuous-Flow Biofilm Column Studies

Two semi-continuous-flow, laboratory-scale biofilm column reactors with oxygen as the primary electron acceptor were operated for 4 months to evaluate biotransformation of the four pesticides. Bacteria were provided acetate as a primary substrate to evaluate the biotransformation of trace levels of pesticides as secondary substrates. The glass columns used were 45 cm long, 40 mm in inner diameter, narrowed on the top and on the bottom (Figure 2), and filled with 3-mm glass beads. The top was closed with a rubber stopper, and the bottom closed with a teflon diffuser bed support. A defined sterile inorganic media was fed to provide nutrients and buffering. The media included the following (mg/L): NH_4Cl (29.8), $CaCl_2$ (27.5), $NaHCO_3$ (20), KH_2PO_4 (8.5), K_2HPO_4 (21.8), Na_2HPO_4 (17.6) (Cobb and Bouwer 1991). Glass beads were used as the biofilm support media in order to minimize sorptive effects while creating porous media flow conditions to simulate the subsurface.

Water from Lake Carl Blackwell, Stillwater, OK, was used to seed the column. All tubings and fittings (except for stoppers) were made of either Teflon or silicone in order to minimize sorption. The columns were visually inspected for uniformity of packing, covered with aluminum foil to prevent growth of photosynthetic organisms, and kept fully water saturated and at room temperature (21 °C) for the duration of the experiment.

The column fluids were exchanged approximately once every twelve hours with 175 mL of new feed solution. A peristaltic (Masterflex) pump was used to exchange the liquid in an upflow direction at a flow rate of 7 mL/min. A steel hypodermic needle penetrating the rubber stopper was connected to silicone tubing and used to collect effluent during pumping. Influent and effluent samples were collected frequently and stored in the dark at 4°C until further analysis.

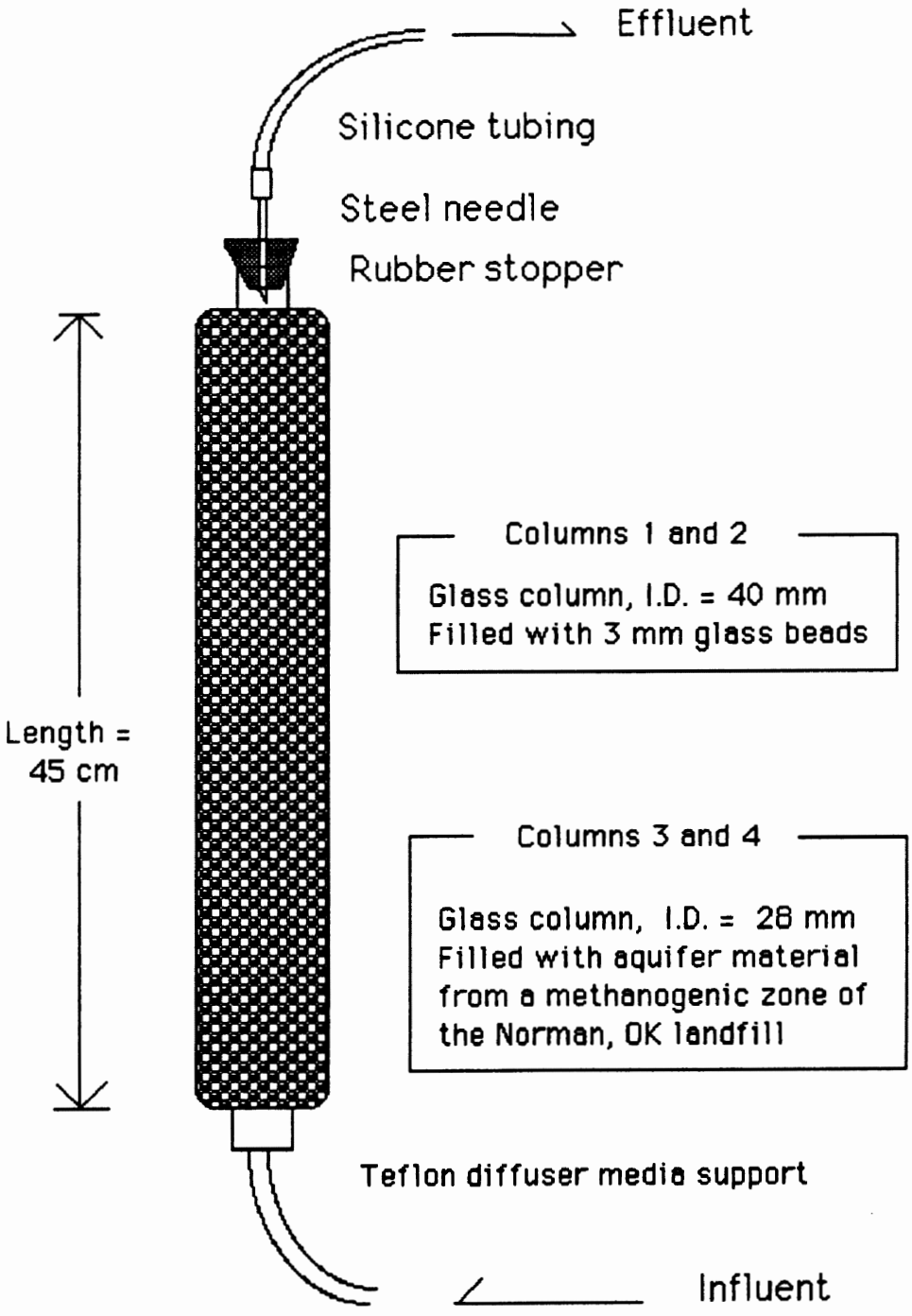


Figure 2. Design of Glass Columns

Semi-Continuous-Flow Soil Column Studies

Two semi-continuous-flow, laboratory-scale anaerobic soil column reactors were operated for 44 days to evaluate biotransformation and sorptive properties of the four pesticides. As with the glass bead biofilm columns, bacteria were provided acetate as a primary substrate in one of the columns to evaluate the biotransformation of trace levels of pesticides as secondary substrates.

The glass columns used were 45 cm long, 28 mm in inner diameter, and narrowed on the top and on the bottom (Figure 2). The top and bottom were closed in the same manner as for the glass bead biofilm columns. The volume of aquifer material placed in each column was approximately 210 mL, corresponding to about 328 grams of dry material. A steel hypodermic needle penetrating the rubber stopper was connected to silicone tubing and used to collect effluent during pumping.

Sediment materials were provided by the Department of Botany and Microbiology, University of Oklahoma, Norman, OK, after collection from a shallow anoxic aquifer site adjacent to the Norman, OK, municipal landfill. This site was previously characterized by Beeman and Suflita (1987), who concluded that methanogenesis appeared to be the primary metabolic process at this site. Aquifer sediments were collected by digging to the top of the water table (1.3 m) and collecting material in sterile jars as previously described (Beeman and Suflita 1987).

During filling of a column, the aquifer material was added with a spoon through the top of the columns, after which a nutrient solution was added from the bottom at 7 mL/min by use of a peristaltic (Masterflex) pump. The nutrient solution fed to both columns contained the same inorganic media fed to the aerobic biofilm columns. For good settling of the aquifer material the glass column was continuously tapped with a plastic rod. This method allowed for good flow conditions in the columns, although some of the fine particle fraction was lost by this procedure. The columns were visually inspected for

uniformity of packing, covered with aluminum foil to prevent growth of photosynthetic organisms, and kept fully water saturated and at room temperature (21°C) for the duration of the experiment.

Aquifer material sufficient to fill one column was sterilized in an autoclave at 15 psi and 248 °F for 30 minutes. This column was operated to determine the sorptive and desorptive properties of the pesticides onto the soil. No acetate was added to its feed solution.

The column fluids were exchanged approximately once every forty eight hours with 85 mL of new feed solution (based on assumed soil porosity). Prior to exchange, the feed water was stripped of oxygen by bubbling vigorously with nitrogen gas. A peristaltic (Masterflex) pump was used to exchange the liquid in an upflow direction at a flow rate of 7 mL/min. Influent and effluent samples (50 mL) were collected at each exchange and diluted with 50 mL of distilled water to produce the 100 mL of aqueous sample necessary for extraction. The GC peak areas were adjusted appropriately to obtain the actual concentration.

Abiotic Sulfide Experiment

An experiment was conducted to investigate the kinetics of an abiotic sulfide reaction with each of the four pesticides. In each of two batch tests, a solution containing the same inorganic media fed to the aerobic and anaerobic columns was stripped of oxygen by bubbling vigorously with argon gas and dosed with a phosphate buffer (170 mg/L KH_2PO_4, 109 mg/L K_2HPO_4, and 89 mg/L Na_2HPO_4) at pH 7.1 (the pKa of hydrogen sulfide). In one batch, the media was then dosed with 200 µg/L bromacil and 21 mg/L total sulfide. The solution was then quickly distributed among a series of 120-mL serum bottles. In the other batch, the media was prepared as described and then dosed with 300 µg/L alachlor, propachlor, and atrazine, and 19 mg/L total sulfide.

In each batch test, the serum bottles were sealed without headspace with 1-cm thick PTFE-faced silicone septa (Supelco) and capped with aluminum crimp seals, preventing any volatilization of the hydrogen sulfide. The bottles were stored in the dark in a 21°C incubator . Pesticide and sulfide concentrations were measured over time, and from this data a rate constant could be calculated. Total sulfide concentration was measured by the Iodometric Method (Method 4500E, Standard Methods).

CHAPTER IV

RESULTS

Four columns were initially operated as described in Table 1, and the subsequent modifications that were made are described below.

TABLE 1

THE INITIAL EXPERIMENTAL DESIGN

Column number	*Initial operating conditions*
1	Aerobic biofilm column, acetate, nutrient supplements, Propachlor, and Atrazine.
2	Aerobic biofilm column, acetate, nutrient supplements, Propachlor, Alachlor, and Bromacil.
3	Anaerobic, "live" biological soil column, acetate, nutrient supplements, Propachlor, Alachlor, Atrazine, and Bromacil.
4	Anaerobic, autoclaved soil column, nutrient supplements, Propachlor, Alachlor, Atrazine, and Bromacil.

As mentioned in Chapter I, a key objective of this research was to evaluate the biotransformation of pesticides. Before results are reviewed, a brief discussion of

terminology is included to avoid confusion. *Biotransformation* is used in this thesis to describe any biological transformation of a pesticide. It does not necessarily mean that the pesticide was completely mineralized, although this could be possible. The term *biodegradation* would be more appropriate to describe complete mineralization of the pesticide. It was the intent of this study to confirm the extent of initial transformation, not mineralization, of the pesticides.

Column Experiment Results

Biofilm Column 1

Biofilm Column 1 was exchanged approximately once every twelve hours, which provided adequate time for the biomass to consume the acetate, while preventing the organisms from experiencing anaerobic conditions for extended periods. This column was fed acetate for 42 days before any pesticides were added to the influent. Day 0 on all graphs represents the day propachlor and atrazine were added, at a target influent concentration of 250 μg/L. Biofilm Column 1 was operated under the initial experimental conditions (see Table 1) for 28 days. During this period, acetate was fed as the primary substrate. The target influent concentration for acetate was 20 mg/L, a concentration slightly in excess of the stoichiometric amount required to deplete dissolved oxygen levels. Influent acetate values to the column averaged 20.6 mg/L (s.d. 22%, n=13). Actual acetate influent and effluent concentrations appear in Appendix A. Ten days after the pesticides were added, acetate removal was consistently at or near 100%.

Pesticide influent and effluent data for Biofilm Column 1 appear in Figures 3 and 4 for atrazine and propachlor, respectively. Over this period, measurable differences in transformation rates between the two pesticides were observed. Under these conditions, atrazine proved to be less susceptible to biological transformation. Actual atrazine influent and effluent concentrations during this period appear in Appendix B. Influent

