

LONG-TERM CAPACITY FOR ORGANIC-SUBSTRATE
REMOVAL BY BACTERIAL FILMS

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ABSTRACT

When wastewater is discharged to small streams, the effluent quality normally obtained from a sewage treatment plant is often not good enough to prevent serious water-quality deterioration. Hence, enhanced removal of organic pollutants is required. Efficient and economic removal of organics to very low concentrations is best achieved by biofilm processes, in which bacteria are attached to a fixed media and remove the organic compounds from the wastewater flowing past. Laboratory-scale reactors were utilized to evaluate the ability of biofilms to remove low levels of organic contaminants in water during extended operation. Nonsteady-state operation, in which trace concentrations of organic substrate were treated with a biofilm previously grown on a relatively high concentration feed, demonstrated that a slowly decaying biofilm was able to bring about high efficiency removal of the trace compound for extended periods, up to 7 months in this study. A kinetic model to describe the transient growth and decay of the biofilm was developed, and it predicted the growth and steady-state phases of the biofilm when input parameters were determined independently. The observed slow decay rate of the biofilm prolonged the usefulness of the nonsteady-state biofilm and was explained by adaptation to oligotrophic (low concentration) conditions and the growth of nitrifying bacteria which produced supplemental organic substrate to sustain the organic-utilizing bacteria. The results of this study demonstrated that nonsteady-state-biofilm processes can sustainably achieve organic concentrations much lower than conventional wastewater treatment.

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INTRODUCTION

BACKGROUND

Bacterial growths attached to solid surfaces, commonly referred to as biofilms, can be used advantageously in the treatment of valuable water resources. Biofilms are the predominant form of bacterial growth in environments of low nutrient concentrations and high specific surface areas. Currently biofilms are being used in water and wastewater treatment with methods such as the rotating biological contactor, the trickling filter, submerged filters, land treatment, bank filtration, aquifer recharge, and biological activated carbon. Biofilms also exist freely in nature and can be both useful and a nuisance to man. The most common forms of natural biofilms are found in stream beds, groundwater aquifers, water pipes, ship hulls, heat-exchange surfaces, plants, animal intestines, and plaque on teeth (Rittmann and McCarty, 1981; Brock, 1979; Characklis, 1973).

With the ever-growing concern about trace-level concentrations of organics entering the hydrological cycle and the adverse effects they may have on health and natural environments, methods for their removal to the lowest level achievable become highly desirable. Among possible methods to remove organic compounds, the biological processes are often advantageous. Physical-chemical processes merely exchange the pollutant of concern from the aqueous phase to another phase, which must still be disposed of in some manner. Biological processes not only separate the organic pollutant from the aqueous phase, but also can degrade the potentially harmful substances into benign compounds, such as carbon dioxide, water, and harmless organic cell matter.

When the concentration of biodegradable organic substances is a few milligrams per liter or less, the most practical biological treatment system is a fixed-film process. A fixed-film process has the advantage over a suspended growth system when the organic concentration is low, because the cells remain within the system by attachment and are continually exposed to a fresh supply of nutrients. In addition, fixed-film processes are usually more energy and cost efficient, compared to suspended-growth biological processes and physical-chemical processes.

BIOFILM PROPERTIES

A biofilm generally consists of about 95 to 99 percent water which is either part of the biomass or held within the biofilm structure. The biomass is characteristically comprised of organic cell matter, both active and inactive, organic secretions used for adhesion, and inorganic components. The inorganic portion of the biofilm consists of major and trace nutrients and comprises up to 10 percent of the dry biomass weight. The fraction of organic matter is about 90 percent by dry weight, and its key components are the active and dead bacterial cells. About 80 percent of the active biomass is biodegradable (McCarty, 1972) and can be oxidized by the actively growing bacterial cells. Active cells utilize their own cytoplasm as a source of energy. In addition, when some cells die and lyse, they release biodegradable nutrients that can be used by the other organisms. The inorganic fraction of the biofilm and a portion of the organic fraction are refractory; if they are not washed out of the biofilm system, they tend to accumulate, increasing the biofilm mass and thickness.

APPLYING BIOFILM PROCESSES TO WATER AND WASTEWATER TREATMENT

Although biofilm processes are usually most advantageous for removal of low concentrations of organic contaminants, some potential problems exist. Two of the most important problems are the build-up of refractory cell material and the impossibility of growing and sustaining a biofilm when organic concentrations are very low.

Refractory Material Build-Up

Rittmann and McCarty (1980a) and Rittmann (1982) demonstrated that biomass decay is the main biomass loss mechanism for many reactor types. When biomass decays, it gradually accumulates nonactive, refractory cell material. A build-up of refractory material potentially can reduce the effectiveness of a biofilm process by clogging the reactor pores, causing short circuiting, or reducing the active-cell density of the biofilm.

Experimental evaluation of biofilm processes for advanced biological treatment has not yet addressed the long-term build-up of refractory cell material. Although several studies have demonstrated good potential for removal of trace-organic contaminants (Rittmann and McCarty, 1980a, 1980b, 1981; Stratton *et al.*, 1982; Namkung *et al.*, 1982), none was carried out for long enough to test the impact of refractory build-up.

Very Low Substrate Concentrations

Heterotrophic bacteria grow and sustain themselves through utilization of organic substrates. The kinetics of cell growth are proportional to the concentration of the rate-limiting substrate; as that concentration becomes very low, the growth rate becomes less than the biomass loss rate and the biofilm either does not form or it disappears. The problem of low concentration is especially cogent for advanced biological treatment because the organic material is at low concentration and often comprised of slowly biodegradable compounds which yield a slow growth rate.

For a steady-state biofilm--one for which growth and losses just balance--the minimum concentration for sustaining the biofilm is called S_{\min} (Rittmann and McCarty, 1980a) and is defined as

$$S_{\min} = K_s \frac{b}{Yk-b} \quad (1)$$

in which

K_s = Monod half-velocity coefficient ($M_s L^{-3}$);

Y = true to yield ($M_x M_s^{-1}$);

k = maximum specific substrate utilization rate ($M_s M_x^{-1} T^{-1}$); and

b = biomass decay coefficient (T^{-1}).

Typical values of S_{\min} are 0.1-1.0 mg/l in aerobic systems (Stratton *et al.*, 1982). Since trace organic contaminants are often present at concentrations well below 0.1 mg/l, the direct use of a steady-state biofilm to bring about biodegradation seems impossible.

Nonsteady-State Biofilm Process

One technique for achieving biodegradation of organic substrates present at sub- S_{min} concentration is to employ a nonsteady-state biofilm technique. In the nonsteady-state technique, a biofilm is grown for a relatively short time through utilization of a high (i.e., greater than S_{min}) concentration of substrate; subsequently, the substrate feed concentration is reduced to the (normal) trace level. Since the biofilm is already present and cannot decay away as quickly as it was grown, substrate removal is achieved for extended periods as the nonsteady-state biofilm slowly decays. Rittmann and McCarty (1981) showed that the nonsteady-state technique can bring about high removal efficiencies of trace-level organics for short-term experiments; however, the long-term capability was not investigated.

Internal Substrate Supplementation

A second means by which biofilm mass can be sustained when the feed concentration is less than S_{min} is internal substrate supplementation. The most likely example is the production of organic products by autotrophic nitrifying bacteria. The nitrifiers oxidize ammonium nitrogen to obtain energy to fix inorganic carbon for cell synthesis. Some of that organic carbon is released and potentially can be utilized by heterotrophs. Thus, nitrification activity can supplement the organic material in the feed and reduce heterotrophic decay rates. Nitrification is favored by a low organic concentration in the feed, which would be typical of a nonsteady-state operation (McCarty *et al.*, 1981).

RESEARCH OBJECTIVES

1. To determine the long-term effects that the accumulation of refractory biomass has on substrate removal.
2. To determine the long-term capability of a nonsteady-state biofilm to allow efficient removal of trace-level substrate.
3. To develop and evaluate a mathematical model that predicts the transient growth, decay, and substrate utilization of a nonsteady-state biofilm.

4. To determine the role of nitrification in reducing the rate of heterotrophic biofilm decay under nonsteady-state conditions.

EXPERIMENTAL MATERIALS AND METHODS

EXPERIMENTAL APPARATUS

The laboratory set-up used to complete the biofilm studies is illustrated in Figure 1. Glass columns, 2.5-cm in diameter and 12-cm or 36-cm in length, were operated in an upflow manner. The columns possessed sampling ports sealed with rubber serum caps and spaced 1, 2, 4, 6, 8, 10 . . . cm from the inlet. Two different column lengths were used for the two types of experiments. A 36-cm column was used for the long-term steady-state tests, and a 12-cm column was used for the nonsteady-state tests. The columns were packed with 3-mm diameter glass beads, which served as the attachment media for the biofilm. The end caps on the 12-cm column were tapered glass with baffling on the influent side to maintain nearly plug-flow conditions throughout the reactor. Teflon screening was placed between the end caps and packing media to prevent the displacement of glass beads. The end caps were connected to the transport tubing and column with quick-release fittings to allow for rapid cleaning. The end caps on the 36-cm column consisted of a teflon plunger device that could be adjusted within the column to produce reactors of different lengths.

The columns were operated by gravity flow from 8-liter, glass aspirator-bottle feed reservoirs. Tygon tubing was used to transport the feed solution to the biofilm reactors and to the effluent reservoir. The flow rate was controlled by a Dial-a-Flow valve (Sorenson) connected to the effluent tubing. Before the effluent passed into the effluent reservoir, it passed through a 300-ml BOD bottle used for dissolved-oxygen analysis. The flow rate through the column was maintained at 3 l/day during the length of the biofilm experiments. Some important column parameters are presented in Table 1.

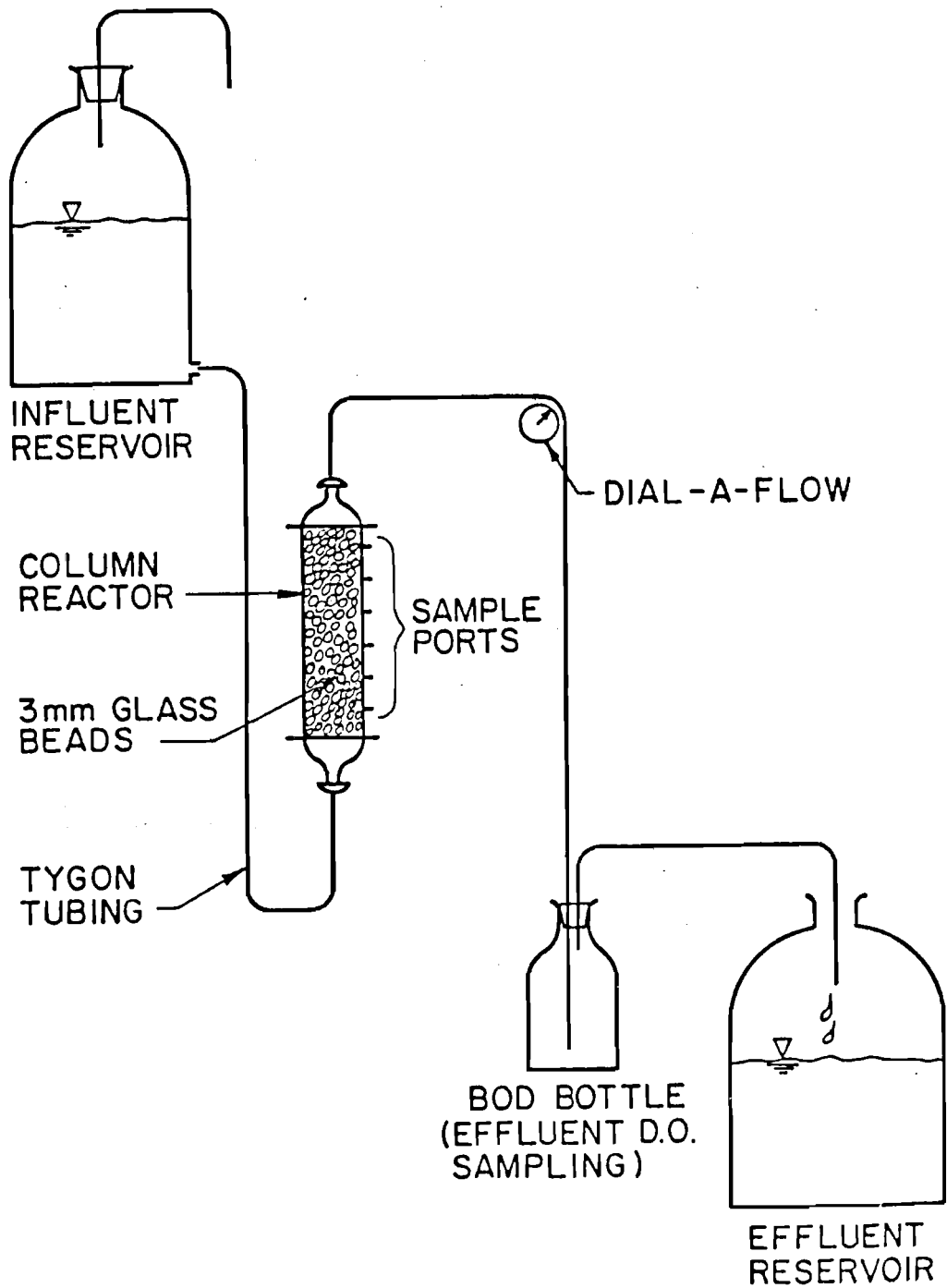


Figure 1. Experimental Biofilm Reactor System

Table 1. Characteristics of Biofilm Reactors

Parameter	12-cm Column	36-cm Column
Volume of reactor	58.9 cm ³	176 cm ³
Porosity, ϵ	~ 0.4	~ 0.4
Volume of voids, V_v	23.6 cm ³	70.7 cm ³
Volume of one glass bead	0.01414 cm ³	0.01414 cm ³
Area of one glass bead	0.282 cm ²	0.282 cm ²
Flow rate, Q	125 cm ³ /hr	125 cm ³ /hr
Theoretical detention time, V_v/Q	11.3 min	33.9 min
Velocity of fluid	1.06 cm/min	1.06 cm/min
$R_e = (vd_{pp}/\mu)$	0.0056	0.0056

FEED SOLUTIONS

The feed solutions used were prepared with 3 mg COD/l for phenol in the steady-state column and 0.03 to 3 mg/l D-galactose in the nonsteady-state column. These concentrations are typical of those found in tertiary effluents and some surface and groundwaters. Since little oxygen would be consumed at these low organic levels, the possibility of dual limitation between electron donor and electron acceptor was eliminated. Also added to the feed solution were essential inorganic nutrients. The nutrients and their concentrations in the basic medium were: 8.5 mg/l KH_2PO_4 , 21.8 mg/l K_2HPO_4 , 17.8 mg/l Na_2HPO_4 , 1.7 mg/l NH_4Cl , 11.0 mg/l MgSO_4 , 27.5 mg/l CaCl_2 , 0.15 mg/l FeCl_3 , and 1.0 mg/l NaHCO_3 . The pH was 7.1. Solutions were prepared with deionized and distilled water that was subsequently filtered through a Multi-Q purification system and sterilized by autoclaving.

COLUMN START-UP

The procedure followed to start up a column was to first wash the column, the end caps, glass beads, and tubing with either chromic acid or a concentrated chlorine solution and then to thoroughly rinse them each with sterile water. The glass beads were sterilized in the autoclave for 15 minutes at 15 psi. In order to allow biofilm growth

to occur only on the glass beads and column wall, the tygon tubing, column end caps, and the teflon screens were sterilized with a chlorine solution each time the feed was changed. The column was initially packed by filling the column about halfway with sterile water and pouring the sterile glass beads in a few at a time and then vibrated with a vortex mixer to insure that settling of the glass beads would not occur after the experiment began. After the column was entirely packed, a settled domestic sewage solution, diluted 1:10⁶ with a medium of 8.5 mg/l KH_2PO_4 , 21.8 mg/l K_2HPO_4 , 17.8 mg/l Na_2HPO_4 , and 11.0 mg/l MgSO_4 , was passed through the column. After the solution had passed through the reactor for several detention times, the influent and effluent lines were crimped to stop the flow. The column remained in this state for 24 hours to allow the bacteria to attach to the glass beads. After 24 hours the influent and effluent tubings were sterilized and the column was attached to the feed reservoir so that continuous flow could begin. The flow remained continuous and constant during the entire experiment except for the short time periods when the tubings were cleaned.

EXPERIMENTAL AND ANALYTICAL PROTOCOLS

After the columns had been in operation for approximately three weeks and steady-state conditions were reached, the nonsteady-state experiments began. The procedure followed to test the nonsteady-state D-galactose column was to initially grow the biofilm at a galactose concentration of 3.0 mg/l in the basic nutrient medium. After steady-state conditions were achieved and an S_{\min} was determined, the galactose feed concentration was reduced to 0.3 mg/l, which was below S_{\min} . Using a radio-labeled galactose tracer (Brunner, 1982), short-term tests at galactose concentrations of 0.3 and 3.0 mg/l were conducted. The reason that the galactose concentration was increased to 3.0 mg/l for some short-term tests was to test if the decay of the original biofilm had reduced the substrate removal capacity from its original steady-state value. It was especially important to keep the tests as short as possible when a higher concentrated test feed solution was used, since long-term exposure to a higher concentration would allow regrowth

of the biofilm. While the column was operated with a feed solution concentration of 0.3 mg/ℓ galactose, nonsteady-state tests were conducted at least once per week at both concentrations of 0.3 and 3.0 mg/ℓ galactose.

