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# Biodegradation, sorption and transport of 2,4-D under saturated and unsaturated soil conditions

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# BIODEGRADATION, SORPTION AND TRANSPORT OF 2,4-D UNDER SATURATED AND UNSATURATED SOIL CONDITIONS

by

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**ABSTRACT**

Researchers have traditionally viewed sorption, degradation and transport as separate processes and only recently have these processes viewed as coupled. 2,4-D was chosen as a model system to study the interaction between these processes. A series of laboratory batch and column experiments with a sandy loam soil were conducted to determine the relative contributions of sorption and degradation to transport of 2,4-D under both saturated and unsaturated conditions. The sorption contribution to 2,4-D transport was not significant under saturated (Kd= 0.249 mg/g) nor unsaturated conditions (Kd= 0.566 mg/g). Degradation however, was very significant, specially under unsaturated conditions where the estimated first order biodegradation rate ($\mu$) constant was 4.39 d-1. Rate constants under the saturated transport experiment were restricted by oxygen limitations. There was an order of magnitude difference between $\mu$ of batch and column experiments which were attributed to differences in aeration and mixing conditions.

# INTRODUCTION

Biochemical and physical transformation of pesticides are usually simultaneous processes in soils and although 2,4-D (2,4-Dichlorophenoxyacetic acid) has been extensively studied, sorption, degradation, and transport are rarely investigated concurrently. Researchers have traditionally viewed these as separate processes and only recently have these processes begun to be viewed as coupled.

Ultimately, the fate and persistence of any pesticide is determined by the end result of the interactions of the retention, transformation and transport processes. These interactions are controlled by the properties of both the organic chemical and the porous media and by the environmental conditions (e.g. temperature, moisture) (Yaron, B., 1989).

2,4-D was chosen as a model system to study these interactions. 2,4-D is an aromatic chlorinated molecule which is structurally similar to numerous other compounds of current interest such as the halogenated aromatics, PCB's, dioxins or other phenolic compounds.

The main objective of this work was to conduct a series of laboratory batch and column experiments with a sandy loam soil to determine the relative contributions of sorption and degradation to transport of 2,4-D under both saturated and unsaturated conditions.

# LITERATURE REVIEW

## 2,4-Dichlorophenoxyacetic Acid (2,4-D)

The chlorine-substituted phenoxyacetic acid, 2,4-D, was introduced as selective weedkiller at the end of World War II (Peterson, 1967). It has high activity against many broadleaved weeds but not against graminaceous species. Thus, it is used for the control of weeds in cereal crops, grass pastures and lawns (Fryer and Evans, 1968) as well as in many other situations. The effectiveness of 2,4-D is thought to derive from its ability to mimic plan growth auxins; its application on leaves, steams or root causes unnatural elongation and abnormal growth, which causes the plant to die (Loss, 1975).

2,4-D is commonly prepared by combining monochloroacetic acid with 2,4-dichlorophenol or by the chlorination of phenoxyacetic acid. The second method leads to more impurities among which are highly toxic dioxins (WHO, 1984).

Because of its low water solubility (Table 1), 2,4-D is rarely used in the form of the free acid. It is typically formulated as a water soluble amine salt or oil soluble ester. Subsequent to application the esters undergo hydrolysis and the amine salts dissolve to form the free acid again (Figure 1). Normal field application rates are from 0.2 to 2.4 kg/ha (Bovey, 1980). Some physical and chemical properties of 2,4-D are summarized in Table 1.

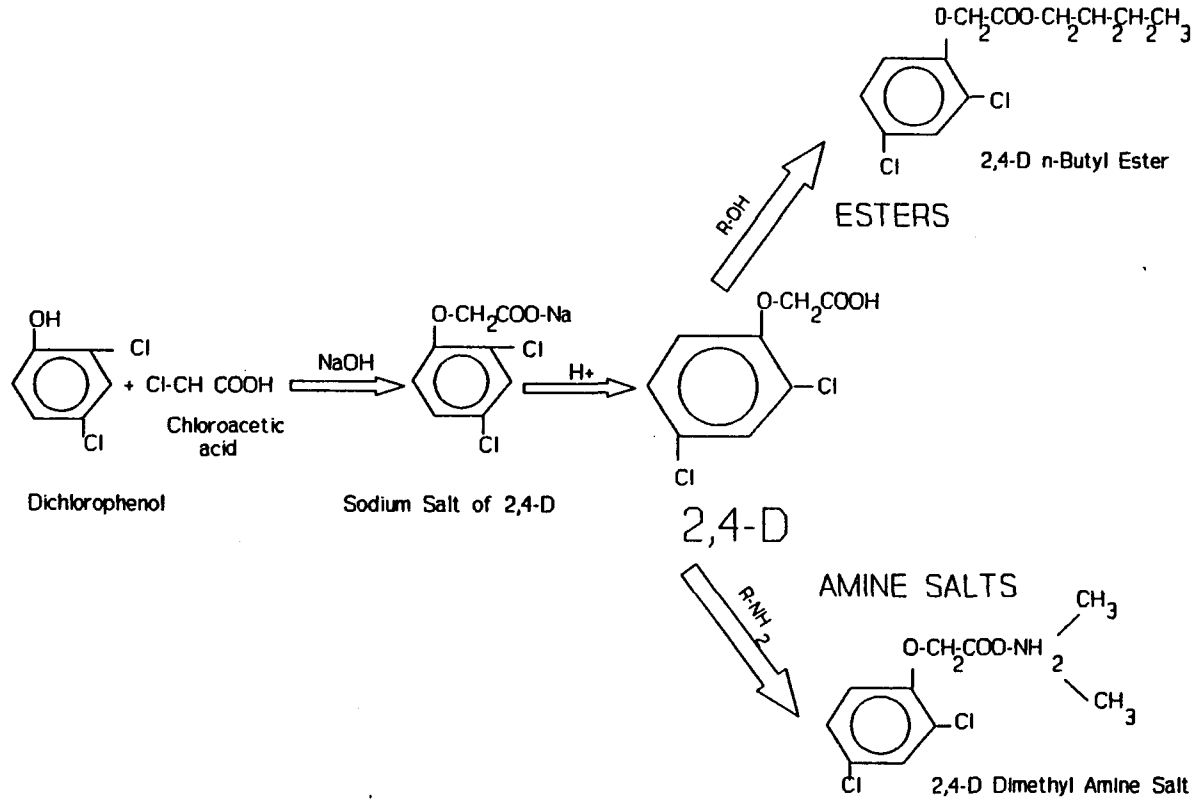


**Figure 1. Synthesis and forms of 2,4-D.**

**Table 1. Selected physical and chemical properties of 2,4-D**

| | |
|---|---|
| Molecular formula | $C_8H_6Cl_2O_3$ |
| Relative molecular mass | 221.0 |
| Specific gravity, C | 1.565 |
| Boiling point | 160 °C at 0.4 mm Hg |
| Melting point | 140-141 °C |
| Solubility in water | 620 mg/l |
| ethanol | 1300 g/l |
| acetone | 850 g/l |
| ether | 27 g/l |
| Diesel oil | 0.1 to 0.35 g/100 ml |
| Vapour pressure | 52.3 Pa at 160°C |
| pKa at 25 °C | 2.64 - 3.31 |

## Retention Processes

The term retention describes the phase distribution or adsorption-desorption of the pesticide in soils (Yaron,B., 1989). Adsorption refers to the bonding of a solute to adsorption sites on the soil solids, either soil mineral surfaces or organic surfaces (Jury et al., 1986). By definition then, adsorption removes a compound from the bulk solution. Conversely, the release of adsorbed solute into the soil aqueous phase is usually expressed as desorption.

The distribution of any solute into the different phases is known to be a function of the specific soil parameters (nature and properties of soil colloids), the partition coefficient of the chemical itself and the features of the soil environment (external factors).

### Soil Parameters Affecting Phase Distribution

Individual soil properties responsible for significant adsorption capacities are often difficult to assess since they are usually related to each other. Nevertheless, the specific soil parameters that are commonly known to dramatically affect the extent of sorption and desorption are clay and organic matter content in the soil.

Clay and organic matter form the mineral and organic colloidal fraction of the soil. This colloidal fraction has a large physico-chemically active surface area and contains

the sites for both retention and surface reactions (Saltzman and Yaron, 1986). The 1:1 mineral (kaolin group), because of their low cation exchange capacity and low surface area, have very limited adsorption capacities. The 2:1 minerals which can expand (montmorillonite and vermiculite) have very high cation exchange capacity and surface areas. These values are 10 to 100 times greater than those for the kalolinite. As a result the extent of adsorption is much greater and readily measured (Bailey and White, 1970). Many chemicals have a greater affinity for organic rather than for mineral (clay) adsorptive surfaces. Saltzman et al. (1972) studied adsorption-desorption of parathion, finding that parathion adsorbed preferentially to organic surfaces over mineral ones. A number of studies have shown that pesticide adsorption can be directly correlated to soil organic matter content (Huang et al., 1984; Cheng and Koskinen, 1986; Rao and Davidson, 1981) and adsorption constants have been statistically correlated with indicators of soil organic matter such as organic carbon content ($K_{oc}$) (Abdul et al., 1987).

The work by Saltzman et al. (1972) studying the adsorption-desorption of parathion emphasized that parathion has a greater affinity for organic adsorptive surfaces than for mineral ones. However, the important finding suggested by these authors is that adsorption was dependent on the type of association (specific interactions) between organic and

mineral colloids, which determine the nature and the magnitude of the adsorptive surfaces.

A study by Gaillardon et al. (1977) of the adsorption-desorption of terbutryn by montmorillonite, humic acids, and their mixtures also points out that the organic matter-mineral colloids must be considered in relation to each other, rather than as isolated parameters to provide a better understanding of pesticide activity in soils.

Because acidic pesticides can ionize in aqueous solutions forming anion species, they are not likely to be adsorbed by clay particles. Hence, they are mainly adsorbed by the soil organic matter (Grover, 1971). Studies by Weber et al. (1965) demonstrate this showing that 2,4-D is not significantly adsorbed by clays. This phenomenon has been attributed to the fact that at the pH of most agricultural soils (6-8), the carboxyl functional group of the side chain in 2,4-D can readily ionize to produce an anion and thus, be repelled by the negatively charged clay particles. Reddy and Gamrell (1987) studied the factors affecting adsorption of 2,4-D and methyl parathion in 19 different soils and sediments. They found that organic matter content was the most important single factor affecting adsorption but if the organic matter content was less than 1%, oxalate extractable Mn and Ca were well associated with adsorption of 2,4-D and methyl parathion. They also reported that soil pH and CEC were reasonably well

associated with 2,4-D adsorption. Their work is also supported by the findings of Moreale and Bladel (1980). The later found that 2,4-D adsorption was dependent not only on organic matter but also on pH and exchangeable aluminum.

**Adsorption-Desorption Measurements**

The sorption of pesticides is usually quantitatively expressed in terms of isotherms, which indicate the relationship between the concentration of the chemical in the sorbent (soil) and its equilibrium concentration in solution at a constant temperature (Rachinskiy et al., 1985). The extent of adsorption of a compound may be expressed by an adsorption coefficient obtained from an adsorption isotherm.

Various mathematical relationships have been used to parametize the laboratory adsorption-desorption measurements for organic chemicals. Among the most successful is the Freundlich equation that can be stated as follow

$$S=K_fC^n \qquad (1)$$

where S is the adsorbed pesticide concentration in mass per mass of soil (ug/g soil), C is the solution phase pesticide concentration in mass per volume of solute (ug/ml), $K_f$ (or K) is the Frendlich constant and n is the exponent which lies between 0 and 1. K and n are empirical coefficients that depend on the soil-pesticide system.

The logarithmic form of Equation 1 is

$$\text{Log}S = \text{Log}K + n\,\text{Log}C \qquad (2)$$

where K and n represent the intercept and the slope on a log-log plots of S vs. C. When n = 1, then Equation 1 is linear and an even simpler linear adsorption equation can be used:

$$S=K_dC \qquad (3)$$

where $K_d$ is the distribution coefficient (Rao and Davidson, 1980). Although this is just an approximation to the nonlinear behavior represented in the more general Freundlich relationship, Karickhoff (1091; Karickhoff et al., 1979) has shown that many organic chemicals display linear behavior at low concentrations similar to those associated with pesticides found in agricultural ecosystems. The error introduced by assuming linearity depends on C and n. This error can be represented as the ratio of Equations 1 and 3 and is equal to $C^{n-1}$. Such error may be tolerable for many practical applications, but for high solution concentrations (waste disposal sites) the amount adsorbed could easily be overestimated by an order of magnitude or more (Rao and Davidson, 1980).

2,4-D sorption is commonly described by the Freundlich isotherms with the slope (n) equal to 1 or slightly less (Que Hee and Sutherland, 1981).

The most common method used to measure adsorption-desorption is the "batch" equilibrium technique, in which soil

samples (adsorbent) are suspended by shaking until equilibrium with a series of pesticide (adsorbate) concentrations and the changes in the solution concentration are attributed to sorption. The equilibrium solution is separated from the adsorbent by centrifugation (Green R.E. et al., 1980). The batch method has been specified in the "Protocol of Adsorption Tests" published by the United States Environmental Protection Agency in its guidelines for registering pesticides in the United States (EPA, 1975).

In some cases the adsorption isotherm is nearly identical to the desorption isotherm. But, with some soils hysteresis (non-singular sorption-desorption isotherms) occurs (DiToro and Horzempa, 1982: Swanson and Dutt, 1973). The adsorption-desorption processes may be non-singular or even irreversible and assuming that adsorption and desorption have the same characteristics may lead to the wrong conclusions. However, in some instances hysteresis may not be a real phenomena in which case three major possible sources for non-singularity may be (1) an artifact created by the particular methodology, (2) a failure to establish complete equilibrium during adsorption phase, or (3) chemical and/or microbiological transformation of the pesticide during the experiment (Rao and Davidson, 1980).

In addressing the question of methodology, Rao et al. (1978) and Bowman and Sans (1985) found that the

centrifugation-resuspencion steps in the normal batch-type method might be responsible for at least part of the hysteresis. They suggested the use of a modified batch equilibration method, the "dilution-desorption" method. This avoids non-singular sorption-desorption isotherms by eliminating repeated centrifugation and resuspencion steps. In the common batch method, desorption is achieved by replacing the equilibrated supernatant solution by equal amounts of solute-free solution several times. In the "dilution-desorption" method, desorption is done by replacing the equilibrated supernatant solution by an excess of solute-free solution just once.

**External Factors**

The extent to which sorption occurs is affected not only by the soil media and the properties of the organic chemical itself but also by external factors, mainly climatic condition and agricultural practices.

Among these external factors, the most important are soil moisture content and temperature. The soil moisture content affects the adsorption process by modifying the accessibility of the adsorption sites and the surface properties of the adsorbent. Adsorption processes are exothermic and desorption processes are endothermic in nature, thus, an increase in temperature would normally be expected to reduce adsorption

and favor the desorption process. This corresponds to a weakening of the attractive forces between the solute and the solid surface with increasing temperature, and corresponding increase in solubility of the solute in the solution phase (Yaron, 1985; Bailey and White, 1970; Mills and Biggar, 1969; Yaron and Saltzman, 1972).

## Transformation Processes

Photochemical and chemical degradation and biological transformations are the principal causes of pesticide degradation in soils.

### Photodecomposition

Photodecomposition is one of the pathways of pesticide conversion in the environment and is induced mainly by ultraviolet (UV) sunlight radiation. Organic molecules can absorb energy in the ultraviolet region causing excitation of electrons and resulting in transformations (eg. break down and/or formation of chemical bonds, fluorescence) (Yaron et al., 1985). 2,4-D can be transformed by photolysis near the soil surface but the pathway and final product of photodecomposition depend on the form in which 2,4-D is applied and the irradiation wavelength (Que Hee and Sutherland, 1981).

**Chemical Degradation**

Trying to distinguish between chemical and biological degradation reactions is not easy and, in general, the experimental approach is to compare the transformation which occurs in natural and sterile soils (Yaron et al., 1985; Torstensson, 1988). Pesticides can undergo chemical degradation in soils either in solution phase or while adsorbed to the solid phase. Chemical degradation can occur as a hydrolytic process or as a result of oxidation or other non-hydrolytic reactions (Yaron, 1989). Even though non-biological reaction have been proposed as the main degradation mechanism of only a few herbicides in soils, they can play a role in mediating biological reactions (Torstensson, 1988). Hydrolysis is by far the most important chemical reaction that pesticides undergo in the soil media and it refers to any reaction that involves the dissociation of water into $H^+$ and $OH^-$ by the action of a substance that will combine with either species (Yaron et al., 1985). All forms of 2,4-D, once introduced into soil, undergo hydrolysis to either the free acid or the anionic species. The rate of hydrolysis depends on the form of 2,4-D and the pH of the aqueous phase, but in general this reaction occurs on the order of days or less (Que Hee and Sutherland, 1981). Concurrent with hydrolysis is the pH dependant disassociation reaction whereby the free acid or anionic species of 2,4-D is formed. At pH less than the pKa,

the free acid will form, and at pH greater than the pKa, the anionic species will form. Therefore, at the pH of most natural systems (6-9) the anionic species will dominate regardless of the initial form of 2,4-D.

**Biological Transformation**

Biodegradation is the most important transformation process for 2,4-D in the soil environment. Biodegradation refers to any biologically induced structural transformation in the parent compound that changes its molecular integrity (Scrow, 1982). Like most processes occurring in nature, biodegradation is dependent upon numerous chemical and environmental factors (eg. soil type, pH, temperature, moisture, chemical concentration).

Soils with high organic matter content tend to have greater microbial activity but also tend to adsorb greater amounts of herbicides and possibly protect them from degradation. Soil pH can affect degradation either through its effect on the chemical's stability, by its effect on adsorption, or the makeup of the microflora (Yaron et al., 1985). Temperature and moisture content do not control biodegradation specifically but rather they control the microbial metabolic activities in general. Increasing microbial activity and decreasing adsorption associated with higher temperatures generally enhance pesticide degradation.

Higher adsorption and lower microbial activities may be responsible for reduced degradation as the soil water content is decreased (Rao and Davidson, 1980). In addition, moisture content also controls oxygen levels in the soil, thereby, at high soil water content (close to saturation) pesticide degradation rates are determined by the relative rates of decomposition under aerobic vs. anaerobic conditions (Yaron, 1985).

