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Rates of Sorption and Partitioning of Contaminants in River Biofilm

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Biofilms are believed to play a significant role in the fate and transport of contaminants in aquatic environments. However little is known about the rates of sorption (k) and partitioning (K_d) of contaminants to biofilms. Studies of the latter were performed using mature biofilm cultivated in a roto-torque reactor. The reactor was flushed with river water containing a mixture of tetrabutyltin, *p*,*p*-DDT, diclofop-methyl, triallate, lindane, atrazine, parathion-methyl, and dimethoate in two experiments. The first experiment was conducted at a spiked level of 1 μg/L, and the second was conducted at 10 μg/L for each component. Apart from dimethoate, there was rapid depletion of all contaminants from the water phase within the first 5−10 min with sorption occurring by pseudo-first-order kinetics. In general, the mean values of k (10^{-4} min^{-1}) increased with water solubility and were 8, 70, 110, 180, 230, 370, and 100 for *p*,*p*-DDT, diclofop-methyl, triallate, tetrabutyltin, lindane, atrazine, and parathion-methyl, respectively. The values of log K_d increased linearly with log K_{ow} and decreased linearly with the log of the aqueous solubilities. In general, K_{ow} values were significantly greater than the corresponding values of K_d, indicating that partitioning of contaminants was not limited to lipophilic regions of the biofilm.

Introduction

The behavior, transport, and ultimate fate of contaminants in aquatic environments may be affected significantly by their sorption and remobilization interaction with biofilms. This is due in part to the insulating layer of biofilms that must first be reached and crossed before a contaminant contacts the substrate supporting the biofilm (*1*). This interaction with contaminants can occur on a large scale since biofilms are ubiquitous in the environment. They form on all surfaces in rivers, lakes, and wetlands, such as rocks and sediments, and account for a wide range of microbial organisms on earth (*2*).

Biofilms are dynamic systems in which various components are synthesized, assembled, transformed, degraded, and sloughed off into the environment (*3*). Biofilms can therefore remobilize sorbed or transformation products back into aquatic environments. They can be considered as microenvironments that are distinct in characteristics from the bulk water phase with a local accumulation of cells between 10^9 and 10^{11} cells/mL biofilm mass (*4*). In general, this biofilm mass is predominantly water (85−95% wet weight), exopolysaccharide (EPS, 1−2% wet weight) (*5*), and polypeptide polymers. This EPS matrix is produced by the bacteria as part of their adaptation to growth on surfaces or at interfaces and, in general, encases the majority of biofilm bacteria. The functions of this EPS in bacterial growth and survival are extensive including attachment, microcolony formation, floc formation, protection against heavy metals, protection against predation and environmental fluctuations, increased resistance against anti-microbial agents, and the localization of extracellular enzymes (*6*−*11*). This extensive polymer network has also been shown to be highly reactive, for example, selectively binding metals from the environment (*6, 10, 12*).

In the present study, a mixture of pesticides (*p*,*p*-DDT, diclofop-methyl, triallate, lindane, atrazine, parathion-methyl, and dimethoate) and one organometallic (tetrabutyltin) was selected to cover a range of water solubilities (1×10^{-3}−5×10^4 mg/L) and K_{ow} values (1×10^{-1}−1×10^7), based on their occurrence in the Elbe River and suspended particulate matter (SPM). The occurrence of the pesticide in SPM was also considered because of the similar behavior of this matrix to biofilms (*13*). Less complex systems containing either tributyltin or diclofop-methyl as the sole carbon source have been previously studied using bioreactors (*13*−*15*) and tandem mass spectrometry techniques (*16, 17*), respectively.

In this first phase of the work, we discuss the results obtained for two independent set of experiments and highlight the factors that appear to control the sorption rates and partitioning coefficients of contaminants in natural biofilms. The experimental results are calculated using a first-order kinetic model. Development of more refined numerical models (*24*) is a subject of future research in our laboratories.