After several months of operation with an influent galactose concentration of 0.3 mg/ℓ, the feed concentration was reduced further to 0.03 mg/ℓ galactose. At this concentration, weekly tests were performed for about a 2-hour time period at concentrations of 0.03 mg/ℓ, 0.3 mg/ℓ, and 3.0 mg/ℓ galactose.

Shortly after changing the galactose concentration in the feed to 0.03 mg/ℓ, the inorganic nitrogen source was changed from ammonia to nitrate in order to reduce the biomass formed due to nitrification, a possible source of energy for the heterotrophic organisms using the galactose. The nitrogen source was changed from 0.445 mg-N/ℓ of $\text{NH}_3\text{-N}$ to 0.445 mg-N/ℓ of $\text{NO}_3^-\text{-N}$. All of the other inorganic nutrients remained the same.

Oxygen consumed in the reactor, indicating the degree of biological stabilization and decay, was measured by carefully collecting both influent and effluent samples in 300-ml BOD bottles. The samples were analyzed using the Winkler Method (azide modification) (American Public Health Association, 1980). One important reason for checking the effluent dissolved-oxygen concentration was to ensure that oxygen remained at a high enough concentration to preclude dual limitation.

Nitrite and nitrate concentrations were measured in both columns to test for the presence of nitrifiers. The cadmium reduction method (American Public Health Association, 1980) was used to determine both nitrite and nitrate concentrations.

Total organic carbon, TOC, measurements were made with a Dohrmann DC 80 Carbon Analyzer on samples of distilled water, water processed by the Milli-Q purification system, solutions containing only the inorganic nutrients at their respective concentrations, and feed solutions containing both the inorganic nutrients and the organic substrate at concentrations between 0.03 and 3.0 mg/ℓ. The purpose of the TOC analysis was to determine what background organics were present in the medium and which would interfere with the nonsteady-state tests at the lowest concentrations.

KINETIC PARAMETER MEASUREMENTS

Kinetic parameters were determined for phenol consumed by phenol-grown bacteria. The parameters of concern were Y , K_s , b , and k . Y was determined using batch growth tests. Initially a small inoculum of cells extracted from the phenol-grown column was added to a 2-liter reservoir containing 500 mg/l phenol and an excess of inorganic nutrients. The nitrogen source was in the form of nitrate in order to eliminate the growth of nitrifiers. The uptake of phenol was measured with the direct photometric method (American Public Health Association, 1971) or with C^{14} -labeled phenol, if it was available. To obtain the yield coefficient, Y , samples were filtered through a 0.45- μ m filter after a significant portion of phenol was utilized. The filter was placed in a 103°C oven for a two-hour period, cooled, and weighed. After the concentration of filtered solids was calculated, the following expression was used to determine Y .

$$Y = \frac{X_{fin.} - X_0}{S_0 - S_{fin.}} \quad (2)$$

$X_{fin.}$ is the concentration of solids present when the concentration of phenol, $S_{fin.}$, was measured. S_0 is the initial concentration of phenol in the batch reactor, and X_0 is the original biomass concentration, which was taken to be equal to zero since $X_{fin.} \gg X_0$.

The maximum specific utilization rate, k , was estimated from fundamentals of bioenergetics (McCarty, 1972), while the decay coefficient, b , was assumed to be 0.1 day⁻¹, a typical value.

The half-velocity coefficient, K_s , was determined from the biofilm tests and Eq. 1. The equation was solved for K_s , and previously estimated values of S_{min} , Y , k , and b were substituted.

The kinetic parameters used for galactose were not determined in this study, but were experimentally obtained in previous work (Stratton, 1981).

BIOFILM PARAMETER MEASUREMENTS

The biofilm thickness, L_f , and density, X_f , were obtained for both columns when experimentation was completed and the columns were disassembled. Glass beads with the attached biofilm were removed from representative sections of the columns. Because the weight of the biofilm was about 99 percent water, L_f could be determined by measuring the weight of water evaporated from the biofilm by drying the beads for two hours in a 103°C oven. L_f was determined by the expression,

$$L_f = \frac{W}{\rho n A} \quad (3)$$

where W = weight of evaporated water;

ρ = density of water at 23°C;

n = the number of glass beads from each section; and

A = surface area per bead.

The biofilm density, X_f , was calculated by shearing the biofilm from the glass beads with a vortex mixer and measuring the COD of the cell mass. The COD results were used to obtain a value of X_f (dry cell weight/cm³) by the following expression.

$$X_f = \frac{\text{COD}_{\text{cells}} (\text{mg}) \times 0.706 \frac{\text{mg cells}}{\text{mg COD}}}{n A L_f} \quad (4)$$

The factor 0.706 mg cells/mg COD comes from assuming that cells are represented by $C_5H_7O_2N$ (McCarty, 1972).

THE TRANSIENT-BIOFILM MODEL

Biofilm kinetic models were developed and tested for steady-state and short-term, nonsteady-state conditions (Rittmann and McCarty, 1978, 1980a, 1980c, 1981). However, no models were available to describe the growth and decay of a biofilm during transient conditions that

spanned a time long enough to allow changes in biofilm mass and thickness. The transient model developed below is built upon the steady-state and nonsteady-state models presented by Rittmann and McCarty (1980c, 1981), but allows the biofilm to change thickness as the substrate concentration varies.

The transient-biofilm model requires that five processes occur simultaneously. First, substrate is transported across the liquid diffusion layer according to Fick's first law.

$$J = -D \frac{dS}{dz} = D \frac{S - S_s}{L} \quad (5)$$

in which J = substrate flux across diffusion layer and into the biofilm surface, $M_S L^{-2} T^{-1}$;

D = molecular diffusion coefficient of the substrate in water ($L^2 T^{-1}$);

S = bulk-liquid substrate concentration ($M_S L^{-3}$);

S_s = substrate concentration at biofilm surface ($M_S L^{-3}$); and

L = thickness of diffusion layer (L).

Second, substrate within the biofilm is utilized according to the Monod relation.

$$r_{util} = - \frac{kX_f S_f}{K_s + S_f} \quad (6)$$

in which r_{util} = substrate utilization rate at a point in the film ($M_S L^{-3} T^{-1}$);

k = maximum specific substrate utilization rate ($M_S M_X^{-1} T^{-1}$);

X_f = density of active biomass ($M_X L^{-3}$);

K_s = half-velocity coefficient ($M_S L^{-3}$); and

S_f = substrate utilization at that point in the biofilm ($M_S L^{-3}$).

Third, mass transport within the biofilm is according to Fick's second law.

$$r_{diff} = D_f \frac{\partial^2 S_f}{\partial z^2} \quad (7)$$

in which r_{diff} = rate of substrate accumulation due to molecular diffusion ($M_S L^{-3} T^{-1}$);

D_f = molecular diffusion coefficient of the substrate in the biofilm ($L^2 T^{-1}$); and

z = distance normal to biofilm surface (L).

Combination of Eqs. 6 and 7 and assumption of a steady-state substrate-concentration profile in this biofilm (Rittmann and McCarty, 1981) give the overall mass balance for substrate in the biofilm.

$$D_f \frac{\partial^2 S_f}{\partial z^2} = \frac{kX_f S_f}{K_s + S_f} \quad (8)$$

Fourth, biomass is grown in the biofilm in proportion to substrate utilization.

$$r_{gr} = \frac{YkX_f S_f}{K_s + S_f} \quad (9)$$

in which r_{gr} = biomass growth rate at a point within the biofilm ($M_X L^{-3} T^{-1}$); and

Y = true yield ($M_X M_S^{-1}$).

Finally, biomass losses are primarily caused by cell decay and shear losses. Rittmann (1982) reported that the loss rate from both processes could be described with a first-order loss term.

$$r_{loss} = -b'X_f \quad (10)$$

in which r_{loss} = loss rate of biomass ($M_X L^{-3} T^{-1}$); and

b' = first-order biomass loss coefficient accounting for decay and shear losses (T^{-1}).

Equations for estimating b' were given by Rittmann (1982).

In the steady-state biofilm model (Rittmann and McCarty, 1980c), Eqs. 9 and 10 were combined, integrated, and set equal to zero.

$$0 = \int_{L_f} (r_{ret}) dz = \int_{L_f} \left[\frac{YkX_f S_f}{K_s + S_f} - b'X_f \right] dz \quad (11)$$

in which L_f = thickness of biofilm (L).

The expression for S_{min} (Eq. 1) and for the steady-state-biofilm thickness,

$$L_f = \frac{JY}{b'X_f} \quad (12)$$

when derived from Eq. 11 (Rittmann and McCarty, 1980c).

Transient growth/decay of a biofilm is modeled by combining Eqs. 9 and 10, but without assuming a steady-state biofilm thickness. For a given increment in time,

$$\int_{L_f} r_{ret} dz = \int_{L_f} \left[\frac{YkX_f S_f}{K_s + S_f} - b'X_f \right] dz \quad (13)$$

When simplified, the left side becomes $\frac{dX_f L_f}{dt}$. The simplified right-hand side becomes $YJ - b'X_f L_f$ (Rittmann and McCarty, 1980c). The simplified form of Eq. 13 is integrated with respect to time. To calculate transient growth/decay over a time increment, Δt .

$$\int_{\Delta t} d(X_f L_f) = \int_{\Delta t} [YJ - b'X_f L_f] dt \quad (14)$$

If Δt is small enough that J , b' , X_f , and L_f does not change much, then Eq. 14 becomes

$$\Delta(X_f L_f) = (YJ - b'X_f L_f) \Delta t \quad (15)$$

For a constant X_f ,

$$\Delta L_f = \left(\frac{YJ - b'X_f L_f}{X_f} \right) \Delta t \quad (16)$$

Eq. 16 is used in the transient-biofilm model to adjust the biofilm thickness for time steps Δt . J is calculated from the model appropriate

for any biofilm thickness (Rittmann and McCarty, 1981), which was shown to be applicable for nonsteady-state biofilms.

Eq. 16 and the flux equations were incorporated into a solute transport model described previously (Rittmann and McCarty, 1980a, 1981). The kinetic parameters (k , Y , K_s , b' , L , D , D_f), biofilm characteristics (X_f , initial L_f values), flow and reactor conditions (v , porosity, particle diameter, length), and feed substrate concentration (S^0) are input values. The finite-difference solution marches forward in time by increments of Δt . For each $t + \Delta t$, the output parameters are: substrate concentration at points along the reactor, biofilm thicknesses along the reactor, and substrate fluxes along the reactor.

If steady input conditions are maintained, the solution converges on the predictions of the steady-state-biofilm model (Rittmann and McCarty, 1980a,c). Before or between steady states, the transient-biofilm model predicts the nonsteady-state growth or decay of a biofilm and the substrate removals during the transient phase.

RESULTS AND DISCUSSION

ACHIEVING STEADY-STATE SUBSTRATE UTILIZATION

After the columns were seeded with a $1:10^6$ dilution of settled domestic sewage in a solution of phosphate buffer and magnesium sulfate, the feeding of primary substrates, phenol and galactose, began in their respective columns at concentrations of 3.0 mg COD/ ℓ for phenol and 3.0 mg/ ℓ for galactose. The initial substrate utilization was monitored, and the resulting removal profiles are presented in Figures 2 and 3. For the first four days after seeding, the removal of each substrate was minimal and nearly the same in both reactors. The removal was slightly more for the galactose column than for the phenol column at the 12-cm distance along the reactor. Between days four and five, the utilization of both substrates increased significantly, with the utilization of phenol being far greater than the utilization of galactose. After day five for the phenol-fed reactor and day seven for the galactose-fed reactor, the respective effluent substrate concentrations were nearly

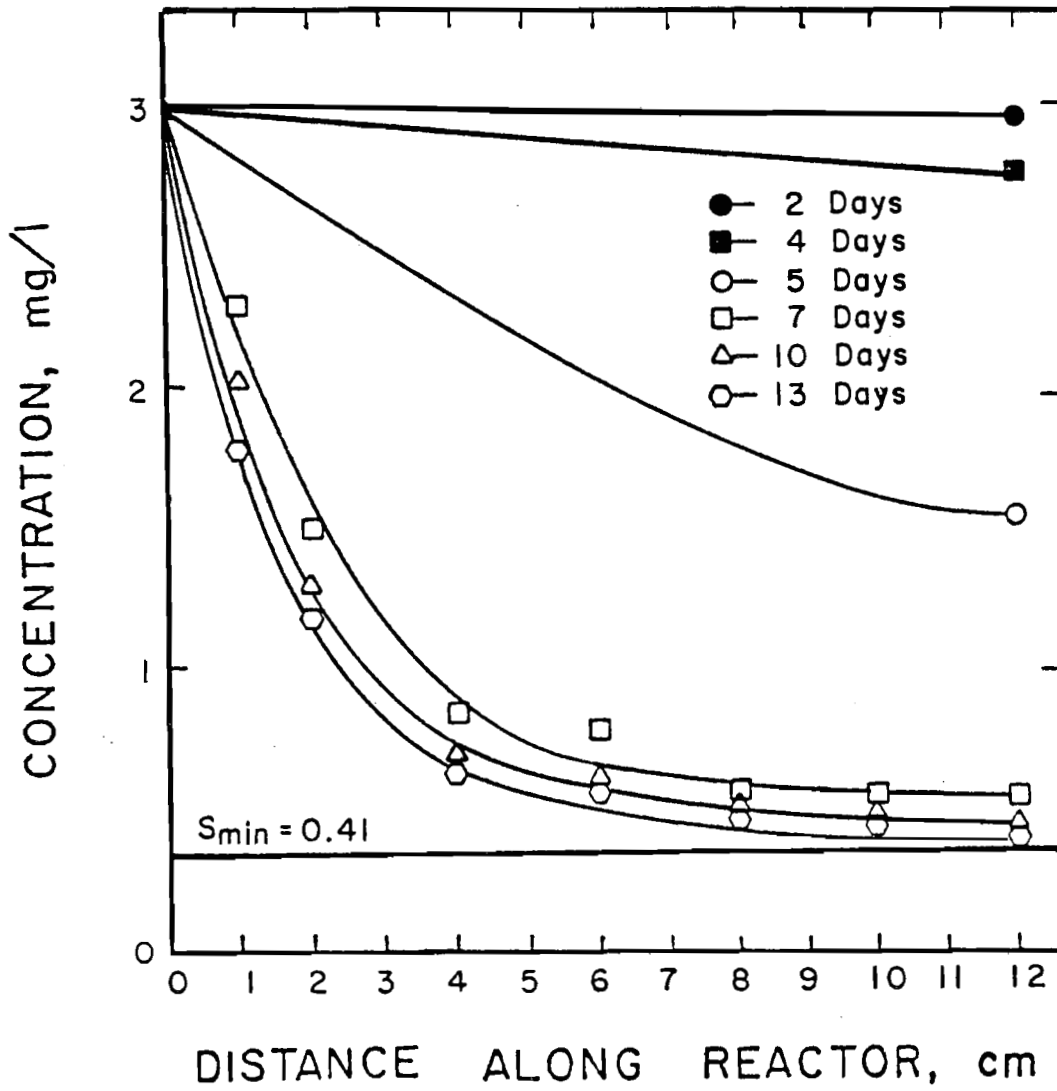
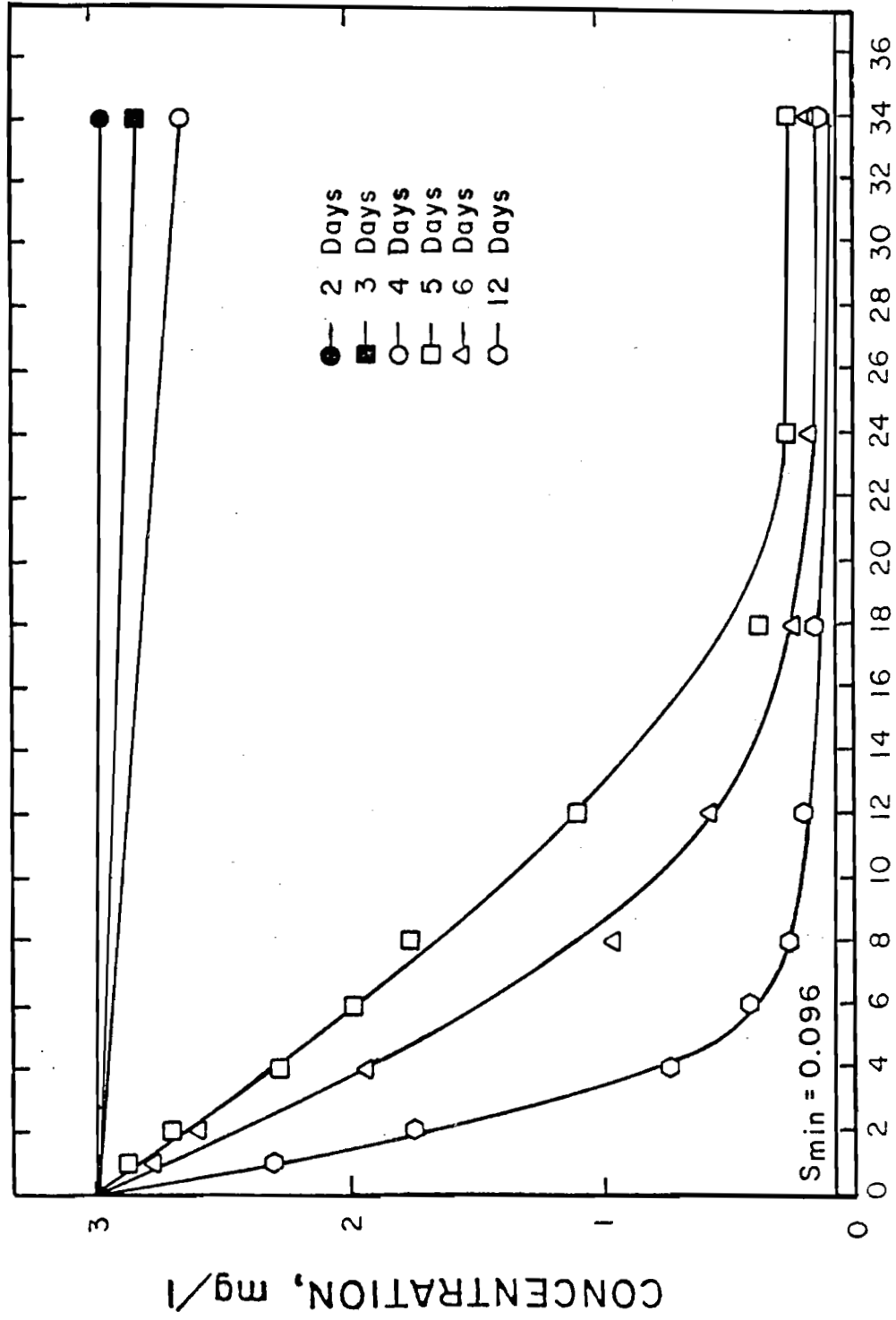


Figure 2. Initial Galactose Utilization Profiles



DISTANCE ALONG REACTOR, cm

Figure 3. Initial Phenol Utilization Profiles

as low as they would reach at steady-state. However, near the inlet of the reactor, increasing substrate removal occurred with time until a steady-state substrate-utilization profile existed throughout the entire reactor. The time for both reactors to reach steady-state conditions was approximately two weeks.