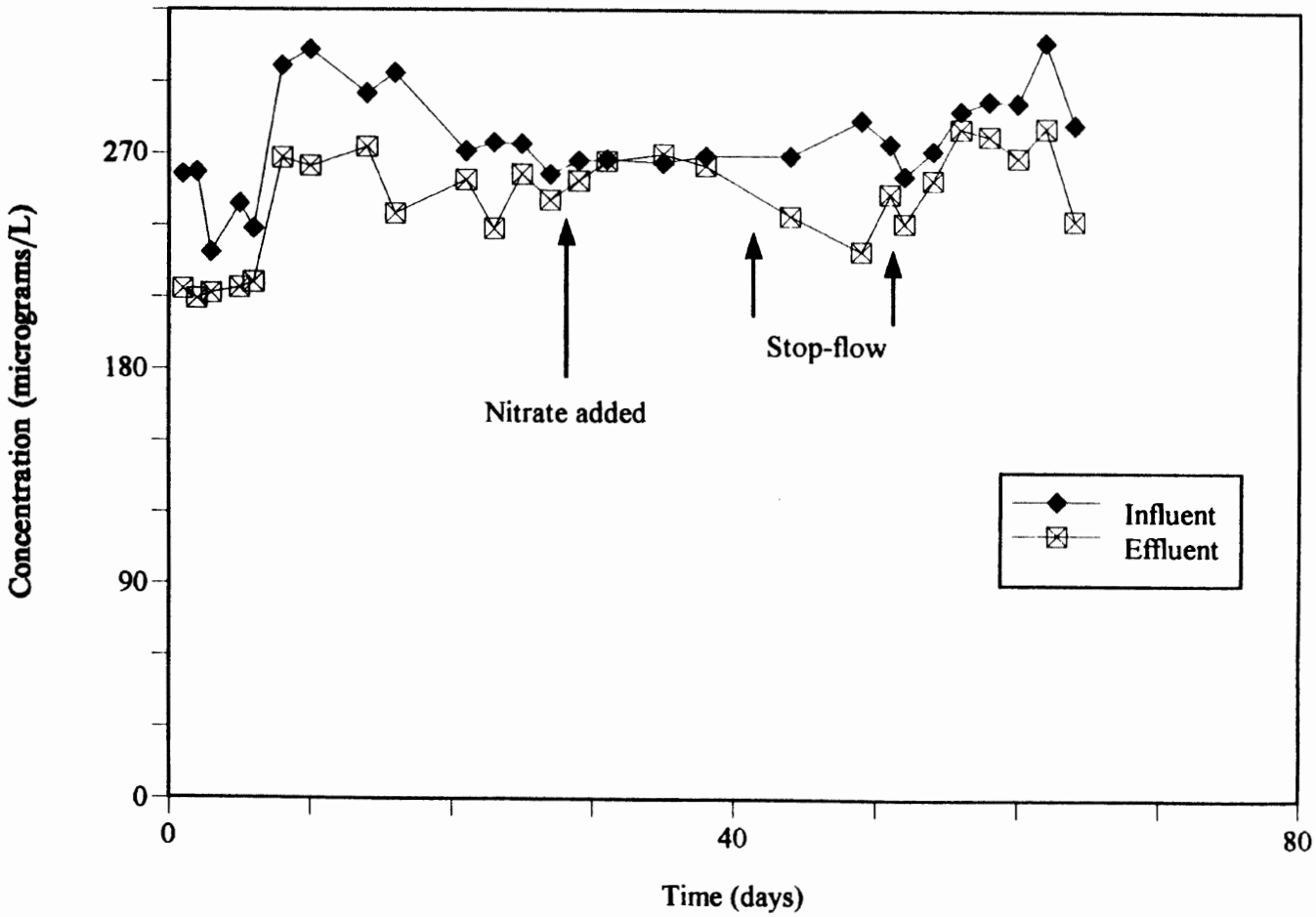


Figure 3. Atrazine Influent and Effluent Raw Data, Biofilm Column 1

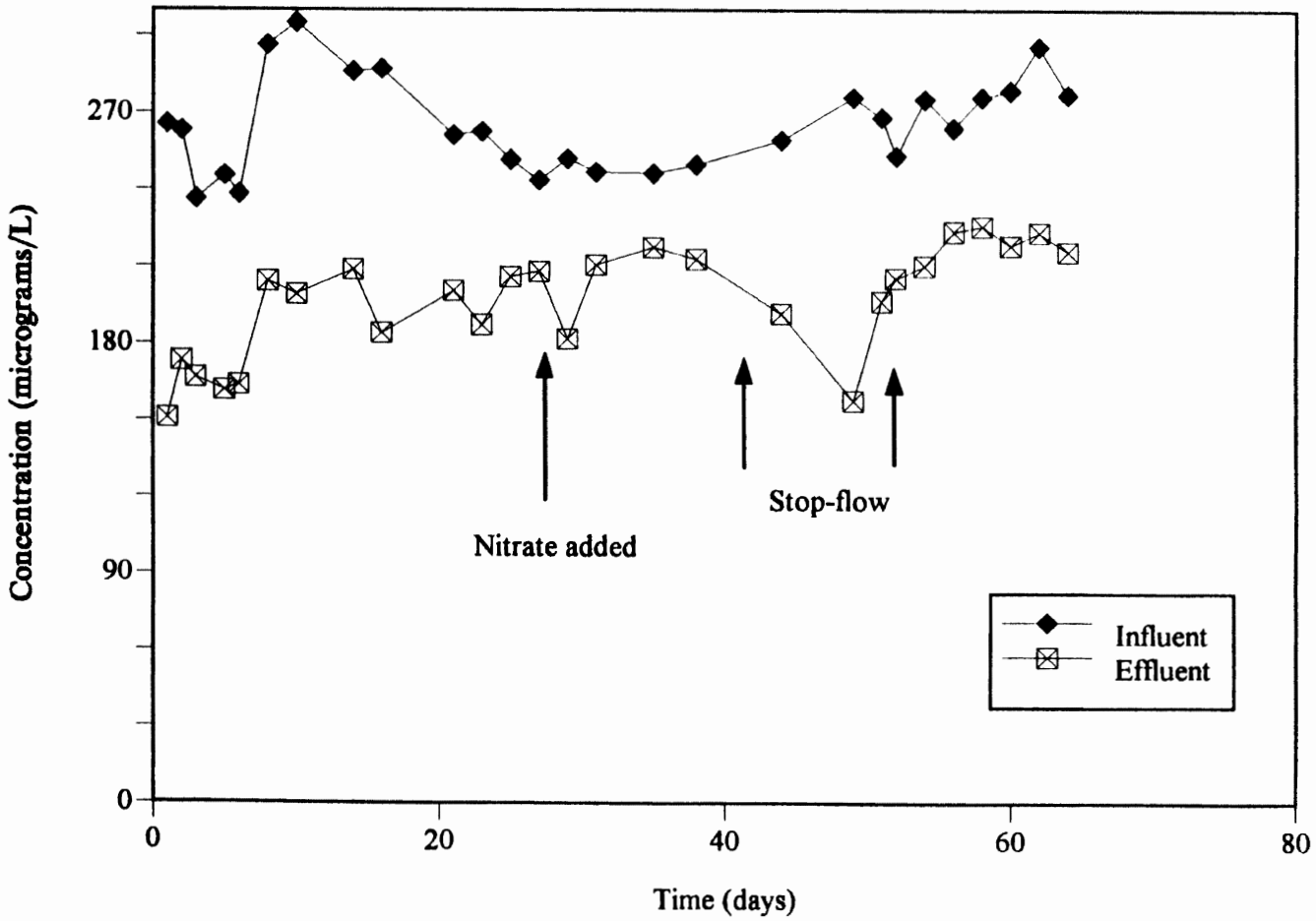


Figure 4. Propachlor Influent and Effluent Raw Data, Biofilm Column 1

atrazine concentrations averaged 272.4 μg/L (s.d. 9%, n=13). C/Co ("Fraction Remaining") values during this same period averaged 0.88 (s.d. 7%). Actual C/Co values are plotted versus time in Figure 5. It should be noted here that maintaining a consistent influent concentration in this column and the others was difficult throughout the entire course of the research. Small volumes of trace-level feed solutions were prepared and exchanged daily, contributing to the concentration variations. Accordingly, this should be kept in mind when reviewing all data in this study.

Propachlor was added to the Biofilm Column 1 influent at the same time as atrazine. Under the conditions of the original experiment, significant biotransformation of propachlor was observed. Actual propachlor influent and effluent concentrations during this period appear in Appendix C.

From the data, it is apparent that the microorganisms present in the column were able to transform the propachlor as a secondary substrate. Influent propachlor concentrations averaged 264.7 μg/L (s.d. 8%, n=13). C/Co values during this same period averaged 0.70 (s.d. 10%). Actual C/Co values are plotted versus time in Figure 5.

From Figure 5, propachlor transformation quickly approached a steady state after introduction of the pesticide to the column. This observation, combined with a reasonable 8% standard deviation, permits a useful basis of comparing transformation rates of propachlor between the original experimental conditions and other conditions (to be discussed later) within the same column, as well as between columns operated under other electron acceptor conditions.

Effect of Nitrate on Biotransformations. The addition of nitrate as a terminal electron acceptor to Biofilm Column 1 was initiated after 29 days of operation to observe the response of the column. The relatively high acetate loading allowed the microorganisms to rapidly consume the dissolved oxygen. Nitrate served as an electron

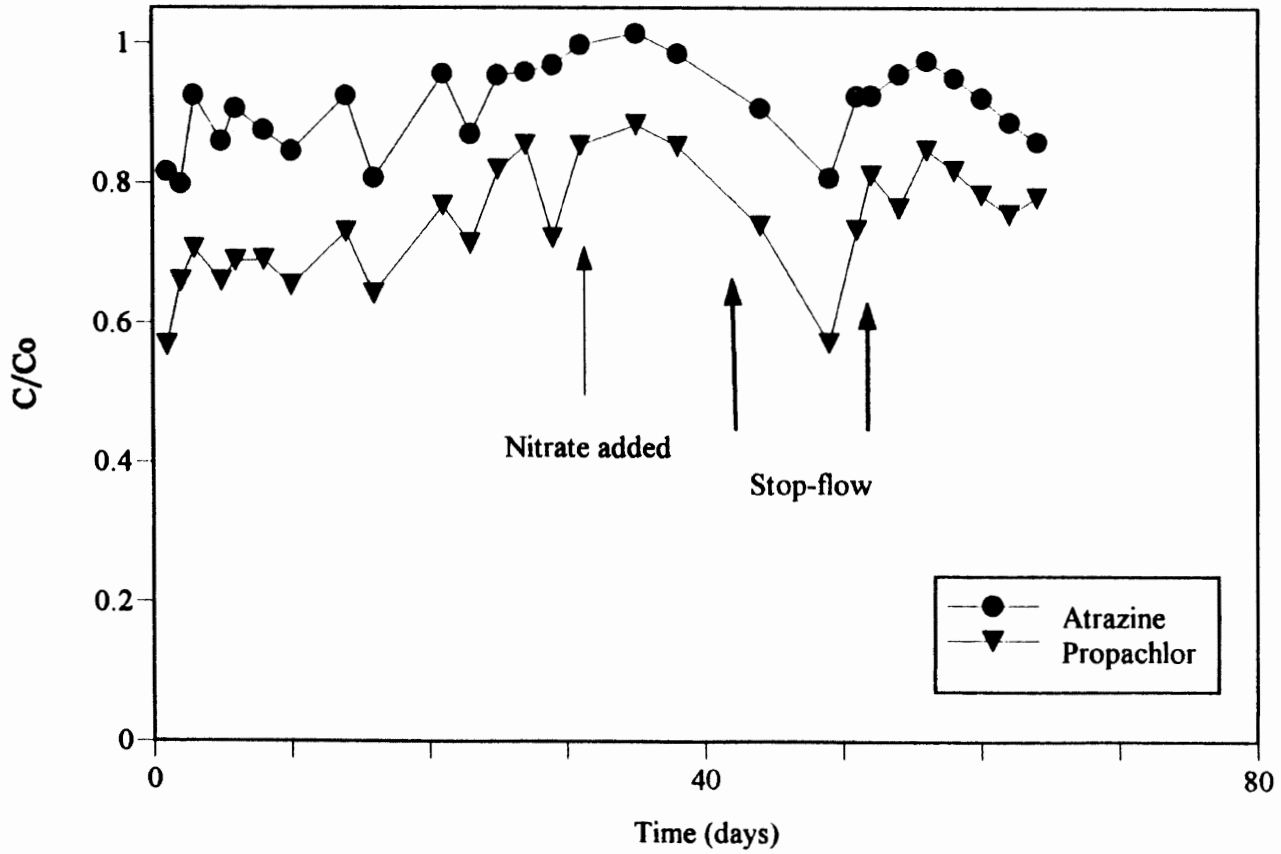


Figure 5. Propachlor and Atrazine, C/Co data, Biofilm Column 1

acceptor for facultative nitrate-reducing bacteria to utilize once oxygen was depleted. The target influent concentration for nitrate was 40 mg/L, a concentration in excess of the stoichiometric amount required to deplete the remaining acetate when coupled with nitrate-reduction. Nitrate influent and effluent samples were analyzed by ion chromatography to ensure the presence of the desired redox conditions. Influent and effluent nitrate concentrations averaged 53.6 mg/L and 42.9 mg/L, respectively.

The addition of nitrate had a negative effect on the ability of the microorganisms to transform atrazine. Atrazine influent concentrations averaged 279.6 μg/L (s.d. 5.7%, n=11) and C/Co values averaged 0.95 (s.d. 4.2%), up from 0.88 for the initial experimental period. This would indicate that atrazine can be biotransformed under aerobic conditions somewhat better than under nitrate-reducing conditions. Similarly, Nair and Schnoor (1992) found atrazine was mineralized much more slowly under nitrate-reducing conditions than under aerobic conditions.

As with atrazine, transformation rates of propachlor worsened after the addition of nitrate. Propachlor influent concentrations averaged 265 μg/L (s.d. 6%, n=12) and C/Co values averaged 0.81 (s.d. 5.9%), up from 0.70 for the initial experimental period. This would indicate that aerobic conditions are more favorable than nitrate-reducing conditions for propachlor biotransformation as well.

Biofilm Column 2

This column was fed acetate at a target influent concentration of 20 mg/L for one month before any pesticides were added to the influent. Day 0 on all graphs represents the day bromacil was added to the influent. Biofilm Column 2 was operated under the initial experimental conditions (see Table 1) for 54 days. During this period, acetate was also fed as the primary substrate. Influent acetate concentrations fed to the column averaged 24.1 mg/L (s.d. 24%, n=17) and C/Co values averaged 0.35 (s.d. 28%). Actual acetate

influent and effluent concentrations appear in Appendix D.

Pesticide influent and effluent data for Biofilm Column 2 appear in Figures 6 through 8 for bromacil, alachlor, and propachlor, respectively. Over this period, wide variability in biotransformation rates between pesticides was observed. For example, under these conditions, bromacil proved to be the least susceptible to biological transformation. Actual bromacil influent and effluent concentrations during this period appear in Appendix E. Visual inspection of Figure 6 yields the conclusion that microorganisms present in the column were unable to transform the bromacil as a secondary substrate. Influent bromacil concentrations averaged 108 µg/L (s.d. 14%, n=21). C/Co values during this same period averaged 0.93 (s.d. 11%). Actual C/Co values are plotted versus time in Figure 9.

Alachlor was added to the Biofilm Column 2 influent feed on Day 16. Under the conditions of the initial experiment, significant biotransformation of alachlor was observed. Actual alachlor influent and effluent concentrations during this period appear in Appendix F.

From the data, it is apparent that the microorganisms present in the column were able to transform the alachlor as a secondary substrate. Influent alachlor concentrations averaged 114 µg/L (s.d. 6.7%, n=16). C/Co values during this same period averaged 0.46 (s.d. 20%). Actual C/Co values for alachlor are also plotted versus time in Figure 9.

The precise degree to which alachlor was transformed is difficult to quantify. The analysis is complicated by the difficulty in pinpointing the time required to reach steady state in pesticide removal levels. These findings do confirm work cited previously (Lynch, 1990; Pothuluri, *et al* 1990; Wilber and Parkin, 1991) which indicated alachlor could be transformed under aerobic conditions.

Propachlor was added to the Biofilm Column 2 influent feed at the same time alachlor was added. Under the initial experimental conditions, biotransformation of