Parker and Doxtader (1983) studied the effects of temperature and moisture content on 2,4-D biodegradation kinetics. They found that the optimum temperature and moisture tension were 27°C and 0.1 bar, respectively. Generally, the rate of decomposition of 2,4-D decreased with increasing soil moisture tension for temperatures between 20°C and 35°C. This is attributed to reduce activity of the 2,4-D degraders as a result of decreased water availability. These results confirm the findings of Ou (1982); 2,4-D degradation decreased with increase moisture tension and the disappearance rates in soils incubated at 35°C were generally smaller than those incubated at 25°C.

Under conditions favorable for degradation, 2,4-D disappears from soil within one to three weeks following normal agricultural applications (Audus, 1960).

Ever since the mid 1940's, the disappearance of 2,4-D from soils has been documented to be primarily microbially

mediated (Loss, 1975). A number of microorganisms capable of degrading 2,4-D have been isolated from soils mainly Arthrobacter sp. and Pseudomonads (Loss, 1971). Degradation pathways of 2,4-D have been elucidated by several groups (Loos et al, 1967 a, b and c; Bollag et al., 1968 a and b; Tiedje et al., 1969; Evans et al., 1971; Gamar and Gaunt, 1971; Gaunt and Evans, 1971). The degradation pathway involves the removal of the acetic acid side chain to yield dichlorophenol followed by orthohydroxylation by a monooxygenase to produce catechol. Cathecol is then converted to muconic acid by ortho cleavage of the aromatic ring. Muconic acid is then transformed into 3 and 4 carbon acids (eg. succinic acid) that can readily be used in the Krebs cycle (Loss, 1975). The degradation pathway is shown in the following figure after Evans et al., 1970.

The mineralization of many organic compounds by microorganisms is often preceded by an acclimation period or lag phase. The lag phase is the time interval during which biodegradation is not detected. The lag phase can be attributed to one or a combination of (1) induction or repression of enzymes specific for degradation pathways; (2) a random mutation in which new metabolic capabilities are produced; or (3) an increase in the number of organisms in the degrading population (Aelion et al., 1987).

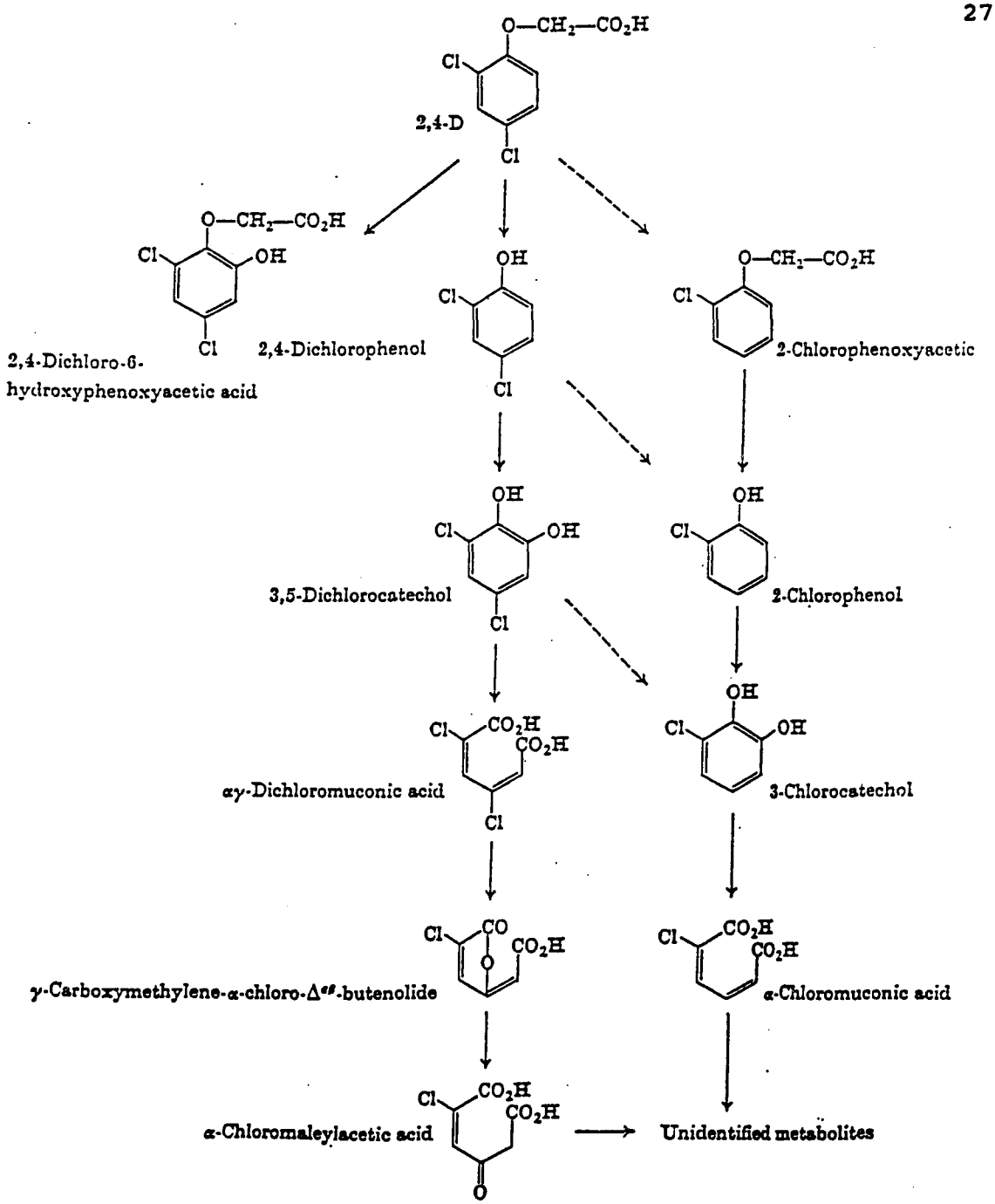


**Figure 2. Proposed pathways of 2,4-D degradation (Evans et al., 1970)**

Degradation of 2,4-D in soils is generally characterized by a lag period before biodegradation begins. Subsequent additions of 2,4-D then disappear rapidly without the lag phase (Audus 1960; Torstensson, 1988; Sinton et al., 1986).

**Energy Production**

The following is a summary of chapter 4 in Brock (1979): Organisms use a wide variety of energy sources and mechanisms to perform their basic function of growing in one or another environment. Chemical energy is the energy that can be released from organic or inorganic compounds by chemical reactions and it is the primary source of energy for non-photosyntetic organisms. The free energy (F) is the energy released that is available to do useful work. The change in free energy during the reactions is expresed as $\Delta F$ . The more negative the $\Delta F$, the more favorable the reaction would be. The utilization of chemical energy in living organisms generally involves oxidation-reduction reactions. The energy released as a result from oxidation reduction reaction is commonly transfered to a variety of phosphate compounds in the form of high energy phosphate bonds which serve as intermediaries in the conversion of energy into useful work. The most important high energy phosphate compound in living organisms is adenosine triphosphate (ATP) which serves as the prime energy carrier. The pathways of oxidation of organic compounds and

conservation of energy in ATP can be divided into three major groups: (1) fermentation, in which oxidation occurs in the absence of any added electron acceptor (an organic compound serves as both the electron donor and electron acceptor); (2) respiration, in which molecular oxygen serves as the electron acceptor and (3) anaerobic respiration, in which an electron acceptor other than $O_2$ is involved ($NO_3^-$, $SO_4^{2-}$ or $CO_3^{2-}$ ).

Looking at the yield of useful energy ($\Delta F$) from the aerobic and anaerobic oxidation of glucose (table 2) , aerobic organisms are more efficient; aerobic respiration yields about 10 times more free energy and 19 times more ATP per mole of glucose metabolized (Davis et al, 1967)

Assuming an energy value for the high energy phosphate bond in ATP of 7.4 Kcal/mol, aerobic respiration is highly efficient converting about 41% of the total free energy released into ATP, while fermentation converts only about 25%.

In fermentation the growth yield (mass of bacteria/ mass of substrate metabolized) represents only less than 1/10 , however in respiration it may be as much as 2/3 (Davis et al., 1967). A facultative anaerobic microorganism will exhibit a larger growth yield under aerobic than under anaerobic conditions; however the difference will be 3-5 fold more rather than the expected 19 fold (Davis et al., 1967).

**Table 2. Energetics of metabolism (Davis et al., 1967)**

| Reaction | No. of ATP generated | - ΔF (Kcal) |
|---|---|---|
| Glucose→ 2 lactic acid | 2 | 58 |
| Glucose→ 2 ethanol + $CO_2$ | 2 | 57 |
| Glucose + $6O_2$→ $6CO_2$ + $H_2O$ | 38 | 688 |

Table 3 shows different reaction models for the chemical oxidation of 2,4-D. When molecular oxigen is used as the terminal electron acceptor (aerobic respiration), the free energy yield (ΔF) is generaly twice as much than under anaerobic respiration. Microorganism can derive maximum energy (ΔF is most negative) and thus generate more ATP, from the oxidation of 2,4-D under aerobic conditions.

**Biodegradation Kinetics**

Numerous models have been proposed to express the growth dynamics of a population that is limited only by the concentration of a single substrate. Some of these formulations have been reviewed by Simkins and Alexander (1984). They discuss these models as a specific or degenerated form of the Monod equation and describe them to be appropriate under unique situations of substrate concentration and population density.

**Table 3. Reaction models for 2,4-D (Que Hee et al., 1981)**

| Redox reaction model | ΔF (Kcal) |
|---|---|
| $2C_8H_6O_3Cl_2 + 15O_2 = 16CO_2 + 4H^+ + 4Cl^- + 4H_2O$ | - 1600.78 |
| $2C_8H_6O_3Cl_2 + 15Cl_2 + 13H_2O = 8CO_2 + 32H^+ + 32Cl^-$ | - 915.33 |
| $2C_8H_6O_3Cl_2 + 11ClO^- + 10OH^- = 4C_2O_4^{-2} + 13Cl^- + 8H_2O$ | - 910.69 |
| $2C_8H_6O_3Cl_2 + 6MnO4^- + 16H^+ = 8CO_2 + 11H_2O + 6Mn^{2++} + 2Cl^-$ | - 992.64 |

Growth rate, like a chemical reaction, is a function of chemical concentration. The relationship between growth rate and substrate concentration can be described by the Monod model:

$$\mu = (\mu_{max} * S) / (K_s + S)$$

where $\mu$ is the growth rate at a substrate concentration S (specific growth rate), $\mu_{max}$ is the maximum growth rate, S is the substrate concentration and $K_s$ is a constant, equal to the substrate concentration when $\mu = 0.5\ \mu_{max}$ (affinity of the substrate). Simkins and Alexander (1984) state that Monod kinetics with no growth requires that the initial cell concentration has to be much higher than the

substrate concentration but that Monod kinetics with growth requires no specific condition.

Zero order kinetics are observed where the initial cell density is much greater than the number of organisms that can be produced from the substrate (growth is insignificant) and where the initial concentration of substrate is much greater than Ks.

At constant biomass (growth insignificant) and limited substrate levels (substrate concentration much smaller than Ks), the rate is directly proportional to the concentration of the substrate and reaction rates conform to first order (Cork and Krueger, 1991).

When the concentration of a degradable organic substrate is considerably in excess of the bacterium's Ks value, logarithmic or exponential growth occurs (Cork and Krueger, 1991).

**Biodegradation-Sorption**

It has long been recognized that organic pollutants become less biologically available through sorption. Thus the extent of sorption may determine both the rate and degree of degradation (Gree et al., 1989). Because of sorption of a contaminant to soil, only a fraction of the total amount of a pollutant in a contaminated soil may be available for degradation at any given point in time. This does not mean

that extensive or total degradation can not occur, but it may occur at a rate limited by desorption. It appears that, in some cases, material sorbed to soil particles must desorb first before degradation occurs. Ogram et al. (1985) studied the effects of sorption on biological degradation rates of 2,4-D in soils. They tested three mathematical models that assumed different mechanisms for 2,4-D degradation to see which one best described the results of a series of laboratory experiments. They concluded that only compounds in the soil solution are available for degradation and that sorbed and solution-phase bacteria degraded solution phase compounds. More recent work by Speitel et al. (1989) showed that decreased liquid-phase concentration by microorganisms was necessary prior to the biodegradation of sorbed compound. These researchers showed that biodegradability of the compound in solution was a necessary but not a sufficient condition for biodegradation of the sorbed compound. Significant biodegradation of sorbed compound occurred only where desorption could also occur.

## Transport Processes

As has already been discussed, retention and transformation are the two major processes that affect the amount pesticide present and available for transport. Retention processes do not affect the total amount of

pesticide in the soil system but rather decrease or eliminate the amount available for transport. Transformation processes can actually reduce or totally eliminate the amount of pesticide present and available for transport (Cheng and Koskinen, 1986). Predicting the fate of organic chemicals in soils requires not only the understanding of these individual processes but also of the interactions among them. Therefore, coupled-process research looking at the interactions of sorption and degradation on transport is necessary (Angley et al., 1992)

To better understand the transport process the following recapitulation of chapter 9 in Freeze and Cherry (1979) has been made:

To describe the transport of solutes in a porous media, the flux of a solute into and out of a given elemental volume is based on conservation of mass. The net rate of change of mass of solute within a given elemental volume is equal to the flux of solute out of the element minus the flux of solute into the element plus/minus the gain or loss of solute mass due to reactions. The physical processes that control the flux into and out of the element volume are advection and dispersion. The loss or gain of solute mass can occur due to chemical or biological reactions. Advection (mass flow or convective transport) is the process by which solutes are transported by the bulk motion of the flowing water. The rate

of transport for nonreactive solutes is equal to the average pore water velocity. Solutes however, tend to spread out of the flow path that would be expected according to advection. This "spreding" is called hydrodynamic dispersion and it occurs because of mechanical mixing during fluid advection and because of molecular diffusion of the solute particles. Diffusion is the process by which solutes move under the influence of their kinetic activity in the direction of their concentration gradient. Axial diffusion is important only under low velocities.

**Nonequilibrium Processes**

In the past, local equilibrium conditions during solute transport were assumed but, recent studies have questioned this assumption. These recent investigations suggested that the observed nonideal behavior of solute transport in several induced-gradient experiments was due to nonequilibrium (Brusseau et al., 1989).

Brusseau and colleagues (Brusseau et al, 1989; Brusseau et al., 1991) grouped the rate-limiting (or non-equilibrium) procesees into two general classes: transport realted and sorption related. Transport related or physical non-equilibrum results from the existence of a heterogeneous porous medium in which there are regions of minimal advective flow ("immobile" regions). This type of nonequilibrium

affects the transport of nonsorbing and sorbing solutes. Sorption related nonequilibrium may result from chemical nonequilibrium (chemisorption) or from intrasorbent diffusion. Chemical nonequilibrium is caused by rate-limited interactions between the solute and sorbent. Nonequilibrium resulting from intrasorbent diffusion is due to diffusive mass transfer of solute within the sorbent ( e.g. organic matter -intraorganic- or mineral -intramineral- surfaces). Sorption nonequilibrium influences only sorbing solutes.

**Transport Models**

Traditionally, analysis of contaminant transport in the subsurface has employed the convective dispersive equation with terms for adsorption and degradation. In these models, the sorption process has often been idealized by assuming instantaneous equilibrium and linear, singular isotherms. However, nonideal sorptive behavior is often the case. Brusseau and Rao (1989) have critically reviewed sorption nonideality. Nonideal sorptive behavior has been attributed to several different factors like kinetic sorption reactions (nonequilibrium or rate-limited sorption), diffusive mass transfer, isotherm nonlinearity and sorption-desorption nonsingularity.

Recently, coupled-process transport models accounting for rate-limited sorption (sorption kinetics) have been developed.

Van Genuchten and Wagenet (1989) have presented theoretical development and analytical solutions for two-site/two-region models for pesticide transport and degradation. The basic development of these transport models however, was done by van Genuchten and Wierenga (1976) and van Genucten et al. (1977). Their models assume that the nonequilibrium processes influencing transport include the possible division of the sorption sites and/or the soil water. The two-site sorption concept, refers to the exchange (sorption) sites being either sites of instantaneous sorption or sites in which sorption is rate limited (time dependent). Two-region or "bi-continuum" models refer to systems in which sorption rate is limited by the rate at which solutes are transported by diffusion to the exchange sites (mobile- immobile soil water concept).

Studies of the sorption of 2,4-D and Atrazine indicate that the two-site models may well be suitable for these pesticides (Rao et al., 1979).

# MATERIALS AND METHODS

## Chemicals

Uniformly ring-labeled $^{14}C$-[2,4-D], with a specific activity of 12.8 mCi/mmol was purchased from Sigma Chemical Co. (St. Louis, MO) with a purity of > 98% . Analytical grade 2,4-D was obtained also from Sigma Chemical Co. (St. Louis MO). Tritiated water ($^3H_2O$ ), with a specific activity of 1 mCi/ml was purchased from DuPont (Surbank, CA) .

## Soil

A sandy loam soil was collected from the top 10 cm of an agricultural site in the Yaqui Valley (state of Sonora, Mexico) that had a previous history of repeated 2,4-D applications. The soil was sieved through a 2 mm mesh sieve and stored at 4°C to maintain the indigenous microbial population. Preliminary 2,4-D sorption experiments with this soil showed very limited sorption, thus it was amended with a sandy loam soil with an organic carbon content of 12.6%, collected from Mount Lemmon, AZ. An 80:20 (g/g) mixture of the Mexican sandy loam and Mount Lemmon soil was used for all the experiments. Selected soil characteristics are given in Table 4 and different soil treatments shown in Table 5.