Figures 4 and 5 represent predicted removal profiles for both phenol and galactose for a series of days after the start-up. The initial biofilm thickness used was 0.05- μm , which is a reasonable estimate, since the seeding procedure would be unable to cover the glass beads with even a monolayer of bacterial cells. The 1- μm organisms would at most only cover a fraction of each glass bead. The other parameters used in the nonsteady-state model are presented in Table 2. The biofilm kinetic parameters for phenol were determined as described previously, and the parameters for galactose were determined experimentally and reported by Stratton (1981). The diffusivity coefficient, D , was calculated from a physical-chemistry handbook (Perry and Chilton, 1973), and D_f was taken to be equal to 0.8 D (Williamson and McCarty, 1976).

Table 2. Physical and Kinetic Parameters Used in the Nonsteady-State Biofilm Model for Galactose and Phenol

Parameter	Galactose	Phenol
X_f , mg/cm ³	16.4	10.6
K_s , mg/cm ³	0.0227	0.000891
k , day ⁻¹	15.0	6.35
Y , mg cells/mg substrate	0.54	0.35
b , day ⁻¹	0.08	0.1
b' , day	see Rittmann (1982)	
D , cm ² /day	0.575	0.795
D_f , cm ² /day	0.460	0.635
τ , cm	0.0092	0.0041

The predicted removal profiles somewhat overestimated the removal of both substrates from days 2-10 after seeding. Part of the reason

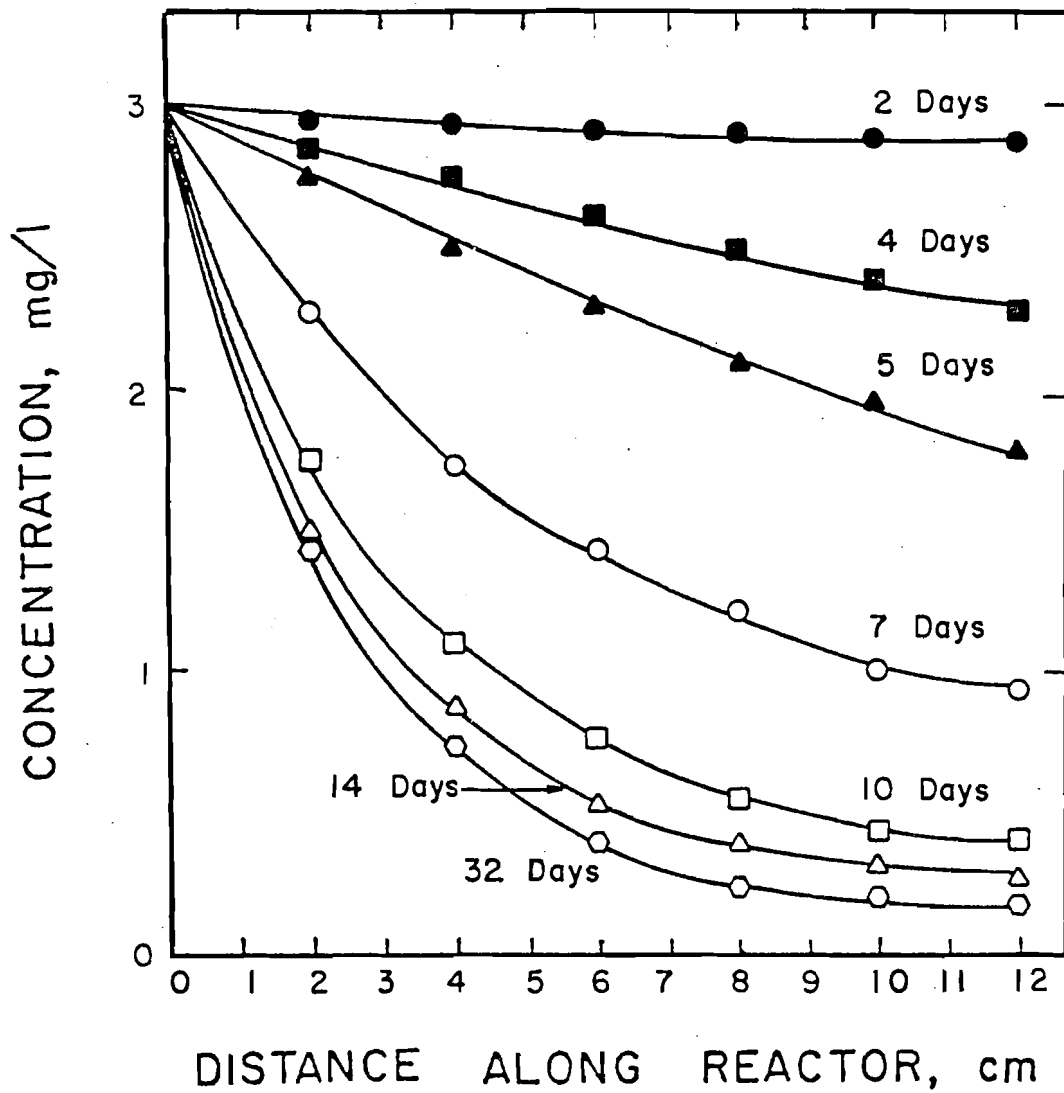


Figure 4. Predicted Galactose Utilization Profiles. Symbols are Model-Predicted. See Fig. 2 for Experimental Results.

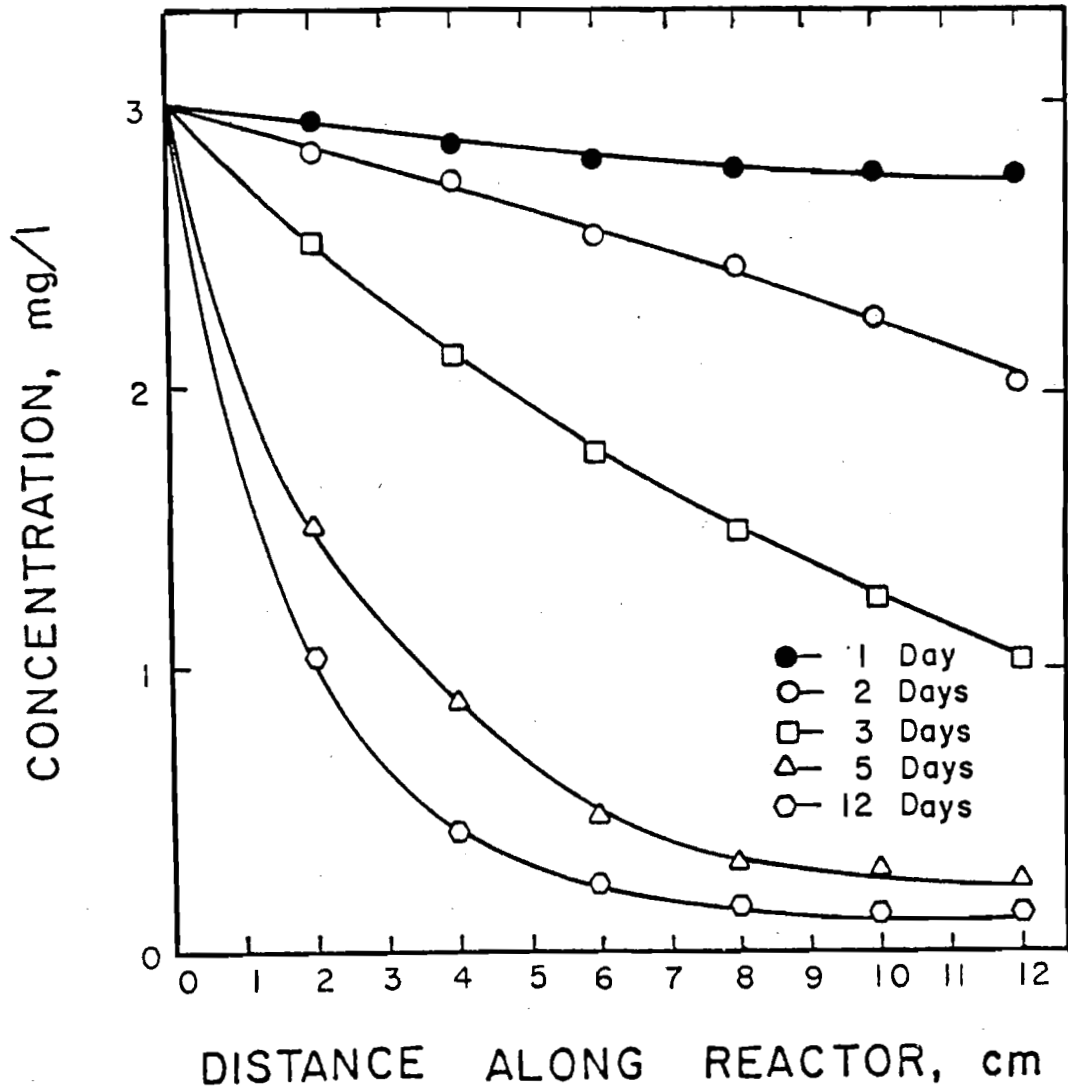


Figure 5. Predicted Initial Phenol Utilization Profiles. Symbols are Predicted. See Fig. 3 for Experimental Results.

lies in the choice of an initial biofilm thickness of $0.05 \mu\text{m}$. A smaller value would have slowed the initial phases. Unfortunately, measuring the initial attachment is practically impossible.

Although the predicted removal profiles are not perfect representations of the experimental data, the predicted results show the observed growth trends: greater substrate removal and biomass accumulation in the first portion of the reactor and the ability to reach a steady-state S_{min} value in less than two weeks. Having a better set of experimentally determined kinetic parameters would have improved predictions. Predicted profiles for galactose, for which a complete set of parameters was determined experimentally, were superior to the profiles for phenol, for which several parameters were estimated.

Figures 2-5 clearly indicate that once a population of bacteria reached a certain size, the amount of substrate utilized increased very rapidly. Batch experimental and modeling results demonstrated the following scenario for biofilm growth to steady-state. The initial biofilm community existed throughout the entire column. As the population of organisms increased, more substrate was utilized in each segment of the column, resulting in less substrate in the latter portions of the biofilm reactor. When the concentration of these latter portions reached a critical value, S_{min} , the organisms present could not maintain themselves, since the energy required for maintenance was equal to the energy available from the substrate. The minimum concentration moved rapidly from the outlet portion of the column toward the inlet of the column, where the active microorganisms remained. When the S_{min} concentration ceased to move further toward the inlet of the column, a steady-state biofilm was defined to exist. For both reactors, the largest population of viable bacteria inhabited the first few centimeters of the column where the substrate concentration was still greater than S_{min} .

The steady-state biofilm conditions existed in each column before much visible biofilm growth could be seen on the glass beads. Before seeding the column, the glass beads were shiny. After the steady-state substrate utilization profiles were determined to exist, the glass beads had turned to a duller texture from the growth of the biofilm.

EFFECTS OF REFRACTORY CELL ACCUMULATION

After one year of operation, the glass beads near the inlet of the column could barely be seen due to the build-up of biomass on and between them. The biomass was a dark brown color and was present mainly in the first five or six centimeters. The glass beads present in the rest of the reactor obtained a brownish color, but even after one year, the glass beads were still quite visible. Since a biofilm was hardly visible when steady-state substrate utilization was reached, the increasing amount of biomass with time was partly a build-up of dead cell matter, refractory cell mass, and bacterial secretions used for biofilm attachment. Substrate utilization did not increase as the biomass built up. The activity of the biofilm after long-term substrate utilization remained the same as it was when it first reached steady-state conditions after about two weeks of operation. Despite being barely visible, the biofilm at two weeks was essentially deep with respect to phenol. After long-term substrate utilization, the active portion of the biofilm was a smaller fraction of the total biofilm than initially when nearly the entire biofilm was active.

One of the purposes of operating a steady-state biofilm for a long period of time was to determine if the build-up of refractory cell matter would adversely affect substrate removal. As the void space between glass beads decreased due to the build-up of biomass, the flow velocity would increase. Possibly short circuiting would occur. Also, the build-up of refractory materials might increase mass transfer resistance within the biofilm.

The phenol-grown biofilm remained at steady-state for 13 months. Since the greatest quantity of cell matter built-up in the first few centimeters of the column, these would be the points where substrate utilization efficiency would be most affected. Figure 6 presents results obtained by measuring the uptake of phenol at the different sampling ports along the column as a function of time. Linear regression analysis was used to obtain the best fit lines illustrated in Figure 6 from fourteen sample points. The slopes of the eight lines, which indicate if long-term removal increased or decreased, turned

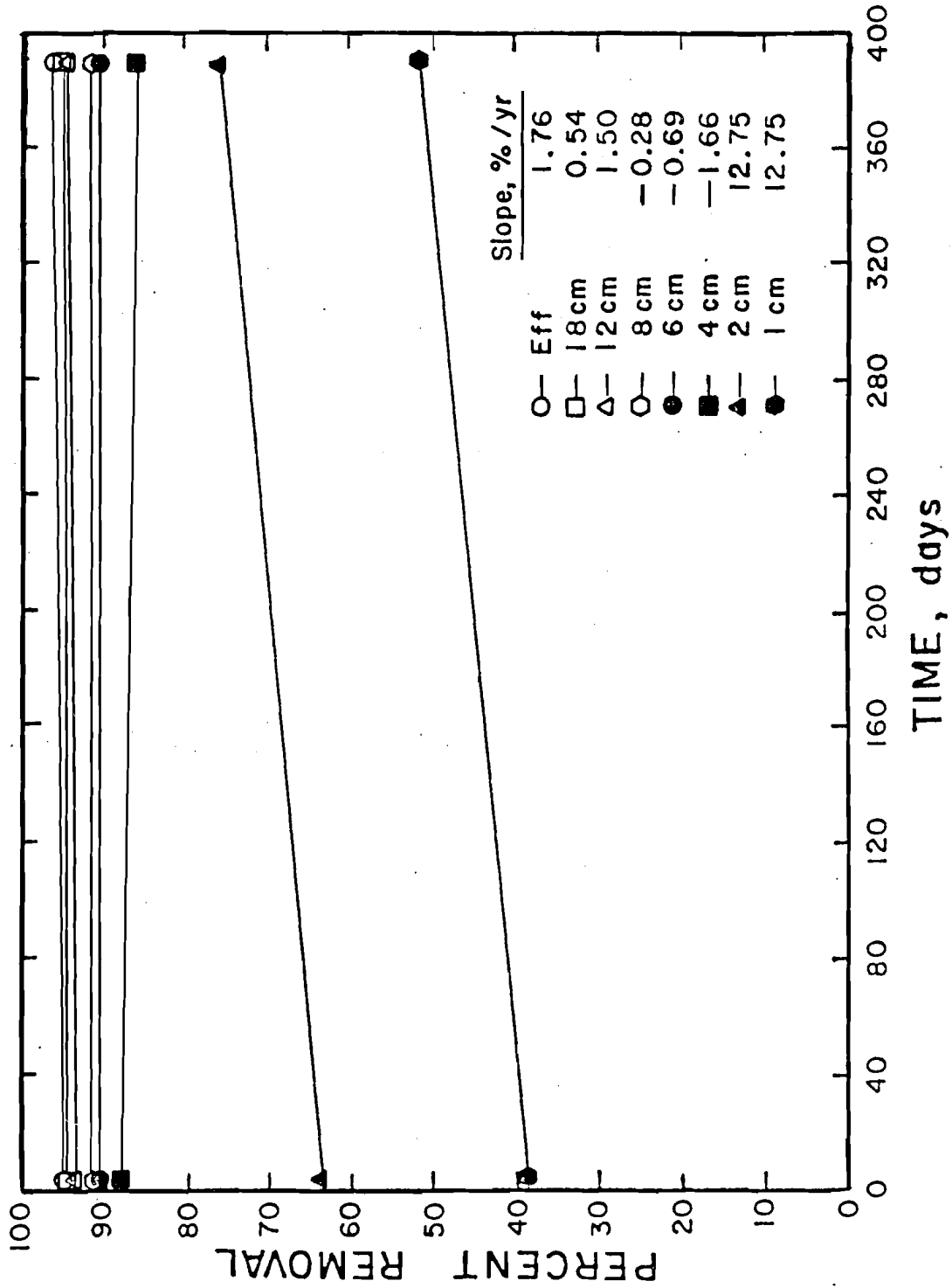


Figure 6. Overall Phenol Utilization Efficiencies at Various Biofilm Reactor Depths. Lines are Results of Linear Regression Analysis for 14 Sets of Data During the 375-Day Period.

out negative for four lines and positive for the other four. The negatively sloped lines indicate less removal efficiency at that section of the biofilm reactor. The results from the inlet end of the reactor clearly indicate that the build-up of refractory cell mass had no adverse effects on the removal of phenol as a primary substrate. Actually the removal of phenol slightly increased over a long term in the first two centimeters of the reactor where the cell mass was the greatest. The increase could be the result of enhanced density of viable organisms in the active portion of the biofilm. Slightly denser viable organisms could exist after long-term operation because the biodegradable portion of the cell matter in the nonactive portion of the biofilm added to the concentration of organic electron donor and provided sufficient energy for slightly more organisms in the active zone than were present when the only source of energy was the substrate added to the feed solution.

