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ENVIRONMENTAL RESEARCH BRIEF

Degradation Kinetics of Chlorinated Aromatic Compounds in Saturated Subsurface Environments

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Abstract

Results are presented to support the use of Monod kinetics in describing the anaerobic degradation of chlorinated aromatic compounds in the saturated subsurface environment. For all compounds studied, a lag period was observed before loss of the compound was detected. However, subsequent additions of compound were dechlorinated without a lag and at an increased rate. The length of the lag was dependent on both the characteristics of the fresh water sediment and the compound investigated. Preliminary studies indicated that the population size of dechlorinating organisms in some sediments can increase in response to the removal of a single chlorine from the chemical, 2,4-dichlorophenol.

Background

The regulation of hazardous and solid waste is continually being refined as the understanding of the properties and fates of chemical waste components increases. This increased knowledge has resulted in a number of adopted and proposed amendments to the Resource Conservation and Recovery Act (RCRA) (PL 98-616). The Hazardous and Solid Waste Amendments of 1984 directed EPA under RCRA section 3001(h) to develop additional hazardous waste characteristics for identifying hazardous wastes. In June 1986, EPA proposed to modify the Toxicity Characteristic. The existing characteristic uses the National Interim Primary Drinking Water Standards (DWS) as toxicity thresholds for listed individual chemical components of the waste. These thresholds are, in turn, combined with a generic dilution/attenuation factor (100 times) to determine the regulatory threshold. The proposed characteristic would use chronic toxicity reference levels, combined with a compound-specific dilution/attenuation factor, to calculate the regulatory threshold for individual waste toxicants in the waste leachate. A subsurface (unsaturated and saturated zone) fate model is used to calculate the dilution/attenuation factors.

In the proposed rules, the model includes mathematical equations that rely on compound-specific hydrolysis and soil adsorption data; these equations are coupled to others using parameters describing a wide range of subsurface environments. The resulting model calculates the degree of attenuation and dilution a compound would undergo as it migrates to a subsurface drinking water source. Chemical hydrolysis is the only transformation mechanism currently considered in the proposed rule. Although the EPA recognizes that biodegradation is an important transformation process, it was considered insufficiently understood to be included in the model at that time.

Research at EPA's Environmental Research Laboratory at Athens, GA, has been aimed at identifying key kinetic expressions that describe anaerobic degradation in the saturated zone. The main emphasis of this research is to increase our understanding of the kinetics of anaerobic degradation to provide the scientific basis necessary for reliably including biodegradation in the future regulation of hazardous and solid wastes.

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Laboratory Procedures

Collection and Treatment of Samples

Sediment and water samples were collected from five ponds near Athens, GA, in September 1986. Bolton's Pond and Cherokee Trailer Park Pond were sampled periodically over the 3 year period beginning September 1986. The samples were collected and treated as follows. Sterile Mason jars were filled to capacity with sediment (0-10 cm) and overlying water and capped near the sediment/water interface. Additional site water was collected near the sediment surface in sterile 1-L Erlenmeyer flasks. Both water and sediment samples then were transported to the laboratory and placed in an anaerobic glovebox that maintained an atmosphere of 95%N_2:5%H_2. All further manipulations of the water and sediment were conducted in the chamber. Sediments were washed with site water through a 1-mm sieve (U.S. Standard Testing Sieve No.18) to remove organic debris and stones. Sediments subsequently were stored in crystallizing dishes for 3 weeks before being used in serum bottle microcosms. Such treatment allowed removal of residual oxygen from the sediments and restoration of methanogenic activity.

Transformation Assays

Wet sediment containing the equivalent of 10 g of dry sediment weight was added to individual 125-ml serum bottles. Sufficient site water then was added to bring the final volume to 100 ml. One-to-five milliliters of an aqueous stock solution (200 ppm) of chlorinated substrate was added to separate reaction vessels to yield a final concentration of 2 to 10 ppm. Bottles were capped with butyl rubber stoppers, crimp sealed, and incubated in the dark in the anaerobic chamber at 25°C. The loss of the substrate then was followed over time. At specific intervals, 1.0-ml subsamples were removed and combined with 1.0 ml of hexane (PCBs), pentane (chlorinated benzenes), acetonitrile (chlorinated anilines, phenols and benzoates), or ethanol (PCP, 2,4-D and 2,4,5-T) to terminate biological activity and to dissolve any test chemical sorbed to the sediment. The subsamples combined with acetonitrile or ethanol were centrifuged at 3500 rpm (IEC HN-S CENTRIFUGE) and subsequently filtered through 0.22-µm filters before analysis or storage (4°C). Subsamples treated with hexane and pentane were also centrifuged, with the solvent phase subsequently analyzed for residual test chemicals.