**Table 4. Selected Soil Characteristics**

| | Mexican Sandy Loam | Mt. Lemmon Soil | 80:20 Mixture |
|---|---|---|---|
| Sand (%) | 76.7 | 60.3 | 77.7 |
| Silt (%) | 14.2 | 24.0 | 18.1 |
| Clay (%) | 9.1 | 15.7 | 4.2 |
| Total N (%) | 0.029 | | 0.072 |
| Organic Matter (%) | 0.466 | 25.2 | 2.74 |
| pH | 7.76 | | 7.92 |
| EC (dS/m) | 0.188 | | 0.198 |
| Cl (mg/l) | 8.31 | | 8.04 |
| $NO_3$ (mg/l) | 34.4 | | 31.4 |
| $SO_4$ (mg/l) | 10.2 | | 11.1 |
| Bacteria (CFU/g dry soil | $1.2x10^6$ | | |
| Fungi (CFU/g dry soil) | $3.2x10^4$ | | |

**Table 5. Soil Treatments**

| Soil | Treatment |
|---|---|
| Saturated | 0 mbars tension (100% moisture) |
| Unsaturated | -30 mbars tension (approx. 30% moisture) |
| Sterile | autoclaved 80:20 mixture soil for 90 minutes at 120 °C and 21 psi |
| Non-Sterile | 80:20 mixture soil |
| Non-sterile Spiked | 80:20 mixture soil spiked twice with 100 ppm 2,4-D two and four weeks prior to experiment |

## Adsorption-Desorption Studies

Two methods, differentiated by the way in which desorption was achieved, were used to obtain sorption-desorption isotherms.

The first method was the widely used "batch" method in which the adsorbent (soil) was suspended by shaking until equilibrium with an adsorbate solution was reached. The change in the adsorbate solution concentration was attributed to adsorption (Green et al., 1980). Specifically, 10 g soil samples and 10 ml of 2,4-D solutions at different initial concentrations were placed in Corex teflon-lined screw-cap (50 ml) centrifuge tubes and shaken until sorption equilibrium was reached. The 2,4-D was labeled with uniformly ring labelled $^{14}C$-[2,4-D] (specific activity equal to 20-50 nCi/ml). Preliminary sorption experiments had shown no increase in pesticide adsorption after 72 hours. After sorption equilibrium (72 h), the samples were centrifuged (Beckman Instruments, Inc., GP Tabletop Centrifuge, Palo Alto, CA.) at 1800 rpm for 10 minutes and the 2,4-D concentration in the supernatant was determined by liquid scintillation (Packard, Tri-Carb liquid Scintillation Analyzer, Model 1600TR, Meriden, CT.). Desorption was initiated by removing the supernatant and replacing it whith equal amounts of solute free solution (0.01 N $CaCl_2$). The samples were shaken again until desorption equilibrium (24 hours) and the concentration of

2,4-D in the supernatant was measured. This desorption process was repeated twice more for a total of three resuspension-centrifugation steps.

The second method used was a modified batch equilibration method, the "dilution desorption" method (Rao et al., 1978; Bowman and Sans, 1985). The adsorption of 2,4-D was measured as described for the "batch" method above. However, in this modified method, desorption was initiated by replacing the equilibrated supernatant solution with an excess (dilution) of solute-free solution (0.01N $CaCl_2$). Soil samples were shaken again until desorption equilibrium, 24 hours, and centrifuged. The concentration of 2,4-D in the supernatant was then measured again by liquid scintillation. For the sorption isotherms 1:1 (g/g) soil-solution ratios were used (10 g of soil and 10 ml solution) and for the desorption isotherms 1:2.5 (g/g) were maintained (10 g of soil and 25 ml of solution).

All sorption-desorption experiments were conducted under sterile conditions and constant temperature in an environmental shaker (Lab-Line Instruments, Inc., Orbit Environ-Shaker model 3527, Melrose Park, IL.) kept at 24°C ± 1°C. Soil was sterilized by autoclaving it once for 90 minutes at 120°C and 21 psi pressure. Solutions were filter sterilized (0.45 micron filters).

Sterility of soil and solutions was verified by streaking samples on nutrient agar and observed for growth prior to their use.

## Biodegradation Studies

### $CO_2$ Evolution Experiments

Mineralization of 2,4-D was determined by quantification of $^{14}CO_2$ evolved from batch soil experiments. Soil samples (10 g) were placed into 125 ml microfernbach flasks and amended with 100 ppm $^{14}C$-[2,4-D] solution (specific activity of 0.5 uCi/100 g soil). The flasks were periodically flushed with air through a series of six traps (see Figure 3); traps 1 and 4 were empty in case of backflow, traps 2 and 3 contained Scintiverse BD (Fisher Scientific, Fair Lawn, NJ.) to scrub out $^{14}C$-labeled volatile organics and traps 5 and 6 contained Oxosol $C^{14}$ (National Diagnostics, Manville, NJ.), a phenethylamine-based cocktail which traps $^{14}CO_2$ (Marianucci and Bartha, 1979). Mineralization of 2,4-D was determined under both, saturated and unsaturated conditions. For unsaturated conditions, soil moisture tension was adjusted by adding enough 0.01 N $CaCl_2$ solution to reach approximately 30 % moisture by volume, equivalent to a soil water tension of -30 mbars. Each experiment was run in triplicate and compared to sterile (autoclaved) controls.

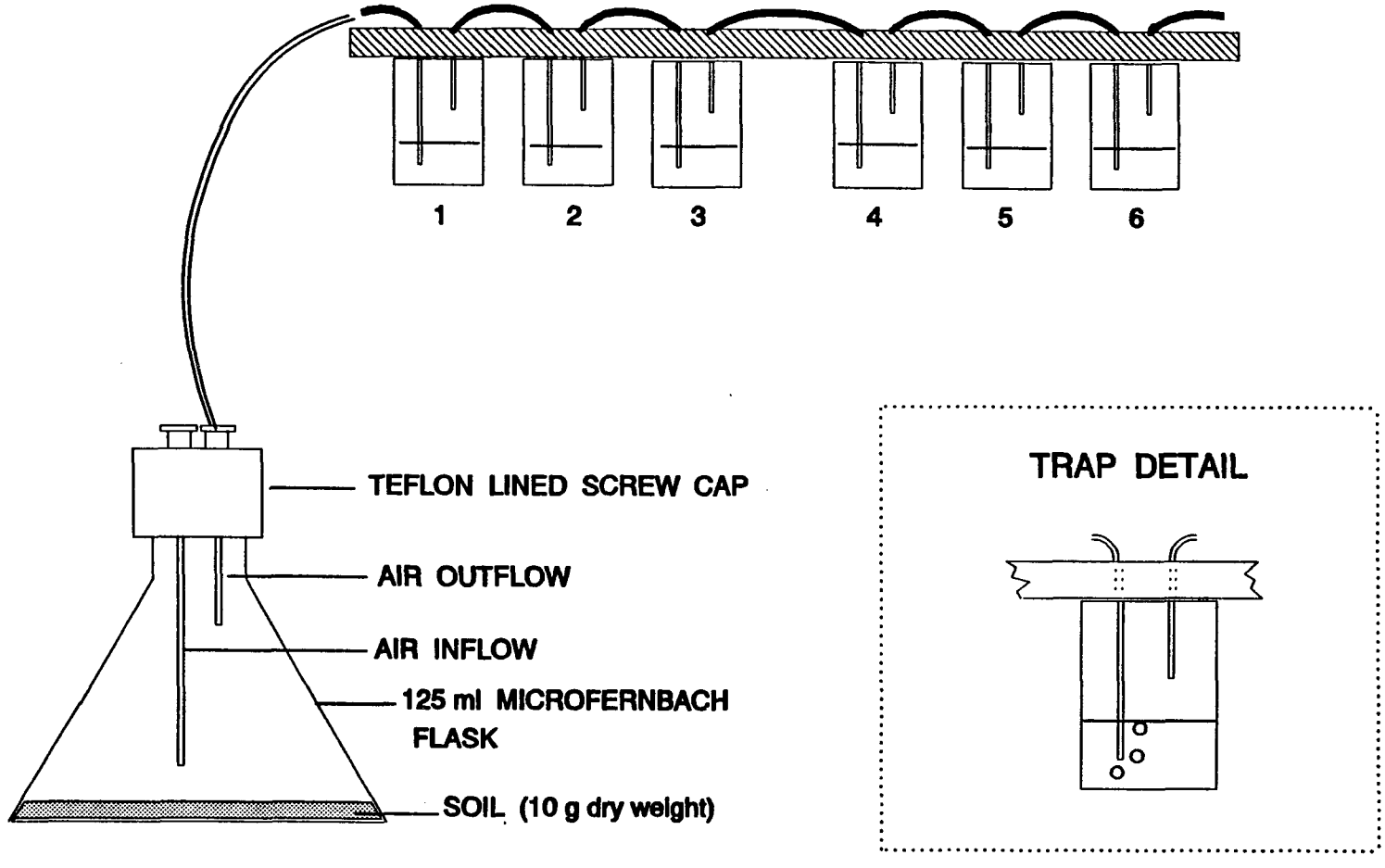


Figure 3. $CO_2$ trapping system (Marianucci and Bartha, 1979)

**Parent Compound Disappearance**

Biodegradation of 2,4-D was also determined by following concentrations in soil. To extract the 2,4-D from the soil samples, 10 ml of acetone were added to each flask and the pH was adjusted to < 2.5 with HCl. Samples were shaken for 10 minutes and then 50 ml of chloroform and 20 g of sodium sulfate were added. Samples were shaken overnight and then filtered. The filtered solution was concentrated on a rotoevaporator (Brinkmann Instruments, Inc., Buchi model RE-140A, Westbury, NY.) and volume adjusted to 10 ml with acetonitrile-acidified water (50:50 v/v - mobil phase). 2,4-D concentration was determined by High Performance Liquid Chromatography (HPLC). The HPLC system (Waters, Division of Millipore Corporation, Milford, MA) consisted of a programmable multiwavelength detector (Waters 490E), a sample processor (Ultra Wisp, Waters 715), and a multi-solvent delivery system (Waters 600E). The column used was a Spherisorb ODS-2 5 Micron (250 x 4.6 mm) column (Allteck Associates Inc., Deerfield, IL.). The isocratic mobile phase used was acetonitrile-acidified water (50:50 v/v) (Both, acetonitrile and water were HPLC grade from Baker Inc., Phillipsburg, NJ). Water was acidified to pH 2.6 with acetic acid. The flow rate was 1 ml/min. The multiwavelength UV detector was set at 235 nm.

## Transport Studies

### Saturated Conditions

Pesticide movement through saturated columns under sterile and non-sterile conditions was analyzed using the miscible displacement technique described by Brusseau et al (1990) (Figure 4). Chromatography columns (Kontes, Vineland, NJ.), 2.5 cm internal diameter and 5 cm length were used in these experiments. Columns were packed and initially saturated with 0.01 N $CaCl_2$ solution until steady-state was established. Then, 100 ppm $^{14}C$-[2,4-D] solution was introduced into the column at constant flux until C/Co= 1. The applied pesticide solutions were collected in 1 ml aliquots using an automatic fraction collector (Isco, Inc., Foxy 200, Lincoln, NB.). Tritiated water was also displaced through each column to characterize the hydrodynamic properties of the column (specific activities of both $^{3}H$ and $^{14}C$ were 5 nCi/ml). Amounts of $^{14}C$ and $^{3}H$ in the effluent samples were assayed by liquid scintillation. Simultaneous sorption and degradation data were analyzed using a coupled process transport model that includes rate limited sorption and first order degradation (van Genuchten and Wagnet, 1989).

### Unsaturated Conditions

Transport under unsaturated sterile and non-sterile conditions was analyzed by the method described by Van Genuchten and Wierenga (1986) (Figure 5).

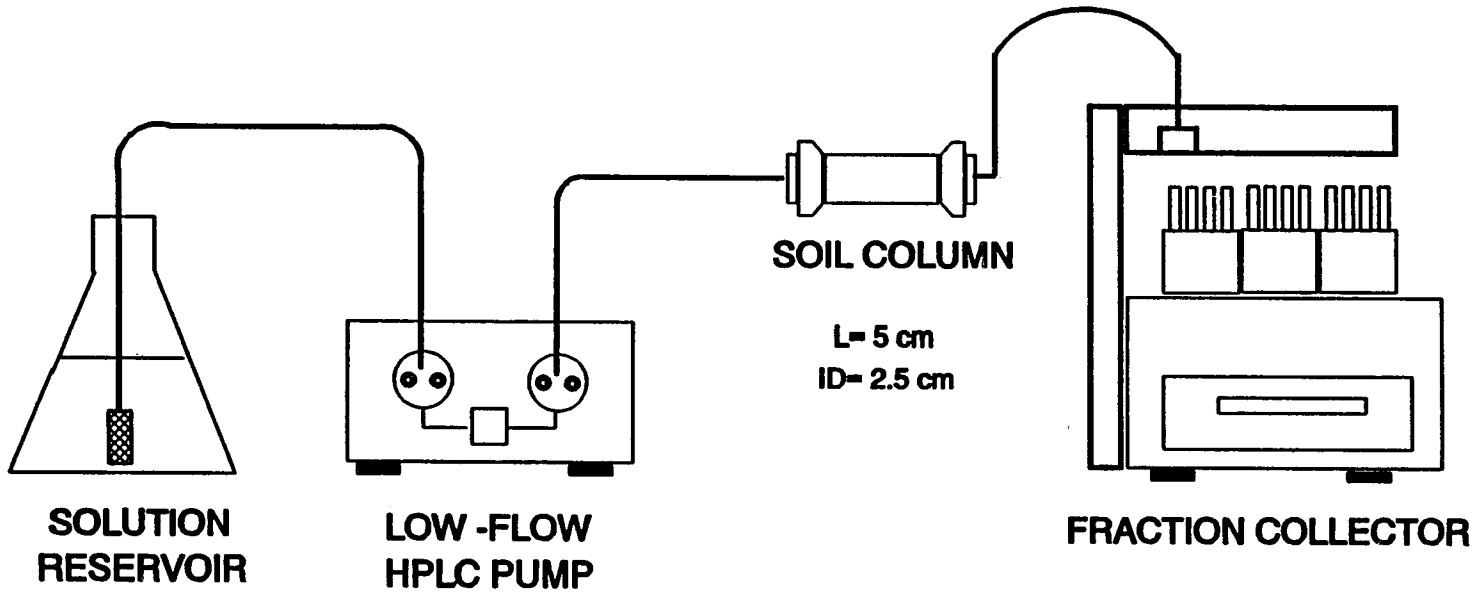


Figure 4. Schematic diagram of miscible-displacement apparatus for saturated column experiments (Brusseau et al., 1990)

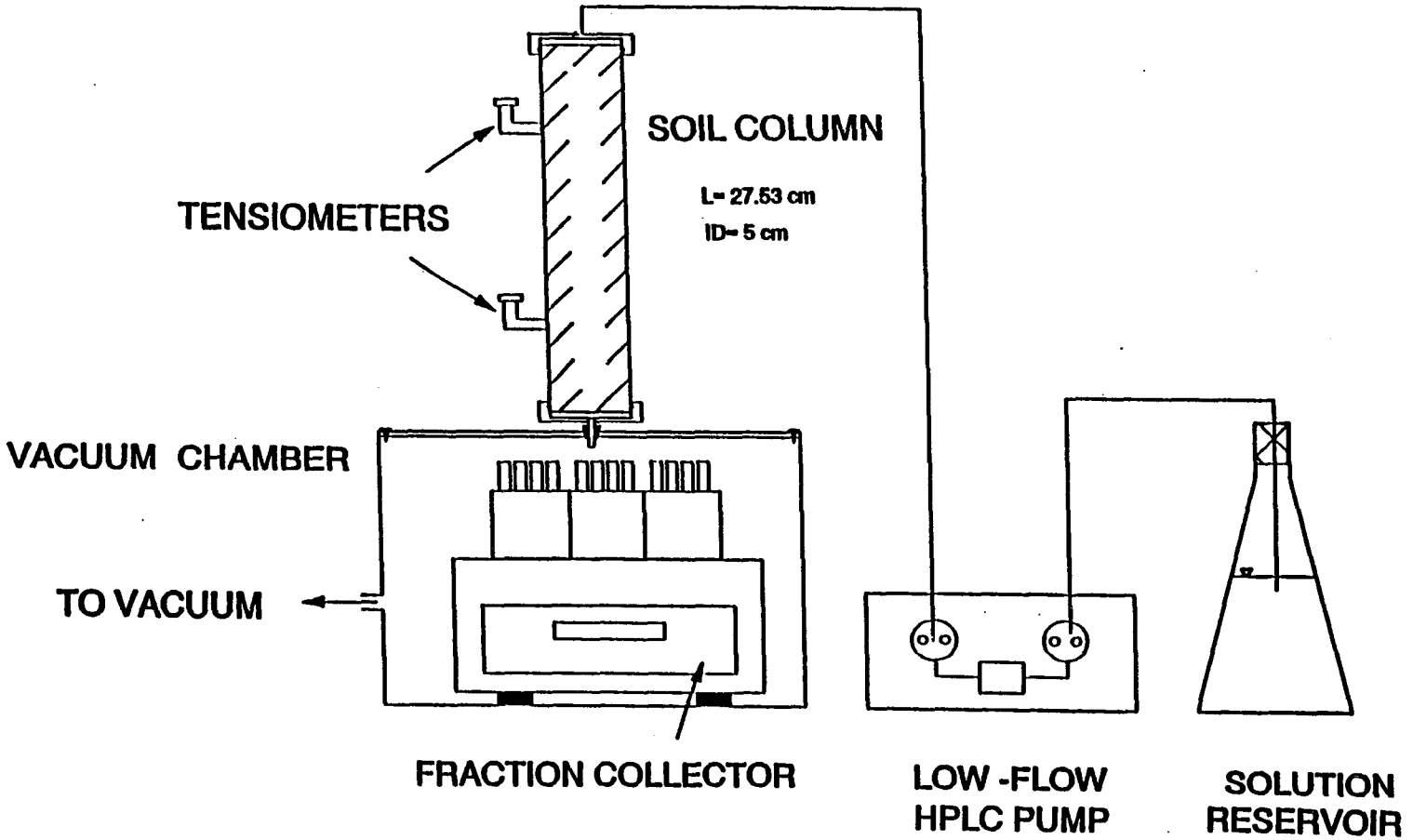


Figure 5. Schematic diagram of experimental apparatus for unsaturated column experiments (Van Genuchten and Wierenga, 1986)

Unsaturated column experiments were performed using a glass column 5 cm internal diameter and 30.5 cm long with a porous stainless steel plate (.5 micron) on the bottom. After the column was packed with soil, two tensiometers were installed 5 cm from the top and bottom of the column. The column was then connected to a vacuum chamber with an automatic fraction collector inside. A 0.01 N $CaCl_2$ solution was flushed through the column until steady state was reached. Steady state was assumed to be established when 1) pressure heads on the two tensiometers were equal, 2) influent and effluent flow rates were equal and 3) the mass of column remained constant. A handheld pressure transducer (or Tensicorder™, Soil Measurement Systems, Tucson AZ.) was used to measure suction on the two tensiometers. Pressure on the two tensiometers stabilized at approximately -30±2 mbars. A flow rate of 100 $cm^3\ d^{-1}$ was maintained during these experiments. Once steady state was reached, the input solution was replaced with a solution containing tritiated water and $^{14}C$-[2,4-D] at a concentration equal to 100 ppm. Specific activity of both $^{3}H$ and $^{14}C$ was 5 nCi/ml. Effluent solution was collected in 7 ml aliquots and subsamples analyzed by liquid scintillation. When C/Co=1 or close to 1, the $^{14}C$-[2,4-D] applied was displaced with a $CaCl_2$ solution.