Figure 7 illustrates how the accumulation of biomass was distributed throughout the column at the end of 13 months of experimentation with the phenol column. The thickest biomass measurement was at a reactor depth of 1.5 cm. At depths less than 1.5 cm, the biofilm thickness was not as large because of disturbances that occurred when the end caps were removed from the reactor for cleaning. The biofilm density was rather constant at about 10 mg VSS/cm^3 , except for near the inlet, where density was 45 mg VSS/cm^3 . The average biofilm density was found to be $10.6 \text{ mg-dry cell weight/cm}^3$.

NITRIFICATION AND COMPETITIVE SUBSTRATE UTILIZATION

Since ammonia was added to supply the required nitrogen for the biofilm bacteria, the possibility of nitrification was tested in the steady-state phenol column. The normal input concentration of ammonia was 0.445 mgN/l , but for a twenty-one day period the concentration of ammonia was doubled to 0.89 mgN/l . Figure 8 presents the concentration of nitrite and nitrate as a function of column depth, along with a steady-state phenol utilization profile. The phenol utilization profile

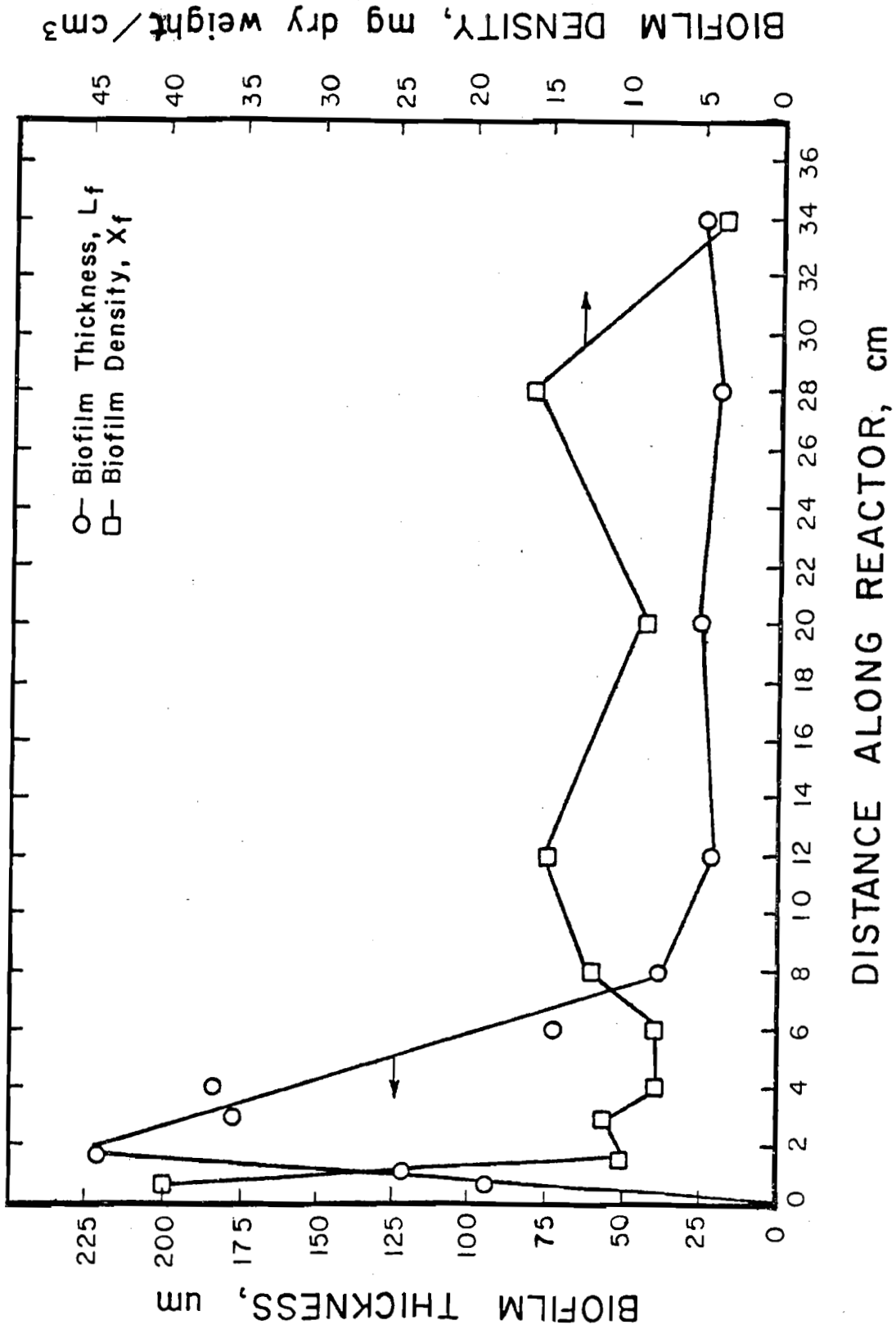


Figure 7. Biofilm Thickness and Density as a Function of Depth into the Reactor for the Phenol-Grown Biofilm After 13 Months of Operation.

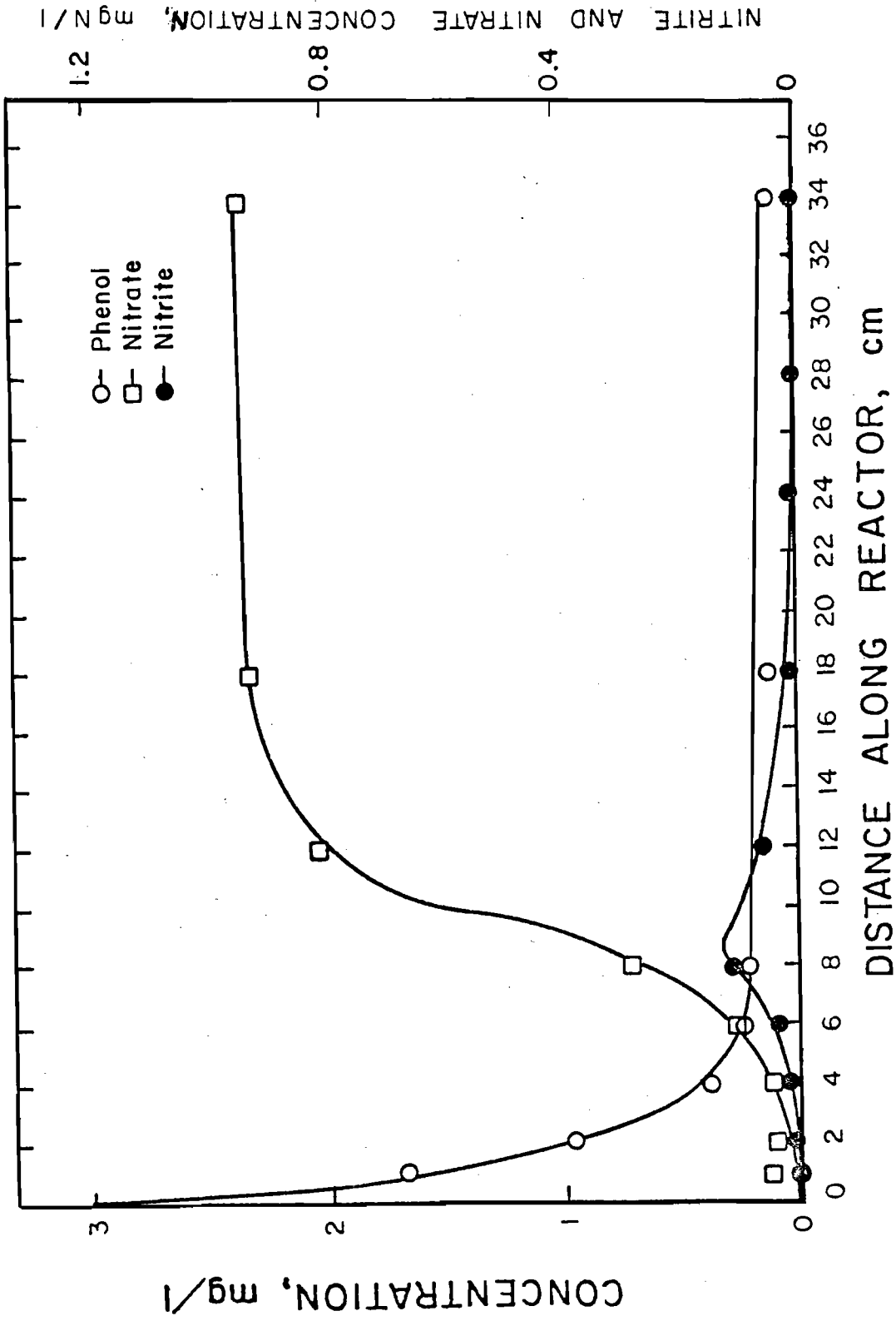


Figure 8. Nitrite and Nitrate Concentrations as a Function of Column Depth. A Steady-State Phenol Utilization Profile is also Presented.

demonstrates competitive substrate utilization. The autotrophic nitrifiers oxidized ammonia to nitrite and nitrate in the presence of oxygen as their source of electrons. Because of the slower growth rate for the nitrifiers, the faster growing heterotrophs were able to dominate the habitat closer to the column entrance, where the organic electron donor was present at the highest concentrations. When the concentration of phenol was reduced to approximately 0.3 mg COD/l, there was not enough organic electron donor left to support a population of heterotrophs large enough to completely dominate the particular environment, and the presence of nitrifiers became apparent. The population of nitrifiers slowly increased from a depth of 4-cm to 8-cm and then increased significantly between 8-cm and 12-cm, as indicated by the largest amounts of nitrate production. Between 12-cm and 18-cm the rate of nitrification decreased indicating a smaller number of autotrophic nitrifiers in this segment. At a depth of 18-cm nitrification was complete and virtually all of the initial ammonia had been oxidized to nitrite and then to nitrate. The phenol example clearly indicates how the different kinetic reaction rates can define how the organisms utilizing different substrates compete for space within the reactor until they find the habitat most suited for them (McCarty *et al.*, 1981).

In general competition can occur for any combination of substrates. The organism that can grow fastest on the input medium will dominate the habitat closer to where the feed solution enters the column. Competition would have to be included in any model used for the purpose of predicting the removal profiles for an individual substrate when the organisms utilizing that substrate are out competed by others. The presence of ammonia was not a problem for studying the removal of the primary organic substrate because the heterotrophs dominated the reactor under steady-state conditions.

NONSTEADY-STATE SUBSTRATE UTILIZATION

A biofilm method that can be used to achieve the removal of substrates to levels below S_{min} is nonsteady-state operation. The way in which a nonsteady-state biofilm can be utilized to obtain

concentrations well below S_{min} is by growing the biofilm at a concentration elevated above S_{min} for a short time period and then reducing the concentration for an extended period of time. During the time in which the biofilm is slowly decaying, it can still achieve removals of substrates present below their S_{min} value.

The 12-cm column was used for the nonsteady-state testing procedures and was initially fed with 3.0 mg/l of galactose. The biofilm was allowed to reach steady-state conditions, and after three to four weeks of operation as such, the influent galactose concentration was below the experimentally determined S_{min} value of 0.42 mg/l. According to theory, the biofilm established when the influent galactose concentration was 3.0 mg/l would not be supported by the lower substrate concentration, and the biofilm thickness would naturally decrease due to a net loss occurring within the biofilm reactor.

The biofilm established at 3.0 mg/l of galactose initially was able to reduce the substrate concentration from 0.3 mg/l to approximately 0.04 mg/l. This removal corresponded to an efficiency of nearly 87 percent, which was identical to the steady-state galactose utilization when the galactose was present at 3.0 mg/l. Little decrease in substrate removal efficiency occurred during four and one-half months of operation at 0.3 mg/l of galactose. Figure 9 presents several removal profiles for the nonsteady-state conditions in which the influent substrate concentration was 0.3 mg/l of galactose. The figure clearly indicates that even after 138 days of operation at a substrate concentration below S_{min} , the overall utilization efficiency remained nearly equal to the original efficiency. The removal rates near the inlet of the reactor segments are illustrated in Figure 10 in which removal efficiency is plotted as a function of time at the different reactor depths. The lines presented for each segment of the reactor were calculated using linear regression analysis for fifteen sample points. The actual sample points are indicated for the depths of 1 and 2 cm for the purpose of revealing the amount of scatter encountered. The slopes of the lines are clearly negative for the depths of 4 cm, 2 cm, and 1 cm. The slopes on the effluent, 8-cm, and 6-cm depths remained nearly constant. Since the biofilm reactor was being operated at nonsteady-state

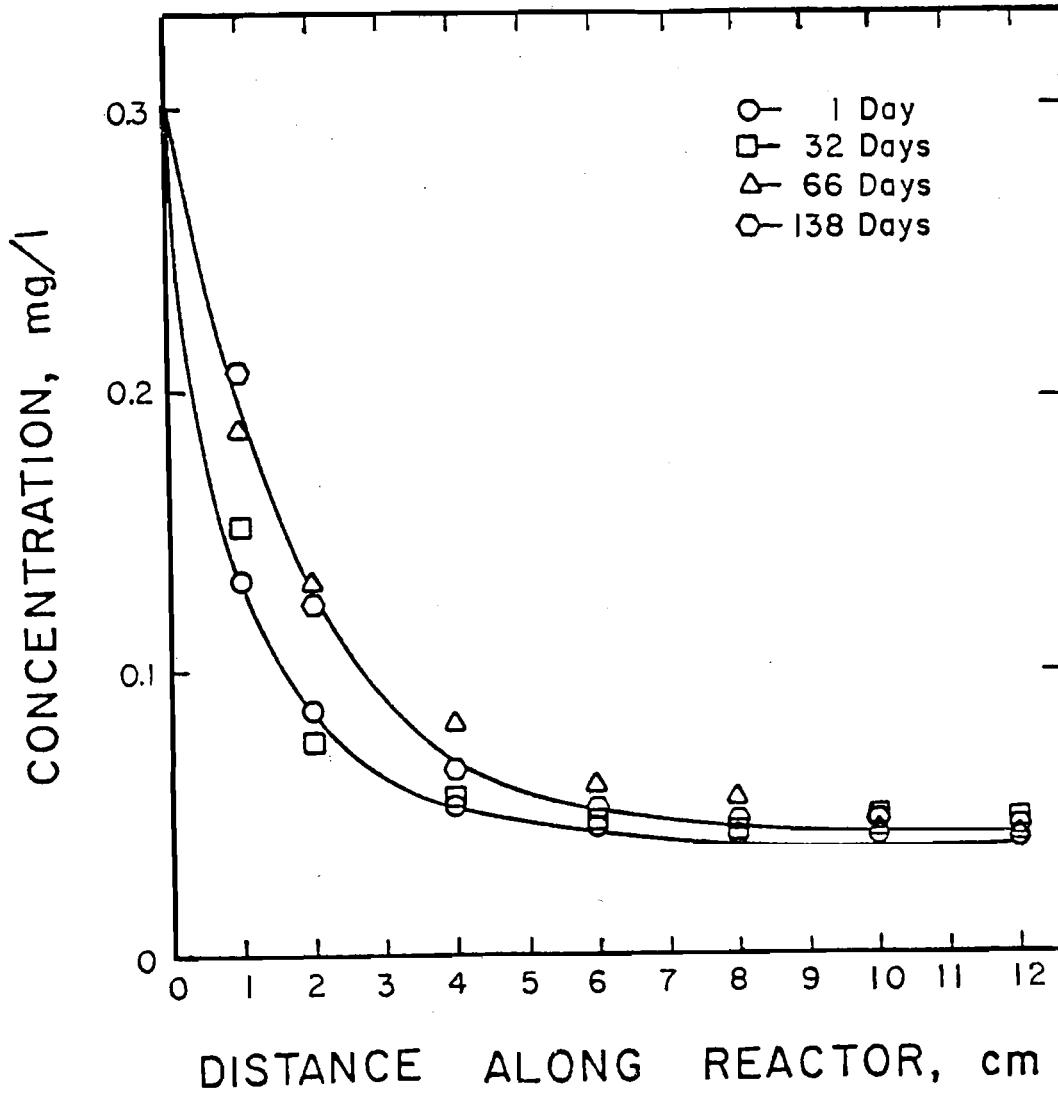


Figure 9. Nonsteady-State Galactose Utilization Profiles at Subsequent Times After Reducing the Influent Galactose Concentration from 3.0 mg/l to 0.3 mg/l. $S_{\min} = 0.41$ mg/l.