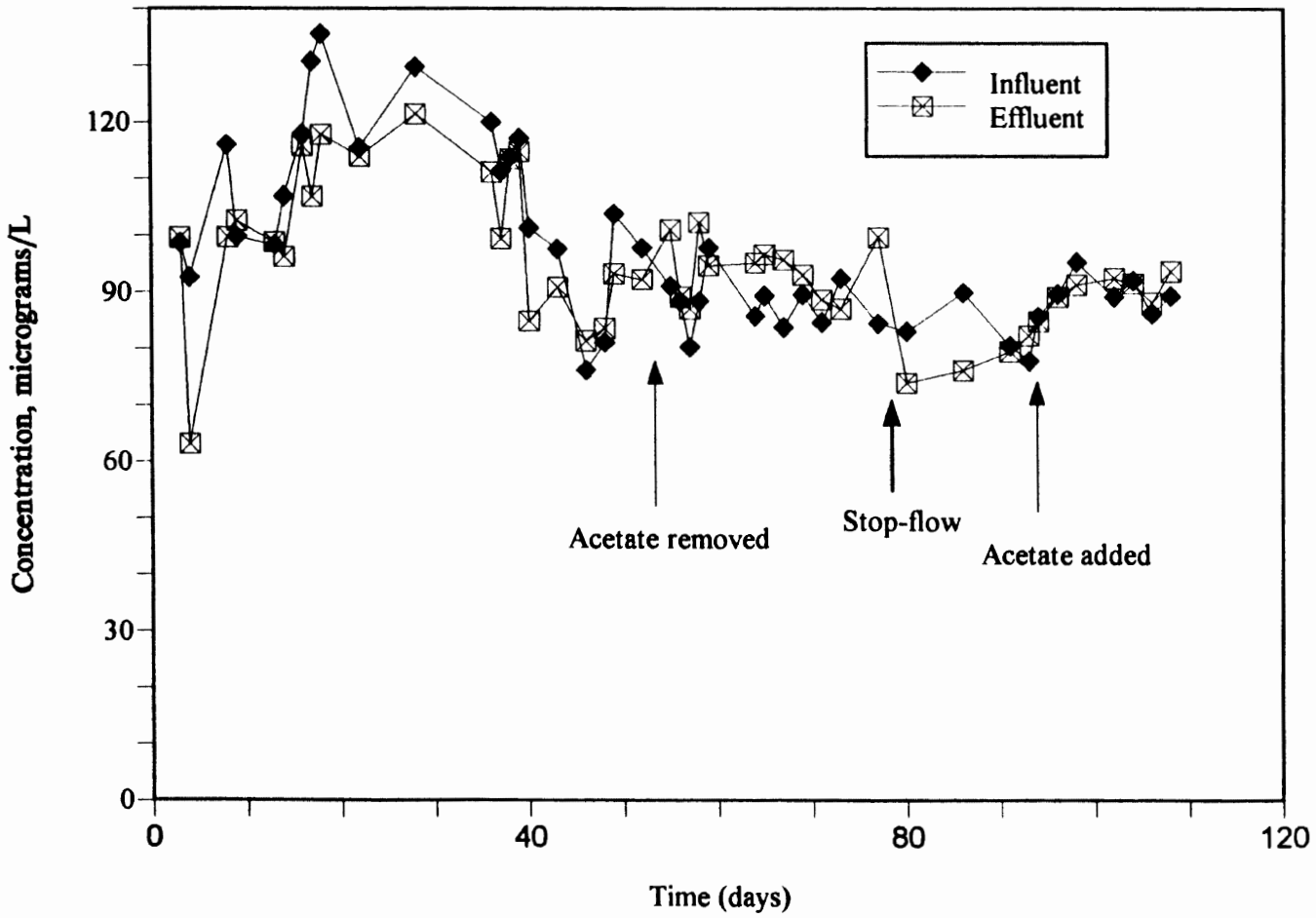


Figure 6. Bromacil Influent and Effluent Raw Data, Biofilm Column 2

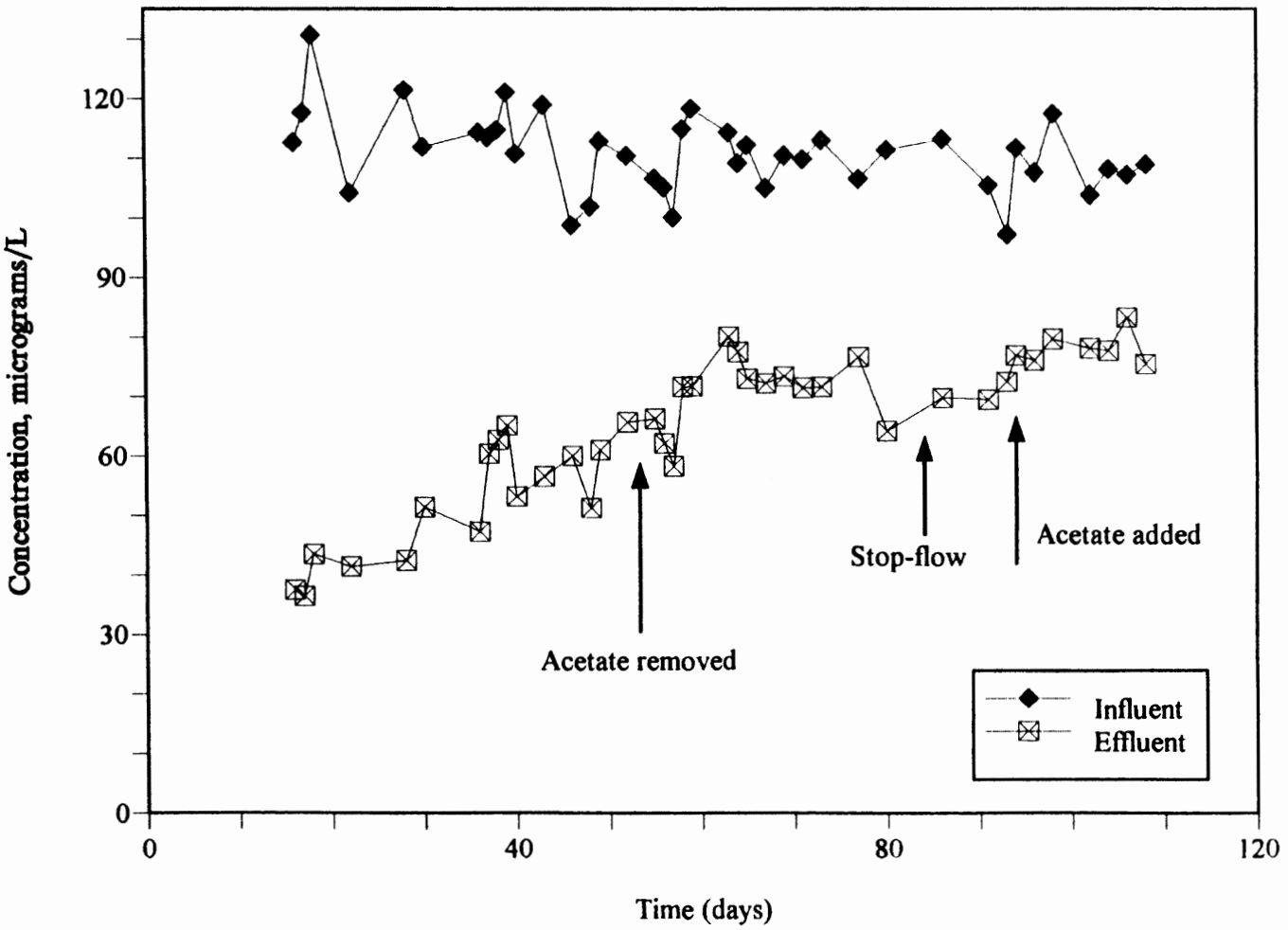


Figure 7. Alachlor Influent and Effluent Raw Data, Biofilm Column 2

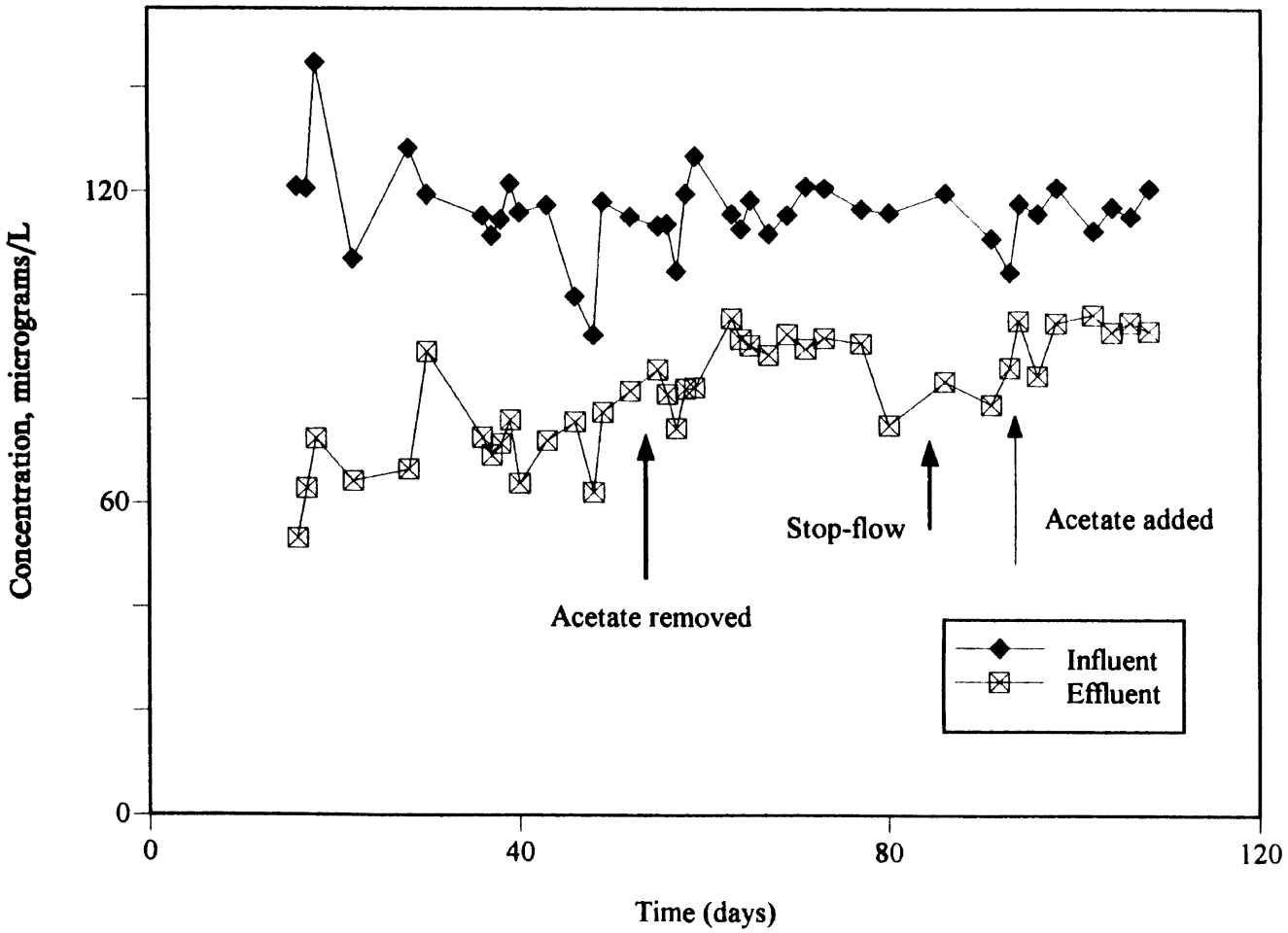


Figure 8. Propachlor Influent and Effluent Raw Data, Biofilm Column 2

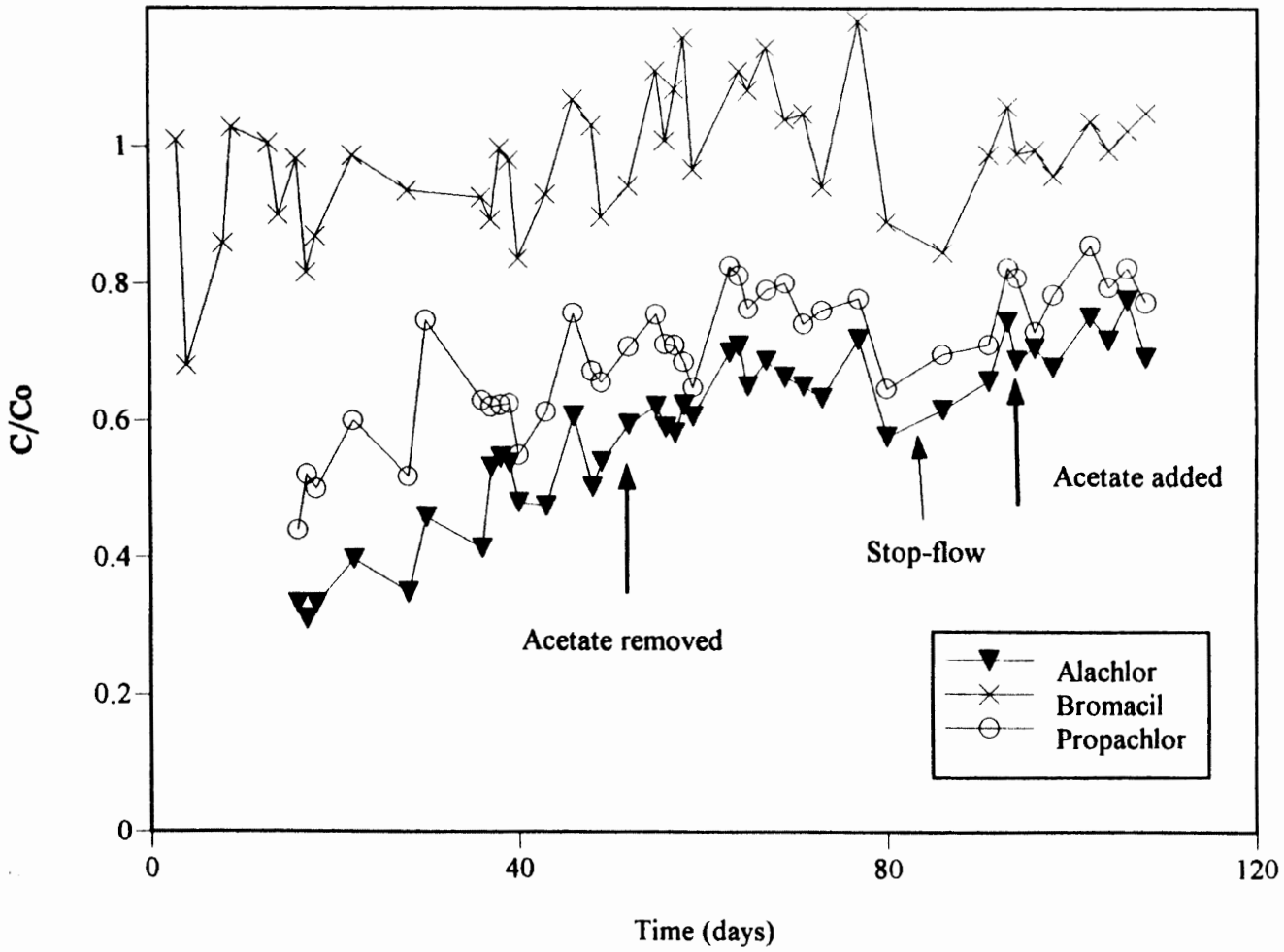


Figure 9. Propachlor, Alachlor, and Bromacil C/Co Data, Biofilm Column 2

propachlor was observed, although to a lesser extent than for alachlor. Actual propachlor influent and effluent concentrations during this period appear in Appendix G. As with alachlor, it is apparent that the microorganisms present in the column were able to transform the propachlor as a secondary substrate. Influent propachlor concentrations averaged 116 μg/L (s.d. 10%,n=16). C/Co values during this same period averaged 0.61 (s.d. 13%). Actual C/Co values for propachlor are also plotted versus time in Figure 9.

From Figure 9, it is evident that the microorganisms found alachlor to be the most favorable secondary substrate, propachlor the next most favorable, and bromacil the least favorable. The difference in transformation rates between alachlor and propachlor is approximately 25% (C/Co = 0.61 versus 0.46). This finding contrasts with Novick and Alexander (1985), who report propachlor being transformed in lake water more readily than alachlor. The lack of removal of bromacil allows it to act as an unintended control, providing incremental assurance that no errors were present in sampling techniques and analytical methods.

Effect of Acetate on Biotransformations. The addition of acetate to Biofilm Column 2 was discontinued after 54 days of operation and initiated again 40 days later to observe the response of the column to the loss and return of the primary substrate.

The pattern of no significant bromacil removal, observed under the initial experimental conditions, continued after acetate was removed as a primary substrate. During the period in which no acetate was fed, bromacil influent concentrations averaged 88 μg/L (s.d. 5%, n=13). Concurrently, C/Co values averaged 1.0 (s.d.=10%). Actual influent and effluent concentrations for this period appear in Appendix E. A plot of this data versus time appears in Figure 6. Actual C/Co values are plotted versus time in Figure 9.

Significant biotransformation of alachlor and propachlor during the initial experimental period led to expectations that once acetate was removed, C/Co values

would eventually increase to levels at or near 1.0 as microbial metabolism slowed. This, however, was not the case, as only slightly higher C/Co values were gradually observed for both pesticides. Thus, in the absence of an external energy source, the transformation of alachlor and propachlor appears possible under endogenous metabolism by the microorganisms.