Autoclaved sediment slurries served as sterile controls. In most cases, sediments were autoclaved (Sybron/Castle 3020) at 120°C (1.4 atm) for 30 minutes on 3 consecutive days. Some sediment samples, however, were autoclaved only once for 30 minutes before the addition of a chlorinated substrate. The more rigorous autoclaving changes the sediment properties and causes the production of compounds that interfere with the chromatographic determination of some chlorinated substrates and products.

HPLC Analysis

Chlorinated substrates in sediment slurries were quantitated as follows. Sediment slurries mixed with equal volumes of acetonitrile or ethanol were centrifuged for 10 minutes at 3500 rpm (IEC HN-S CENTRIFUGE). The supernatant solutions were filtered (0.22 µm, Millipore, GVWP) before analysis by reversed phase HPLC. The chromatographic system consisted of a Rainin pump system coupled to a C-18 Dynamax Microsorb column (0.46 x 25 cm), a Knauer UV absorbence detector operated at 280 nm (chlorinated phenols, anilines and benzoates, and 2,4-D) or 290 nm (PCP and 2,4,5-T) and a Shimadzu C-R3A integrator. The chromatography solvent for chlorinated phenols and benzoates and 2,4-D was methanol:water:acetic acid (60:38:2 v/v/v). For chlorinated anilines, the solvent composition was 70:28:2 (v/v/v). The chromatographic solvent for PCP and 2,4,5-T was acetonitrile:water:acetic acid (60:38:2 v/v/v). Residual substrates and products were identified by comparing their retention times with those of authentic standards.

Gas Chromatograph Analysis

Concentrations of chlorobenzenes were quantified by gas chromatography. A 1.0-ml sediment slurry sample was added to 1.0 ml of *n*-pentane containing 5 ppm lindane as an internal standard. The mixture was vigorously mixed for 30 seconds and then centrifuged at 3900 X g. A 5-µl sample from the solvent phase was injected into an HP 5890 gas chromatograph connected to an HP 3399A integrator. The temperature of the injection port was 270°C and the temperature of the ECD detector was 300°C. Nitrogen was used as the carrier and the auxiliary gas. An OV-I column (30 m X 0.25 mm with 0.5 µm film) was used. The column temperature was maintained under isothermal conditions at 180°C for quantification of substrate. A programed temperature gradient (initial temperature of 40°C for 4 minutes, followed by 10°C/min increase to 180°C which was held for 15 minutes) was used in the identification of degradation intermediates.

Identification of Products

The identities of intermediate metabolites were established by using a combination of co-chromatography (HPLC and GC) with authentic standards and gas chromatography-mass spectrometry (GC-MS). GC-MS was used to confirm molecular weights and numbers of chlorine substituents. Analyses were conducted on iso-octane extracts of sediment slurries. Sediment slurry samples (5 ml) were mixed with 1 ml of isooctane, and after centrifuging (3500 rpm), the isooctane layer was separated from the water phase and used without further purification. The extracts were analyzed with a Finnigan 4500 GC-MS, interfaced to the Finnigan Incos data system. The gas chromatograph was equipped with a DB-5 30-m x 0.25-mm capillary column.