Theory

There have been only a few reports on the theory describing competitive sorption and remobilization of organic contaminants in biofilms. Arcangeli and Arvin (*19, 20*) modeled the cometabolic transformation of *o*-xylene with toluene as a primary carbon source in a denitrifying biofilm system. It was concluded that only a minor part of the biofilm was active, in which there was competition between toluene and *o*-xylene for the same enzyme. It is not known whether these findings are generally applicable where the carbon source is a complex mixture of dissolved organic matter from natural waters. In the present study, the mature biofilm was exposed to a mixture in solution of eight contaminants at levels of environmental significance in which there was potential competition among this mixture and also competition with the components of the dissolved organic matter in the natural river water. Quantification of sorption rates and partitioning presents many challenges in such complex systems, and consequently, the mechanisms controlling sorption in biofilms are poorly understood.

As a first approximation, the sorption rate constant for binding of contaminants to the biofilm is equal to the effective rate constant (k) for the sorption of a given contaminant from the river water in the bioreactor. The latter is made up of primarily k_1 (the rate constant for adsorption to biofilm) − k_2 (the rate constant for desorption from biofilm). Both adsorption and desorption processes occur at the same time

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with the initial rates of adsorption being greater than the rate of desorption. The effective sorption rate will therefore be dependent on the physical and chemical properties of both the analytes and the nature of the biofilm. The key factors affecting the latter include the maturity or age and thickness of the biofilm.

For a flow rate in the bioreactor of Q (L/min) and inlet and outlet concentrations C_o and C (μg/L), respectively, the concentration of chemicals sorbed by the biofilm can be considered as $(M/V)C^*$ where M is the dry mass of the bacteria (kg), V is the volume of the reactor (L), and C^* is the amount of the chemicals sorbed in the biofilm per gram (μg/g). Under these conditions, the total mass of chemicals sorbed by the biofilm is given by the mass balance equation:

$$\int_0^t (QC_o - QC)\, dt = C^*M + CV \quad (1)$$

Differentiating both sides of eq 1 gives

$$QC_o - QC = M\, dC^*/dt + V\, dC/dt \quad (2)$$

Under equilibrium conditions, the concentration of chemicals adsorbed in the biofilm can be expressed by the Freundlich equation $C^* = K_d C^N$, where K_d is the Freundlich constant (L/Kg), and N is a measure of the nonlinearity. The Freundlich equation can be used to obtain kinetic data in a manner similar to that described by Brusseau et al. for the partitioning of solutes under equilibrium conditions given by Henry's law (*25*). Rates of adsorption and desorption are integrated for an infinitely small time interval close to the equilibrium condition.

For N equal to 1 at low solute concentration, $dC^*/dt = K_d\, dC/dt$, and eq 2 can be written as

$$QC_o - QC = K_d M\, dC/dt + V\, dC/dt \quad (3)$$

$$dC/dt = Q(C_o - C)/(K_d M + V) \quad (4)$$

Integrating eq 4 gives

$$C = C_o - A \exp[-Qt/(K_d M + V)] \quad (5)$$

Based on the initial experimental conditions, it can be shown that the integration constant A is equal to $C_o - C_o V/(MK_d + V)$. Rewriting eq 5 gives

$$C = C_o + [(C_o V)/(K_d M + V) - C_o] \exp[-Qt/(K_d M + V)] \quad (6)$$

Experimental Section

Equation 6 is of the form $Y = b + (a - b)\exp(-kt)$ where b equals C_o; a equals $C_o V/(K_d M + V)$, and k equals $Q/(K_d M + V)$. The values of the constants a, b, and k were estimated using a curve-fitting program, Table Curve 2D Version 2.00 (Jandel Scientific). For the calculation of k and K_d, the analytical value of C_o determined at 180 min was substituted for the constant a, and the constants b and k were obtained using the best fit of eq 6 to the analytical data. For the experiments performed, the values of V, M, and Q were 0.65 L, 1.635×10^{-3} kg, and 0.105 L/min. respectively. In view that $k = Q/K_d M + V$, this rate constant is a function of the experimental parameters: V, Q, and M. However, since these values were held constant, the calculated rate constant k provides not only a direct measure of the reactor kinetics but also a measure of the difference in the rate constants for adsorption and desorption, defined as the effective sorption rate constant in this investigation.

Biofilm Reactor. A modified roto-torque bioreactor (Figure 1) was used to develop native Elbe biofilms (*4*). Operation was based on a well-mixed liquid phase and even shear over the reactor walls, resulting in optimal conditions for the formation of homogeneous biofilms. Despite these attractive features, the heterogeneity and position differences in surface growth can make representative sampling of the attached biomass difficult (*21*).