During the period in which no acetate was fed, alachlor influent concentrations averaged 110 μg/L (s.d. 4.2%, n=14). Concurrently, C/Co values averaged 0.64. Thus, on average, biotransformation rates of alachlor were slowed by nearly 40% when acetate was removed (C/Co = 0.64 versus 0.46). This is probably overestimating the impact, however, since the C/Co values during the original period varied widely while a steady state level of pesticide removal was being reached. Actual influent and effluent concentrations for the period with no acetate appear in Appendix F. A plot of this data versus times appears in Figure 7. Actual C/Co values for alachlor are also plotted versus time in Figure 9.

On Day 94, acetate was reintroduced to the influent. Acetate influent concentrations averaged 20.3 mg/L (s.d. 11.8%, n=7). It was expected that re-introduction of acetate to the influent would eventually result in the return of the pesticide removals to their previous levels. Returning acetate to the influent feed did not affect the biotransformation of bromacil. Between Day 94, when acetate feed was initiated again, and Day 108, when the experiment was concluded, bromacil removal remained minimal. The data presented here support the conclusion that, under aerobic respiration, transformation rates of bromacil are not affected by the presence or absence of acetate as a primary substrate to support microbial growth. Returning acetate to the influent feed did influence the removal rate of alachlor, albeit in a surprising fashion. Influent alachlor concentrations averaged 109.3 μg/L (s.d. 3.6%, n=7). C/Co values for the same period averaged 0.72 (s.d. 4.1%), up from 0.64 for the period with no acetate and 0.46 from the initial period with acetate. Since no sharp results were observed, the effects on alachlor

biotransformation in the presence and absence of acetate are inconclusive. Two suggestions are offered as possible explanations. First, it is possible that acetate feed to the influent was shut off before a steady-state level of pesticide removal was reached under the initial experimental conditions. Second, it is possible that some bacteria responsible for patterns of transformation (observed prior to the re-introduction of acetate) were killed during the stop-flow experiments (described below) and insufficient time was allowed to replenish their population.

Propachlor influent concentrations averaged 116 μg/L (s.d.=4.3%) during the period in which no acetate was fed. C/Co values during the same period averaged 0.74 (s.d.8%). Thus, on average, transformation rates of propachlor were slowed by 21% (C/Co = 0.74 versus 0.61). Given these findings for alachlor and propachlor, it is interesting to compare transformation rates for the pesticides between periods of acetate presence and absence.

As mentioned previously, alachlor removal was 25% greater than propachlor under the initial experimental conditions. When acetate was removed, this difference was almost halved to 13% (C/Co = 0.64 versus 0.74). These results suggest that while transformation rates of alachlor and propachlor are both sensitive to the presence of the primary substrate, alachlor transformation is the more sensitive.

The same phenomenon observed for alachlor after re-introduction of the acetate was observed for propachlor. Average C/Co values actually rose during this period. Influent propachlor concentrations averaged 117.2 μg/L (s.d. 2.3%, n=7). C/Co values averaged 0.80 (s.d. 4.6%), up from 0.74 for the period with no acetate and 0.61 from the initial period with acetate. Reasons cited earlier as possible explanations for alachlor's results would also be applicable here.

Stop-Flow Experiments

During the column experiments described above, the feed flow to Biofilm Columns 1 and 2 was occasionally discontinued for up to 72 hours in order to investigate the effect of longer detention time on the biotransformation of the pesticides. The short-term semi-batch conditions within the biofilm columns allowed additional contact time between the biomass and the pesticides. These experiments were conducted for a period of 8 days and not continued further. It should be noted that influent and effluent data collected during the stop-flow experiment for Biofilm Column 1 (conducted after nitrate was added) has been excluded from any discussion on the effects of nitrate as a terminal electron acceptor. Furthermore, influent and effluent data collected during the stop-flow experiment for Biofilm Column 2 (conducted under aerobic conditions in the absence of acetate) has been excluded from any discussion on the effects of acetate as an added carbon source.

A significant increase in propachlor transformation was observed in Biofilm Column 1 under nitrate-reducing conditions when exposure was increased to 72 hours. Unfortunately, only one data point was collected at this exposure time, so definite conclusions cannot be reached. The data for Day 49 represents a 72-hour exposure. The C/Co value for that point is 0.57, a marked improvement in biotransformation compared to the average of all other data in the same period. C/Co values for propachlor averaged 0.81 prior to the stop-flow experiments. Thus, these results do support the conclusion that propachlor can be biotransformed under nitrate-reducing conditions, and that increased contact time results in increased removal.

Minor changes (if any) were observed in C/Co values for propachlor, alachlor, and bromacil in Biofilm Column 2. The extended exposure time between the biomass and the pesticides did not significantly increase biotransformation rates. The most likely explanation is that no other terminal electron acceptor was present once oxygen was consumed, effectively inhibiting the metabolic processes of the aerobic culture. The

results indicate that oxygen is a rate-limiting constituent in the process of biological transformation of these pesticides under the conditions in Biofilm Column 2. For example, if exposure times were extended to 5 days, the additional 48 hours would yield only slight reductions in C/Co values (compared to 72 hour tests). This assumes, of course, that the oxygen is consumed relatively rapidly soon after the column exchange. The column used in this experiment was not equipped to test such an assumption. However, during periods of normal, 12-hour exchange periods, oxygen in the effluent was found to be at or near detection limits (approximately 1 mg/L). As mentioned earlier, the stop-flow experiment for Biofilm Column 2 was conducted in the absence of acetate. It is not known how the presence of acetate would affect an identical stop-flow experiment in this column, except that oxygen depletion would likely be even faster.

Based on the preceding discussion of results, it would be reasonable to expect further transformation of pesticides in a column with 72 hours of contact time if another terminal electron acceptor were available to the organisms after oxygen was depleted. In Biofilm Column 1 nitrate served this purpose, while in Biofilm Column 2, no inorganic electron acceptor was available. Propachlor provided a useful test of this expectation, since it was fed to both the aerobic and nitrate-reducing columns tested during the stop-flow experiments.

Abiotic Sulfide Experiment Results

An experiment was conducted to investigate the kinetics of an abiotic sulfide reaction with each of the four pesticides. In each of two batch tests, a solution containing the same inorganic media fed to the biofilm columns was stripped of oxygen and then dosed with a phosphate buffer (pH=7). The media was dosed with trace concentrations of pesticides and sulfide and then quickly distributed among a series of 120-mL serum bottles. The bottles were sealed and stored in an incubator at 21°C. Pesticide and sulfide

concentrations were measured over time. The total dissolved sulfide concentration (approximately 20 mg/L) was chosen to reflect environmentally relevant conditions. Barbash and Reinhard (1989) report that sulfide concentrations up to 34 mg/L are commonly encountered in ground waters containing landfill leachate and/or sulfate-reducing bacteria.

Raw data resulting from the sulfide experiments with bromacil, alachlor, propachlor, and atrazine appear in Appendices H through K, respectively, and are plotted together versus time in Figure 10. Throughout the discussion of this experiment's results, it will be implicitly assumed that it is the bisulfide ion ($[HS^-]$), not sulfide, reacting abiotically with the pesticides. It should be noted that no experiment was conducted to confirm this assumption. This kinetic study was conducted at a pH of 7. At this pH (the pKa of hydrogen sulfide), half of the total dissolved sulfide is in the form of $[HS^-]$. Based solely upon this observation, it cannot be argued conclusively that the pesticides reacted exclusively with $[HS^-]$. Identical experiments conducted under acidic conditions (pH=2, for example), in which all of the sulfide would be in the hydrogen sulfide form, would be required to test this assumption. Wilber and Parkin (1991) conducted such a study under acidic conditions for alachlor and atrazine, and found no significant removal of either. These results suggest that the assumption is correct for alachlor and atrazine. Given the chemical similarities between all four pesticides studied, it is likely that bromacil and propachlor would behave in a similar manner.

Similarly, identical experiments conducted under extremely high pH conditions could be conducted to confirm that the pesticides were reacting with bisulfide ion and not the sulfide ion. For example, at pH=14 (the pKa of $[HS^-]$), half of the $[HS^-]$ is in the form of $[S^{2-}]$. This condition was not tested since pH levels this high are not considered environmentally relevant.

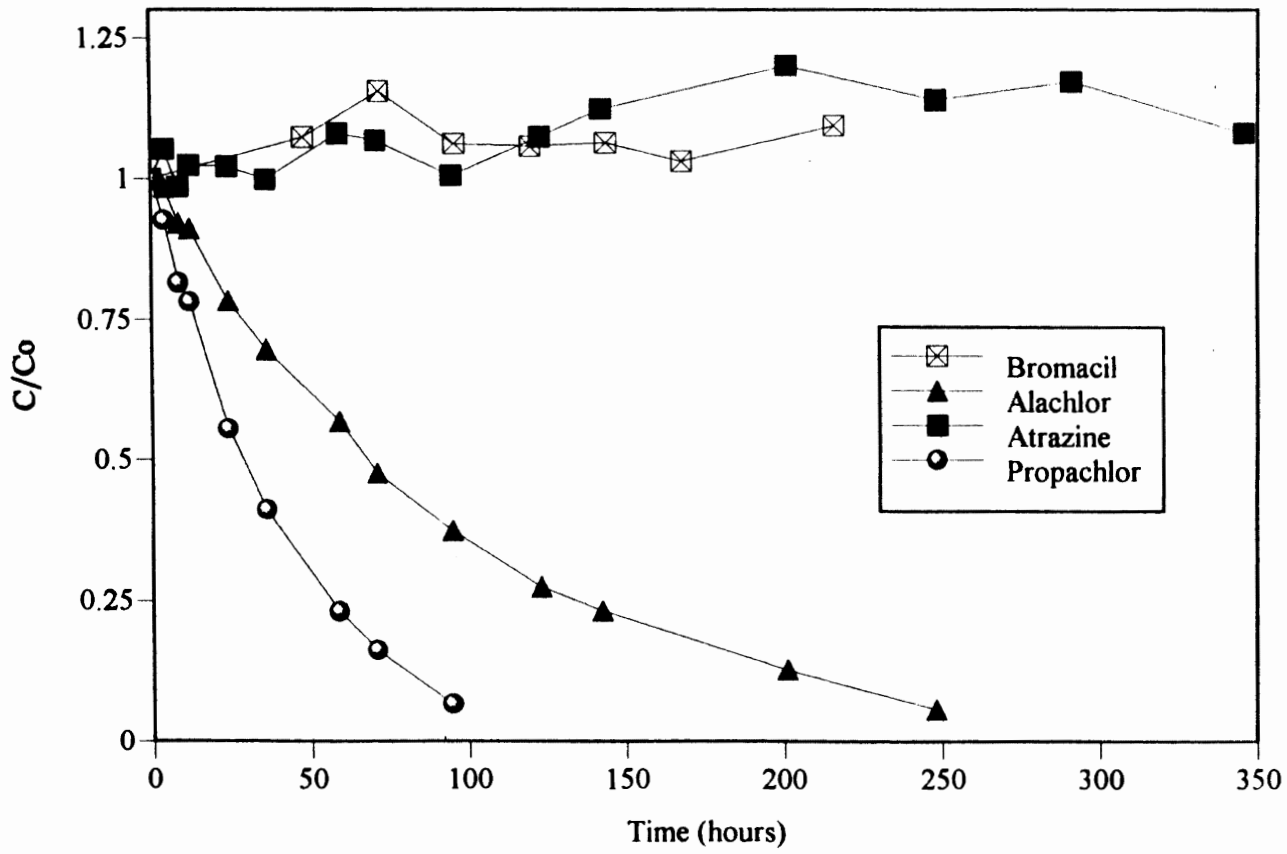


Figure 10. C/Co Values, Abiotic Sulfide Reaction with Pesticides.

The following rate law was used to interpret the kinetic results of this study:

(1) $dC/dt = - k[HS^-]C$

where

dC/dt = change in pesticide concentration with respect to time (mg/L-h)
C = pesticide concentration at time t (mg/L)
k = second-order rate constant (1/h-mg/L $[HS^-]$)
$[HS^-]$ = bisulfide concentration (mg/L)

Second-order rate constants were determined by forcing the overall reaction to proceed in a pseudo-first-order fashion by maintaining one constituent in great excess. In this case, the bisulfide concentration was in great excess, and therefore, was assumed to be constant. Measurements of total sulfide and pH over time confirmed this assumption.

Replacing the product $k[HS^-]$ in Equation 1 with k' results in

(2) $dC/dt = -k'C$

where k' is expressed in units of 1/h.

Rearranging and integrating yields

(3) $\ln(C/C_o) = -k't$

where Co is the pesticide concentration at time zero. Thus, the pseudo-first-order reaction rate constant (k') can be obtained if a plot of ln(C/Co) versus time yields linear results. The second order overall reaction rate (k) is then calculated by dividing k' by the bisulfide concentration.

From Figure 10, it is evident that bromacil and atrazine did not react, as C/C_o values stayed at or near 1.0 for the duration of the experiments. It is also evident that alachlor and propachlor did react. From the figure, it is clear that propachlor disappeared at a much faster rate, as it took less than half the time of alachlor to fall below detection limits.

A plot of ln(C/Co) values for propachlor versus time is found in Figure 11. Since the plot yields linear results ($R^2 = 0.991$), the pseudo-first-order reaction rate constant k' can be obtained from the slope of the line. From Figure 11, a rate constant of 0.02673/h is shown. As discussed earlier, the second order overall reaction rate can be obtained by dividing the pseudo-first-order rate constant by the bisulfide concentration. The resulting rate constant is 0.0028/h·mg/L [HS^-].

Alachlor was analyzed in a similar manner. A plot of ln(C/Co) values appears in Figure 12. As with propachlor, the linearized plot yielded excellent results ($R^2 = 0.993$). From the figure, a rate constant of 0.0108/h is shown. The second-order overall reaction rate constant, obtained as described previously, is 0.0011/h·mg/L [HS^-].

It should be noted that the preceding discussion of results is based on an implicit assumption that the reaction is second-order. A series of batch experiments where [HS^-] concentrations were varied would be required to confirm this assumption. The reaction rate constant determined for alachlor in this experiment is slightly lower than the constant found in previous work. Wilber and Parkin (1991) determined a rate constant of 0.0015/h ·mg/L [HS^-]. No other studies were found describing the reaction of sulfide or bisulfide with alachlor or any of the other pesticides.