Most Probable Number (MPN) Determinations

Numbers of 2,4-dichlorophenol (DCP) dechlorinating microorganisms in pond sediments were estimated with most probable number (MPN) techniques (1). The dilution and incubation medium was prepared by adding 2,4-DCP to sterile pond water to give a final concentration of 10 mg/L. Sterile pond water was prepared by filtration through 0.22-µm membranes followed by autoclaving (30 minutes). Either 4-fold or 10-fold dilution series were used with 3 replicate MPN tubes. Dilution series were prepared directly from sediment slurries used in transformation assays. Autoclaved control tubes were prepared using autoclaved sediment. Following a 4-week incubation period, a 1-ml subsample was removed from each MPN tube, mixed with a 1-ml volume of acetonitrile and analyzed for 2,4-DCP. Tubes were considered positive if the 2,4-DCP peak area was not

more than three times the peak area of the 4-chlorophenol (CP) product. Numbers of dechlorinating organisms (with a 95% confidence interval) were estimated from the number of positive tubes in selected consecutive dilutions using an MS-DOS turbo pascal program utilizing American Society of Microbiology guidelines for MPN determinations.

Computer Simulations

Theoretical simulations of the fate of chlorinated aromatic compounds in sediment slurries were prepared using a Monod growth model (26). Two basic assumptions were made. First, the growth rate of the dechlorinating microorganisms can be expressed by

$$u = umax\ S / (K_s + S) \qquad (1)$$

where u is the specific growth rate, S is the concentration of chlorinated substrate, umax is the maximum specific growth rate, and K_s is the half saturation constant (2). Second, the following mass balance equation applies,

$$S_o + B_o/Y = S + B/Y \qquad (2)$$

where S_o is the concentration of substrate at zero time, B_o is the concentration of bacteria at zero time, and Y is the growth yield factor. Y is treated here as a constant, as has been the practice of others (2-5). Because only changes in S are of interest, the term X can be substituted for B/Y. If B is given in cells/L and Y in cells/mg (number of cells formed per mg parent compound dechlorinated) then X has the units of mg (parent compound)/L. Thus, X corresponds to the amount of "substrate" required to produce a population density of B. By analogy, X_o corresponds to the amount of "substrate" that could be equated to the formation of the initial population of specific degraders at time zero, i.e. B_o. Because Y is considered a constant, u can be represented as

$$u = 1/X\ dX/dt \qquad (3)$$

Combining Equation 3 with Equation 1 yields

$$dX/dt = umax\ S\ X/(K_s + S) \qquad (4)$$

Because of the mass balance Equation 2, dX/dt can be assumed to equal -dS/dt, yielding

$$- dS/dt = umax\ S\ X/(K_s + S) \qquad (5)$$

Plots of S versus t were obtained by simultaneously solving Equations 4 and 5 using TUTSIM software.

Results and Discussion

The object of this project was to develop kinetic models for predicting the anaerobic degradation of hazardous organic chemicals in the saturated zone, and in particular, the degradation of chlorinated aromatic compounds. Studies on the anaerobic degradation of dichlorinated phenols were conducted to develop the appropriate kinetic models. Where possible, we refer to the work of others to illustrate how Monod kinetics may also appropriately describe the degradation of a wide variety of compounds in the saturated zone.

To provide perspective and to ensure that our conclusions have breadth of application, the degradation of the six dichlorophenol isomers was investigated in the sediments from five ponds near Athens, GA. In addition to the dichlorophenol isomers, the degradation of PCP, 2,4-D and 2,4,5-T was investigated using sediments collected throughout the United States (in Georgia, Florida, and New York) and the Soviet Union. Although the data are not presented here, similar results also were observed with chlorinated anilines and benzenes. In all cases, the initial dechlorination of the test compounds was preceded by a lag period. Similar results have been observed for a variety of chlorinated (3,6,9,10) and nonchlorinated aromatic compounds (6,8,11,2,17,14,19). The length of the lag was observed to be both sediment- (Tables 1 and 3) and compound- (Tables 2 and 3) dependent. In those cases where the test compound was added a second and third time (Figures 1 and 2) following the complete dechlorination of the previous addition, dechlorination was faster and no lag was apparent. When such an increase in the onset and rate of activity is observed, the sediments are considered to have adapted to the dechlorination or degradation of the particular compound under investigation (3,6,9,10,23).

In some adapted sediments or sludges, the compound loss (3,6,9) and the formation of methane and carbon dioxide is immediate (no lag) . Few, if any, degradation intermediates are observed. At the other end of the spectrum are those adapted sediments that remove only a single chlorine from the compound to form a stable intermediate (9,10,23). In this research, for example, 2,4-DCP was converted to 4-CP in the sediment from one pond, and 2,3-, 2,4- and 2,6-DCP were converted to 3-, 4-, and 2- CP in the sediment from another. None of the monochlorophenols was further degraded. The conversion of 2,3- and 2,6-DCP to a mixture of monochlorophenols and phenol in some sediments was indicative of an intermediate level of adaptation. The direct conversion of dichlorophenols to methane and carbon monoxide was not indicated with either sediment.