## Dissolved $O_2$ Analysis

Dissolved oxygen measurements of the effluent solution of some of the saturated column experiments was determined by using CHEMets (self-filling ampules for colorimetric analysys-CHEMetrics, Inc., Calverton, Virginia).

## Mathematical Models

### Biodegradation Model

The kinetics of 2,4-Degradation were analyzed by using the Monod equations for growth of cell mass and single substrate utilization (Monod, 1949):

$$\frac{\partial x}{\partial t} = \mu_{max} \frac{X S}{K_s + S + \frac{S^2}{K_i}} - K_d X \qquad (4)$$

$$-\frac{\partial S}{\partial t} = \frac{1}{Y} \mu_{max} \frac{X S}{K_s + S} \qquad (5)$$

where X is the cell mass concentration, t is time, S is the substrate concentration, $\mu_{max}$ is the maximum specific growth rate coefficient, $K_s$ is the Monod coefficient for 2,4-D (half saturation coefficient), $K_d$ is the endogenous decay coefficient, $K_i$ is the inhibition coefficient and Y is the yield coefficient. For the analyses, it was assumed that $K_d$=0 and $K_i$ was very large.

The program T2 (courtesy of W.J. Maier, Dept. of Civil and Mineral Eng., Univ. of MN) can simultaneosly solve the equations describing cell mass (4) and substrate (5) as a function of time and allows the estimate of kinetic coefficients from batch test data. $CO_2$ production data from batch experiments were converted to cell mass data by estimating the cell yield (Y) and calculating a carbon mass balance according to the relationship:

$$cell\ mass produced\ (g) = [Y\ (g/g)\ ]\ [\ substrate\ consumed\ (g)]$$

The cell yield was estimated to be 0.53 g/g from Figure 13 which shows that approximately 34% of the added $^{14}C$-[2,4-D] is evolved as $^{14}CO_2$. For modeling of the experimental data, the data sets were cut off at 200 hours. This was done to eliminate effects of endogenous decay ($K_d$) on the simulation. Since data sets were cut off at 200 hours, a lower value of Y (0.45) was used for estimation of $X_o$.

**Two-Site Sorption and First Order Degradation Transport Model.** To analyze the results of the saturated miscible displacement experiments and simulate one-dimensional solute transport under steady-state water flux, rate limited and linear sorption, and first order degradation, the following non-dimensional equations have been proposed (Van Genuchten and Wagenet, 1989):

$$\beta R \frac{\partial C^*}{\partial T} + (1-\beta) R \frac{\partial S^*}{\partial T} = \frac{1}{P} \frac{\partial^2 C^*}{\partial X^2} - \xi C^* - \frac{\partial C^*}{\partial X} \qquad (6)$$

$$(1-\beta) R \frac{\partial S^*}{\partial T} = \omega (C^* - S^*) \qquad (7)$$

where

$$C^* = \frac{C}{C_o} \qquad (8)$$

$$S^* = \frac{S_2}{[(1-F) K_p C_o]} \qquad (9)$$

$$X = \frac{x}{l} \qquad (10)$$

$$T = \frac{tv}{l} \qquad (11)$$

$$P = \frac{vl}{D} \qquad (12)$$

$$R = 1 + (\frac{\rho_b}{\theta}) K_p \qquad (13)$$

$$\beta = \frac{1 + (\frac{\rho_b}{\theta}) F K_p}{R} \qquad (14)$$

$$\omega = \frac{k_2 l}{v} R(1-\beta) \qquad (15)$$

$$\xi = \frac{\mu_1 l}{v} \qquad (16)$$

and where C is the concentration of solute in the solution ; $C_o$ is the concentration of solute in the influent solution ; t is time; x is distance; $\theta$ is volumetric soil water content; v is the average pore-water velocity; P is the Peclet number, which represents the dispersive-flux contribution to transport; R is the retardation factor, which represents the effect of sorption on transport; D is the dispersion coefficient; $K_p$ is the equilibrium sorption constant; F is the fraction of sorbent for which sorption is instantaneous; $K_2$ is the reverse sorption rate constant; $\beta$ is the fraction of

instantaneous retardation; $\omega$ is the Damkohler number, which is a ratio of hydrodynamic residence time to characterize time of sorption reaction; $\mu_i$ is the first order degradation rate constant for the solution. It was assumed that biodegradation only occured in the solution phase.

To run the model, P, R, $\beta$, $\omega$, and $T_o$ have to be known. $T_o$ is the size of the input pulse in pore volumes. The P value was obtained from the tritium breakthrough curves. Values for the retardation factor (R) were obtained by first moment analysis (Valocchi, 1985) when the frontal and distal portions of the breakthrough curve were available or by calculating the area above the curve when only the frontal breakthrough curve was available. The two unknown parameters were thus $\beta$ and $\omega$ which were determined using the nonlinear least-squares optimization program CFITIM (van Genuchten, 1981).

**Substrate Transport and Biomass Growth Model**

The results of the unsaturated transport with biodegradation (non-sterile soil) experiment were analyzed by Dr. Robert S. Maier (AHPCRC, University of Minnesota). Data from this experiment were fitted with a mathematical model that describes bacterial growth coupled with transport of a substrate. The model formulation is given by a one-dimensional advection dispersion equation describing the transport and fate of the substrate, S(x,t), coupled to an ordinary

differential equation describing the growth of a stationary biological population X(x,t), were x and t denote location in space and time.

$$R\frac{\partial S}{\partial t} = D\frac{\partial^2 S}{\partial x^2} - V\frac{\partial S}{\partial x} - \frac{1}{Y}\mu \frac{S}{K_s+S} X \qquad 0<x<L \quad 0<t\leq T \qquad (17)$$

$$X_t = \mu \frac{S}{K_s+S} X \qquad 0\leq x\leq L \quad 0<T\leq T \qquad (18)$$

$$S(x,0) = S_{initial} \qquad 0<x<L \qquad t=0 \qquad (19)$$

$$X(x,0) = X_{initial} \qquad 0<x<L \qquad t=0 \qquad (20)$$

$$S(0,t) = S_{influent} \qquad x=0 \qquad t>0 \qquad (21)$$

$$\frac{\partial S}{\partial x}(L,t) = 0 \qquad x=L \qquad t>0 \qquad (22)$$

where L is column length; R is the retardation parameter representing reversible adsorption of the substrate onto soil particles; D is de dispersion parameter; V is the velocity parameter; Y is a yield coefficient representing the production of cell mass per unit mass of substrate utilized; $\mu$ represents the maximum rate or proportion of substrate utilized by the bacterial population and $K_s$ is the half-saturation coefficient.

# RESULTS AND DISCUSSION

## Sorption Studies

### Batch Experiments

Non-singular adsorption-desorption isotherms (hysteresis) have often been reported (Bowman and Sans, 1977; Rao and Davidson, 1980; O'Connor and Wierenga, 1980) when dealing with pesticide systems. In some instances however, the method used has been found to be responsible for at least part of the hysteresis (Rao et al., 1978). To address the question of methodology, adsorption-desorption isotherms obtained by the common "batch" method (Green et al., 1980; EPA, 1975) were compared to the results of the "dilution-desorption" method (Rao et al., 1978; Bowman and Sans, 1985).

The adsorption data from the two methods were obtained in the same manner, thus both generated very similar results. Adsorption isotherms were accurately described by the linear adsorption model

$$S=K_dC$$

were Kd is the distribution (or partition) coefficient (ml/g), S is the adsorbed concentration (ug/g soil) and C is the solution concentration (ug/ml). The Kd values computed using the least square fit to the adsorption isotherms were equal to 0.238 ($r^2$= 0.995) for the "batch" method (Figure 6) and 0.249

($r^2$=0.986) for the "dilution-desorption" method (Figure 7).

Linear sorption isotherms reflect a partition coefficient being constant or independent of the equilibrium solution concentration. Linear behavior is commonly observed when working with systems where solutes are low in concentration. Karickhoff et al. (1979) determined that if the equilibrium solution concentration is below $10^{-5}$ M, or less than half the solute's water solubility, then, sorption isotherms would be linear. The $10^{-5}$ M concentration has also been suggested by Giddings (1965) as a limit below which linearity can be expected. For 2,4-D with a solubility of 0.62 g/l and a molecular weight of 221, the maximum equilibrium solution concentration would be 2.81 x 10-3 M which is within the "safe limit" range and linearity is expected.

The desorption phase, unlike the adsorption one, provided very different results. The desorption branches from the "batch" method had a lesser slope than the adsorption branch, implying a partial irreversibility (Figure 6). However, the desorption branch from the "dilution-desorption" method (Figure 7) was practically superimposed over the adsorption isotherm.

Singular sorption-desorption isotherms represent the ideal behavior of a system at true equilibrium where the

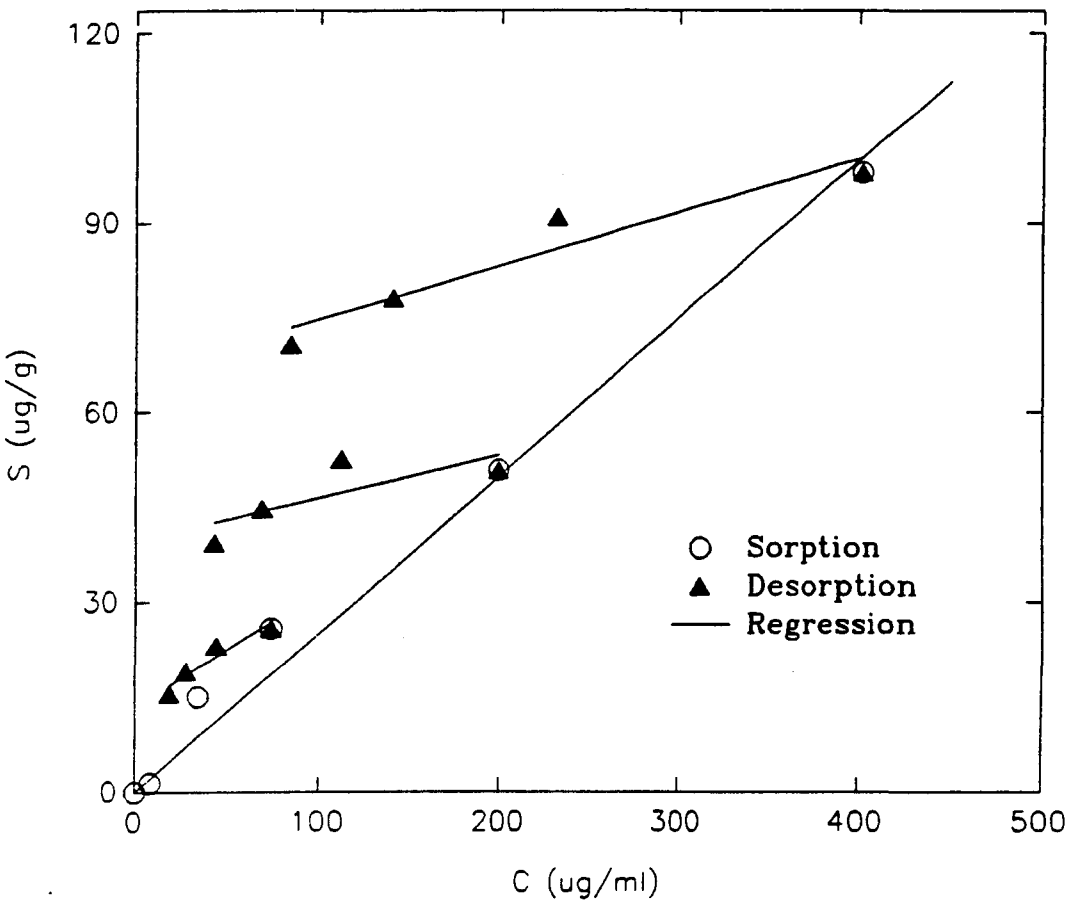


Figure 6. Non-singular 2,4-D sorption-desorption isotherms obtained using the "batch" method. $K_d$ = 0.238 ml/g. Desorption branches show a lesser slope than the adsorption branch, implying a partial irreversibility.

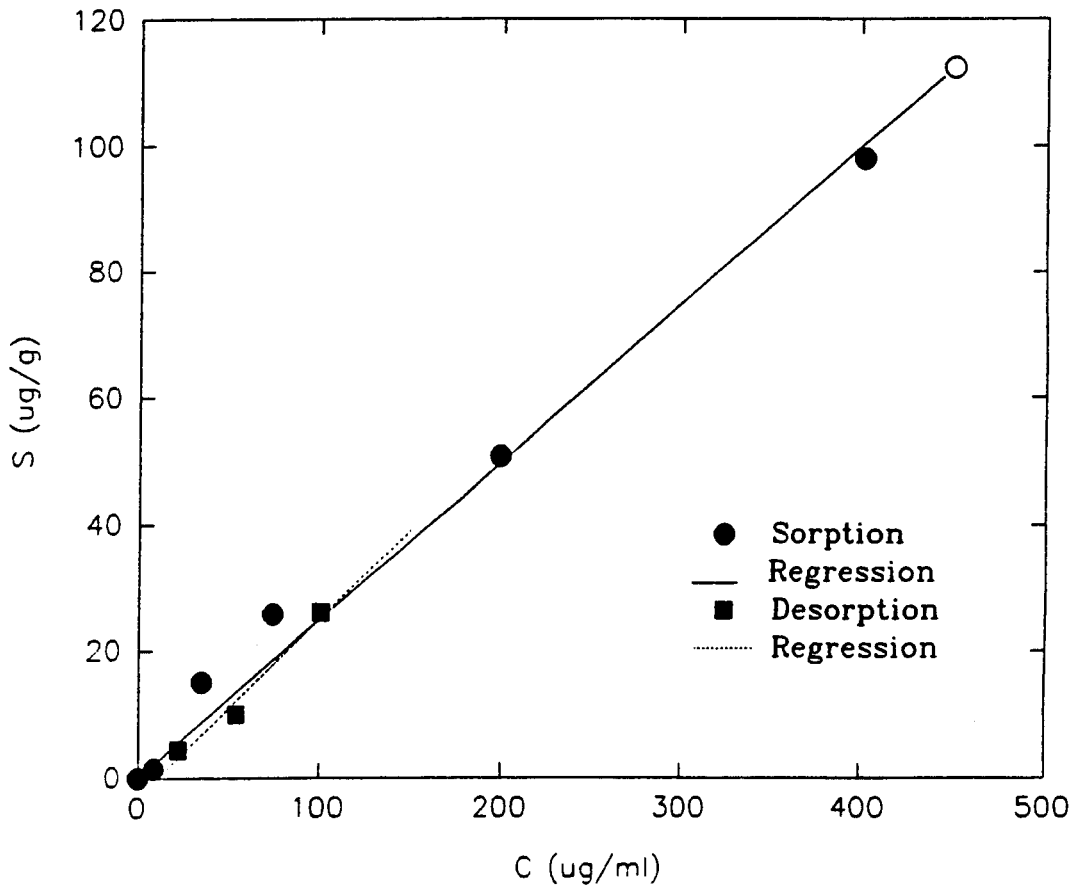


Figure 7. Singular 2,4-D sorption-desorption isotherms obtained by the "dilution-desorption" method. $K_d$ = 0.249 ml/g. The distribution partition coefficient is the same weather equilibrium is approached from an adsorptive or desorptive direction.

distribution or partition coefficient ($k_d$) is the same whether equilibrium is approached from an adsorptive or desorptive direction. That is, equal amounts of pesticide are retained on the soil during both desorption and adsorption phases.

For the system in question, repeated resuspension-centrifugation steps on the "batch" method did seem to be responsible for the apparent hysteresis effect.

**Column Experiments**

Saturated Conditions: Effluent breakthrough curves (BTC) were measured for $^{14}C$-[2,4-D] at an input concentration of 100 ug/ml and tritiated water ($^3H_2O$) under saturated sterile conditions using the miscible displacement technique (Brusseau et al, 1990).

Tritiated water was used to characterize the hydrodynamic properties of the column, that is, it represents a non-adsorbed solute that serves as a reference to compare adsorbed solutes (e.g. 2,4-D).

Sterile column studies were performed to account for all other but biological transformations.

The reproducibility of the miscible-displacement method can be evaluated by comparing the results of replicate experiments. These experiments represent the results of two different packed columns under different pore water velocities performed six months apart. The experimental data from both

experiments were analyzed by a model that regards sorption as a rate limited process (Selim et al., 1976) and the results are shown in Figures 8 and 9.

Backcalculating the partition coefficients (Kd) from the corresponding retardation factors (R), results in equal Kd values (Kd= 0.24). The retardation factor (R) is a function of the bulk density, thus, slight differences in column packing would result in small differences in the retardation factors. However, the equilibrium distribution between the solid and solution phase does not change.

The parameters from the first column, P=57, Kd=0.24, $\beta$=0.80 and $\omega$ (multiplied by the change in velocity for the second column) = 0.48, can be used to accurately predict the second experiment (Figure 10).

The Peclet number (P) obtained from the tritiated water BTC's can be used to calculate and compare dispersivity values. Given that

$$P=\frac{VL}{D} \qquad \text{and} \qquad D=\alpha V+D^*$$

where v is the average pore water velocity (L $T^{-1}$), L is the length of the column (L), D is the coefficient of hydrodynamic dispersion, $\alpha$ is dispersivity (L) and $D^*$ is the coefficient of molecular diffusion for solutes in the porous media ($L^2$ $T^{-1}$).