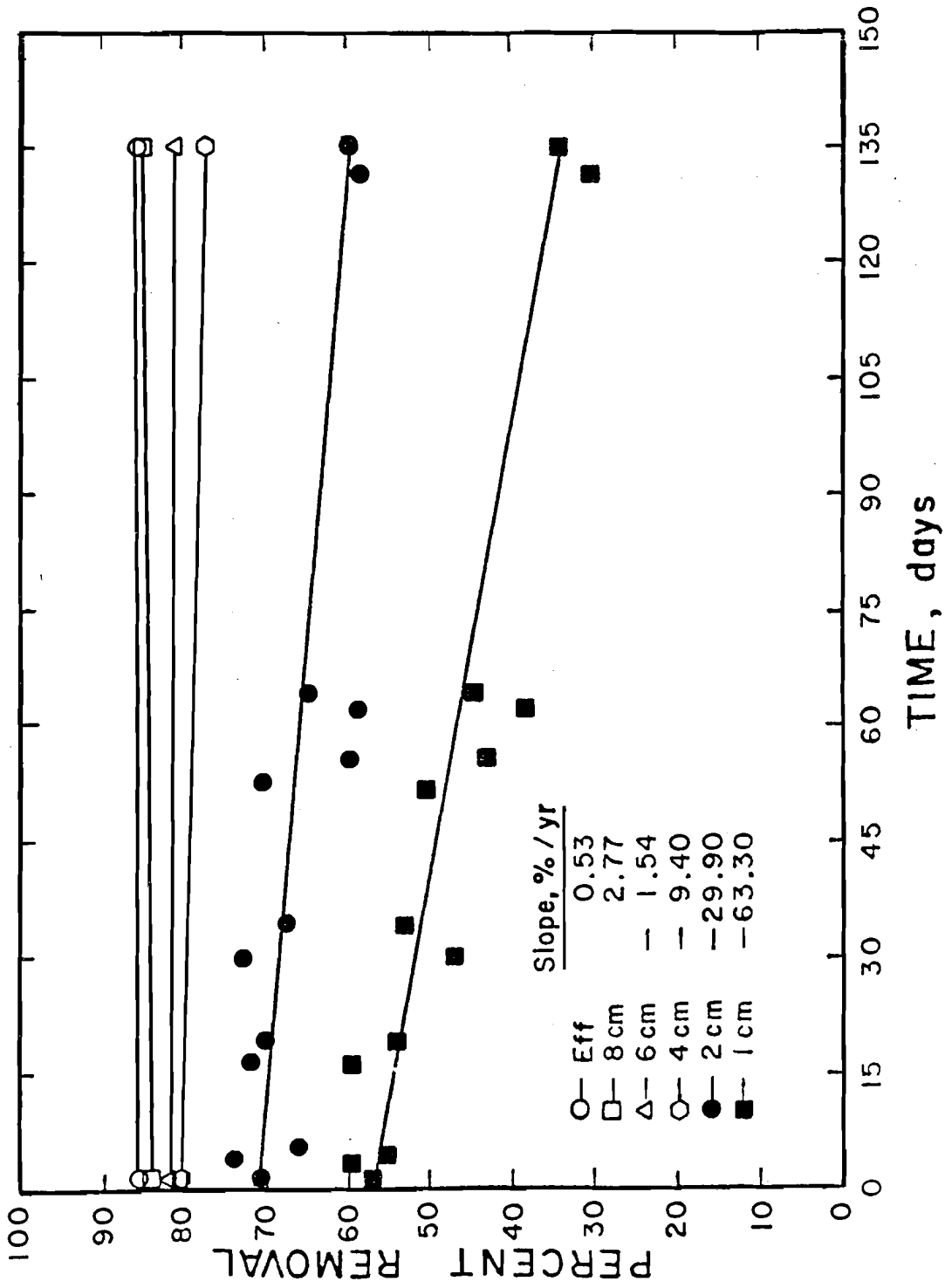


Figure 10. Cumulative Removal Efficiencies as a Function of Time at Different Reactor Depths for the Nonsteady-State Biofilm Feeding on 0.3 mg/l of Galactose. $S_{min} = 0.41$ mg/l.

conditions, the likely explanation for the decreasing removal efficiencies near the column inlet is biofilm loss. After the concentration of galactose was reduced to 0.3 mg/l, the number of active organisms began to decrease, and as a result, the removal rates decreased with time. Since 0.3 mg/l of galactose was less than the S_{min} value for galactose, the biofilm, in theory, would continue to decay until the viable biofilm was completely gone, yielding near zero removal efficiency. With the exception of the decreasing removal efficiencies near the column inlet, the results indicated that the decay of the biofilm was proceeding at an extremely slow rate.

For a biofilm grown with galactose and controlled by the kinetic parameters presented in Table 2, the predicted removal profiles for a galactose feed of 0.3 mg/l and at times 1 and 10 days after reducing the substrate concentration are presented in Figure 11. Clearly, the model predicted a noticeable decrease in the overall removal efficiency after only 10 days. Thus, the observed decay rate was much slower than predicted from the independently measured parameters in Table 2.

Since the decay process was barely noticeable at the new nonsteady-state galactose concentration, the use of short term, elevated concentration tests were conducted to obtain more sensitivity for evaluating the rate at which decay was occurring. Figure 12 presents the removal profiles for galactose when it was present for a 2- or 3-hour time period at a concentration of 3.0 mg/l. The figure indicates that the biofilm was experiencing net decay of its capability to remove galactose. However, the rate of decay measured by the 3.0 mg/l spike tests was still very slow. Even 138 days after the galactose concentration was reduced from 3.0 mg/l to 0.3 mg/l, nearly 50 percent of the 3.0 mg/l galactose concentration was still utilized.

Since the rate of decay was proceeding so slowly, the influent galactose concentration was reduced to 0.03 mg/l from the 0.3 mg/l level. This change occurred after operating the column at 0.3 mg/l for 138 days. The galactose concentration of 0.03 mg/l was well below the S_{min} value determined experimentally (0.43 mg/l). A faster decay rate was expected at this very low organic concentration. The substrate removal profiles for galactose at the normal influent concentration of

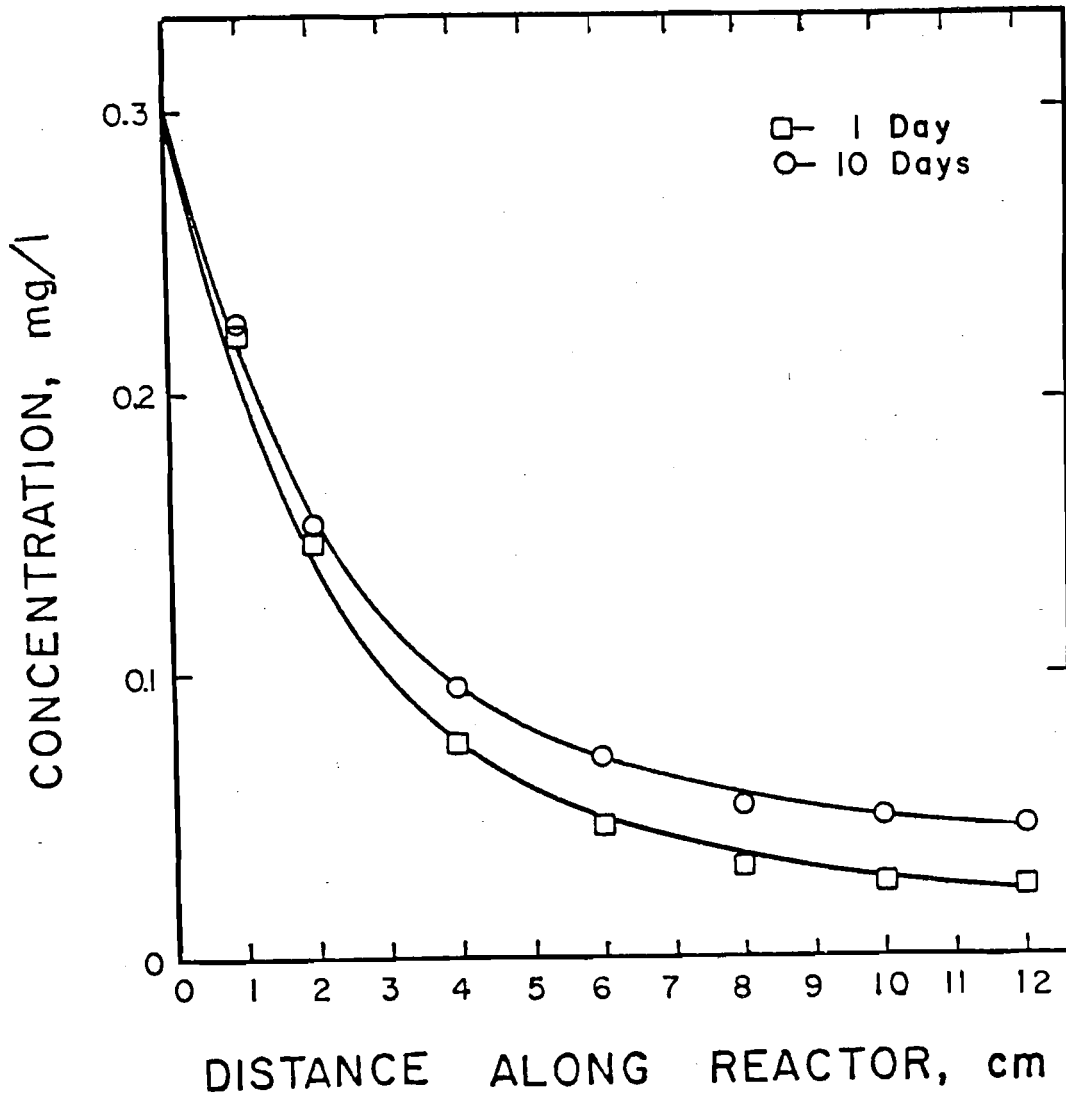


Figure 11. Predicted Galactose Removal Profiles at 0.3 mg/l on the First Day and Tenth Day After Reducing the Influent Concentration from 3.0 to 0.3 mg/l.

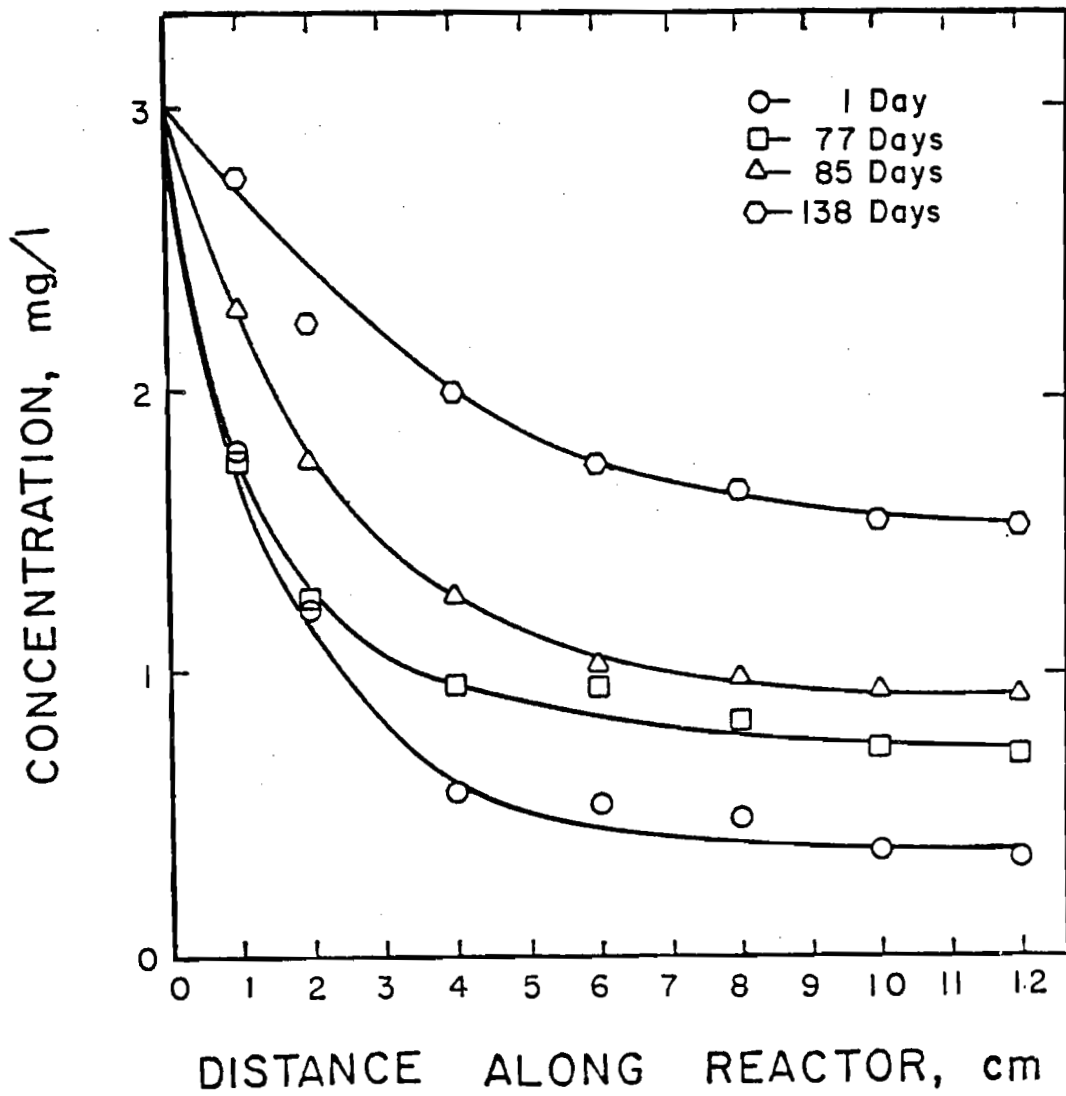


Figure 12. Short-Term Removal Profiles for Galactose at 3.0 mg/l When the Normal Feed Concentration was 0.3 mg/l.

0.03 mg/l, and for short-term elevated concentrations of 0.3 mg/l and 3.0 mg/l were observed with passing time and the results are presented in Figures 13, 14, and 15, respectively, for the first 52 days of operation at 0.03 mg/l. The only set of utilization profiles that indicated that the biofilm was significantly decaying was the set in Figure 15 for the short-term 3.0 mg/l of galactose. During the first 52 days of operation at 0.03 mg/l of galactose, the overall removal efficiency decreased from approximately 47 percent to 37 percent for a feed concentration of 3.0 mg/l. The profiles for galactose concentrations of 0.03 mg/l and 0.3 mg/l remained nearly constant during the same time period. The overall removal efficiencies, around 86 percent, for both of these concentrations for extended time periods once again indicated that trace-level organics could be reduced well below their respective S_{min} values when a nonsteady-state biofilm was used. Although it was quite desirable that the galactose could be removed efficiently for extended periods of time when present at sub- S_{min} levels, the reasons for the slower than predicted decay rates required explanation.

Effect of Nitrification on Prolonging Heterotrophic Activity

After the nonsteady-state biofilm reactor was operated at an influent galactose concentration of 0.03 mg/l for 52 days with little decay observed, the concentration of ammonia in the feed was doubled from 0.445 mgN/l to 0.890 mgN/l. Previously presented results proved that nitrification occurred in the phenol reactor when phenol was fed at 3.0 mg/l, but nitrification was prevented by the rapid growth of heterotrophs near the inlet.