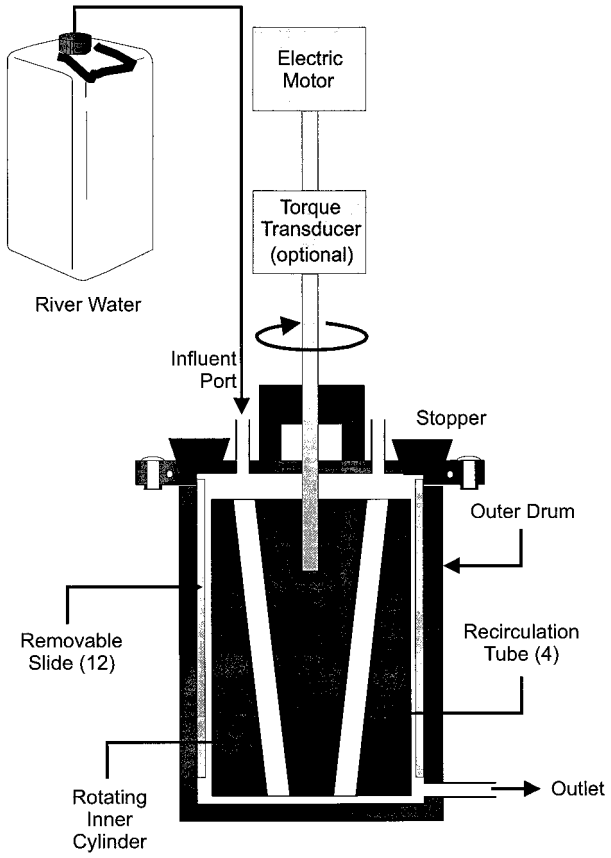

FIGURE 1. Schematic of roto-torque bioreactor.

The reactor consisted of an outer cylinder with a rotating inner cylinder. Recirculating tubes in the inner cylinder ensured optimal mixing of the system and minimized nutrient gradients. Important parameters, such as flow rate, nutrient concentration, shear forces, pH, and types of organisms were regulated and adjusted to natural conditions using the procedures described by Kuballa and Griesse, (*15*). At the inside of the outer cylinder, 12 slides (area of one slide: 3×10^{-3} m^2) could be withdrawn and stored at 4 °C in screw cap 1-L bottles for monitoring the presence of contaminants in the biofilm at the start (2 slides) and at the termination (2 slides) of the experiments.

The biofilm reactor was placed at the Geesthachter Wehr and flooded continuously via a pump with Elbe River water from the nontidal part of the river (flow rate, 105 mL/min; rotating rate, 150 rpm). Under these conditions, the native biofilm developed with a microbial species diversity similar to the suspended matter found in the Elbe River (*15*). The biofilm attained a steady state after approximately 14 days based on measurements of the protein and glucoronic acids in the biofilm (*15*). At this stage, the biofilm covered all surfaces of the reactor with an average thickness of 5−10 mm. Continuous flow centrifugation was used to remove suspended particulate matter from a grab sample of Elbe River surface water, and a 40-L Nalgene container was filled with the centrifuged water. After collecting a grab sample (1.2 L) to monitor ambient levels of contaminants in the water, the 40-L reservoir was spiked at 1 μg/L (1.4 mL of standard containing 30 ng/μL per component dissolved in methanol/water). For the second set of experiments, the

reservoir was spiked at 10 μg/L (1.4 mL of standard containing 300 ng/μL per component dissolved in methanol/water). After the contaminants were added to the river water, the reservoir was agitated vigorously for approximately 5−10 min to ensure even mixing of the spiked standards and preconditioning of active sites on the walls of the vessel. To minimize the effects of dilution, just prior to initiating the flow of contaminants from the reservoir to the bioreactor, the reactor was also spiked with the same mixture of contaminants to establish the same concentration as that present in the reservoir (using a volume of 22 μL spiked into the bioreactor (650 mL)). The bioreactor was then flushed with the spiked contaminants from the reservoir for 5 h. Thus, the concentration of contaminants in the influent was held constant (1 or 10 ppb) at a fixed flow rate throughout the experiments. The effluent was collected continuously in 1200-mL sample bottles and stored at 4 °C until analysis. These samples were used for all measurements of sorption kinetics to the biofilm by monitoring the concentration (ppb levels) of contaminants in the effluent as a function of time. Each determination of k was based on 15 or more measurements of the effluent concentration for each of the eight analytes investigated at both 1 and 10 ppb influent concentration. In total, more than 210 experimental measurements were made using gas chromatography/mass spectrometry (GC/MS) and gas chromatography atomic adsorption spectrometry (GC/AAS).