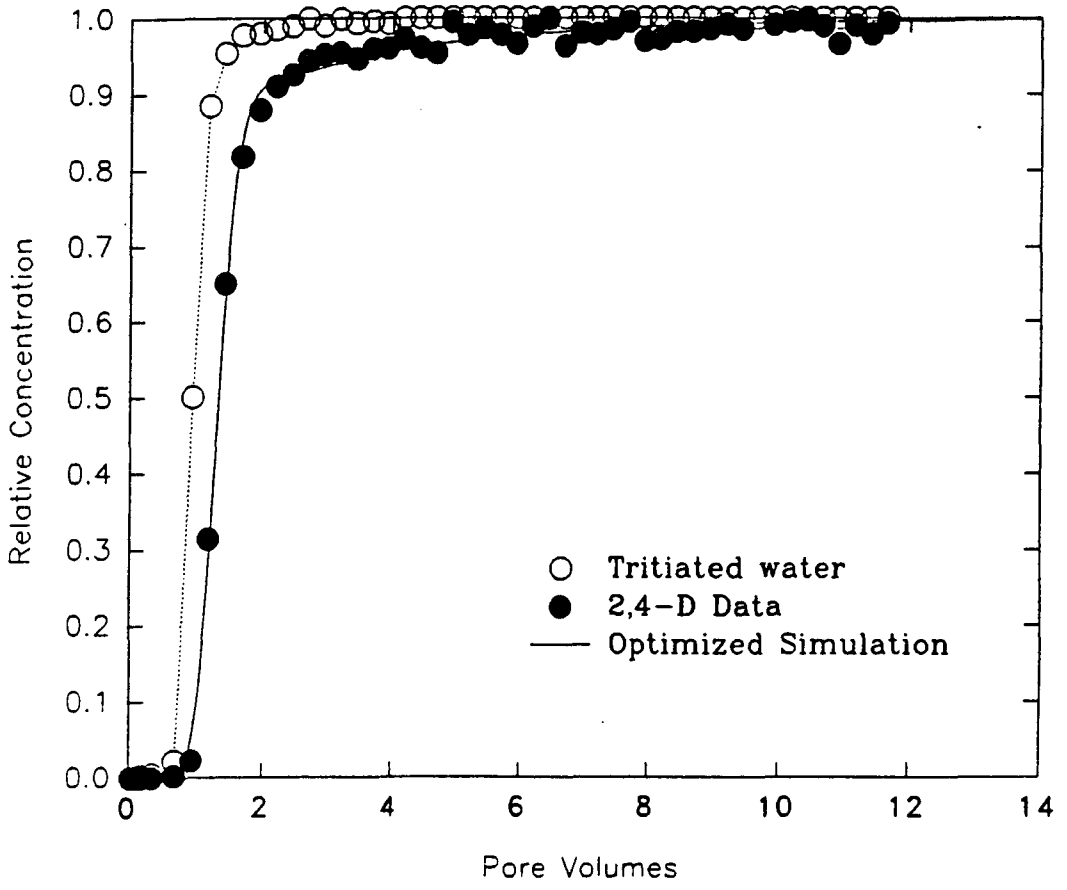


**Figure 8.** Breakthrough curves from saturated sterile experiments (no biodegradation) and the simulation obtained with transport model that includes rate-limited sorption. First experiment: v= 24.9 cm/h, $\rho_b$= 1.39 g/cm$^3$, $\theta_v$= 0.53, P= 57, R= 1.64, $\beta$= 0.80 and $\omega$= 0.12 .

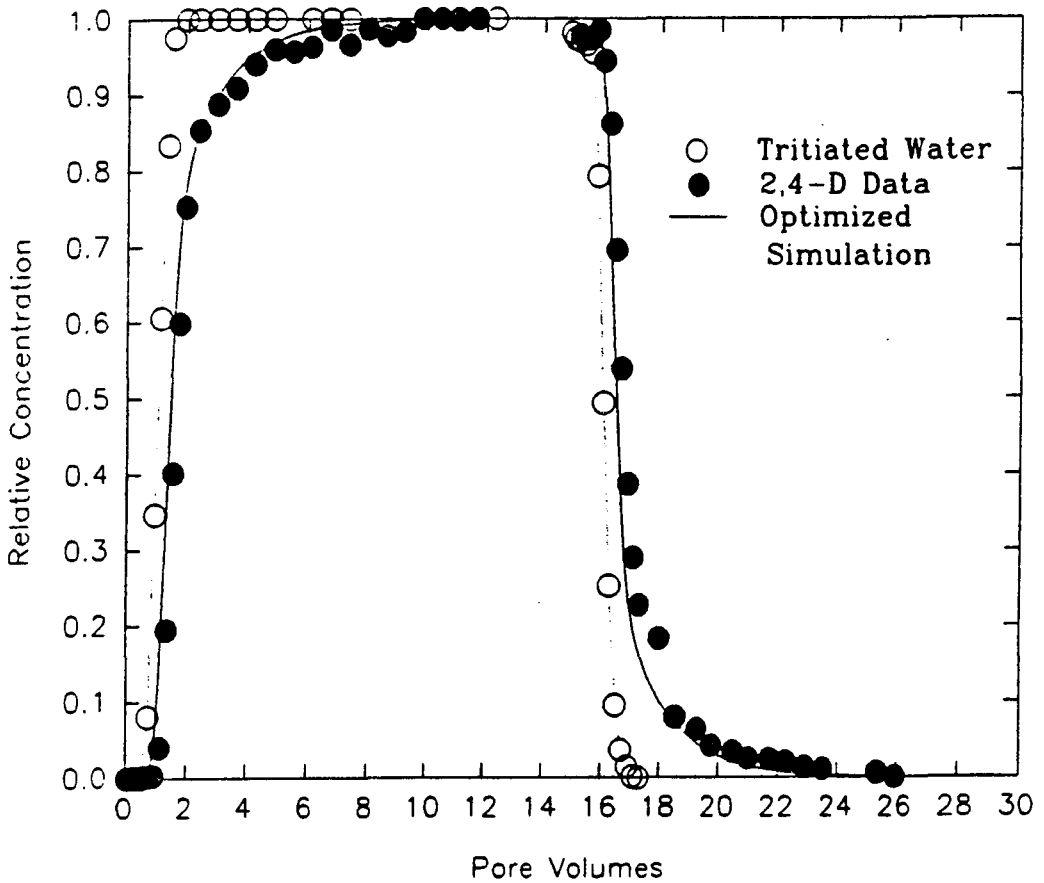


Figure 9. Breakthrough curves from saturated sterile experiments (no biodegradation) and the simulation obtained with transport model that includes rate-limited sorption. Second experiment: v= 6.16 cm/h, $\rho_b$= 1.59 g/cm$^3$, $\theta_v$= 0.47, P= 41, R= 1.80, $\beta$= 0.80 and $\omega$= 0.31 .

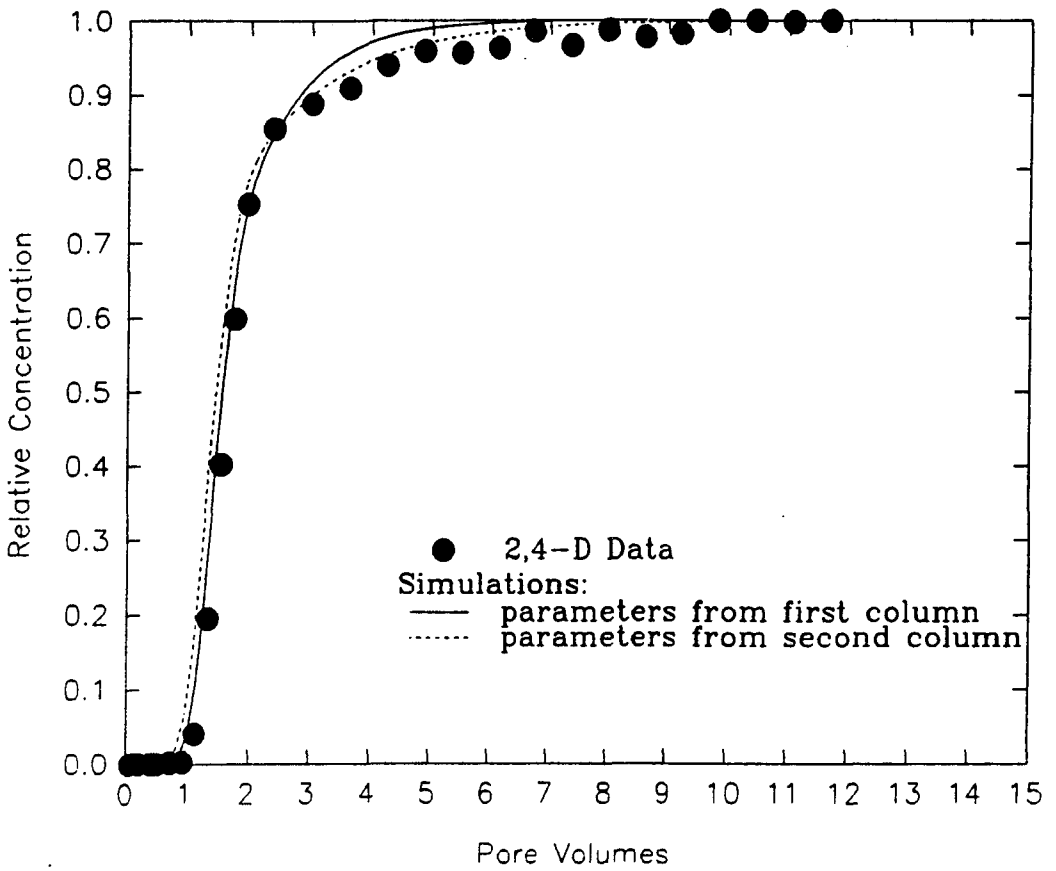


Figure 10. Predicted simulation of the second saturated sterile experiment (fig. 9 data) using the parameters from the first saturated sterile experiment (fig. 8 data). P= 57, $K_d$= 0.24, $\beta$= 0.80 and $\omega$ multiply by the change in velocity of second experiment =0.48 .

For tritiated water $D^*$ can be regarded as equal to zero given the sharp sigmoidal and symmetrical BTC's that reflect diffusion or dispersive flux being negligible. Calculated dispersivity values, $\alpha$ for the first column and the replicate are very similar, 0.12 and 0.09 respectively.

All of the above similarities suggest that dispersion, retardation and sorption kinetics are consistent between different experiments.

BTC's for tritiated water were symmetrical and sigmoidal in shape and independent of velocity. However, BTC's for 2,4-D were asymmetrical, exhibiting tailing (Figures 8 and 9). This implies that any observed non-equilibrium would probably be due to sorption related nonequilibrium (rate limited sorption) processes rather than to any transport related nonequilibrium (Brusseau, 1991; Angley et al., 1992)

Sorption rate constants ($k_2$) can be estimated from the Damkholer number ($\omega$)

$$\omega = \frac{k_2 \, l}{v} R(1-\beta)$$

The Damkholer number ($\omega$) is the ratio hydrodynamic residence time which by definition varies inversely with pore water velocity. The degree of sorption nonequilibrium however, varies directly with velocity (Brusseau, 1992). Sorption rate constants for the first sterile saturated transport experiment

(v=24.9 cm/h) was 1.8 $h^{-1}$ and for the second one (v=6.26 cm/h) was 1.06 $h^{-1}$.

Unsaturated Conditions: Tritiated water and $^{14}C$-[2,4-D] (100 ug/l) effluent breakthrough curves under unsaturated sterile conditions are shown in Figure 11.

Backcalculating the equilibrium partition coefficient from the estimated retardation factor (R= 3.4), results in a Kd= 0.566, which is more than twice the value obtained from the batch isotherm data and the saturated transport experiments. In the batch sorption studies performed, it was assumed that most of the sorption sites would be exposed by the gentle shaking and the slurry conditions and that the $K_d$ obtained would thus be a maximum. However studies have shown that the degree of adsorption is not always the same in slurry studies and in soil water systems with lower water content (Hance, 1977; Bailey and White, 1964). Adsorption of some compounds has been found to be greater as soil moisture decreases. Some possible explanations are that water held at greater tensions may be ineffective or unavailable for equilibration (Green and Obien, 1969; Lambert et al., 1965) or that water and solute might compete for adsorption sites (Yaron and Saltzman, 1972; Hance, 1965).

The sorption rate constant ($k_2$) for this unsaturated sterile transport experiment (v=0.72 cm/h) was 0.01 $h^{-1}$ which

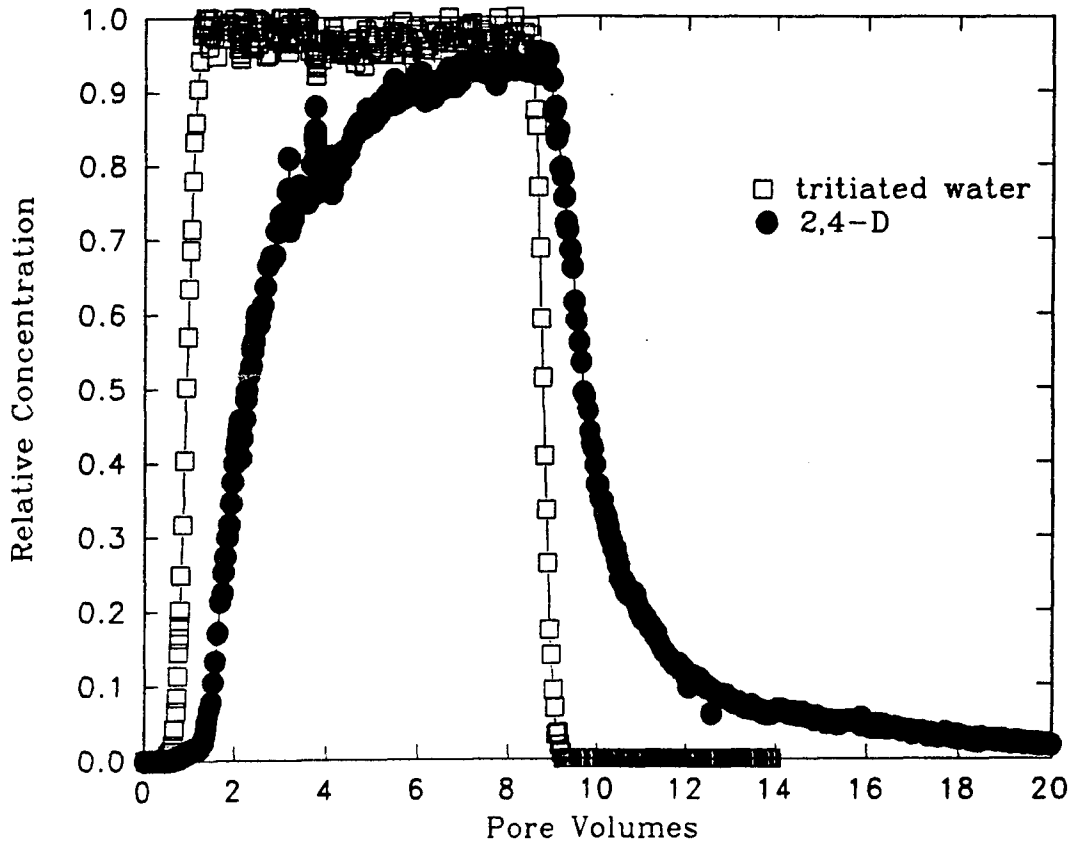


Figure 11. Breakthrough curves from tritiated water and $^{14}$C-[2,4-D] from the unsaturated sterile soil experiment. v=17.41 cm/d, $\rho_b$= 1.25 g/cm$^3$, $\theta_v$= 0.295, R= 3.4, P= 64, R= 3.4, $\beta$= 0.64 $\omega$= 0.47

is two orders of magnitude lower than the $K_2$ from the saturated column experiment.

## Biodegradation Studies

Biodegradation of 2,4-D was studied by conducting a series of soil batch and column experiments under both saturated and unsaturated conditions.

### Batch Experiments

The degradation of 2,4-D was determined by measuring $^{14}CO_2$ evolved from uniformly ring labeled material and by measuring the rate of disappearance of the organic solvent-extractable parent compound. When 2,4-D degradation is determined by measuring the evolution of $^{14}CO_2$ from uniformly ring labelled material, it can be assumed that 2,4-D degrades to its final oxidation products, that is $CO_2$, $H_2O$ and $Cl^-$ (Ou et al, 1978).

Mineralization occurred rapidly under both saturated and unsaturated conditions (Figure 12). After 7 days, 100 % of the parent compound had been mineralized. By the end of the incubation period (26 days), a total of 36 and 52 % of the 2,4-D had evolved as $CO_2$ for unsaturated and saturated conditions, respectively. This difference can be explained by taking into account the different moisture conditions.

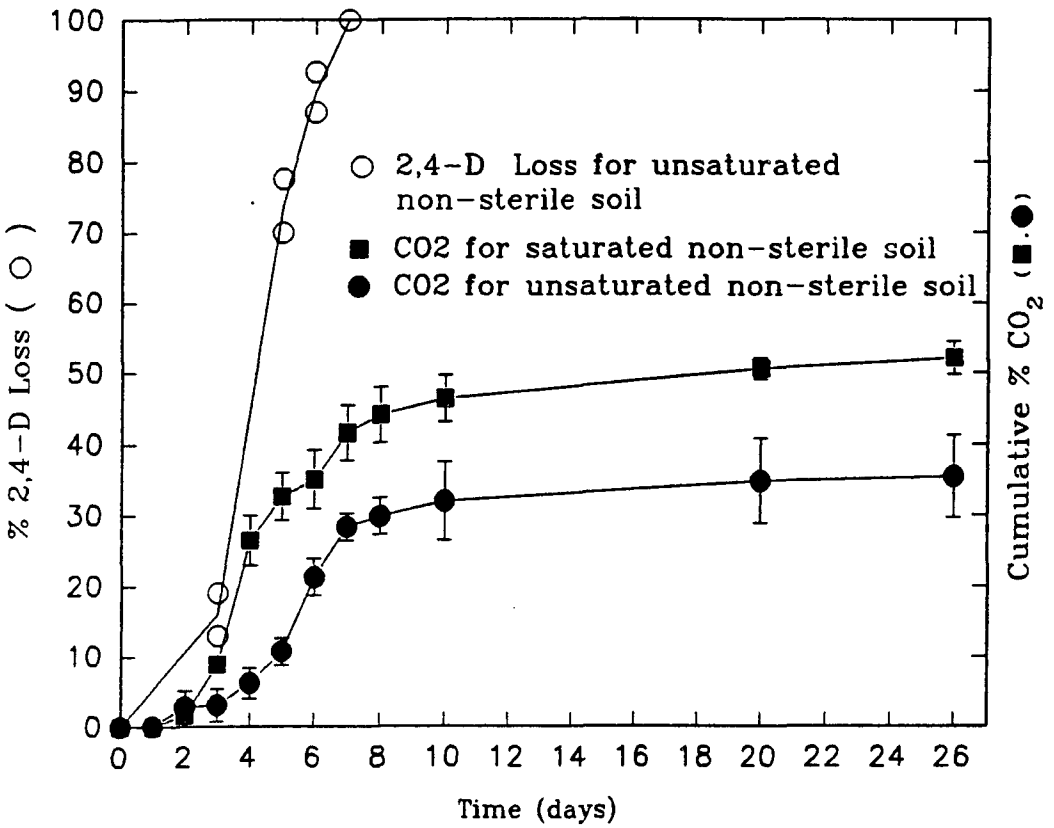


Figure 12. Mineralization of 2,4-D by measuring $^{14}CO_2$ evolved from uniformly ring labelled material and by measuring the rate of loss of the solvent-extractable parent compound under saturated and unsaturated non-sterile conditions.