Soil Column Experiments

Two semi-continuous-flow, laboratory-scale anaerobic soil column reactors (designated as Soil Columns 3 and 4) were operated in parallel. Aquifer sediments used to fill the columns were obtained from a site characterized previously by Beeman and Suflita (1987), who concluded that methanogenesis was the primary metabolic process occurring in these sediments. The columns were operated to evaluate the sorptive properties of each pesticide, the biotransformation of each pesticide under methanogenic conditions, and in the presence of acetate as an added carbon and energy source. To describe the effect of

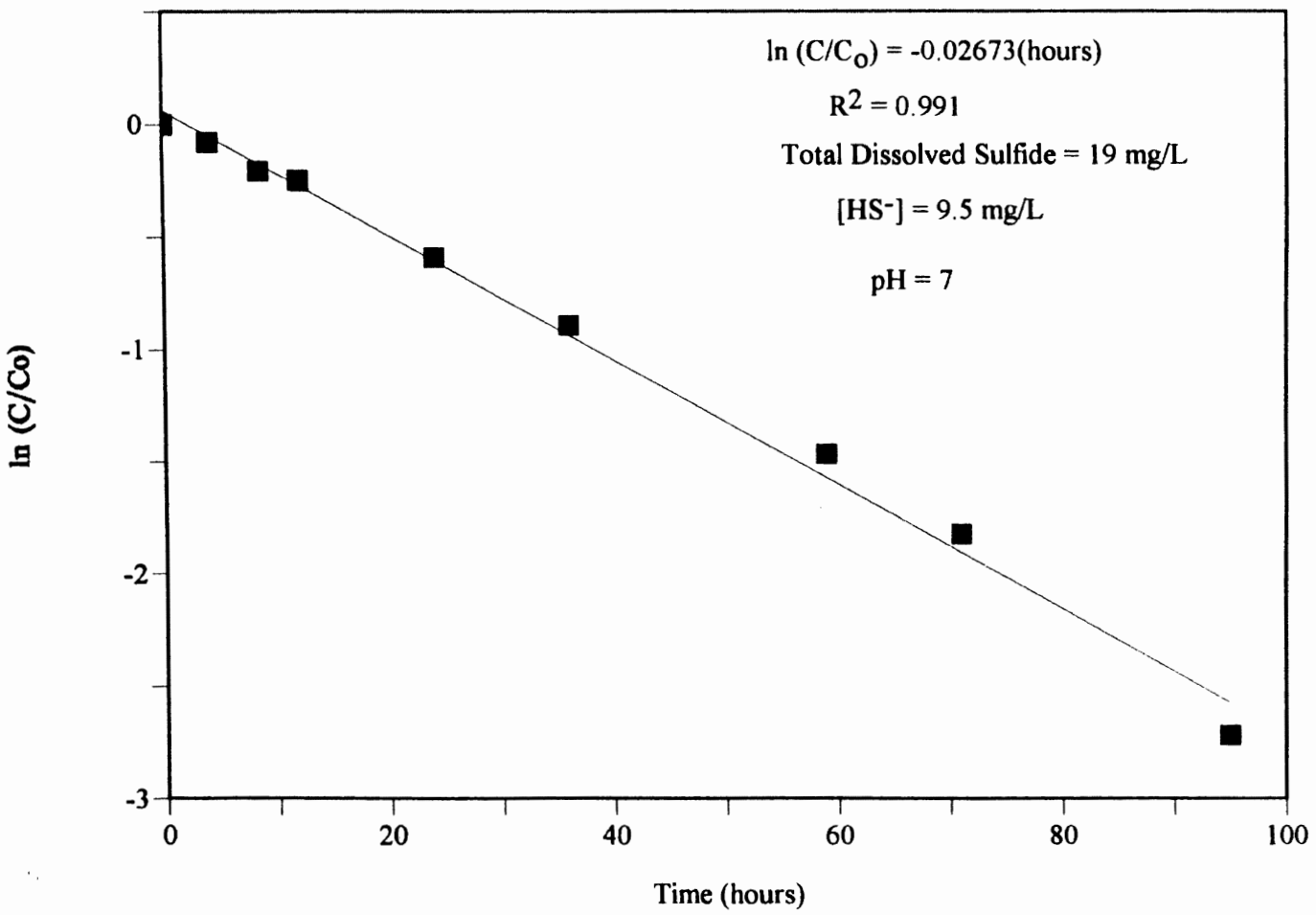


Figure 11. Ln(C/Co) values, Abiotic Sulfide Reaction with Propachlor

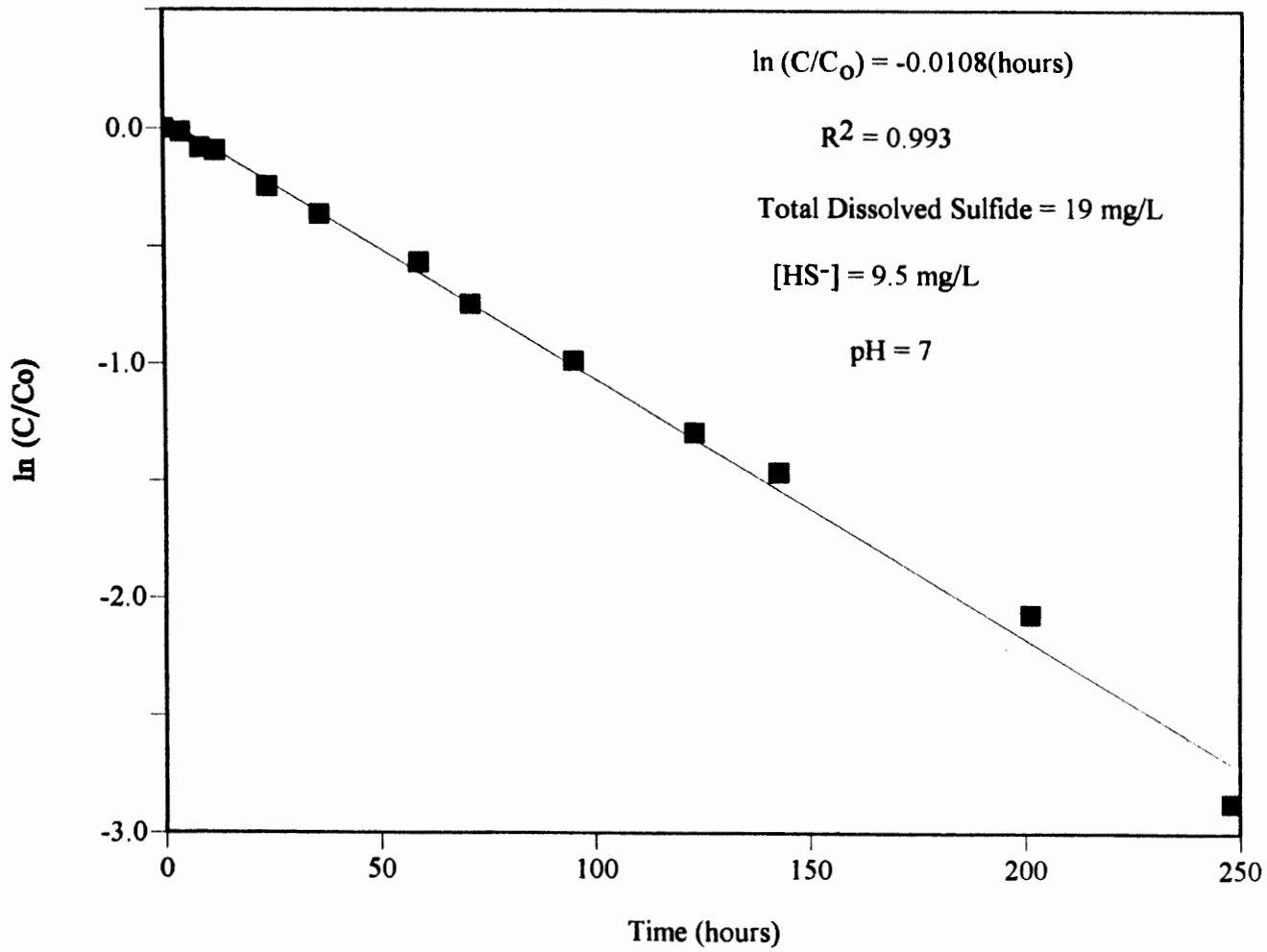


Figure 12. Ln(C/Co) values, Abiotic Sulfide Reaction with Alachlor.

sorption and its impact on the availability of the pesticides for biotransformation, Soil Column 4 and its contents were autoclaved to serve as a sterile control.

Soil Columns 3 and 4 were operated under the initial experimental conditions (see Table 1) for 44 days. Day 0 on all graphs represents the day acetate and the pesticides were added to the influent. The target acetate influent concentration was 50 mg/L and the target concentration for each pesticide was 100 μg/L. Unlike the other biofilm columns, Soil Column 3 was not fed acetate for a period of time prior to the addition of the pesticides. The column fluids were exchanged with 85 mL of new feed solution approximately once every forty eight hours to provide additional time for developing the slower-growing methanogenic culture. Prior to exchange, the feed water was stripped of oxygen by bubbling vigorously with nitrogen gas. Dissolved oxygen influent concentrations were measured and found to be below detection limits.

Within three weeks, it became apparent that Soil Column 4, the sterilized control, was exhibiting signs of biological activity. A decision was made to autoclave the column and its contents again. It is not known how this second sterilization affected the pesticides initially sorbed to the soil. For this reason, it is not possible to quantify the sorption characteristics of the soil. Additionally, when this column was filled for the first time following the second sterilization, it was discovered that the pore volume was actually 50 mL, substantially less than the assumed volume of 85 mL. This discrepancy renders all data collected prior to Day 24 unreliable. Thus, only data collected after Day 24 will be included in the discussion of results. Data collected from Soil Column 4 after this point can still be used as a basis of comparison between sorption and biotransformation, but it is not suitable for a rigorous quantitative analysis.

From Figure 13, which plots C/Co data for all four pesticides in Soil Column 4 (the sterile control), it is clear that propachlor and alachlor were sorbed more strongly than either atrazine or bromacil. This result is not entirely unexpected, as the nitrogen substituted heterocyclic structures of bromacil and atrazine make them less likely than

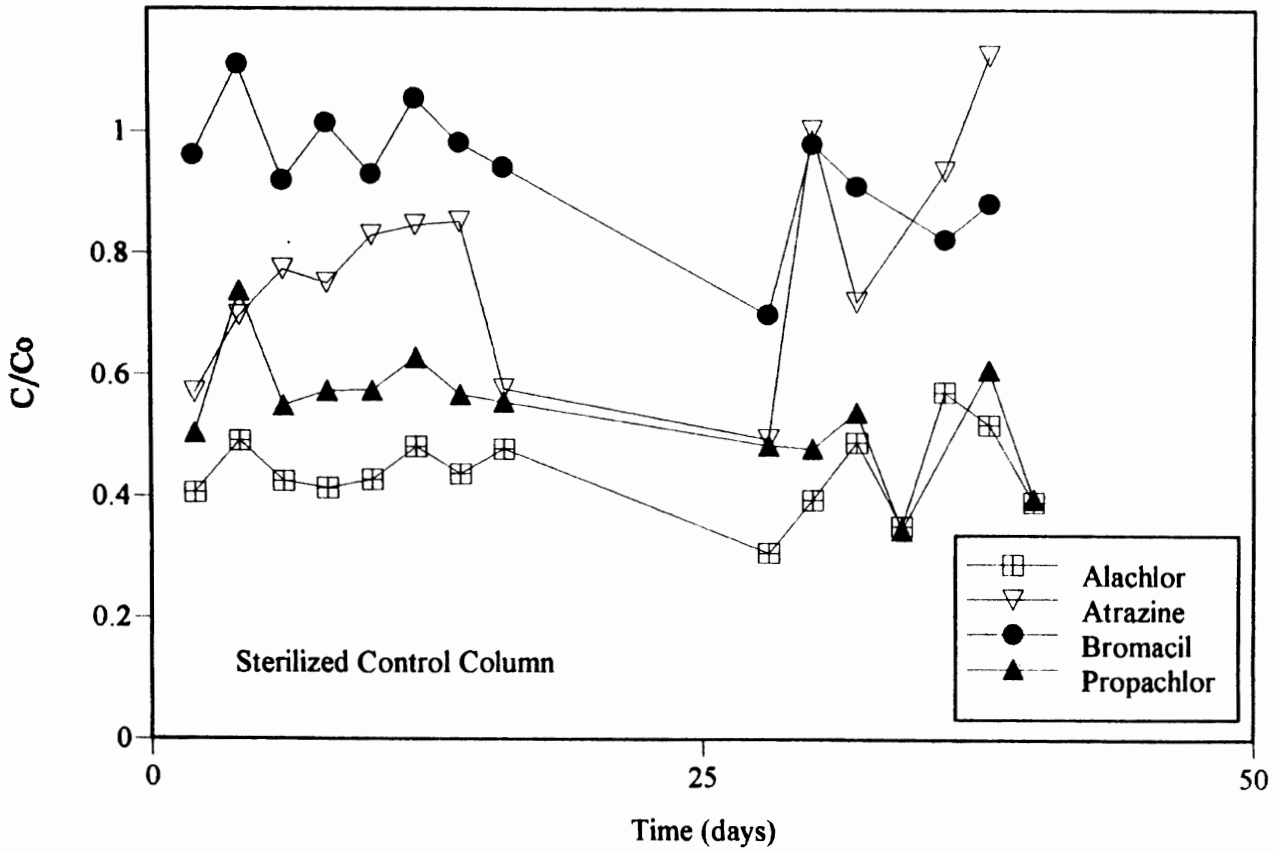


Figure 13. Pesticide C/Co Data, Soil Column 4

propachlor and alachlor to adsorb to carbonaceous material. However, the contradicting results of Bowman (1990) and Alhajjar *et al.* (1990) indicate that sorptive properties are difficult to predict based on structures alone. It appears that breakthrough was achieved for atrazine, and that breakthrough was very close for bromacil.

Sorption was, of course, also a removal mechanism in the "live" column, Soil Column 3. C/Co data for all four pesticides in this column are plotted in Figure 14. Results in this figure also lead to the conclusion that propachlor and alachlor are more strongly sorbed than either bromacil or atrazine. Biotransformation patterns of each pesticide can be interpreted by comparing the differences between the two columns. During this period, acetate was fed to Soil Column 3 as the primary substrate. Influent acetate values to the column averaged 52 mg/L (s.d. 21%, n=9). Actual acetate influent and effluent concentration data appear in Appendix L. C/Co values of acetate (fraction remaining) averaged 0.36. Consumption of acetate indicated the presence of a live bacterial population in Soil Column 3.

The data from these columns yield no evidence of transformation of bromacil. Actual bromacil influent and effluent concentrations appear in Appendices M and N for Soil Columns 3 and 4, respectively. Influent bromacil concentrations averaged 91.1 µg/L (s.d. 9%, n=8). C/Co data for bromacil are plotted in Figure 15. From this figure, which allows a direct comparison of C/Co values for both columns, it appears that no significant microbial removal of bromacil occurred. This result contrasts sharply with the findings of Adrian and Suflita (1990), who observed significant biotransformation of bromacil under methanogenic conditions using soil samples collected from the same site. One possible explanation for such a difference in findings is the primary substrate used to support microbial growth. In the referenced study, glucose was fed, whereas acetate was used in this study. These different substrates may be expected to support different populations, with different secondary utilization properties.