That sediments can adapt to convert dichlorophenols to monochlorophenols suggests that the dechlorination process provides a selective advantage for the survival of dechlorinating organisms. Brown et al. (4) have calculated that the free energy released during reductive dechlorination is exergonic. Therefore, one could expect that if this release of energy could be coupled to the utilization of organic compounds in sediment, the conversion of 2,4-DCP to 4-CP observed here could support biological growth, resulting in adaptation. Results indicate that the number of dechlorinating microorganisms, as measured by MPN techniques, increases following the addition of dichlorophenols to sediment slurries (Table 4). Although an increase in MPN units was not always observed, adaptation was consistently observed. The relative importance of induction (i.e., an increase in enzymatic activity) and growth were not evaluated here. It is apparent from these studies, however, that both are occurring. Considering that the sterile controls in these studies showed no loss of compound, abiotic processes can reasonably be ruled out.

Several other mechanisms have also been considered in explaining the length of lag periods. Others have suggested that a lag period is required for mutation and genetic transfer (16,25). Also, certain environmental factors have been implicated. These include limiting nutrient concentrations (13,24), preferential use of organic (12,13) or inorganic (7) compounds before degradation of the test chemical, recovery from toxic chemicals (21), and the predation of degrader populations by protozoa (26).

Because we observed both adaptation and an increase in the units of biological activity, Monod growth kinetics can be

Table 1. Persistence of 3,5-DCP in Fresh Water Sediment Slurries

Source of Sediment	Initial Concentration (mg/L)	Residual 3,5-Dichlorophenol Concentration (mg/L) Time of Incubation						
		Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 14
Bar H	4.36	3.30	3.48	3.57	4.35	3.01	3.23	2.18
Pond		4.18	4.36	4.36	4.36	4.01	4.14	3.26
Cherokee	4.14	4.14	4.14	3.74	0	0	0	0
Pond		4.14	4.14	4.09	0	0	0	0
2-Boat	4.22	4.22	4.22	0	0	0	0	0
Pond		4.22	4.22	4.18	4.12	3.92	4.04	4.06
Sandy Creek	4.27	4.27	4.27	4.00	4.27	3.84	4.24	4.27
Nature Ctr.		4.27	4.27	3.74	4.27	4.01	4.24	3.48
Bolton's	4.18	3.78	4.18	2.87	0	0	0	0
Pond		4.18	4.18	4.18	4.18	4.18	4.18	3.41

Table 2. Persistence of Dichlorophenol Isomers in Fresh Water Sediment Slurries

Isomer	Initial Concentration (mg/L)	Residual Dichlorophenol Concentration (mg/L) Time of Incubation						
		Week 0	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
2,3-	4.57	4.57	3.60	0	0	0	0	0
		4.57	3.33	0	0	0	0	0
2,4-	4.70	4.70	4.45	0	0	0	0	0
		4.70	4.75	0	0	0	0	0
2,5-	4.91	4.91	3.96	4.30	0	0	0	0
		4.35	4.02	4.20	3.92	0	0	0
2,6-	4.12	2.85	4.02	4.04	0	0	0	0
		3.03	3.25	4.12	3.35	0	0	0
3,4-	4.40	4.32	3.60	4.12	0	0	0	0
		4.40	3.71	4.02	3.98	4.02	3.32	4.02
3,5-	4.14	4.14	4.14	3.74	0	0	0	0
		4.14	4.14	4.09	0	0	0	0

Table 3. Lag Times and $^{a}T_{50}$ Values for PCP, 2,4-D, and 2,4,5-T in Fresh Water Sediment Slurries

Sediment	Lag (days)	$^{a}T_{50}$ (days)
PCP		
East River, NY	19	26
Lake Borek, USSR	14	15
Cherokee Pond, GA	>40	>40
2,4-D		
Wacissa Spr., FL	9	16
Lake Borek, USSR	50	55
Cherokee Pond, GA	22	46
2,4,5-T		
Cherokee Pond, GA	60	60