Moisture content controls the microbial metabolic activities by regulating the oxygen levels in the soil. At high soil water content, close to saturation, pesticide degradation rates and efficiency is determined by the relative rates of decomposition under aerobic vs. anaerobic conditions. (Rao and Davidson, 1980; Yaron, 1985). Under saturated soil conditions oxygen is rapidly depleted by microbial activity and availability is regulated by diffusion of $O_2$ through soil and the soil atmosphere interfacial area. As shown in Table 3 , when molecular oxygen is used as the terminal electron acceptor (aerobic respiration), the free energy yield ($\Delta F$) is at least twice as much as under conditions of anaerobic respiration. Thus, facultative microorganisms generate more ATP, from the oxidation of 2,4-D under aerobic conditions and will exhibit a larger cell yield and less $CO_2$ evolution than under anaerobic conditions. Therefore if a partially anaerobic environment developed under the saturated batch biodegradation experiment conditions, then, less cell mass and more $CO_2$ would indeed be expected as shown in Figure 12.

Degradation of 2,4-D has generally been reported to be characterized by a lag period before biodegradation begins (Pfarl et al, 1990; Ou et al, 1978) and subsequent additions of 2,4-D had been reported to disappear rapidly without the lag phase (Audus, 1960; Torstensson, 1988; Sinton et al.,

1986). The lag phase is defined as the time interval during which biodegradation is not detected. The lag phase can be attributed to any one or a combination of (1) enzyme induction (2) random mutation or (3) an increase in the number of organisms in the degrading population (Aelion et al., 1987).

The Mexican sandy loam soil used in these experiments has a history of 2,4-D treatment suggesting that acclimation was most likely due to increasing the population rather than enzyme induction or random mutation. To confirm this (Figure 13), when the nonsterile soil was spiked with 2,4-D 4 weeks prior to experiment, the lag phase disappeared. To estimate the difference in the degrading population numbers, the program T2 was used to simulate the data in Figure 13 by fitting initial cell mass concentration $X_o$. The curve fitting results and the values for the kinetic coefficients are shown in Figures 14 and 15. $X_o$, cell mass (mg/l), was almost 3.4 times greater for the spiked soil than for the non-sterile soil, suggesting that probably the disappearance of the lag phase was indeed due to a build up of the degrading population. Repeated 2,4-D applications do seem to increase the number of the 2,4-D degraders.

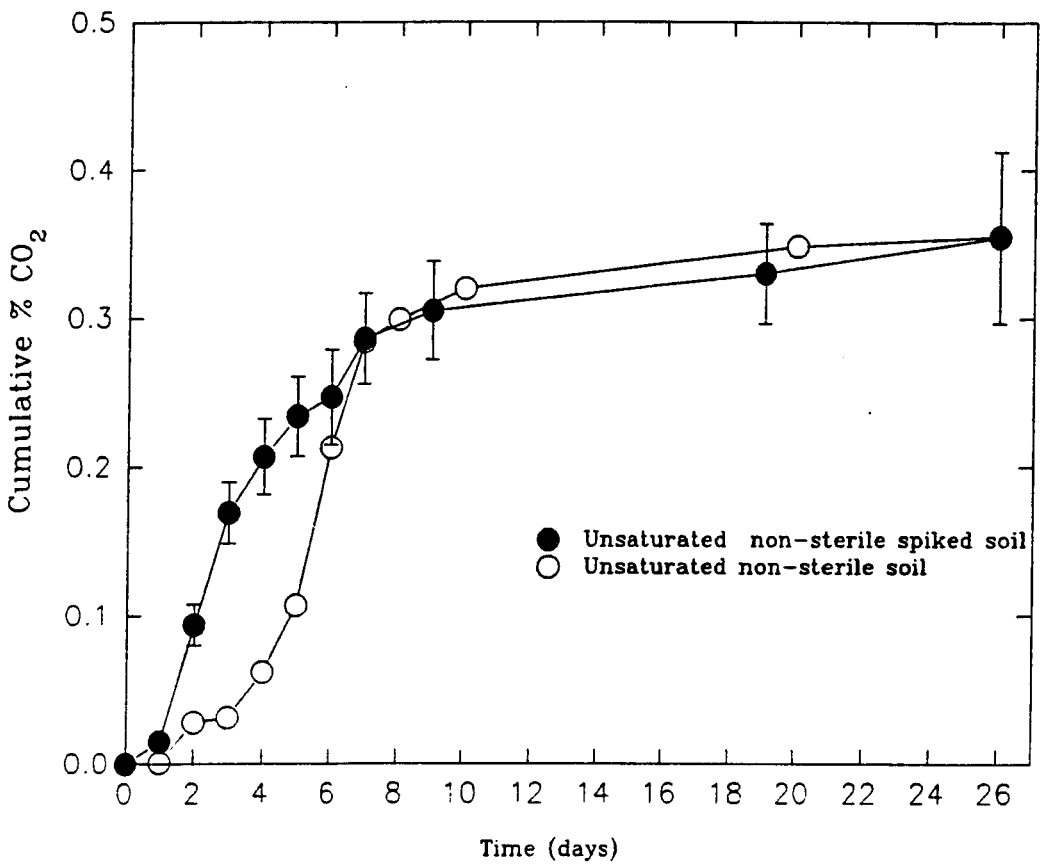


Figure 13. Pattern of 2,4-D mineralization by measuring $^{14}CO_2$ evolved from uniformly ring labelled material for the unsaturated non-sterile and non-sterile spiked soil treatments.

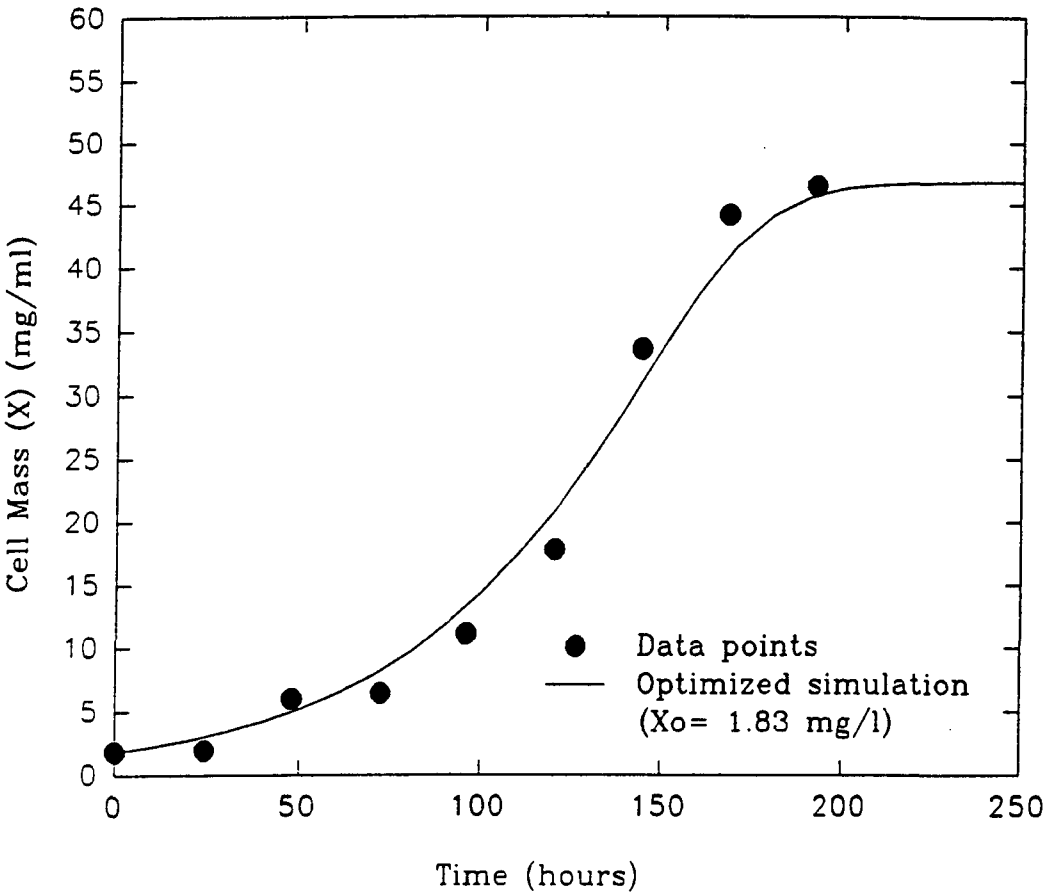


Figure 14. Cell mass vs time batch experimental data from the unsaturated non-sterile soil and predicted simulation obtained with T2. $X_o$= 1.83 mg/l, $S_o$= 100 mg/l, $\mu_m$= 0.028, $Y_d$= 0.45, $K_s$= 32.2 and $K_i$= 100,000 .

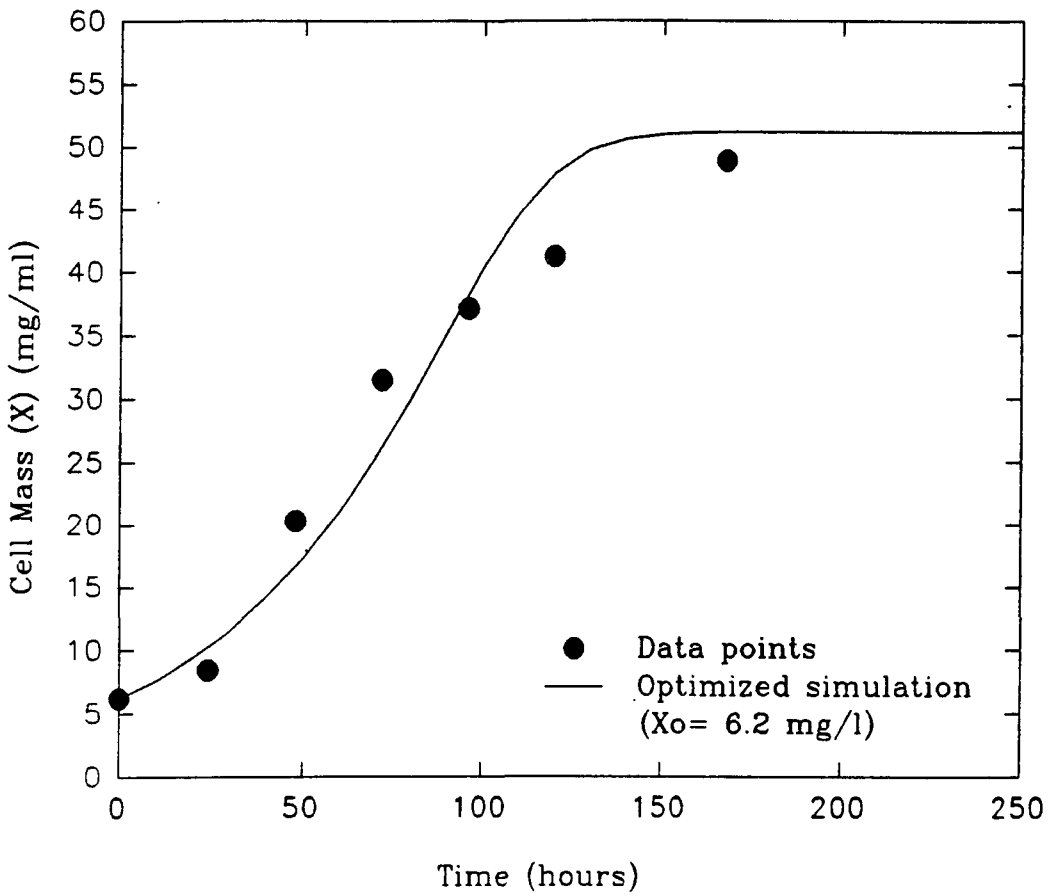


**Figure 15. Cell mass vs time batch experimental data from the unsaturated non-sterile spiked soil and predicted simulation obtained with T2. $X_o$= 6.2 mg/l, $S_o$= 100 mg/l, $\mu_m$= 0.028, $Y_d$= 0.45, $K_s$= 32.2 and $K_i$= 100,000 .**

**Column Experiments**

Saturated conditions: A number of effluent breakthrough curves (BTC's) measured for $^{14}C$-2,4-D at an input concentration of 100 ug/ml and tritiated water under saturated conditions with the non-sterile and non-sterile spiked soils were generated using the miscible displacement technique (Brusseau et al, 1990). All these experiments where biodegradation occurred, exhibited a plateau in the effluent solution concentration characteristic of steady-state behavior (Angley et al., 1992). Simultaneous sorption and degradation data were analyzed using the coupled process transport model (Van Genuchten and Wagenet, 1989) that includes rate limited sorption and first order degradation.

First order degradation assumes that at constant biomass (insignificant growth) and limited substrate levels, the rate of degradation is directly proportional to the concentration of the substrate. Given the time frame of the saturated experiments (maximum of 24 hours), these assumptions seem to be valid and the simulations by the model did provide a close fit to the experimental data.

Rate limited sorption refers to the two site sorption concept. Exchange sites are considered as either sites of instantaneous sorption or sites in which sorption is rate limited or time dependent.

It might be useful to remember that "non-sterile" refers to the 80:20 (g/g) mixture of the Mexican sandy loam soil and the Mount Lemmon soil and that the Mexican sandy loam had a history of repeated 2,4-D applications. "Non-sterile spiked" refers to the same 80:20 mixture soil, which was treated twice with 100 ppm 2,4-D (two and four weeks prior to the experiments).

The breakthrough curve obtained using the non-sterile soil presented a plateau at a relative concentration of 0.96 Figure 16 presents the experimental data and the model simulation ($\xi$= 0.04) . To calculate the first order biodegradation rate constants ($\mu$), the following formula (Angley et al., 1992) can be used:

$$\xi = \frac{\mu l}{v} = -\ln C^* \qquad (22)$$

where $\xi$ is the dimensionless degradation term from the model, l is the length of the column, v is the average pore water velocity , $\mu$ is the first order biodegradation rate constant, and $C^*$ is the steady-state relative effluent concentration. $\mu$ for the saturated non-sterile soil was equal to 0.904 $d^{-1}$.

To evaluate once more the reproduciblility of the method, a replicate experiment was performed and the effluent samples

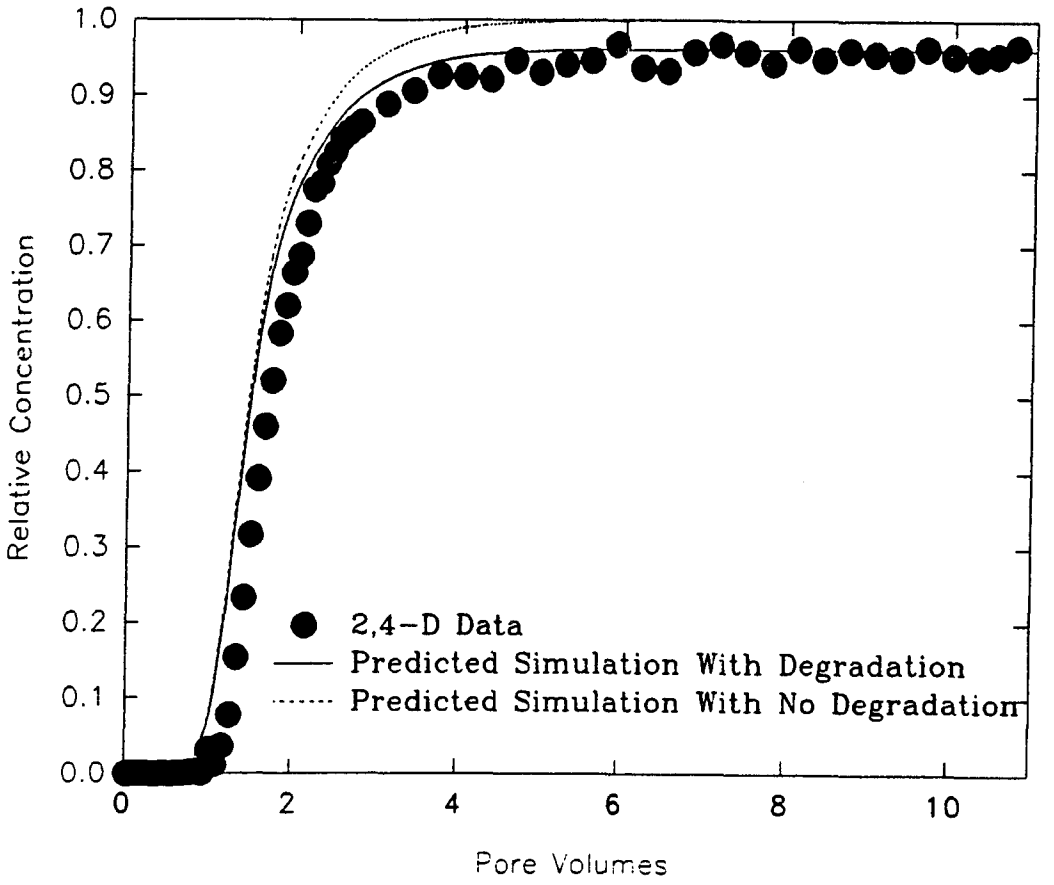


Figure 16. Breakthrough curve from saturated non-sterile soil experiment and the simulation obtained with transport model that includes rate-limited sorption and first order degradation. v= 4.71 cm/h, $\rho_b$= 1.37 g/cm$^3$, $\theta_v$= 0.52, P= 57, R= 1.64, $\beta$= 0.80, $\omega$= 0.62 and $\xi$= 0.04.

were analyzed by HPLC. Results of both experiments are compared in Figure 17.