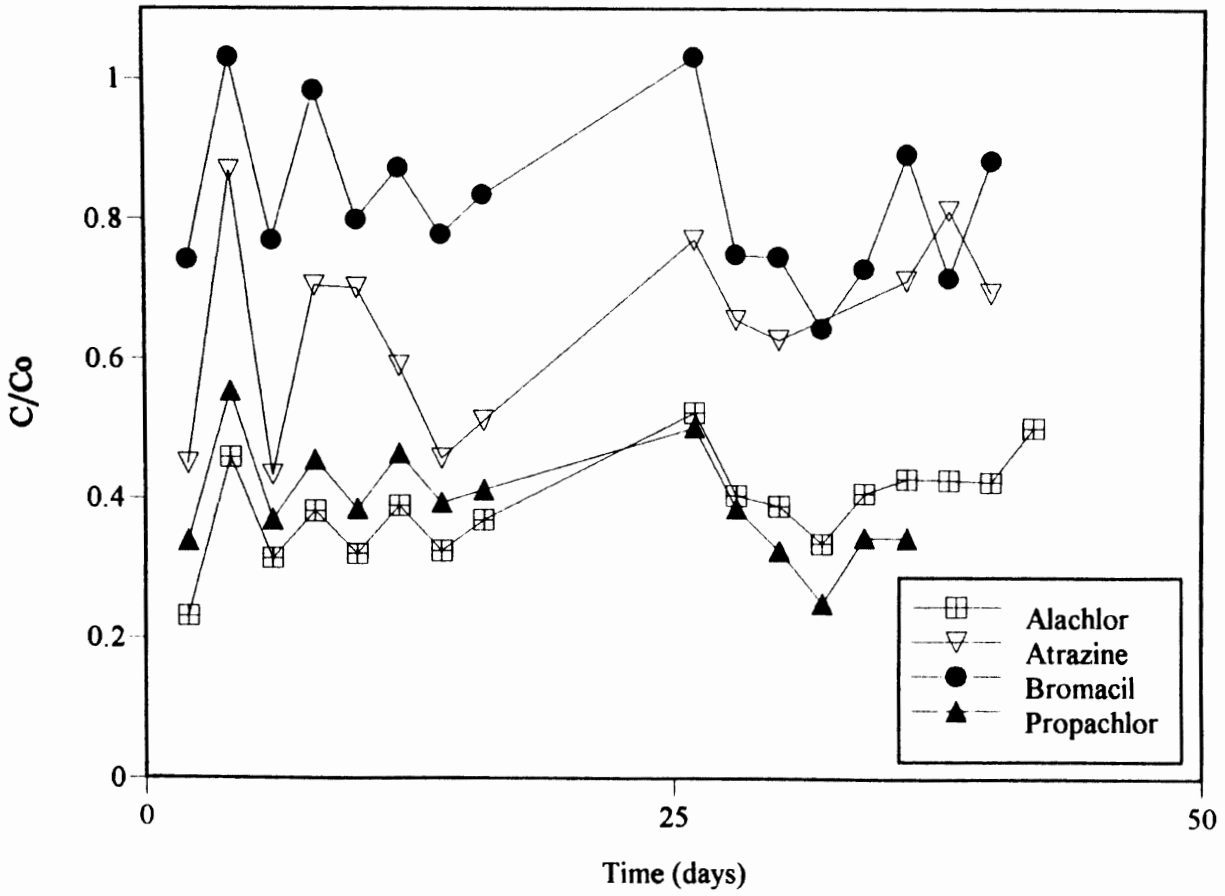


Figure 14. Pesticide C/Co Data, Soil Column 3

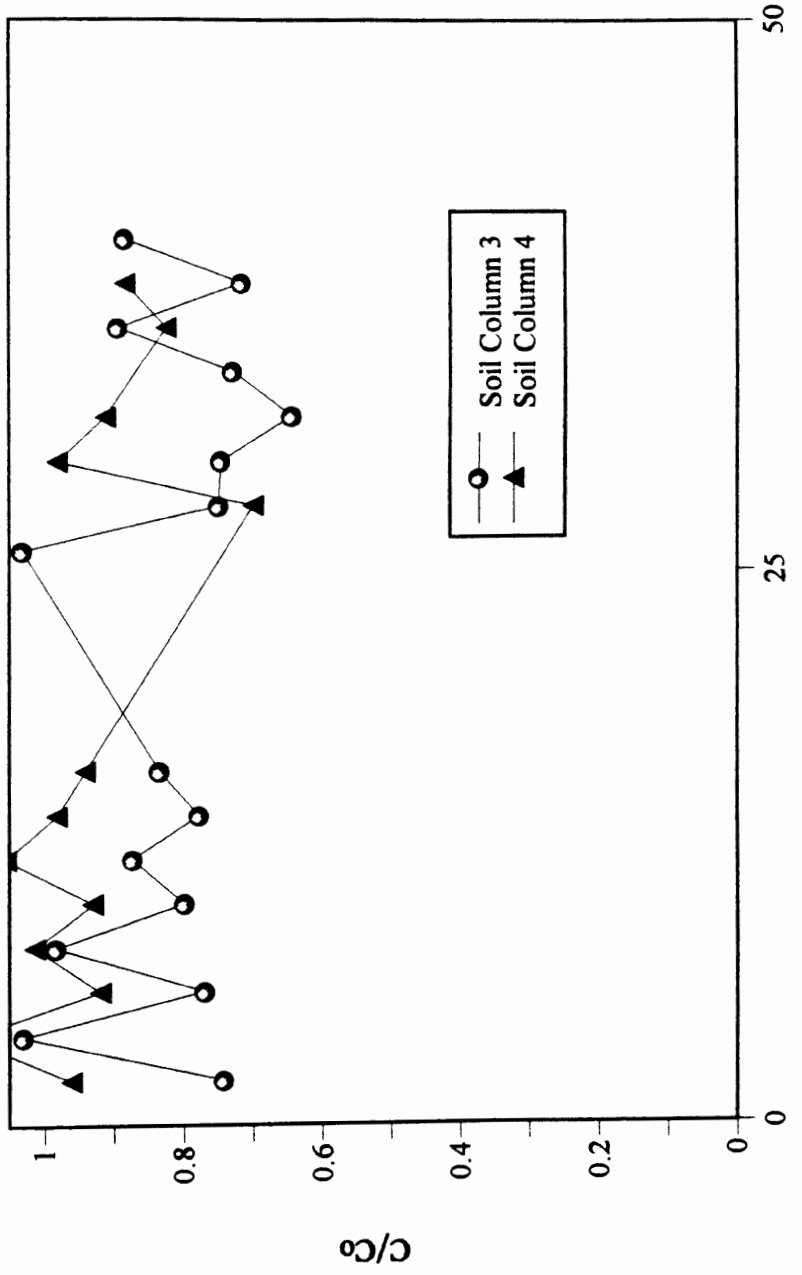


Figure 15. C/Co, Bromacil, Soil Columns 3 and 4

Influent, effluent, and C/Co data for atrazine in Soil Columns 3 and 4 appear in Appendices O and P, respectively. Similar data appear in Appendices Q and R for propachlor and in Appendices S and T for alachlor. Figures 16 through 18 provide a comparison of C/Co data between each column for atrazine, propachlor, and alachlor, respectively.

By comparing C/Co data between columns for each pesticide, it should be possible to evaluate the biotransformation of each pesticide. The relative difference between the two curves would represent the amount of pesticide removed biologically. From Figures 16 through 18, the difference between the C/Co data for the sterile column and the "live" column is not sufficient to comment on the portion of pesticides removed by biotransformation. In each case, other removal factors, which are not yet understood, appear to be involved.

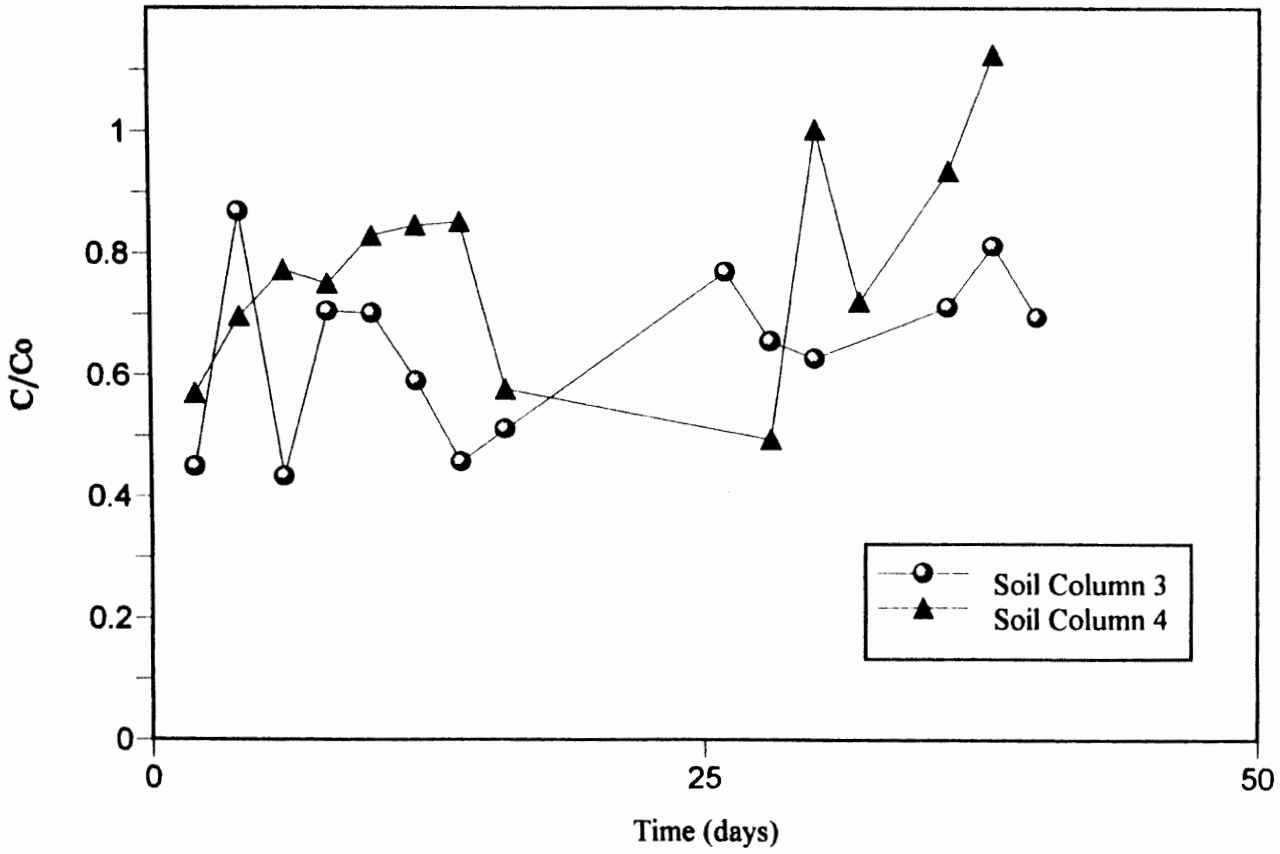


Figure 16. C/Co, Atrazine, Soil Columns 3 and 4

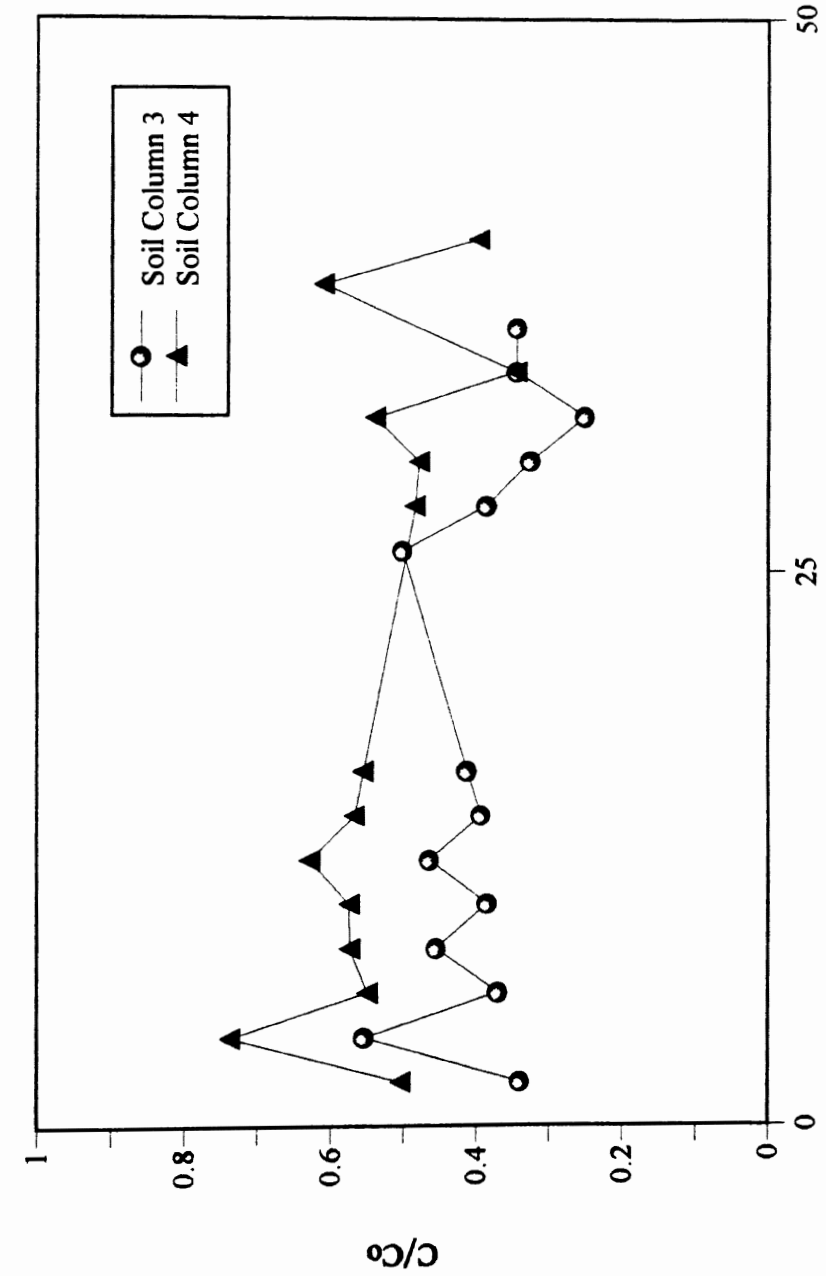


Figure 17. C/Co, Propachlor, Soil Columns 3 and 4

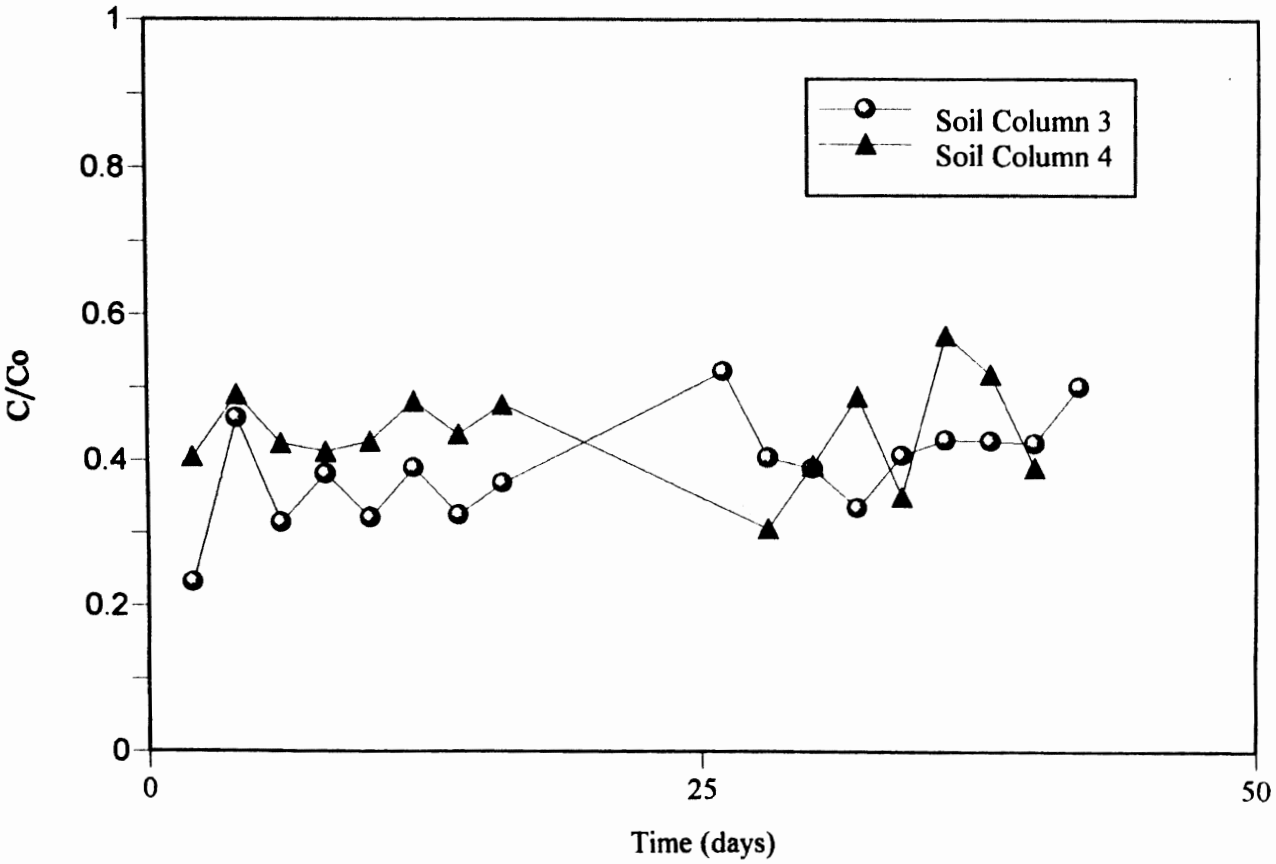


Figure 18. C/Co, Alachlor, Soil Columns 3 and 4

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This study has helped to identify the biotransformation patterns of alachlor, atrazine, propachlor, and bromacil under various electron acceptor conditions. Additionally, the effect of acetate as an added carbon source has been examined. Biofilm columns were operated under conditions of aerobic respiration and nitrate-reduction, and soil columns were operated under anaerobic methanogenic conditions.