[a] T_{50} is the time to observe a 50% decrease in concentration, and should not be mistaken for a half life that is related to first-order kinetics (Moore *et al.*, EPA/600/3-89/080).

useful in investigating the dechlorination of chloroaromatic compounds in anaerobic saturated zone water. The length of the lag period in this case is dependent on the values of K_s, umax and X_o. The effects of increasing K_s and decreasing biomass are shown in Figures 3 and 4. The effects of decreasing umax would be similar to the effects of decreasing biomass. Factors affecting the lag period (such as those described above) would presumably affect one or all of these kinetic parameters. Adaptation is represented by the theoretical curves in Figure 5.

Incorporating Monod kinetics into a subsurface transport and transformation model requires a supporting database. Unfortunately, such a database is currently not available. This fact was recognized in 1988 with the publication of an anaerobic protocol (40 CFR, Part 795, Section 795.54) for developing anaerobic degradation data for organic chemicals of interest in the subsurface environment. A geometric sampling approach was recommended for use in providing data for rapidly and slowly degrading compounds. Samples were to be analyzed at 0, 4, 8, 16, 32, and 64 weeks. The protocol would be used to determine the length of time (lag) before which detectable degradation could be observed and the half-life of the chemical following the lag period. Conceivably these two pieces of information could be incorporated into a fate model as separate entities. The methodology provided here is a crude, but conservative

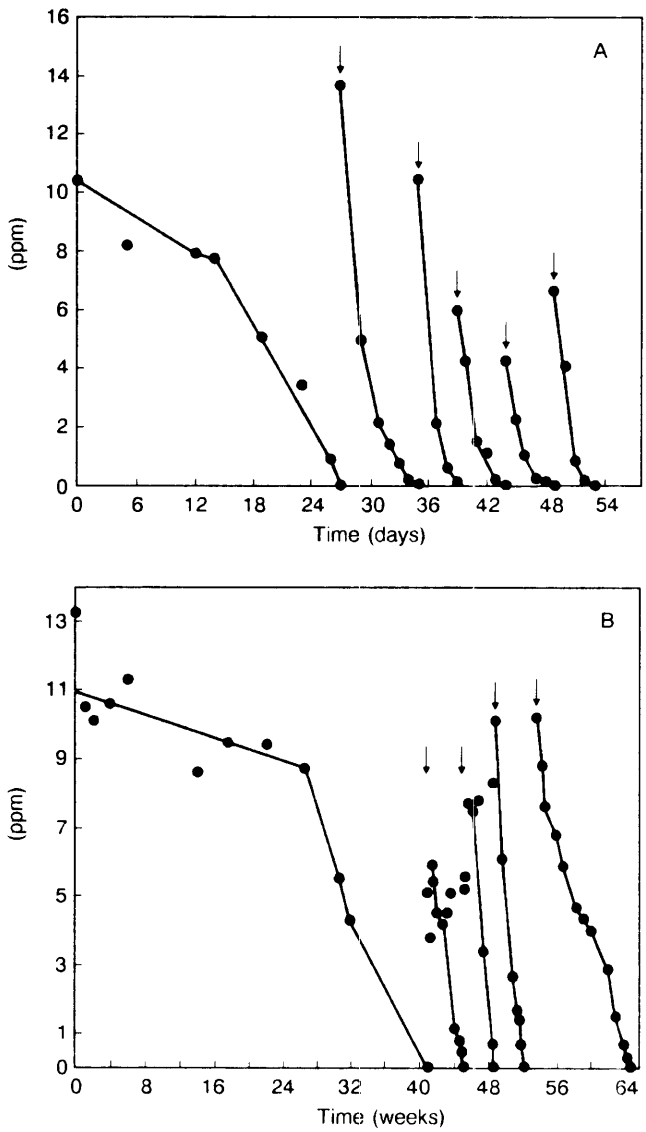

Figure 1. Loss of 2,6-DCP in Cherokee Pond (A) and Bolton's Pond (B) sediment. Arrows denote additions of 2,6-dichlorophenol.

(under estimation of degradation or transformation rate), approximation to the direct use of Monod kinetics.