Experiments with the non-sterile spiked soil presented the plateau at a relative concentration of 0.89 . Figure 18 shows the results of the experimental data and the modeling simulation ($\xi$=0.11). The first order rate constant ($\mu$) was in this case 3.23 $d^{-1}$.

From modeling the batch biodegradation experiments, it was predicted that the initial biomass concentration ($X_o$) of the non-sterile spiked soil was 3.4 times higher than of the non-sterile soil. This greater degrading population would in turn result in a higher rate constant. The difference in biodegradation rate constants (3.57 fold) might very well be the result of the similar difference in $X_o$. It might be possible to correlate these differences by using the relationship of substrate disappearance with time for Monod and first order mineralization kinetics

$$-\frac{\partial S}{\partial t} = \frac{\mu_{max} X_o}{Y K_s} S \equiv \mu S$$

If $\mu_{max}$, Y, and $K_s$ are constant between the two experiments, then, the change in $\mu$ (first order rate constant) would indeed be due to increments in the initial cell mass ($X_o$).

Under the conditions of the saturated column experiments, oxygen levels were strictly regulated by the amounts of oxygen

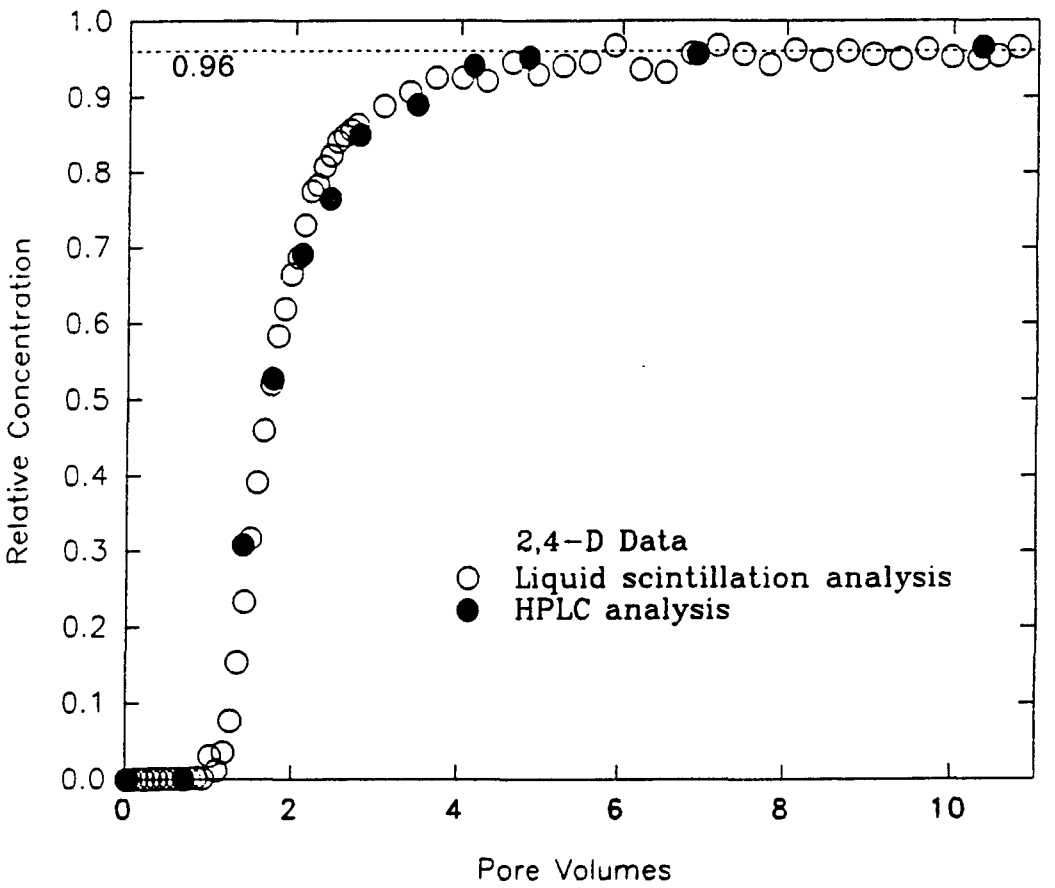


**Figure 17. Breakthrough curve from saturated non-sterile soil experiment (fig. 16) and replicate experiment manalysed by HPLC (v= 4.71 cm/h, $\rho b$= 1.37 g/cm$^3$, $\theta_v$= 0.52).**

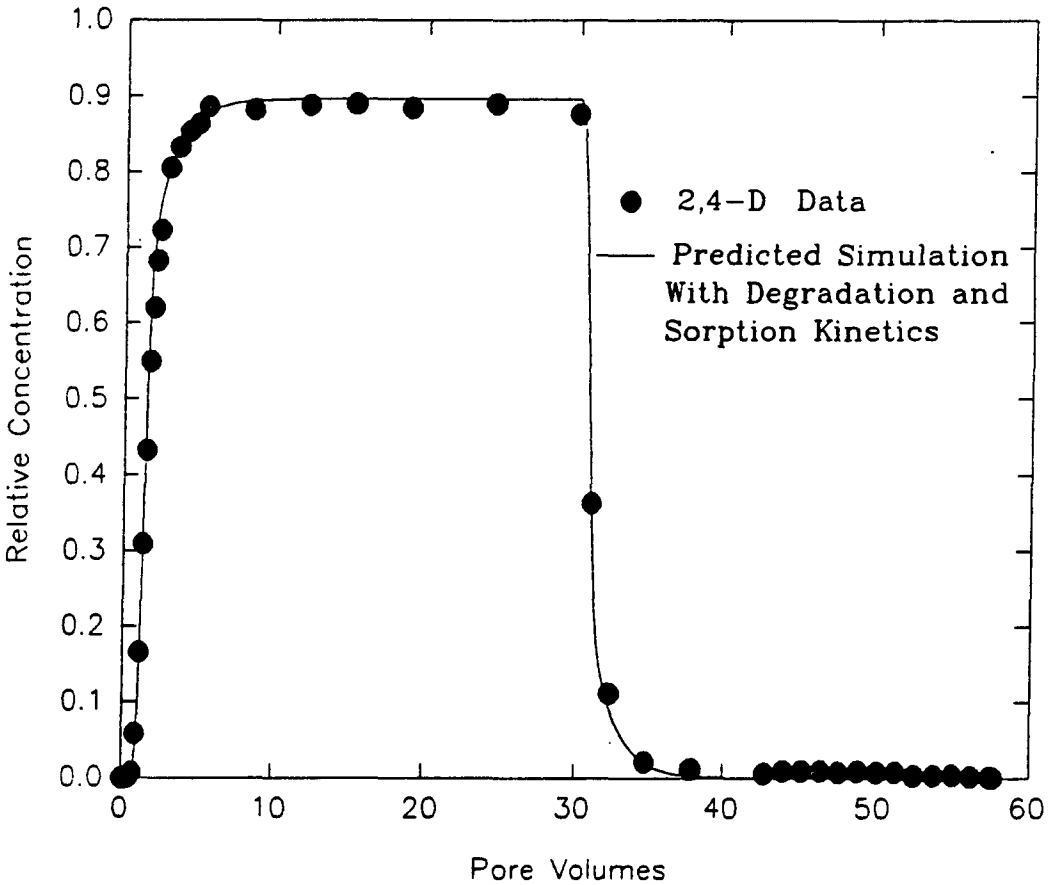


**Figure 18. Breakthrough curve from saturated non-sterile spiked soil experiment and the simulation obtained with transport model that includes rate-limited sorption and first order degradation. v= 6.12 cm/h, $\rho_b$= 1.55 g/cm$^3$, $\theta_v$= 0.48, P= 32.3, R= 1.80, $\beta$= 0.80, $\omega$= 0.30 and $\xi$= 0.11.**

dissolved on the input solution (8-10 mg/l). Thus aerobic biodegradation of 2,4-D for the saturated column experiments was limited to just that given amount supplied during the length of the experiments (maximum of 24 h.). Given that the non-sterile spiked soil was acclimated under unsaturated conditions, any significant contribution of facultative anaerobe microorganisms to the rate of 2,4-D biodegradation during the length of the experiment if possible, is not likely. However, given the time frame (26 days) and aeration conditions of the saturated batch biodegradation experiments, the 2,4-D degradation rate was very likely determined by the relative rates of decomposition of both aerobic and anaerobic conditions.

Figure 18a shows the effect of assuming sorption as an instantaneous process and not considering sorption kinetics. This simulation fails to predict the shape of the BTC, suggesting that indeed sorption kinetics is responsible for the non equilibrium observed.

The breakthrough curves for these two biodegradation experiments (Figures 16 and 18) are compared in Figure 19 to an experiment with a sterile soil. Breakthrough curves from non-sterile and spiked soil experiments are moved to the right of the BTC from the experiment were biodegradation did not occur (sterile soil). However the equilibrium distribution between the solution and the solid phase, that is, the

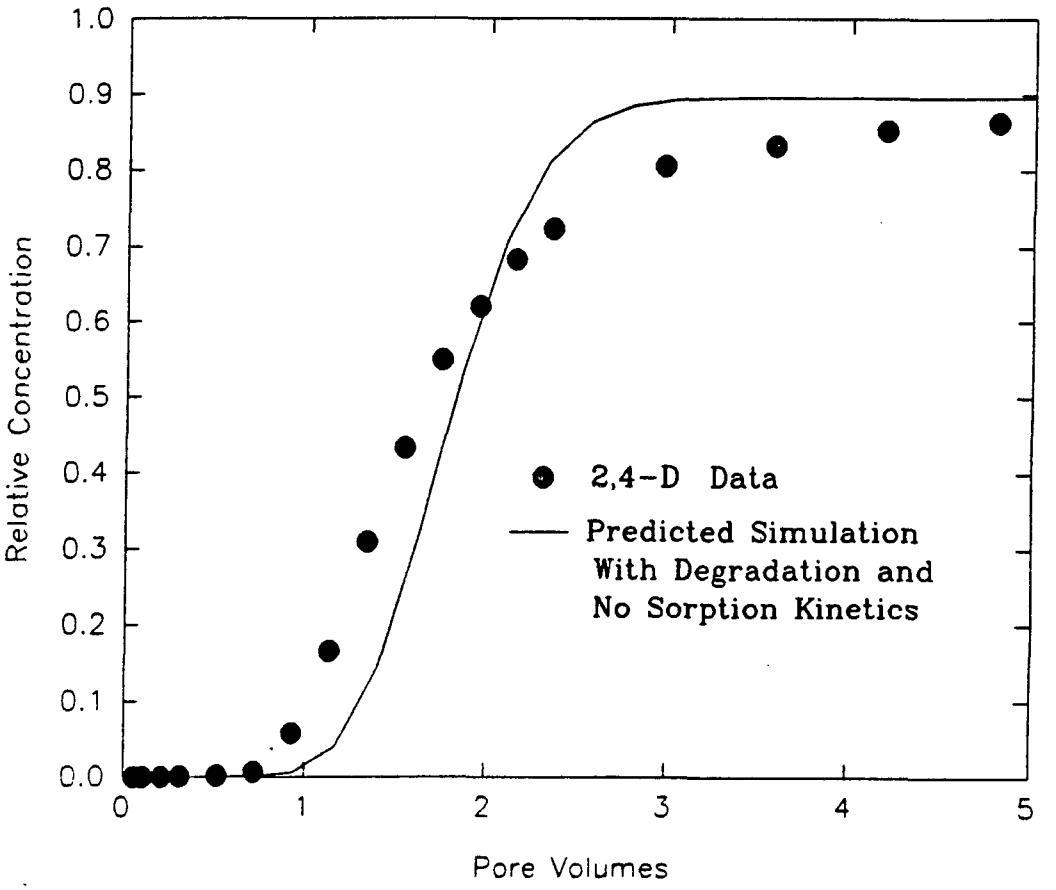


Figure 18a. Breakthrough curve from saturated non-sterile spiked soil experiment and the simulation obtained with transport model that includes no kinetics and first order degradation. v= 6.12 cm/h, $\rho_b$= 1.55 g/cm$^3$, $\theta_v$= 0.48, P= 32.3, R=1.80, $\beta$= 0.80, $\omega$= 99 and $\xi$= 0.11 .

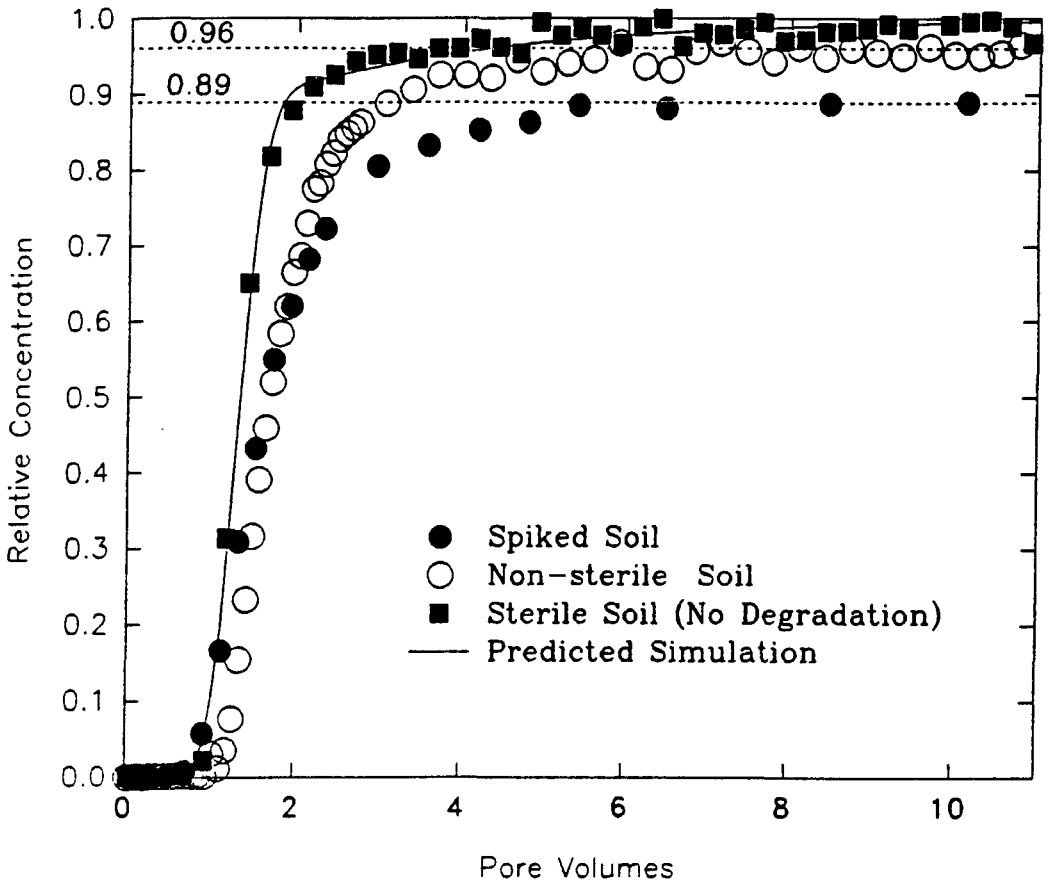


Figure 19. 2,4-D breakthrough curves from saturated sterile, non-sterile and non-sterile spiked soil experiments (data from figures 8, 16 and 18).

equilibrium partition coefficients, are the same for sterile and non sterile conditions (Table 6 ) (Angley et al., 1992). The batch sorption equilibrium partition coefficient ($K_d$= 0.249) was also consistent to the saturated biodegradation column studies ($K_d$= 0.245)

To analyze the effects of pore water velocity on the biodegradation experiments, non-sterile spiked soil, with a higher degrading population was used so that differences in degradation rate constants, if any, would be exaggerated. Consecutive experiments with imposed different velocity were performed on the same packed column (Figure 20) as well as on other columns (Figure 21). Results are in agreement with past observations. Increasing pore water velocity usually creates a leftward shift of the breakthrough curves (Brusseau, 1991; Lee et al., 1988; Bouchard et al., 1988). However, it was also expected to see the plateaus changing with velocity since the substrate residence time decreases with increasing velocity (Angley et al., 1992).