The primary objectives of this research were the following:

1. To investigate the effect of electron acceptor conditions on the biotransformation of atrazine, alachlor, bromacil, and propachlor.
2. To quantitatively describe the effect of sorption on these systems and evaluate its impact on the availability of pesticides for biotransformation.
3. To investigate the effect of acetate as an added carbon source on such systems.
4. To determine the abiotic effect of sulfide on these pesticides.

The influence of electron acceptor conditions on the biotransformation potential of pesticides was the primary focus of this research. Therefore, no conclusions can be drawn about the extent of mineralization resulting from the disappearance of the parent compounds. These results did reveal several important results of varying electron acceptor availability on the biotransformation of pesticides.

- Bromacil was the least susceptible to biotransformation, regardless of electron acceptor condition. The absence or presence of acetate as an added carbon source had

no effect on the transformation of bromacil. Increased contact time between the biomass and bromacil did not increase its susceptibility to transformation.

- Atrazine was only slightly more susceptible than bromacil to biotransformation under aerobic conditions. The addition of nitrate as a terminal electron acceptor negatively affected atrazine transformation rates. Increased contact time between the biomass and atrazine did not significantly increase the transformation of atrazine under nitrate-reducing conditions.
- Propachlor was significantly transformed under aerobic conditions in the presence of acetate. Under aerobic conditions in the absence of acetate, propachlor transformation continued, but at a slower rate. Propachlor transformation also decreased after the addition of nitrate as a terminal electron acceptor, although increased contact time to the biomass lessened this negative effect.
- Alachlor was more susceptible than propachlor to transformation under aerobic conditions in the presence of acetate. Under aerobic conditions in the absence of acetate, alachlor transformation continued, but at a much slower rate than propachlor. Under oxygen-limited conditions, increased contact time between the biomass and alachlor did not increase its susceptibility to transformation.
- Abiotic, sulfide-reaction experiments with each of the pesticides were conducted. Atrazine and bromacil showed no reaction. Alachlor and propachlor disappeared over time, with propachlor disappearing at a much faster rate. A second-order overall rate constant of 0.0028/h-mg/L [HS-] was determined for propachlor. Similarly, a second-order overall rate constant of 0.0015/h-mg/L [HS-] was determined for alachlor.
- Soil column experiments were conducted to evaluate the sorptive properties of each pesticide and the biotransformation of each pesticide under methanogenic conditions and in the presence of acetate as an added carbon and energy source. Sorption appeared to be a significant removal mechanism for propachlor and alachlor, but not for bromacil and atrazine. Due to errors in column operation, it was not possible to

quantify the effect of sorption and its impact on the availability of pesticides for biotransformation. A comparison of the sterile column and the "live" column yielded no significant findings.

Limitations

A comprehensive (and thus, more rigorous) treatment of these objectives could not be obtained given the time and resources allotted to this study. For example, results from the abiotic sulfide experiments are not particularly useful when considered alone; their real value is seen when their results are integrated with biotransformation experiments in a sulfate-reducing column or other systems in which sulfide is present. Since no sulfate-reducing column was operated, results from the abiotic sulfide experiments can be used only as preliminary considerations for other researchers interested in further study of these reactions. Further, errors in column operation during the soil column experiments prevented quantitative analysis of sorption properties of the pesticides.

Recommendations

Further column studies are needed to address a variety of issues. The effect of sorption largely remains unanswered. Additional soil column experiments should be conducted, with rigorous tracer studies conducted initially to characterize flow through the columns. Additional glass-bead biofilm column studies are needed to further understand the effect of acetate as an added carbon source to the biotransformation of these pesticides (other primary substrates could be tested, as well). Column studies under sulfate-reducing conditions should be conducted to provide additional insight into the effect of electron acceptor conditions on the biotransformation of pesticides.

Practical Implications

This study provides additional information about the possibilities of stimulating native bacterial populations in order to enhance *in-situ* biorestoration of ground waters contaminated with bromacil, alachlor, atrazine, or propachlor. Based on the results of this study, *in-situ* biorestoration of ground waters contaminated with bromacil, and to a lesser extent atrazine, is likely to be infeasible, regardless of the electron acceptor condition present. On the other hand, ground waters contaminated with propachlor and alachlor are strong candidates if aerobic conditions are present (and can be maintained) and acetate can be added as a primary substrate. Laboratory-scale tests would be required to determine the potential for transformation in a given ground water and to determine the effect of sorption. Wide applicability of techniques such as an added carbon source remains to be tested.

REFERENCES

Adrian, N.R.; Suflita, J.M. "Reductive Dehalogenation of a Nitrogen Heterocyclic Herbicide in Anoxic Aquifer Slurries." *Applied and Environmental Microbiology* **1990**, 56(1), 292-294.

Alhajjar, B.J. et al. "Fate and Transport of Alachlor, Metolachlor, and Atrazine in Large Columns." *Water Science and Technology* **1990**, 22(6), 87-94.

Barbash, J.E.; Reinhard, M. "Abiotic Dehalogenation of 1,2-Dichloroethane and 1,2-Dibromoethane in Aqueous Solution Containing Hydrogen Sulfide." *Environmental Science and Technology* **1989**, 23(11), 1349-1357.

Beeman, R.E.; Suflita, J.M. "Microbial Ecology of a Shallow Unconfined Ground Water Aquifer Polluted by Municipal Landfill Leachate." *Microbial Ecology* **1987**, 13, 39-54.

Behki, R.M.; Khan, S.U. "Degradation of Atrazine by *Pseudomonas*: N-dealkylation and Dehalogenation of Atrazine and Its Metabolites." *Journal of Agricultural and Food Chemistry* **1986**, 29, 1132.

Berry, D.F.; Francis, A.J.; Bollag, J.M. "Microbial Metabolism of Homocyclic and Heterocyclic Aromatic Compounds under Anaerobic Conditions." *Microbiological Reviews* **1987**, 51(1), 43-59.

Bowman, B.T. "Mobility and Persistence of Alachlor, Atrazine, and Metolachlor in Plainfield Sand using Field Lysimeters." *Environmental Toxicology and Chemistry* **1990**, 9, 453-461.

Bouwer, E.J.; McCarty, P.L. "Utilization Rates of Trace Halogenated Organic Compounds in Acetate-Grown Biofilms." *Biotechnology and Bioengineering* **1985**, 27, 1564-1571.

Bovey, R.W.; Meyer, R.E.; David, F.S.; Merkle, M.G.; Morton, H.L. "Control of Woody and Herbaceous Vegetation with Soil Sterilants." *Weeds* **1967**, 15, 327-330.

Chaudhry, R.G.; Cortez, L. "Degradation of Bromacil by a *Pseudomonas* sp.". *Applied and Environmental Microbiology* **1988**, 54(9), 2203-2207.

Cobb, G.D.; Bouwer, E.J. "Effects of Electron Acceptors on Halogenated Organic Compound Biotransformations in a Biofilm Column." *Environmental Science & Technology* **1991**, 25, 1068-1074.

Cohen, S.Z.; Eiden, C.; and Lorber, M.N. "Monitoring Groundwater for Pesticides." In *Evaluation of pesticides in groundwater*; W.Y. Gamer *et al.*, Eds.; ACS Symposium Series 315; American Chemical Society: Washington, DC; 1986; pp 170-196.

CPP (Crop Protection Chemicals Reference). Chemical and Pharmaceutical Press, 1991.

Erickson, L.E.; Lee, K.H. "Degradation of Atrazine and Related S-Triazines." *Critical Reviews in Environmental Control* **1989**, 19(1), 1-14.

Hallberg, G.R. "Agricultural Chemicals in Ground Water. Extent and Implications." *American Journal of Alternative Agriculture* **1987**, 2(1), 3-15.

Hallberg, G.R. "Agricultural Chemicals and Ground Water in Iowa: Status Report 1985." Cooperative Extension Service, Iowa State University, Ames, Iowa **1985**, Circular CE-2158q.

Hallberg, G.R.; Libra, R.D.; Long, K.R.; Splinter, R.C. "Pesticides, Groundwater, and Rural Drinking Water Quality in Iowa." In *Pesticides and Groundwater: A Health Concern for the Midwest*; The Freshwater Foundation: Navarre, MN, 1987; 83-104.

Hebb, E.A.; Wheeler, W.B. "Bromacil in Lakeland Soil Ground Water." *Journal of Environmental Quality* **1978**, 7, 598-601.

Holden, P. "Pesticides and Ground Water Quality: Issues and Problems in Four States." National Academic Press **1986**.

Junk, G.; Spalding, R.; Richard, J. "Areal, Vertical, and Temporal Differences in Ground Water Chemistry: II. Organic Constituents." *Journal of Environmental Quality* **1980**, 9(3), 479-482.

Kuhn, E.P.; Suflita, J.M. "Dehalogenation of Pesticides by Anaerobic Microorganisms in Soil and Groundwater – A Review." In *Reactions and Movement of Organic Chemicals in Soil*, Soil Science of America and American Society of Agronomy, SSSA Special Publication No. 22, 1989; pp 111-180.

Lanzarone, N.A.; McCarty, P.L. "Column Studies on Methanotrophic Degradation of Trichloroethene and 1,2-Dichloroethane." *Ground Water* **1990**, 28(6), 910-919.

Lynch, N.L. 1990. "Transformation of Pesticides and Halogenated Hydrocarbons in the Subsurface Environment." Ph.D Dissertation, Department of Civil and Environmental Engineering, The University of Iowa, May 1990.

Nair, D.R.; Schnoor; J.L. "Effect of Two Electron Acceptors on Atrazine Mineralization Rates in Soil." *Environmental Science and Technology* **1992**, 26(11), 2298-2300.

NAS (National Academy of Sciences). *Drinking Water and Health*; NAS: Washington, DC, 1977.

Novick, N.J.; Alexander, M. "Cometabolism of Low Concentrations of Propachlor, Alachlor, and Cycloate in Sewage and Lake Water." *Applied and Environmental Microbiology* **1985**, 49(4), 737-743.

Pease, H.L. "Determination of Bromacil Residues." *Journal of Agricultural Food Chemicals* **1966**, 14, 94-96.

Peter, C.J.; Weber, J.B. "Adsorption, Mobility, and Efficacy of Alachlor and Metolachlor as Influenced by Soil Properties." *Weed Science* **1985**, 33, 874-881.

Pontius, F.W. "A Current Look at the Federal Drinking Water Regulations." *American Water Works Ass'n. Journal* **1992**, 3, 36-50.

Pothuluri, J.V.; Moorman, T.B.; Obenhuber, D.C.; Wauchope, R.D. "Aerobic and Anaerobic Degradation of Alachlor in Samples from a Surface-to-Groundwater Profile." *Journal of Environmental Quality* **1990**, 19, 525-530.

Rhodes, R.C.; Belasco, I.J.; Pease, H.L. "Determination of Mobility and Adsorption of Agrochemicals on soils." *Journal of Agricultural Food Chemicals* **1970**, 18, 524-528.

Sabatini, D.A.; Austin, T. "Sorption and Transport of Pesticides in Ground Water." *Journal of Irrig.Drain.* **1990**, 116(1), 3-15.

Siegrist, H.; McCarty, P.L. "Column Methodologies for Determining Sorption and Biotranformation Potential for Chlorinated Aliphatic Compounds in Aquifers." *Journal of Contaminant Hydrology* **1987**, 2, 31-50.

Standard Methods for the Examination of Water and Wastewater, 17th edition, American Public Health Association, Washington DC 1989.

Steen, W.C.; Collette, T.W. "Microbial Degradation of Seven Amides by Suspended Bacterial Populations." *Applied and Environmental Microbiology* **1989**, 55(10), 2545-2549.

Stumm, W.; and Morgan, J.J. *Aquatic Chemistry*, Second Edition; Wiley:New York, 1981.

Thurman, E.M.; Meyer, M.; Pomes, M.; Perry, C.A.; Schwab, A.P. "Enzyme-Linked Immunosorbent Assay Compared with Gas Chromatography/Mass Spectrometry for the Determination of Triazine Herbicides in Water." *Analytical Chemistry* **1990**, 62, 2043-2048.

US. Environmental Protection Agency. "Pesticides in Ground Water Data Base 1988 Interim Report." Office of Pesticide Programs, EPA, Washington DC **1988**.

Wilber, G.G; Parkin, G.F. "Transformation of Pesticides in Groundwater under Anaerobic Conditions." *On-Site Bioreclamation: Processes for Xenobiotic and Hydrocarbon Treatment*. R.E. Hinchee and R.F. Olfenbuttel, editors, Butterworth-Heinemann, Stoneham, MA, 1991, 385-402.