Figure 16 supports the fact that nitrification also occurred within the nonsteady-state reactor. The figure indicates that when galactose was fed at 0.03 mg/l the nitrifiers were able to exist in a habitat very near the column inlet. In fact, over two-thirds of the ammonia had been oxidized to nitrite and then nitrate within the first 2 cm of reactor depth. All of the ammonia in the feed solution had been oxidized to nitrate by the time it had reached a depth of

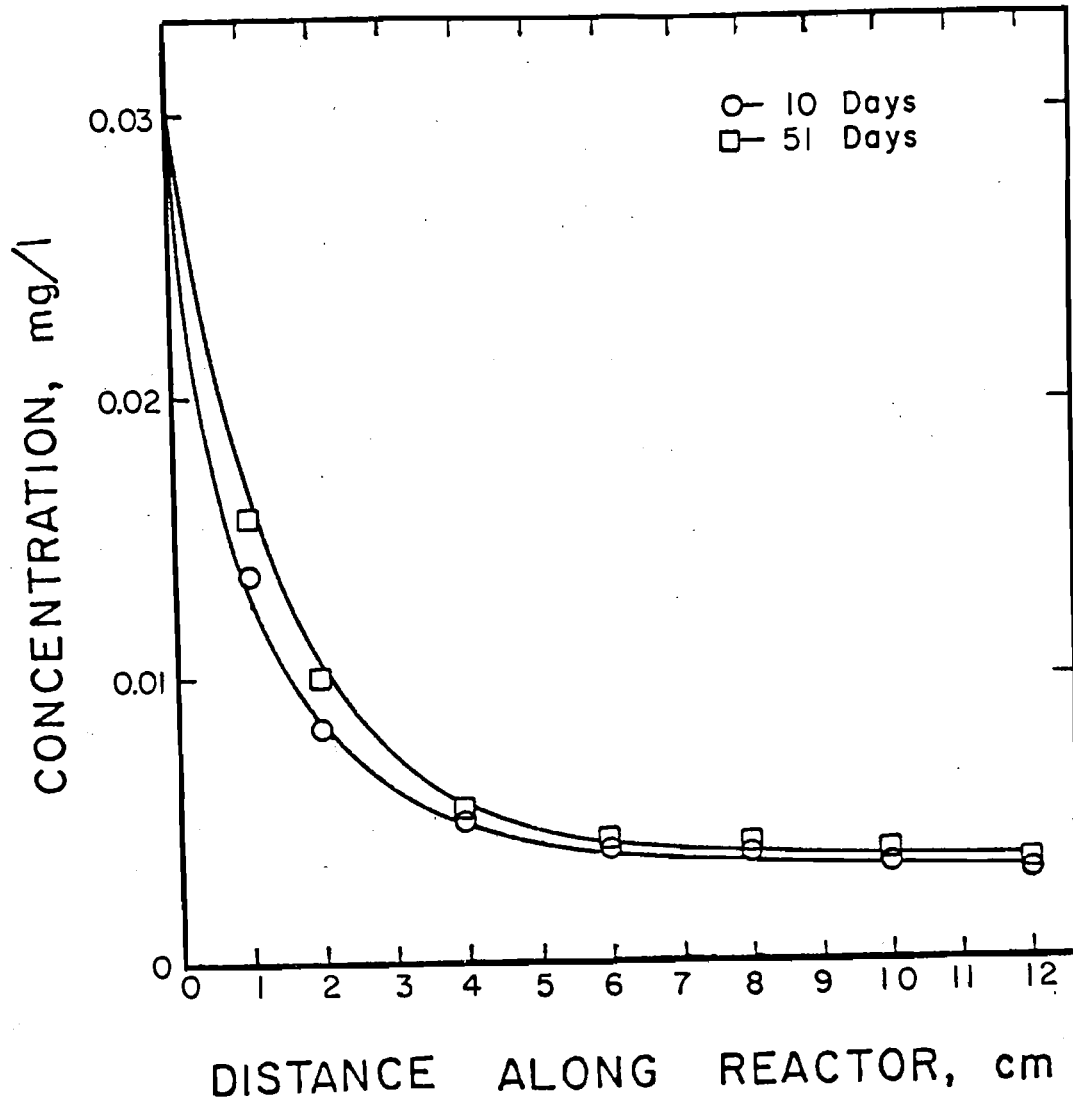


Figure 13. Nonsteady-State Removal Profiles for Galactose at 0.03 mg/l When the Normal Feed Concentration was the Same.

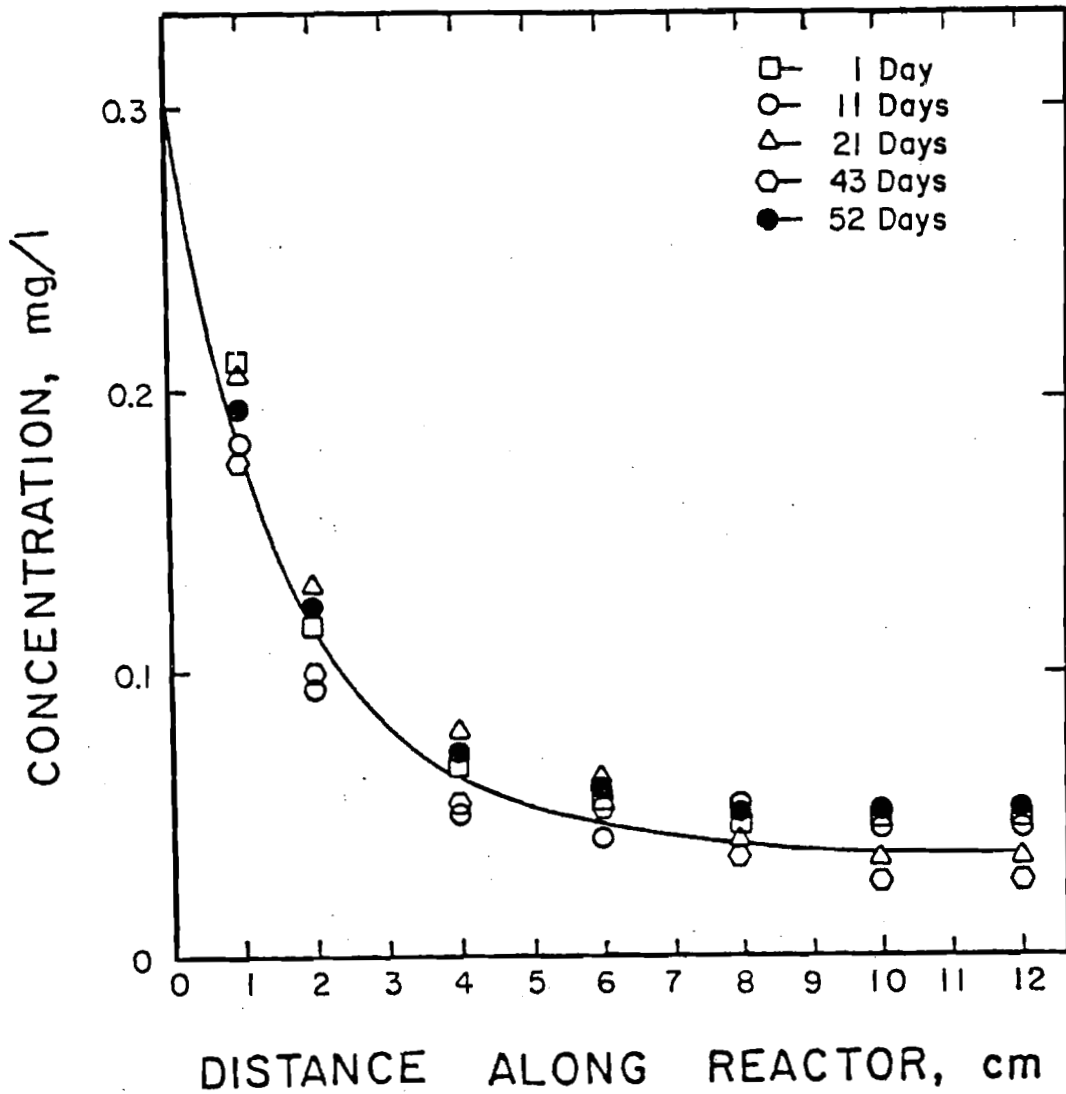


Figure 14. Short-Term Nonsteady-State Removal Profiles for Galactose at 0.3 mg/l When the Normal Feed Concentration was 0.03 mg/l.

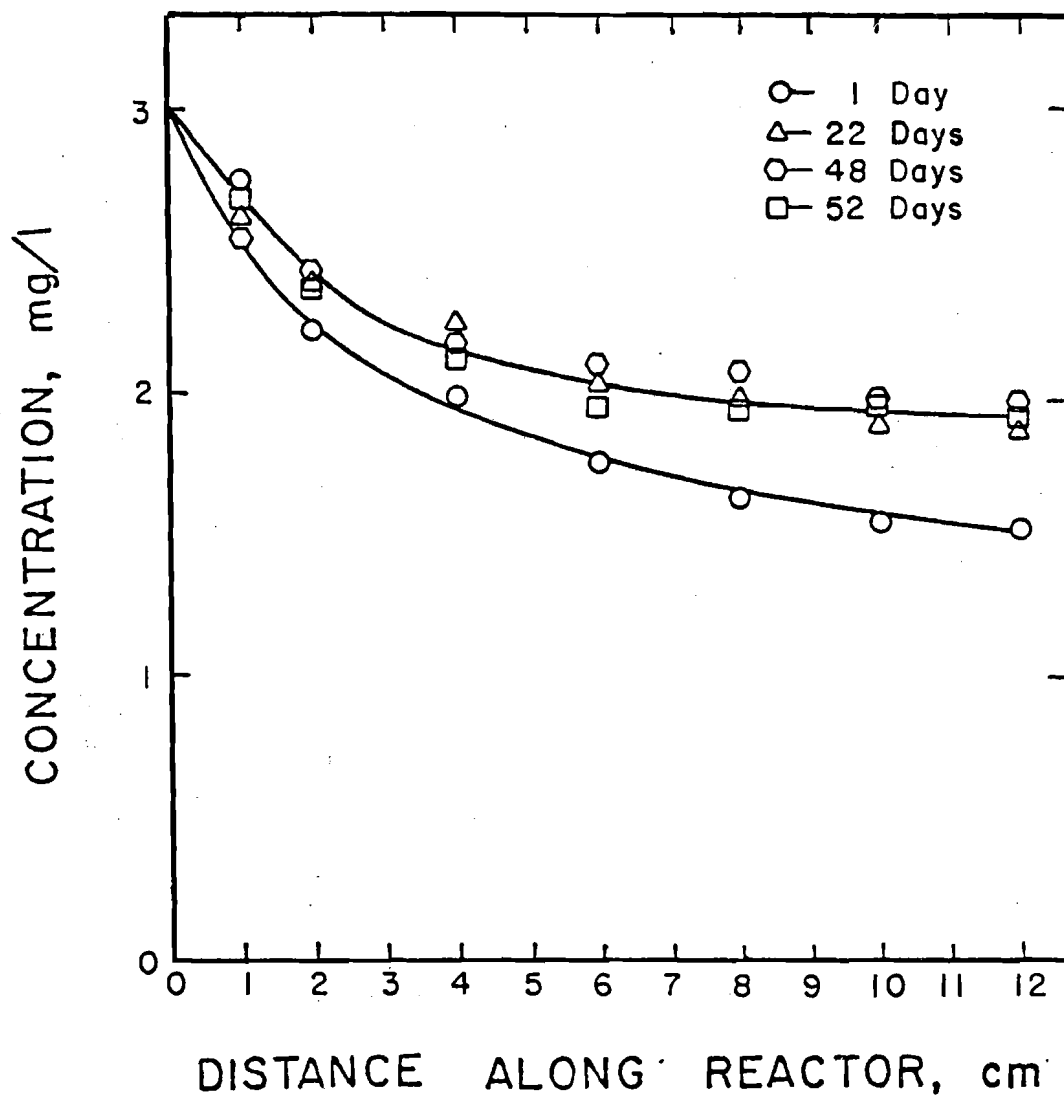


Figure 15. Short-Term Nonsteady-State Removal Profiles for Galactose at 3.0 mg/l When the Normal Feed Concentration was 0.03 mg/l.

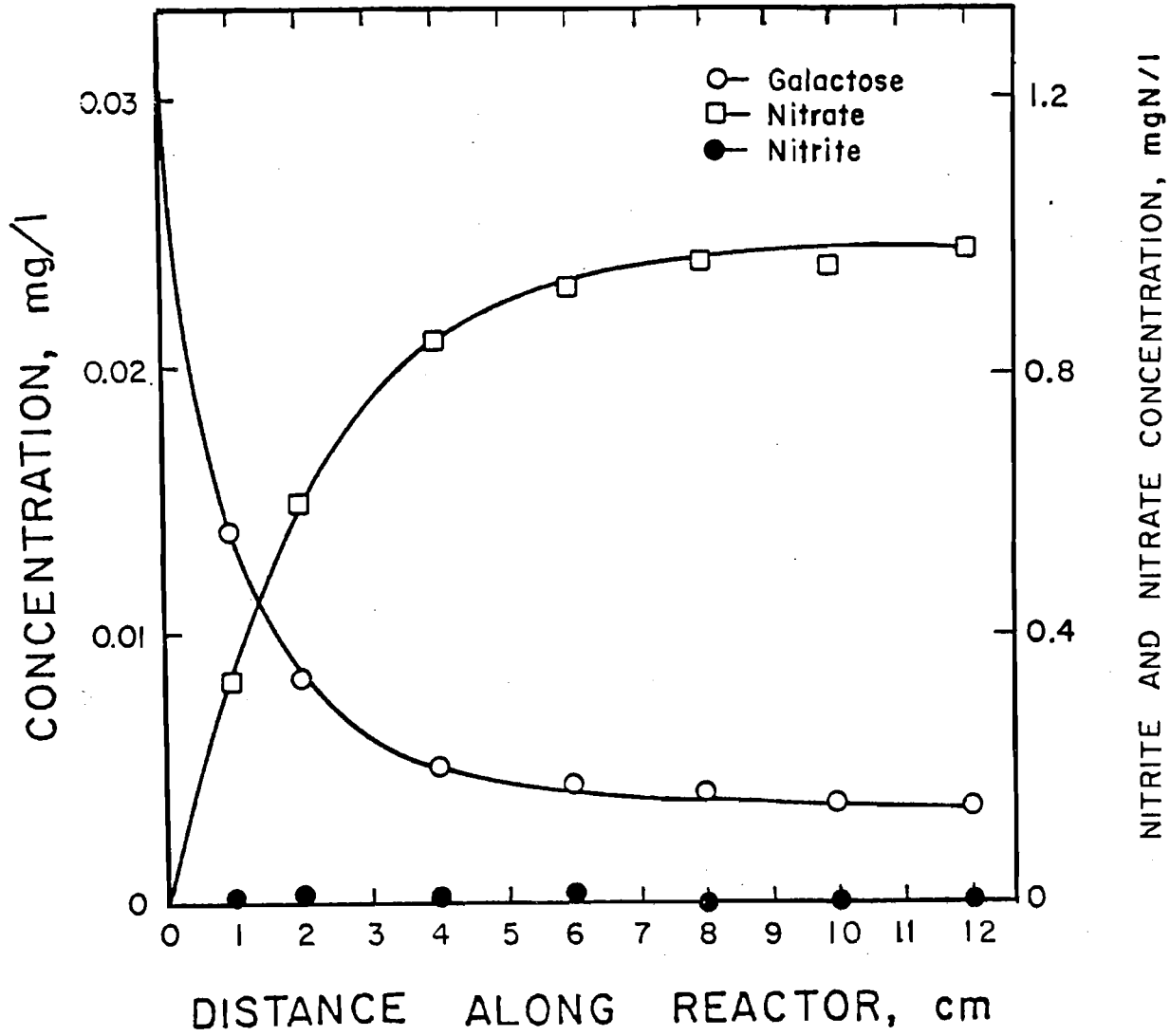


Figure 16. Concentration of Nitrite and Nitrate in the Nonsteady-State Galactose Reactor. A Galactose Removal Profile is Included for 0.03 mg/l.

6 cm in the reactor. Since the potentially faster-growing heterotrophs were exposed to a very low influent organic concentration, they were unable to dominate the inlet section of the reactor. The removal profile for galactose, also illustrated in Figure 16, indicates that the nitrifiers were unable to completely dominate the inlet biofilm region, since the organic compound was significantly utilized in this region.

With the occurrence of nitrification and heterotrophic activity together, the autotrophic nitrifiers produce organic cell matter, part of which the heterotrophs can utilize. If the heterotrophs utilize the organic material synthesized through nitrification, the organic matter supplements the feed galactose, and the heterotrophs would be exposed to a higher concentration of organic electron donor than was fed in the influent.