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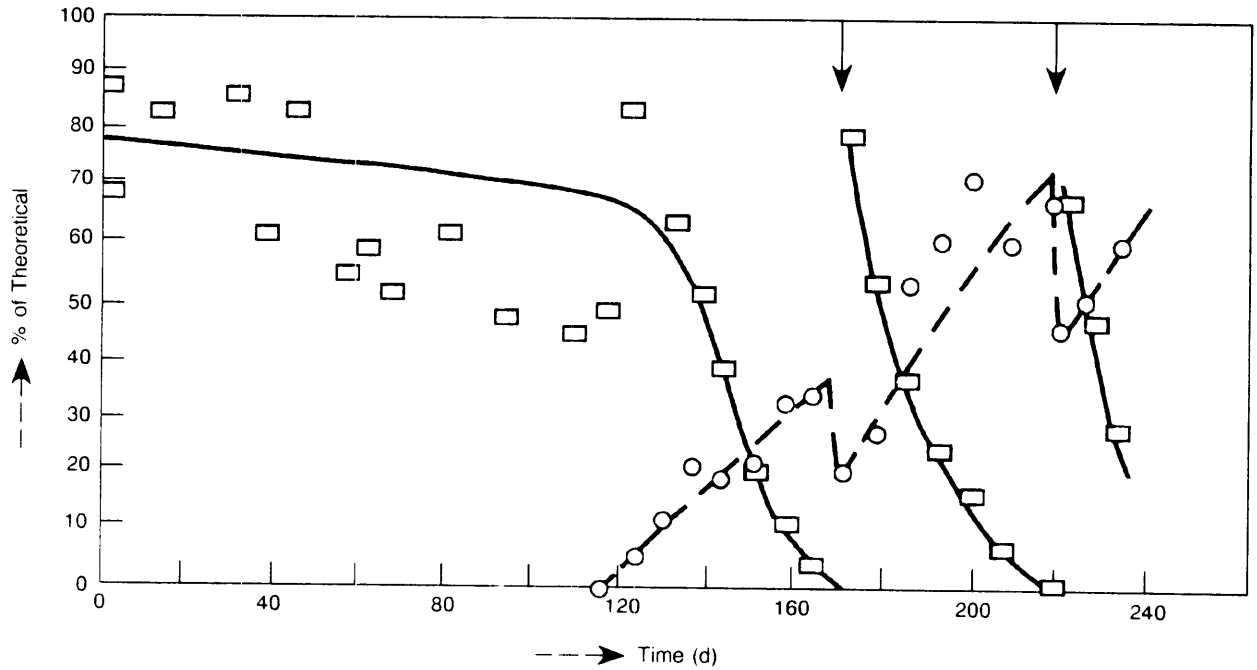

Figure 2. Loss of 2,4-dichlorobenzoate (□ - □, 5 ppm) and the formation of 4-chlorobenzoate (O-O) in Cherokee Pond sediment. Arrows denote additions of 2,4-dichlorobenzoate.

Table 4. Mean Number (Log_{10} ± Standard Deviation, n = 5) of 2,4-Dichlorophenol Dechlorinating Organisms in Cherokee Pond Sediments Collected from Selected Sites During Various Seasons

Sampling Date	T_0[a]	T_{loss}[b] Sample	Control
9/27/88	2.99 ± 0.88	5.18 ± 1.20	3.56 ± 1.01
1/24/89	3.62 ± 0.09	4.54 ± 0.58	3.78 ± 0.39
3/27/89	4.22 ± 0.31	4.68 ± 0[c]	4.13 ± 0.33
5/30/89	4.03 ± 0.55	4.37 ± 0.49	3.65 ± 0.26

[a] Time = 0
[b] Time = complete dechlorination of 2,4-dichlorophenol to monochlorophenol
[c] n = 3