APPENDIXES

APPENDIX A

RAW DATA, ACETATE INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 1

Time (days)	Concentrations (mg/L) Influent	Effluent	C/Co
1	23.1	10.2	0.44
2	24.9	10.0	0.40
3	30.3	10.1	0.33
5	13.7	4.1	0.30
8	17.3	5.1	0.30
10	15.9	0.0	0.00
11	21.1	0.0	0.00
12	16.2	4.7	0.29
14	22.4	0.0	0.00
16	19.7	0.0	0.00
21	24.2	0.0	0.00
23	22.4	0.0	0.00
27	16.2	0.0	0.00
29	14.6	0.0	0.00
31	15.9	0.0	0.00
35	26.5	0.0	0.00
38	15.1	0.0	0.00
44	20.7	0.0	0.00
49	18.2	0.0	0.00
51	20.8	0.0	0.00
52	21.1	0.0	0.00
54	19.0	0.0	0.00
56	18.1	0.0	0.00
60	16.4	0.0	0.00
64	18.8	0.0	0.00

APPENDIX B

RAW DATA, ATRAZINE INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 1

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
1	261.4	213.3	0.82
2	262.2	209.4	0.80
3	228.9	211.7	0.92
5	249.0	214.0	0.86
6	238.6	216.3	0.91
8	306.6	268.2	0.87
10	313.4	264.9	0.85
14	295.2	272.8	0.92
16	303.7	245.1	0.81
21	271.2	259.2	0.96
23	274.8	239.0	0.87
25	274.3	261.6	0.95
27	261.6	250.9	0.96
29	267.3	258.9	0.97
31	267.8	267.1	1.00
35	266.4	270.0	1.01
38	269.4	265.3	0.98
44	269.4	244.4	0.91
49	284.2	229.6	0.81
51	274.4	253.6	0.92
52	261.1	241.4	0.92
54	271.5	259.4	0.96
56	288.3	280.9	0.97
58	292.4	277.8	0.95
60	291.8	268.8	0.92
62	317.0	281.2	0.89
64	282.7	242.7	0.86

APPENDIX C

RAW DATA, PROPACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 1

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
1	265.6	150.8	0.57
2	263.0	173.3	0.66
3	236.2	166.6	0.71
5	245.4	161.7	0.66
6	238.1	163.8	0.69
8	296.2	204.2	0.69
10	305.1	199.2	0.65
14	286.0	208.7	0.73
16	287.0	184.0	0.64
21	261.2	200.5	0.77
23	262.6	187.2	0.71
25	251.5	206.0	0.82
27	243.6	208.1	0.85
29	251.9	181.7	0.72
31	246.8	210.7	0.85
35	246.3	217.6	0.88
38	249.6	212.9	0.85
44	259.3	191.6	0.74
49	276.0	157.5	0.57
51	268.1	196.5	0.73
52	253.1	205.5	0.81
54	275.3	210.3	0.76
56	264.1	223.8	0.85
58	276.1	225.8	0.82
60	278.7	218.3	0.78
62	295.8	223.4	0.76
64	277.1	215.9	0.78

APPENDIX D

RAW DATA, ACETATE INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 2

Time (days)	Concentrations (mg/L) Influent	Effluent	C/Co
8	18.4	3.1	0.17
16	25.2	5.9	0.23
17	37.3	14.6	0.39
18	25.3	9.6	0.38
21	23.9	6.1	0.25
28	23.5	8.9	0.38
30	35.9	13.5	0.38
37	26.1	8.4	0.32
39	21.0	8.6	0.41
40	28.4	11.3	0.40
43	25.2	9.9	0.39
44	24.6	9.0	0.36
46	15.6	9.5	0.61
48	22.9	7.4	0.32
52	20.5	7.1	0.35
53	20.6	4.3	0.21
54	16.0	7.1	0.44
94	21.9	6.9	0.32
95	18.7	0.0	0.00
98	21.7	0.0	0.00
100	17.9	0.0	0.00
104	16.5	0.0	0.00
105	22.4	0.0	0.00
107	23.1	0.0	0.00

APPENDIX E

RAW DATA, BROMACIL INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 2

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
3	98.7	99.6	1.01
4	92.6	63.1	0.68
8	116.0	99.7	0.86
9	99.8	102.6	1.03
13	98.3	98.8	1.01
14	106.9	96.2	0.90
16	117.9	115.8	0.98
17	130.7	106.8	0.82
18	135.5	117.8	0.87
22	115.4	113.9	0.99
28	129.7	121.4	0.94
36	120.0	111.1	0.93
37	111.3	99.4	0.89
38	113.8	113.5	1.00
39	117.1	114.7	0.98
40	101.3	84.8	0.84
43	97.6	90.8	0.93
46	76.1	81.3	1.07
48	81.0	83.5	1.03
49	103.8	93.2	0.90
52	97.8	92.2	0.94
55	91.0	101.0	1.11
56	88.3	89.1	1.01
57	80.2	86.9	1.08
58	88.3	102.3	1.16
59	97.8	94.6	0.97
64	85.7	95.1	1.11
65	89.3	96.6	1.08
67	83.7	95.7	1.14
69	89.5	93.0	1.04
71	84.6	88.6	1.05
73	92.4	87.0	0.94
77	84.4	99.7	1.18
80	83.0	73.9	0.89
86	89.9	76.1	0.85
91	80.5	79.5	0.99
93	77.8	82.3	1.06
94	85.7	84.8	0.99
96	89.6	89.2	0.99
98	95.4	91.3	0.96
102	89.2	92.5	1.04
104	92.1	91.5	0.99
106	86.2	88.1	1.02
108	89.3	93.7	1.05

APPENDIX F

RAW DATA, ALACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 2

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
16	112.7	37.6	0.33
17	117.7	36.5	0.31
18	130.6	43.5	0.33
22	104.3	41.4	0.40
28	121.5	42.4	0.35
30	111.9	51.4	0.46
36	114.4	47.3	0.41
37	113.5	60.3	0.53
38	114.8	62.7	0.55
39	121.1	65.1	0.54
40	110.8	53.2	0.48
43	119.0	56.5	0.48
46	98.8	60.0	0.61
48	101.9	51.2	0.50
49	112.9	60.9	0.54
52	110.5	65.7	0.59
55	106.7	66.2	0.62
56	105.1	62.1	0.59
57	100.2	58.3	0.58
58	115.0	71.6	0.62
59	118.4	71.7	0.61
63	114.4	80.1	0.70
64	109.2	77.5	0.71
65	112.2	73.0	0.65
67	105.1	72.2	0.69
69	110.5	73.4	0.66
71	109.9	71.5	0.65
73	113.1	71.6	0.63
77	106.6	76.7	0.72
80	111.4	64.2	0.58
86	113.3	69.8	0.62
91	105.5	69.5	0.66
93	97.3	72.5	0.75
94	111.7	77.0	0.69
96	107.7	76.1	0.71
98	117.5	79.8	0.68
102	103.9	78.2	0.75
104	108.2	77.8	0.72
106	107.3	83.4	0.78
108	109.0	75.5	0.69

APPENDIX G

RAW DATA, PROPACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, BIOFILM COLUMN 2

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
16	121.0	53.2	0.44
17	120.6	62.9	0.52
18	144.7	72.5	0.50
22	107.1	64.3	0.60
28	128.3	66.5	0.52
30	119.5	89.2	0.75
36	115.4	72.6	0.63
37	111.6	69.2	0.62
38	114.7	71.5	0.62
39	121.6	76.0	0.63
40	116.1	63.8	0.55
43	117.5	72.0	0.61
46	99.9	75.7	0.76
48	92.4	62.1	0.67
49	118.1	77.5	0.66
52	115.3	81.6	0.71
55	113.5	85.8	0.76
56	113.8	81.0	0.71
57	104.8	74.4	0.71
58	119.7	82.1	0.69
59	126.9	82.3	0.65
63	115.8	95.6	0.83
64	112.9	91.7	0.81
65	118.5	90.5	0.76
67	112.0	88.6	0.79
69	115.6	92.6	0.80
71	121.1	89.8	0.74
73	120.8	91.9	0.76
77	116.8	90.9	0.78
80	116.1	75.1	0.65
86	119.8	83.5	0.70
91	111.2	79.1	0.71
93	104.6	86.2	0.82
94	117.8	95.3	0.81
96	115.9	84.7	0.73
98	120.9	94.9	0.78
102	112.6	96.5	0.86
104	117.2	93.2	0.80
106	115.4	95.0	0.82
108	120.7	93.4	0.77

APPENDIX H

RAW DATA, ABIOTIC SULFIDE REACTION WITH BROMACIL

Time (hours)	Concentration (micrograms/L)	C/Co
0	201.0	1.00
48	215.9	1.07
72	232.3	1.16
96	213.6	1.06
120	212.8	1.06
144	214.0	1.06
168	207.4	1.03
216	220.1	1.09

APPENDIX I

RAW DATA, ABIOTIC SULFIDE REACTION WITH ALACHLOR

Time (hours)	Concentration (micrograms/L)	C/Co	Ln(C/Co)
0	278.5	1.000	0.000
4	274.4	0.985	-0.015
8.5	256.7	0.921	-0.082
12	253.7	0.911	-0.093
24	218.1	0.783	-0.245
36	193.8	0.696	-0.363
59	158.2	0.568	-0.566
71	132.5	0.476	-0.743
95	104.0	0.373	-0.985
123	76.5	0.275	-1.292
142.5	64.6	0.232	-1.461
201	35.2	0.126	-2.068
248	15.8	0.057	-2.871

APPENDIX J

RAW DATA, ABIOTIC SULFIDE REACTION WITH PROPACHLOR

Time (hours)	Concentration (micrograms/L)	C/Co	Ln (C/Co)
0	269.2	1.000	0.000
4	249.4	0.926	-0.077
8.5	219.4	0.815	-0.205
12	210.3	0.781	-0.247
24	149.3	0.555	-0.589
36	110.5	0.411	-0.890
59	62.0	0.230	-1.468
71	43.5	0.162	-1.823
95	17.8	0.066	-2.717

APPENDIX K

RAW DATA, ABIOTIC SULFIDE REACTION WITH ATRAZINE

Time (hours)	Concentration (micrograms/L)	C/Co
0	291.2	1.00
4	306.5	1.05
8.5	287.0	0.99
12	298.2	1.02
24	297.6	1.02
36	290.9	1.00
59	314.7	1.08
71	311.1	1.07
95	292.9	1.01
123	313.1	1.08
142.5	327.4	1.12
201	349.7	1.20
248	332.0	1.14
291	341.3	1.17

APPENDIX L

RAW DATA, ACETATE INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 3

Time (days)	Concentration (mg/L) Influent	Effluent	C/Co
2	59.0	0.0	0.00
4	59.9	7.6	0.13
6	59.5	14.1	0.24
8	56.9	36.1	0.63
12	40.6	23.2	0.57
14	56.8	26.2	0.46
16	56.5	25.7	0.46
18	34.4	30.8	0.90
22	55.3	18.4	0.33
26	30.0	4.1	0.14
30	40.1	0.0	0.00
32	66.2	14.7	0.22
34	48.7	9.4	0.19
36	58.8	19.7	0.33
38	58.8	25.0	0.43
40	57.0	39.4	0.69
42	60.4	37.1	0.61
44	47.9	31.6	0.66

APPENDIX M

RAW DATA, BROMACIL INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 3

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
2	84.9	63.0	0.74
4	69.1	71.2	1.03
6	89.5	68.9	0.77
8	72.2	71.0	0.98
10	90.2	72.1	0.80
12	82.3	71.9	0.87
14	88.4	68.8	0.78
16	85.7	71.6	0.84
26	82.9	85.5	1.03
28	95.7	71.8	0.75
30	80.7	60.3	0.75
32	96.2	61.9	0.64
34	92.4	67.4	0.73
36	85.6	76.5	0.89
38	87.9	63.0	0.72
40	107.6	95.2	0.89

APPENDIX N

RAW DATA, BROMACIL INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 4

Time (days)	Concentrations (micrograms/L)		C/Co
	Influent	Effluent	
2	84.9	81.6	0.96
4	69.1	76.7	1.11
6	89.5	82.3	0.92
8	72.2	73.2	1.01
10	90.2	83.9	0.93
12	82.3	86.8	1.05
14	88.4	86.8	0.98
16	85.7	80.7	0.94
28	95.7	67.0	0.70
30	80.7	79.2	0.98
32	96.2	87.8	0.91
36	85.6	70.6	0.83
38	87.9	77.7	0.88

APPENDIX O

RAW DATA, ATRAZINE INFLUENT AND EFFLUENT CONCENTRATIONS. SOIL COLUMN 3

Time (days)	Concentrations (micrograms/L) Influent	Effluent	C/Co
2	136.5	61.3	0.45
4	90.2	78.4	0.87
6	124.1	53.7	0.43
8	102.2	72.1	0.70
10	125.2	87.8	0.70
12	120.5	71.1	0.59
14	119.4	54.5	0.46
16	126.7	64.7	0.51
26	112.3	86.6	0.77
28	117.6	77.1	0.66
30	110.0	69.0	0.63
36	99.8	71.1	0.71
38	96.0	78.1	0.81
40	121.8	84.7	0.70

APPENDIX P

RAW DATA, ATRAZINE INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 4

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
2	136.5	77.9	0.57
4	90.2	62.8	0.70
6	124.1	96.0	0.77
8	102.2	76.8	0.75
10	125.2	103.9	0.83
12	120.5	102.0	0.85
14	119.4	101.8	0.85
16	126.7	73.1	0.58
28	117.6	58.1	0.49
30	110.0	110.4	1.00
32	103.4	74.6	0.72
36	99.8	93.5	0.94
38	96.0	108.1	1.13

APPENDIX Q

RAW DATA, PROPACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 3

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
2	131.8	44.7	0.34
4	92.0	51.0	0.55
6	126.5	46.8	0.37
8	104.6	47.6	0.46
10	134.5	51.7	0.38
12	122.4	56.8	0.46
14	129.1	50.8	0.39
16	130.4	53.7	0.41
26	105.6	52.9	0.50
28	117.2	45.2	0.39
30	112.4	36.6	0.33
32	118.1	29.6	0.25
34	103.3	35.6	0.34
36	96.1	33.1	0.34

APPENDIX R

RAW DATA, PROPACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 4

Time (days)	Concentration (micrograms/L) Influent	Effluent	C/Co
2	131.8	66.4	0.50
4	92.0	67.9	0.74
6	126.5	69.5	0.55
8	104.6	60.0	0.57
10	134.5	77.2	0.57
12	122.4	77.0	0.63
14	129.1	73.2	0.57
16	130.4	72.3	0.55
28	117.2	56.7	0.48
30	112.4	53.8	0.48
32	118.1	63.7	0.54
34	103.3	35.7	0.35
38	93.4	57.0	0.61
40	86.9	34.5	0.40

APPENDIX S

RAW DATA, ALACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 3

Time (days)	Concentrations (micrograms/L) Influent	Effluent	C/Co
2	117.2	27.2	0.23
4	83.8	38.5	0.46
6	120.0	37.7	0.31
8	97.2	37.1	0.38
10	119.5	38.3	0.32
12	111.3	43.3	0.39
14	114.9	37.3	0.32
16	114.0	42.0	0.37
26	101.1	52.8	0.52
28	110.7	44.7	0.40
30	110.3	43.0	0.39
32	102.6	34.4	0.34
34	105.7	43.1	0.41
36	95.1	40.8	0.43
38	97.2	41.6	0.43
40	116.3	49.4	0.42
42	120.9	60.8	0.50

APPENDIX T

RAW DATA, ALACHLOR INFLUENT AND EFFLUENT CONCENTRATIONS, SOIL COLUMN 4

Time (days)	Concentrations (micrograms/L) Influent	Effluent	C/Co
2	117.2	47.5	0.41
4	83.8	41.2	0.49
6	120.0	50.9	0.42
8	97.2	40.1	0.41
10	119.5	51.0	0.43
12	111.3	53.6	0.48
14	114.9	50.1	0.44
16	114.0	54.4	0.48
28	110.7	34.0	0.31
30	110.3	43.5	0.39
32	102.6	50.2	0.49
34	105.7	37.1	0.35
36	95.1	54.5	0.57
38	97.2	50.5	0.52
40	116.3	45.5	0.39

VITA

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