By doubling the concentration of ammonia in the feed solution, more organic matter from nitrification would be present for the heterotrophs and the utilization of galactose should increase from the level when only 0.445 mgN/l was used. Table 3 indicates how the removal efficiencies for galactose changed at the different depths of the biofilm reactor during the time period that ammonia was present at 0.890 mgN/l. The change from 0.445 mgN/l to 0.890 mgN/l occurred 52 days after the galactose concentration was reduced from 0.3 mg/l to 0.03 mg/l and continued for 21 days. The entries in the table at 50, 51, and 52 days represent the last galactose utilization experiment before increasing the ammonia concentration to 0.89 mgN/l. The table indicates that at the normal galactose concentration of 0.03 mg/l, increased galactose removal occurred between the inlet and the 2-cm depth. Beyond the 4-cm depth little galactose was utilized, resulting in an overall utilization efficiency of 88 percent, which was almost identical to the overall removal efficiency when the lower ammonia concentration was used.

When the concentration of galactose was increased to 0.3 mg/l for a short time period, the biofilm grown at a higher ammonia concentration also removed more galactose in the first 2-cm of reactor length, but overall reactor efficiency remained the same for both instances.

Table 3. Incremental Percentages of Influent Galactose Removed by the Specified Segment of the Reactor for the Case When the Ammonia Concentration was 0.445 mgN/l and Approximately 3 Weeks After the Ammonia Concentration was Changed to 0.89 mgN/l

S°, mg/l	Time, days	Distance along reactor, cm						Overall efficiency, %
		0-1	1-2	3-4	4-6	6-8	8-10	
0.03	50	48	18	16	3	1	1	87
0.03	73	55	23	8	1	1	0	88
0.3	51	36	23	19	4	2	0	84
0.3	73	40	24	17	1	2	1	85
3.0	52	12	10	7	6	0	2	37
3.0	73	14	13	13	5	2	1	48

The most sensitive results were obtained for the short-term influent galactose concentration of 3.0 mg/ℓ. For this case, the biofilm grown at a higher ammonia concentration was able to increase the utilization enough in the first 4 centimeters of reactor depth so that it experienced an 11 percent increase in overall utilization efficiency. A larger heterotrophic biofilm thickness at the inlet sections was apparent, since for each of the three galactose concentrations, more substrate mass was removed in the first 2 to 4 cm of reactor depth when the ammonia concentration had been increased for an extended time. The results in Table 3 suggest strongly that nitrification activity supplemented the organic supply of the heterotrophs and enhanced the survival of the nonsteady-state biofilm.

To ensure that nitrification actually did enhance galactose removal near the biofilm reactor inlet, the ammonia was completely removed from the feed solution and replaced with 0.445 mgN/ℓ of nitrate. The removal of ammonia from the feed solution occurred 73 days after the galactose concentration in the feed was reduced from 0.3 mg/ℓ to 0.03 mg/ℓ. Figures 17, 18, and 19 indicate the effect that removing the ammonia from the feed solution had on cumulative removal efficiencies at various depths within the reactor for the three different influent galactose concentrations. The figures indicate that when the ammonia was completely removed from the feed solution, the amount of galactose removed noticeably began to decrease, whereas it had increased during the time ammonia was fed at 0.89 mgN/ℓ.

The most notable change occurred between the inlet and 1-cm depth for a galactose concentration of 0.03 mg/ℓ because the heterotrophic biofilm had been supplemented by the nitrifying activity in the first few centimeters to a much greater extent than had other parts of the reactor. Total removal of the ammonia from the feed significantly decreased the heterotrophic biomass in the early stages and caused the removal of 0.03 mg/ℓ of galactose to drop from its still relatively high percentage. When the ammonia supply was eliminated, the biofilm near the inlet was not supplemented by the autotrophic biomass and began to decay, utilizing a lower percentage of influent

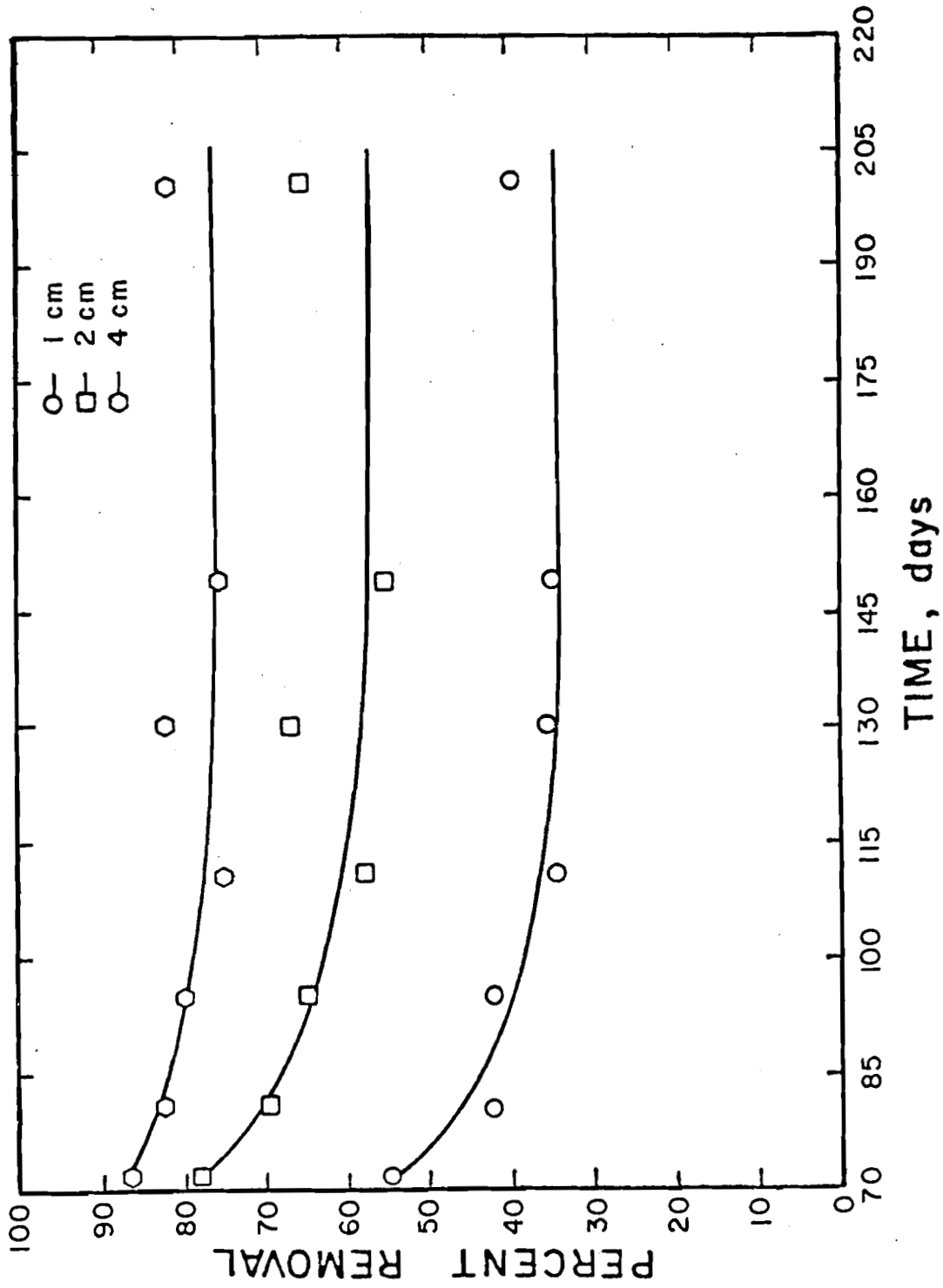


Figure 17. Galactose Utilization Efficiencies at Depths of 1 cm, 2 cm, and 4 cm as a Function of Time After Ammonia Nitrogen was Replaced by Nitrate Nitrogen. The Influent Galactose Concentration was the Normal 0.03 mg/ℓ.

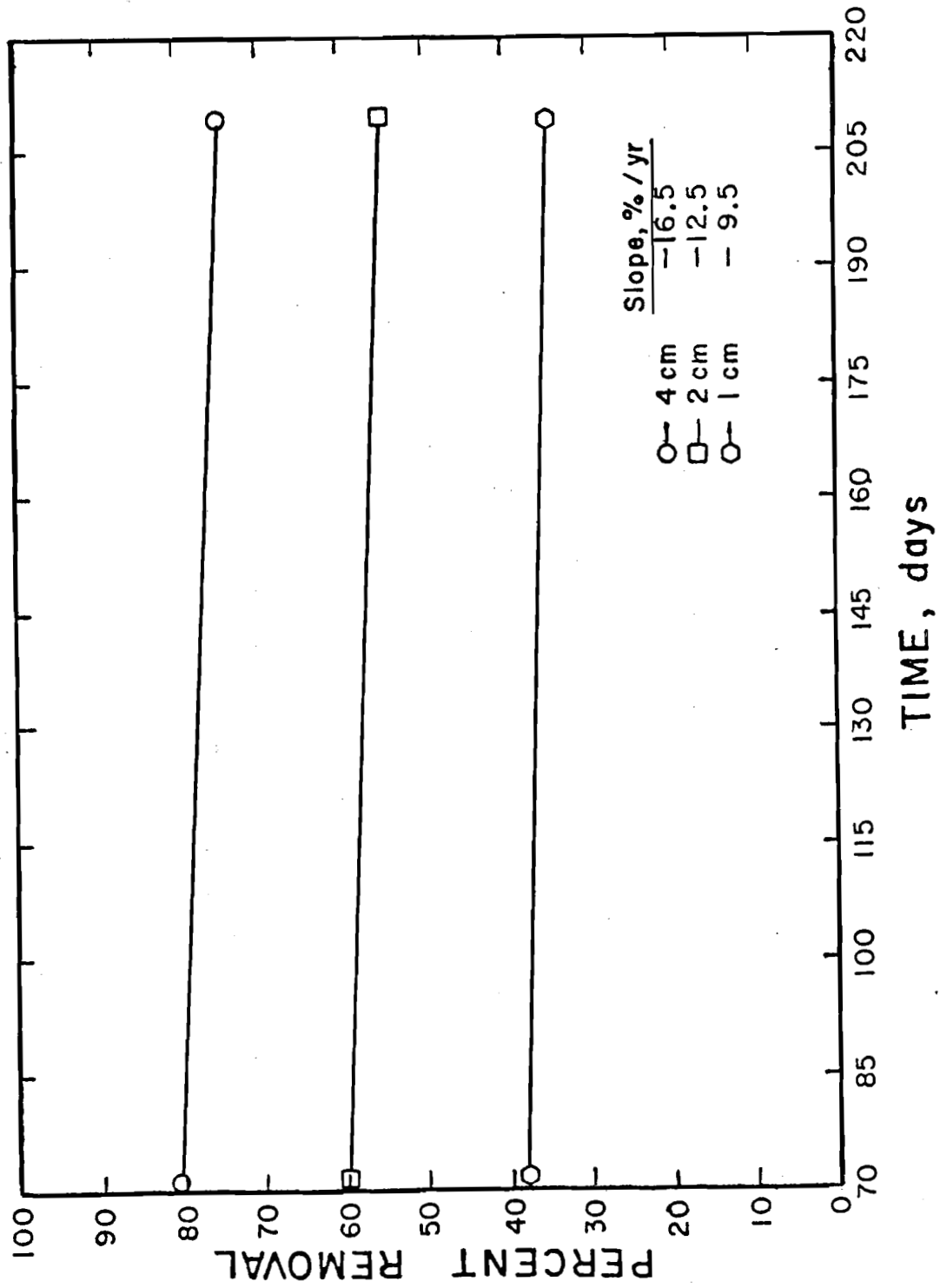


Figure 18. Cumulative Galactose Utilization Efficiencies at Depths of 1 cm, 2 cm, and 4 cm as a Function of Time After Ammonia was Replaced by Nitrate. The Influent Galactose Concentration was Increased to 0.3 mg/l for a Short Time Period.

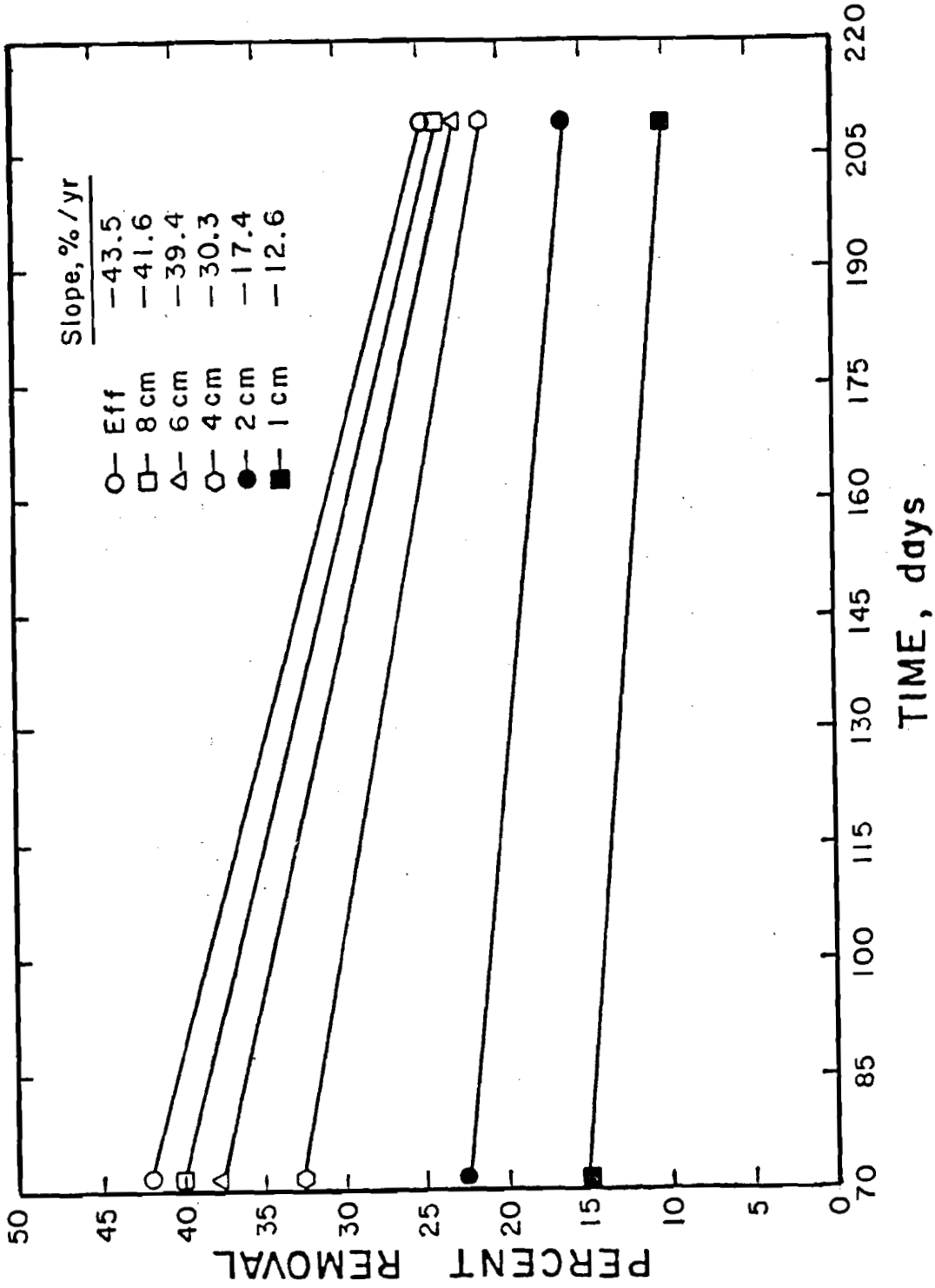


Figure 19. Cumulative Utilization Efficiencies Throughout the Reactor as a Function of Time After Ammonia was Replaced by Nitrate. The Influent Galactose Concentration was Increased to 3.0 mg/ℓ for a Short Time Period.

galactose and leaving more for the biofilm further in the reactor. As the biofilm at greater reactor depths was exposed to higher galactose concentrations, it was able to decay more slowly and also remove more substrate. The fact that the biofilm was still decaying beyond the 4-cm point is shown in Figure 19, which shows that the mass of galactose removed decreased with time in each segment.

Background Organics

Even after the ammonia was removed, the effluent galactose concentration was not significantly higher when 0.03 mg/l and 0.3 mg/l of galactose were fed into the reactor. This suggests that although nitrification enhanced galactose removal, other factors were also acting to reduce the decay rate. The possibility of a supplemental organic substrate concentration in the feed solution was tested by measuring the total organic carbon, TOC, present in the feed solution background. The background solution measured consisted of the cleanest laboratory water (distilled and filtered through a Milli-Q system) with the inorganic nutrients added at their normal concentrations. Between 0.1 and 0.3 mg-C/l of background TOC was constantly present. The organic carbon consisted of impurities existing in the inorganic reagents and a small amount that remained in the cleanest laboratory water. Of the additional organic substances, about 10 percent were utilized within the biofilm reactor when only the medium (no galactose) was passed through the reactor. If the 0.01 to 0.03 mg-C/l of biodegradable organic substances are represented as galactose, they correspond to an additional 0.025 to 0.075 mg/l of galactose. Biodegradable organics at these levels would have affected the decay rate significantly when the long-term galactose feed concentration was 0.3 mg/l, as the additional organic material could have increased the overall influent substrate concentration above S_{min} . If this were true, the overall removal of galactose still should have decreased, as the combination of the effluent galactose concentration and background organic concentration would approach the S_{min} value. Measurement of galactose alone obscures the fact that the background organics (not measured) are part of S_{min} . Taking into account the background organics cannot by itself explain the slowness of the decay rate.