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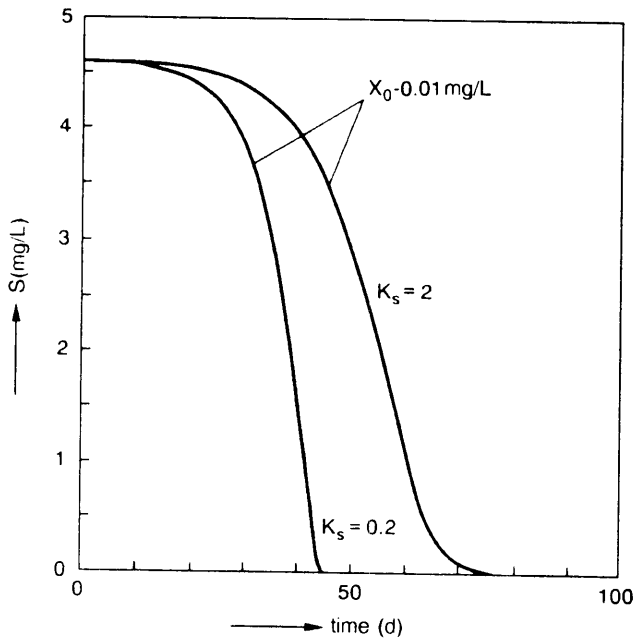

Figure 3. Theoretical plot representing the effect on the lag of increasing the half saturation constant K_s. S_o (4.6 mg/l); umax (0.15/d); X_o and K_s (mg/l) as indicated.

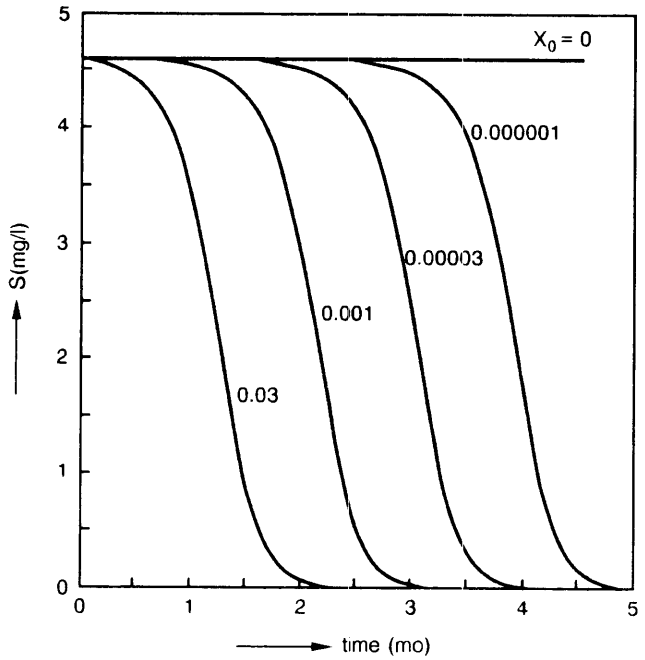

Figure 4. Theoretical plot indicating the effect of decreasing bacterial population on lag. S_0 (4.6 mg/l); umax (0.35/d); K_s (8 mg/l); X_0 (mg/L) as indicated.

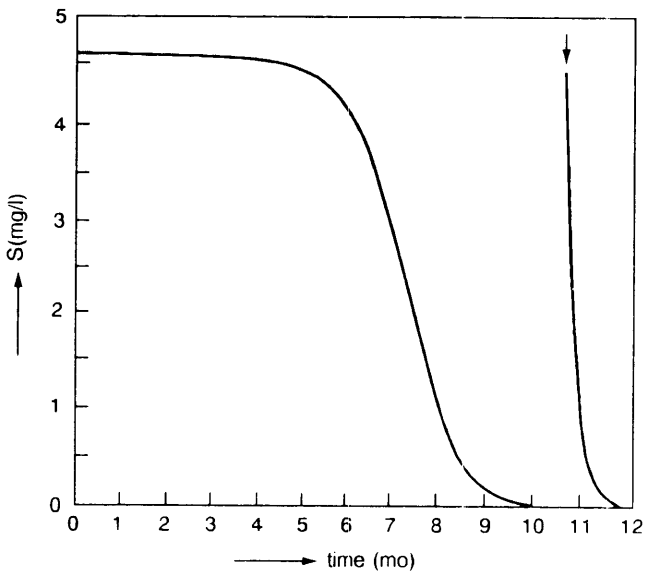

Figure 5. Theoretical plot indicating adaptation. S_0 (4.6 mg/L) each addition; umax (0.15/d); K_s (10 mg/l); X_0 (0.1 µg/l).

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