Neither plateau nor leftward shift were very significant in either Figures 20 or 21. In the column where consecutive experiments were performed (Figure 20), it can be seen that the first and third experiments were performed at the same velocity, yet breakthrough curves were not the same. One explanation could be that further acclimation took place

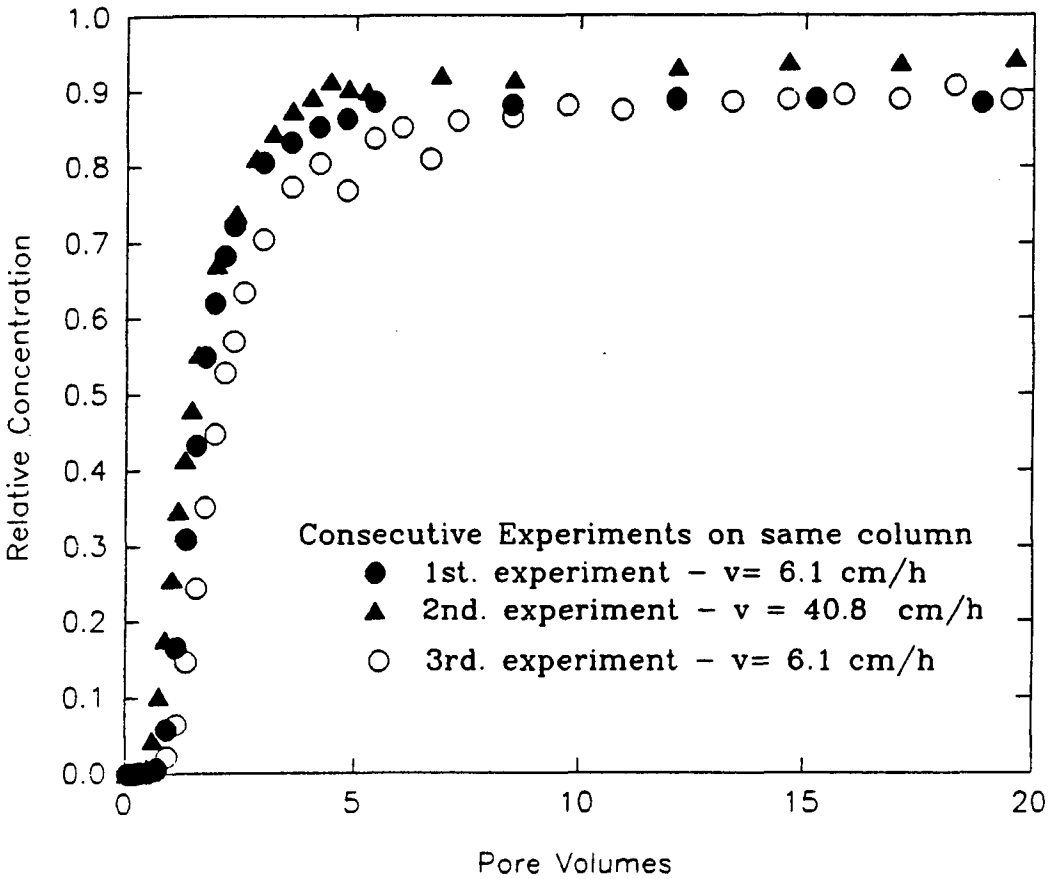


**Figure 20. 2,4-D breakthrough curves from consecutive saturated non-sterile spiked soil experiments (same paked columns).**

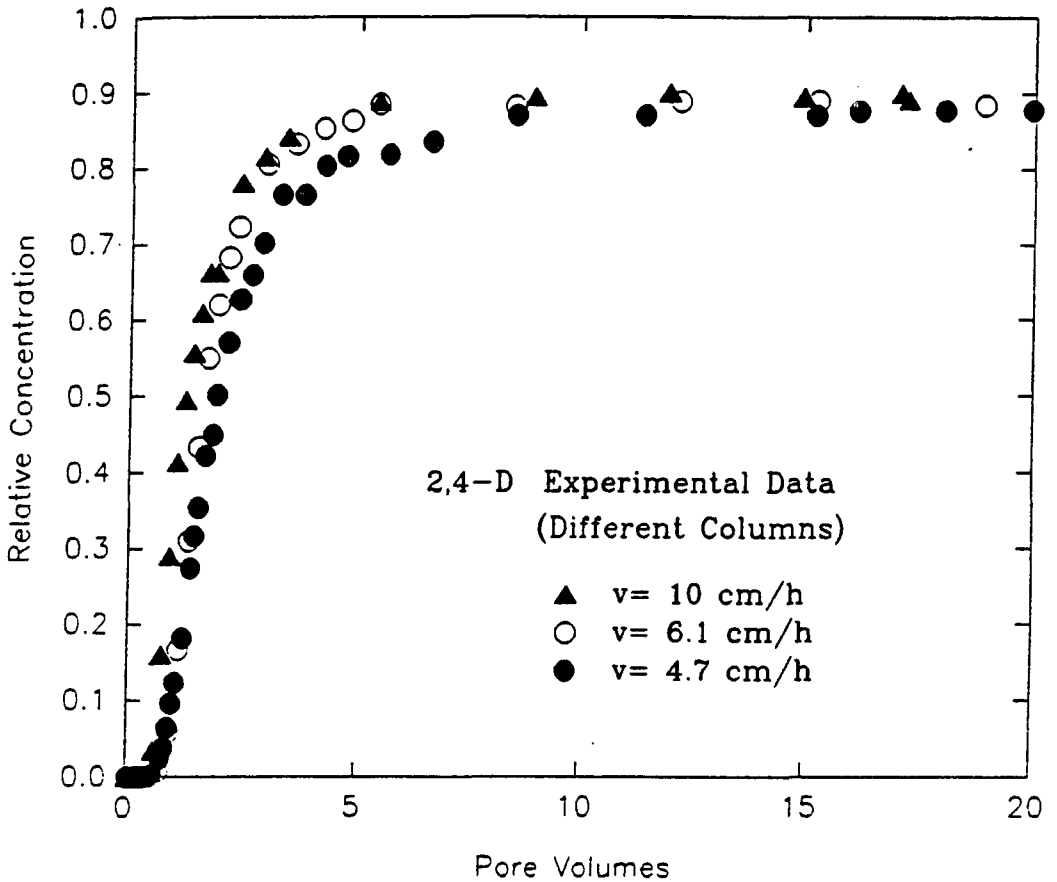


Figure 21. 2,4-D breakthrough curves from saturated non-sterile spiked soil experiments (different columns).

between the first and third run resulting in a larger degrading population. However, if this was the case, then, in addition to the later breakthrough, the plateau should have been established at an even lower relative concentration except if oxygen was a liming factor. Since the plateau remained constant, it was postulated that oxygen might be a limiting factor. Therefore, the dissolved oxygen concentration of the effluent solution was measured for the experiments performed at 6.1 and 4.7 cm/h on Figure 21. Typical oxygen concentration in aqueous solution is in the order of 8-10 mg/l. Oxygen in the effluent solution was indeed found to be limiting with a concentration of $\leq$ 1 mg/l. Since oxygen was limiting, the plateau of these curves are higher than expected and rates of degradation may be lower (smaller rate constants) than under aerobic conditions.

Unsaturated Conditions: Tritiated water and $^{14}C$-[2,4-D] (100 ug/ml) breakthrough curves from the unsaturated non-sterile column experiment are shown in Figure 22.

The soil moisture tension was maintained at -30 mbars (approximately $\theta_v$= 29%) by adjusting the pressure on the vacuum chamber (refer to Figure 5). The porosity was approximately 52%, thus, only 56% of the porosity was filled with solution insuring that air was being interchanged

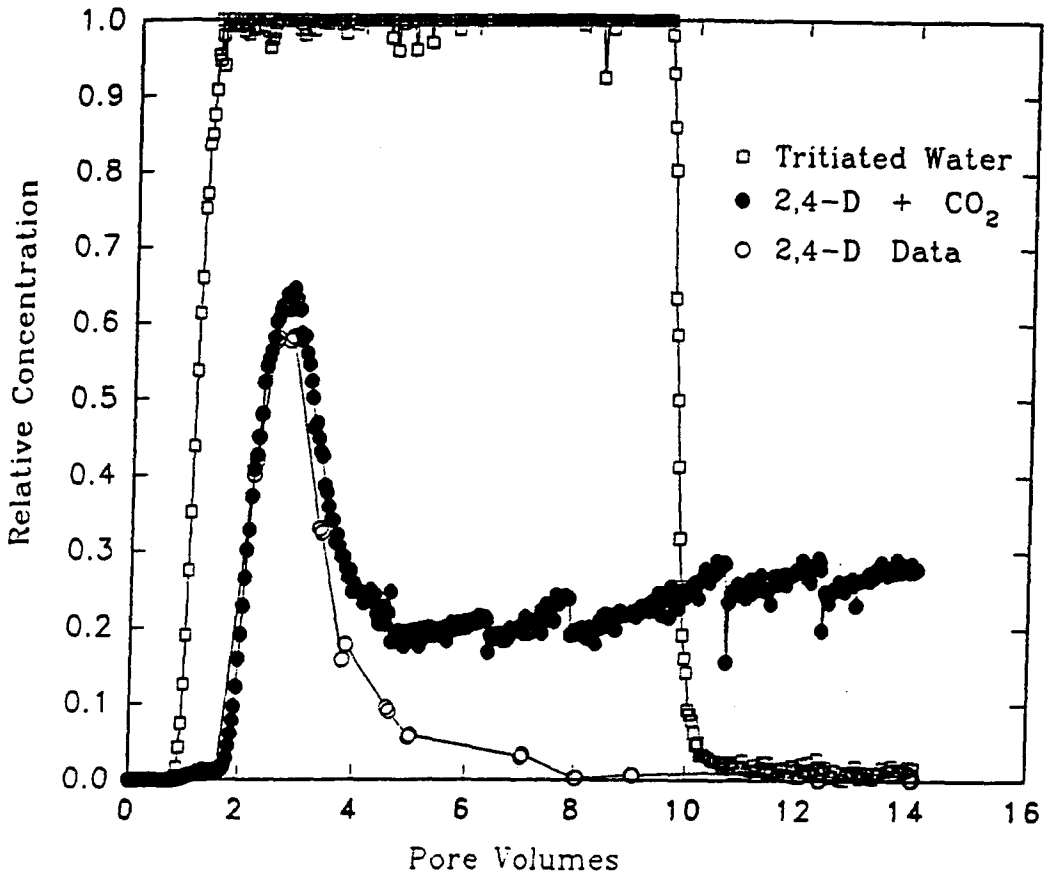


Figure 22. Breakthrough curves from tritiated water and $^{14}C$-[2,4-D] from the unsaturated non-sterile soil experiment. v= 17.41 cm/d, $\rho_b$= 1.25 g/cm$^3$, $\theta_v$=0.29, R=3.4, $\beta$= 0.64 , $\omega$= 0.47.

continuously within the column. As can be seen from Figure 22, the relative concentration of 2,4-D in the effluent solution initially increased in the column until approximately 3 pore volumes after which it started to decrease until C/Co=0 at 8 pore volumes. The initial increase in the 2,4-D effluent concentration was probably due to a low 2,4-D degrading population and as the population increased, the effluent concentration started decreasing. The lag time of approx. 2.75 PV (4.3 days) is consistent with batch biodegradation results which showed a lag of approx. 3-4 days (Figure 13).

The 2,4-D biodegradation data from Figure 22 were fitted with a mathematical model of substrate transport and biomass growth by Dr. Robert S. Maier (AHPCRC, University of Minnesota). This model was used to determine whether or not it could provide a significantly better prediction of the observed experimental data than a standard linear model of substrate transport with first order decay. The simulation results are shown in Figure 22a. The model used does provides a significantly better description of the data ($r^2$= 0.99) than a model of substrate transport with first order decay ($r^2$= 0.61).

At an average pore water velocity of 17.41 cm/day, the residence time of one molecule of 2,4-D would be of at least 1.58 days (1 PV). At 8 PV (12.65 days) steady state biodegradation was reached in the column and all of the 2,4-D

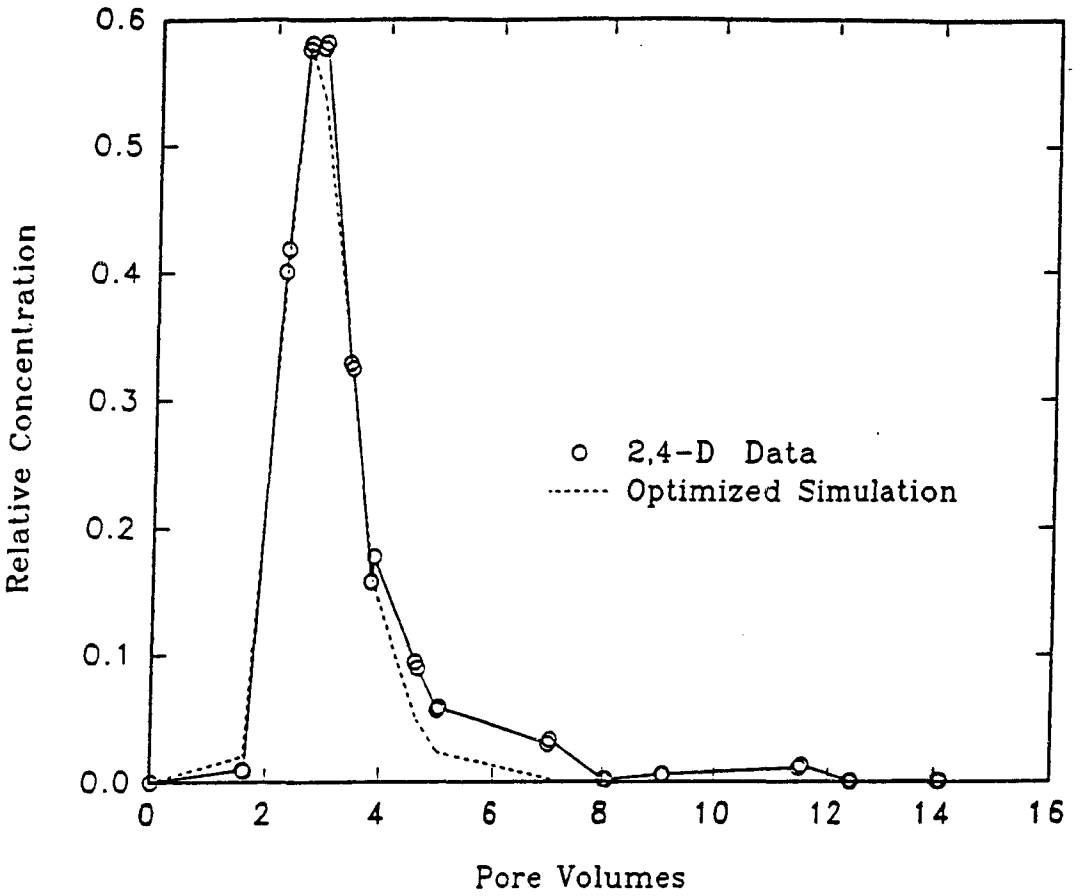


Figure 22a. Observed and model substrate effluent concentration vs. time for unsaturated non-sterile soil experiment. Solid line denotes measured effluent concentration, dotted line denotes approximation using fitted parameters. v= 17.41 cm/d, $\rho_b$= 1.25 g/cm$^3$, $\theta_v$= 0.29 . Fitted parameters are Y= 0.116, $K_s$= 287, R= 2.25. Fixed parameters are $\mu_{max}$=0.15, D=0.23, $X_{init}$= 1.8 .

being applied at that point was being completely mineralized in 1.58 days or less.

To estimate the degradation rate constant it was assumed that at 10 half lives ($t_{1/2}$) all the compound is degraded. Then, for the 2,4-D under the conditions of the unsaturated soil column, 1 $t_{1/2}$ would be equal to 0.158 days and from

$$\mu = \frac{\ln 2}{t_{1/2}}$$

the biodegradation rate constant, $\mu$, under the unsaturated non-sterile transport experimental conditions would be equal to 4.39 d-1.

**Table 6. Adsorption partition coefficients and biodegradation rate constants.**

| | $K_d$ (ml/g) | $\mu$ ($d^{-1}$) |
|---|---|---|
| SORPTION STUDIES | | |
| BATCH (Saturated sterile soil) | | |
| "Dilution-desorption" method | .249 | ----- |
| COLUMN | | |
| Saturated sterile soil | .244 | ----- |
| Unsaturated sterile soil | .566 | ----- |
| BIODEGRADATION STUDIES | | |
| BATCH | ----- | .198 |
| Saturated non-sterile soil | ----- | .198 |
| Unsaturated non-sterile soil | ----- | .198 |
| Unsaturated spiked soil | | |
| COLUMN | | |
| Saturated non-sterile soil | .243 | .904 |
| Saturated spiked soil | .247 | 3.23 |
| Unsaturated non-sterile soil | .566 | 4.39 |

# SUMMARY AND CONCLUSIONS

## Sorption Studies

### Batch Experiments

The "dilution-desorption" method avoided non-singular adsorption-desorption isotherms. This method eliminates repeating the resuspension-centrifugation steps used in the "batch" method (Figures 6 and 7). The adsorption partition coefficient found with the "dilution-desorption" method was $K_d$= 0.249.

### Column Experiments

The miscible-displacement method used for the saturated column experiments showed a high degree of reproducibility. Dispersion and retardation results were consistent between replicate experiments (Figures 8, 9 and 10). The adsorption partition coefficients obtained from the saturated sterile column experiments ($K_d$= 0.244) were very similar to the kd for the batch experiments.

The adsorption partition coefficient for the unsaturated column experiment (Kd= 0.566) was more than twice that obtained from batch isotherm (Kd= 0.249) data and saturated transport experiments ($K_d$= 0.244). This agrees with past studies which have shown that the degree of adsorption might not be the same in soil water systems under different moisture tensions (Hance, 1977: Bailey and White, 1964).

## Biodegradation Studies

### Batch Experiments

Mineralization occurred rapidly under both saturated and unsaturated conditions (Figure 12). The difference in $CO_2$ evolved can be explained by taking into account the different moisture content conditions; facultative anaerobe microorganisms generate more ATP from the oxidation of 2,4-D under aerobic than anaerobic conditions (higher efficiency), thus less cell mass and more $CO_2$ would indeed be expected.

The lag phase for 2,4-D mineralization disappears after repeated applications (Figure 13). The program T2 estimated an initial cell mass difference of 3.4 fold between the non-sterile soil and the non-sterile spiked soil (Figures 14 and 15) suggesting that the disappearance of the lag phase was due to a build up of the degrading population on the non-sterile spiked soil.

### Column Experiments

The first order biodegradation rate constant ($\mu$) from the saturated column experiments with the non-sterile spiked soil was 3.23 $d^{-1}$ and for the non-sterile soil was .904 $d^{-1}$. The 3.57 fold difference in $\mu$ between non-sterile spiked and non sterile soil was probably due to the initial cell mass difference between the soils. The biodegradation rate constants for the saturated column experiments however, might

have been underestimated since it was shown that these columns were operated under oxygen limiting conditions. The first order biodegradation rate constant for the unsaturated column experiment was estimated to be 4.39 $d^{-1}$.

In general, it can be seen (Table 6) that there is 1 order of magnitude difference between the first order biodegradation rate constant of batch and column experiments. That difference might be due to 2,4-D mass transfer limitations in the static conditions of the batch studies. Angley et al. (1992) found that the rate constant for their batch biodegradation experiments were at least one order of magnitude smaller than those obtained from column experiments. They attributed the discrepancy to the difference in aeration and mixing in the batch and column systems. In addition, it would also be expected to find lower rate constants under the batch experiment conditions since the substrate concentration is decreasing with time unlike the column experiments where a constant input substrate concentration is maintained.

The relative contribution of sorption to 2,4-D transport was in general not significant whereas degradation was very significant to 2,4-D transport specially under unsaturated conditions.

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