Bacterial Adaptation

Another explanation for the very slow rate of decay is that the organisms adapted to the extremely low nutrient levels after first being established when the organic concentrations were higher. Microorganisms that can exist when organic nutrients are extremely low have been classified into two groups according to their particular survival strategy (Koch, 1979). One group of organisms, referred to as the r-strategist, is able to grow very rapidly when a substrate is available, but must enter a dormant state to persist during periods of very low nutrient concentrations. When the substrate increases in concentration, the r-strategist has the ability to revive and grow very rapidly because of its high maximum growth rate. The r-strategist group, however, is not well adapted when substrate concentrations are always low. The K-strategist, on the other hand, can use its very high affinity for nutrients and highly efficient metabolism to grow continually, even at very low nutrient concentrations. The K-strategist is also characterized by a relatively low maximum growth rate, the ability to utilize multiple and varied compounds, and possibly the ability to accumulate reserve energy resources when carbon is available in excess in the environment. Some strains of bacteria have demonstrated that they can utilize both survival strategies. Poindexter (1979) has reported that under extreme nutrient limitation, some organisms are able to change from less efficient forms to a metabolically more efficient one. For instance, an organism could change shape and produce different chemical constituents within the cell structure over several generations when the availability of nutrients was altered.

The existence of an adaptive survival strategy can explain why the actual decay of a galactose-grown biofilm did not agree with the predicted decay using the nonsteady-state biofilm model. In the predicted case, all of the bacterial kinetic parameters were assumed to remain constant as the feed concentration of galactose was decreased. If the biofilm bacteria established at 3.0 mg/l were able to adapt to the lower nutrient environments, the possibility exists that more than one of the kinetic parameters changed as the nutrient level decreased. Since

substrate removal continued at all times, the dormant r-strategy seems unlikely. The K-strategists remain in an active but more efficient state, which would mean that their half-velocity coefficient, K_S , and decay coefficient, b , probably were less during nutrient limitation, yielding a lower S_{min} value. Since the K_S and b values used for the decay predictions were evaluated under plentiful nutrient conditions (Stratton, 1981), their effectiveness at accurately predicting decay rates under extreme nutrient limitation was questionable. Since the laboratory was not equipped with instruments that could be used to determine kinetic parameters at extremely low substrate concentrations, experimentally determined values for them could not be inserted into the nonsteady-state biofilm models. However, the effect of changing b and K_S can be demonstrated with the use of the nonsteady-state biofilm model. Figure 20 illustrates the effect different decay coefficients have on the predicted galactose utilization profiles over a period of nonsteady-state decay. Each curve represents the same simulated biofilm established with a galactose concentration of 3.0 mg/l for a period of three weeks. After three weeks, the galactose concentration was reduced to 0.3 mg/l. Ten days after this reduction in galactose concentration, the removal profiles were predicted for various decay coefficients. The typical values for decay coefficients, when measured under nonextreme nutrient conditions, are 0.1 to 0.2 day⁻¹. Figure 20 indicates that for decay coefficients this high, significant reduction in the biofilm's ability to utilize galactose would have occurred in just over one week. If the decay coefficient decreased to 0.008 day⁻¹, 10 times less than measured for this study, very little decrease in the galactose utilization would be predicted over 10 days.

Figure 21 shows the effect of an increasing affinity for galactose by the existing bacteria, as different removal profiles are presented for various K_S values. The profiles were developed under a set of conditions identical to those for the development of the profiles in Figure 20, except that b was held constant at 0.08 day⁻¹ for each case. As the organisms increase their affinity toward a substrate, indicated by lower values of K_S , a new S_{min} value would result. A lowered S_{min} could be responsible for the observations of little decay.

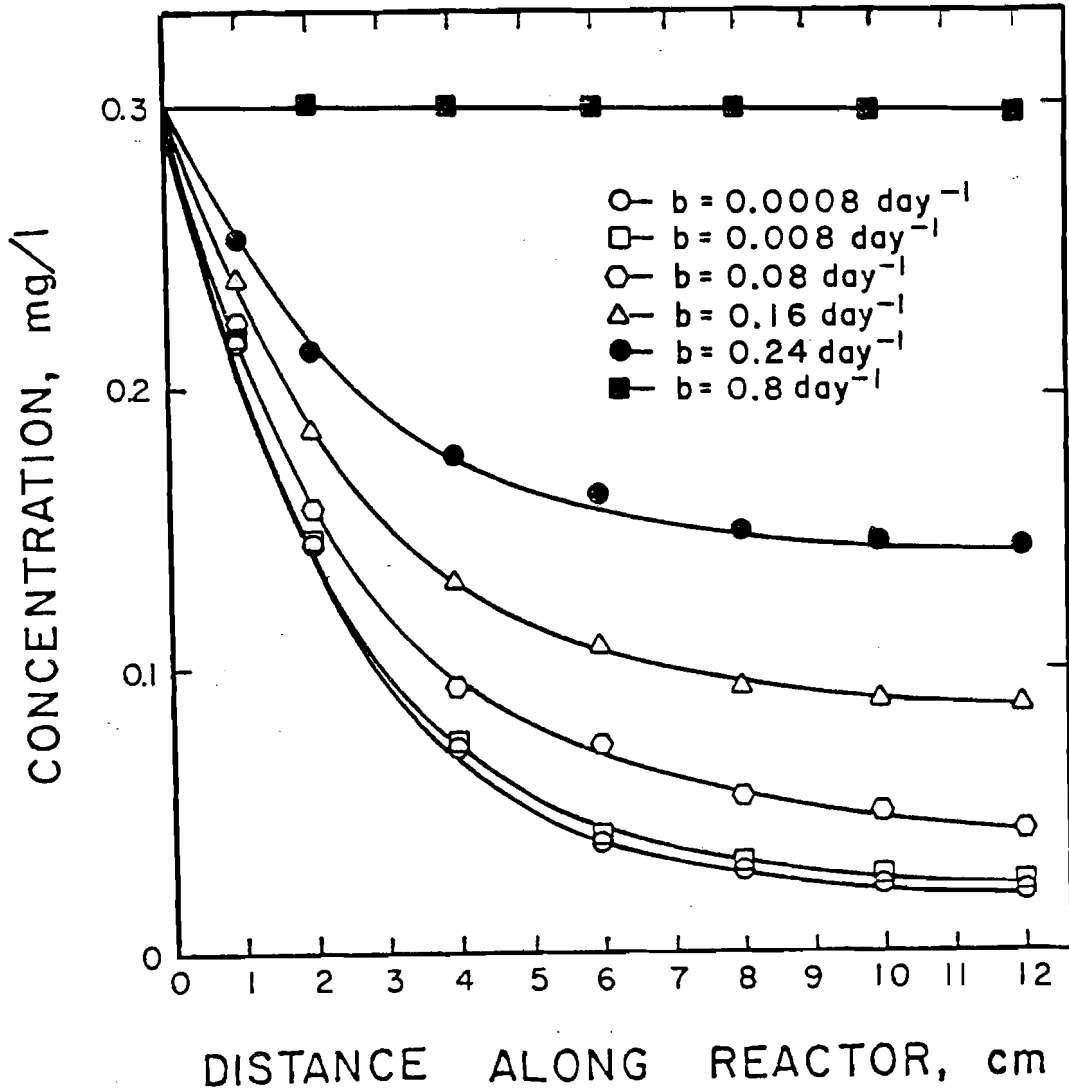


Figure 20. The Effect that the Decay Coefficient, b , has on Galactose Utilization Profiles During Nutrient Limitation as Predicted by the Nonsteady-State Biofilm Model. Time = 10 Days After Decreasing Feed Galactose from 3.0 to 0.3 mg/l.

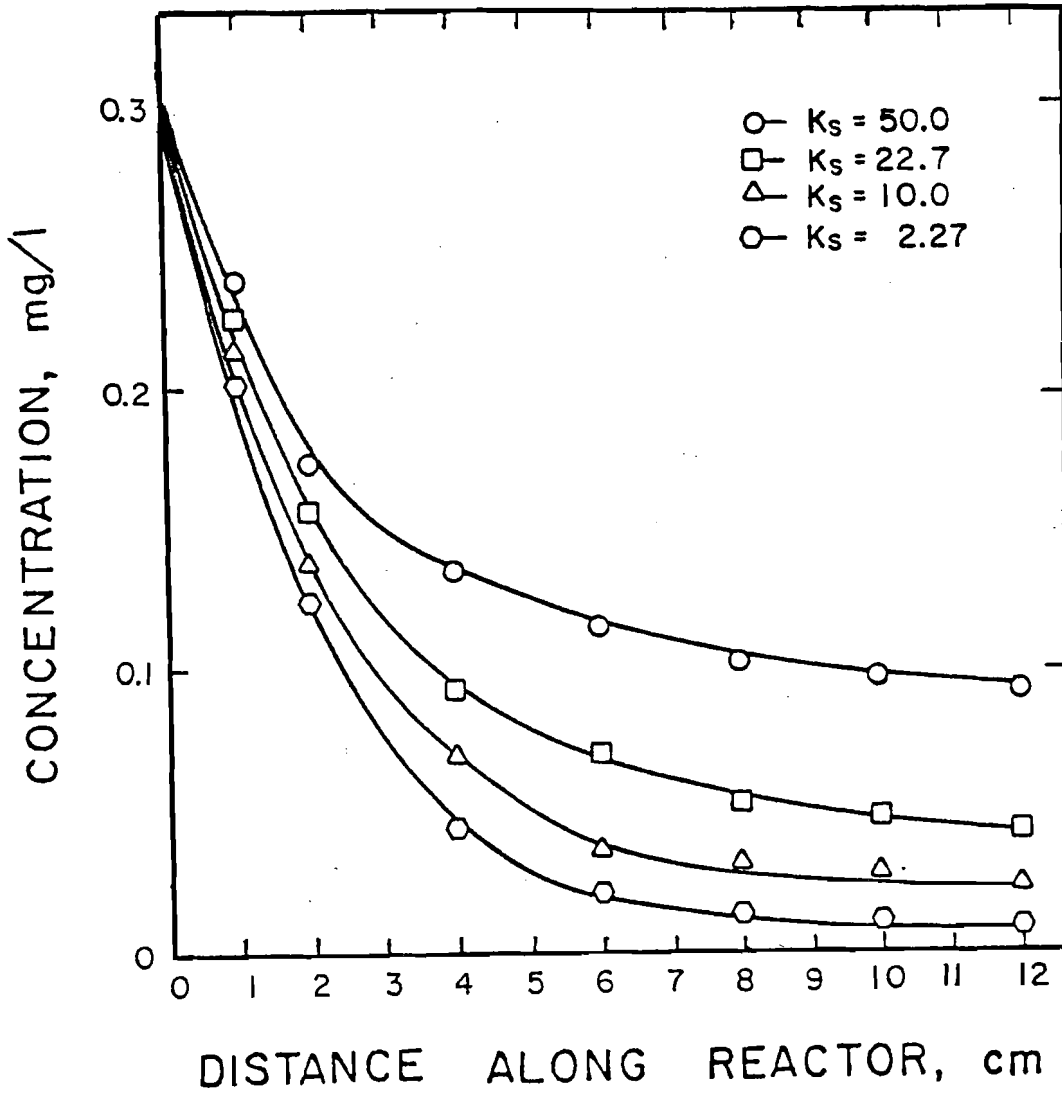


Figure 21. The Effect that the Half-Velocity Coefficient, K_s , has on Galactose Utilization Profiles During Nutrient Limitation as Predicted by the Nonsteady-State Biofilm Model. Time = 10 Days After Decreasing Feed Galactose from 3.0 to 0.3 mg/l.

The results in Figures 20 and 21 indicate that increased organism efficiency is one way to enhance nonsteady-state operation. Kobayashi and Rittmann (1982) described the potential advantages of so-called oligotrophic microorganisms, especially for fixed-film biological treatment of dilute wastewaters. The changes in kinetic parameters that describe any oligotrophic adaptations could not be ascertained in this study. It is likely that b and K_S decreased somewhat. However, the complicating factor of organic supplements from background organics and nitrifying bacteria made quantitation impossible.

In summary, the nonsteady-state biofilm showed a very slow loss rate and gave effective removal of a trace-level substrate for over 200 days. Organics supplementation caused by nitrifying activity clearly played a role, as did background organic material. Evidence also suggests that the heterotrophic bacteria adapted to the low-nutrient situation, probably by decreasing b and K_S .

CONCLUSIONS

Some general conclusions that can be made from the completed research are listed below.

1. The initial removal profiles for substrates after the reactor has been inoculated were predicted reasonably well with the newly developed transient-biofilm model, which allows biofilm growth and decay with time.
2. Long-term effects of refractory biomass build-up on the removal of organics were negligible.
3. Galactose, when present at sub- S_{min} levels, was removed consistently for prolonged periods of time by a biofilm established at a galactose concentration above S_{min} . Although the biofilm was decaying, it could still bring about 88 percent removals of 0.03 mg/l levels of galactose for over 200 days.
4. The slow decay rate of the nonsteady-state biofilm was enhanced by the presence of nitrifying bacteria, which supplemented the organic substrate near the inlet of the reactor.

5. The possibility of kinetic parameters such as b and K_s changing with time under oligotrophic environments must be more clearly developed. Simulating more efficient bacteria by decreasing b and K_s allowed the nonsteady-state model to predict results similar to those obtained experimentally.
6. The slow decay of the nonsteady-state biofilm, whether caused by nitrification or bacterial adaptation, suggests that a nonsteady-state biofilm process should be able to effect trace-organic removals for extended periods of time.

REFERENCES

- American Public Health Assn. 1971. Standard Methods for the Examination of Water and Wastewater, 13th ed., Washington, DC.
- American Public Health Assn. 1980. Standard Methods for the Examination of Water and Wastewater, 15th ed., Washington, DC.
- Brock, T. D., 1979. Biology of Microorganisms, 3rd ed., Prentice-Hall, Inc., Englewood Cliffs, NJ.
- Brunner, C. W. 1982. "Trace Organic Removal with Steady-State and Nonsteady-State Biofilms." Thesis submitted in partial fulfillment of the Master of Science Degree, Dept. of Civil Engineering, University of Illinois at Urbana-Champaign, Urbana, IL.
- Characklis, W. G. 1973. "Attached Microbial Growth-I. Attachment and Growth." Water Research 7:1113.
- Kobayashi, H. and B. E. Rittmann, 1982. "Microbial Removal of Hazardous Organic Compounds." Environmental Science & Technology 16:170A.
- Koch, A. L. 1979. "Microbial Growth in Low Concentrations of Nutrients." Strategies of Microbial Life in Extreme Environments, Dahlem Konferenzen, Berlin.
- McCarty, P. L. 1972. "Stoichiometry of Biological Reactions." Presented at the International Conference, Towards a Unified Concept of Biological Waste Treatment Design, Atlanta, GA.
- McCarty, P. L., B. E. Rittmann, and M. Reinhard, 1981. "Trace Organics in Groundwater." Environmental Science & Technology 15:40.
- Namkung, E., R. G. Stratton, and B. E. Rittmann, 1982. "Predicting Removal of Trace Organic Compounds by Biofilms." Presented at the Annual Conference of the Water Pollution Control Federation, St. Louis, MO.
- Perry, R. H. and C. H. Chilton, 1973. Chemical Engineers Handbook, 5th ed., McGraw-Hill Book Co., New York.
- Poindexter, S. S., 1979. "Morphological Adaptation to Low Nutrient Concentrations." In Shilo, M., ed., Strategies of Microbial Life in Extreme Environments, Dahlem Konferenzen, Berlin.
- Rittmann, B. E., 1982. "The Effect of Shear Stress on Biofilm Loss Rate." Biotechnology and Bioengineering 24:501.

- Rittmann, B. E. and P. L. McCarty, 1978. "Variable-Order Model of Bacterial Film Kinetics." Jour. Environ. Engr. Div., ASCE 106(EE3):753.
- Rittmann, B. E. and P. L. McCarty, 1980a. "Evaluation of Steady-State Biofilm Kinetics." Biotechnology and Bioengineering 22:2359.
- Rittmann, B. E. and P. L. McCarty, 1980b. "Utilization of Dichloromethane by Suspended and Fixed-Film Bacteria." Applied and Environmental Microbiology 39:1225.
- Rittmann, B. E. and P. L. McCarty, 1980c. "A Model of Steady-State Biofilm Kinetics." Biotechnology and Bioengineering 22:2343.
- Rittmann, B. E. and P. L. McCarty, 1981. "Substrate Flux into Biofilms of Any Thickness." Jour. Environ. Engr. Div., ASCE 107(EE4):831.
- Stratton, R. G. "Secondary Utilization of Trace Organics by Steady-State Biofilms." Thesis submitted in partial fulfillment of the Master of Science Degree, University of Illinois at Urbana-Champaign, Urbana, IL.
- Stratton, R. G., E. Namkung, and B. E. Rittmann, 1982. "Biodegradation of Trace-Organic Compounds by Biofilms on Porous Media." Jour. Amer. Water Works Assn., submitted for publication.