The input concentrations from the 40-L reservoir were checked during the course of the experiments (at the start and midpoint) to monitor possible changes from sorption/desorption to the walls of the vessel. Upon preconditioning of the reservoir surfaces by vigorous agitation of the spiked river water prior to commencement of the kinetic runs, such wall effects were found to be negligible. No further controls on reactor sorption/desorption with and without an active biofilm were deemed necessary since the material of the bioreactor walls, polycarbonate, has been shown to be inert for tributyltin, a compound with much lower water solubility (0.5 mg/L) (*15*) than most of the contaminants investigated. The one exception, *p*,*p*-DDT, has a lower solubility (0.001 mg/L) than tributyltin.

Sample Extraction. All solvents were trace analytical grade (E. Merck, Darmstadt, FRG), NaOH (E. Merck, Darmstadt, FRG, No. 6498), and HCl (E. Merck, Darmstadt, FRG, No. 317). Water samples from the bioreactor were not filtered prior to extraction to avoid possible losses of analyte to the filter material. Measured concentrations thus reflect the sum of analytes in the dissolved phase and small amounts of sloughed off biofilm. The amount of the latter in the various 1-L samples varied from nil to visible amounts (∼2−5 mg). This source of error in the determination of the partitioning coefficients is estimated to be ≤10% RSD, based on the overall precision of the analytical methodology.

Extractions of diclofop-methyl and the hydrolysis product (diclofop acid) were performed using a liquid−liquid extraction procedure (*22*). The pH of a volume of 500 mL of surface water was adjusted to pH 2, and extractions were performed serially with 3 × 50 mL dichloromethane. The combined extracts were reduced in volume to 1 mL, solvent exchanged with diethyl ether, and derivatized using diazomethane. Extractions for the determination of tetrabutyltin were based on the procedure described by Kuballa et al. (*13*). In brief, a volume of 10 mL of hexane was added to a 100-mL sample, and the mixture was shaken for 20 min. The organic phase was decanted, and the volume was reduced to 1 mL over a gentle N_2 flow. Other pesticides were extracted using a solid-phase extraction procedure described in detail by Gandrass et al. (*23*).

Instrumental Analyses. Instrumentation used included a gas chromatograph HP5890 and mass spectrometer HP5989 (Hewlett-Packard, Palo Alto, CA), a Fisons AutospecQ tandem mass spectrometer, and a TRIO 1000 GC/MS (Fisons, now Micromass, Manchester, England), equipped with a capillary column DB1701 30 m × 0.32 mm i.d. × 0.25 μm (J&W Scientific, Folsom, CA).

GC/MS Analysis. The extracts from the solid-phase extraction were analyzed using a gas chromatograph HP 5890 equipped with an on column injector and a DB1701 column interfaced to a HP 5989 mass spectrometer. A 2-μL volume of extract was injected, using helium as the carrier gas at 0.3 bar and temperature program: 40 °C (held for 2min), followed by an increase at a rate of 40 °C/min to 160 °C with a second temperature increase by a rate of 5 °C/min to 270 °C (held for 10 min). The ion source was operated using electron impact ionization at an electron energy of 70 eV in the selected ion-monitoring mode. The recoveries of the pesticides from both fortified river water and fortified laboratory water (1−10 μg/L) were 80−90% ±10−15% RSD with a detection limit of approximately 25 pg/μL. The corresponding conditions for the GC/MS analyses of diclofop-methyl utilized a gas chromatograph HP 5890 equipped with a split/splitless injector and a DB1701 column interfaced to Fisons TRIO 1000 mass spectrometer. All other GC/MS conditions were the same as those described above. The combined recovery of diclofop-methyl and the hydrolysis product diclofop acid from fortified water samples (1−10 μg/L) was 90% ±10% RSD with a detection limit of 25 pg/μL.

Instrumental analyses of tetrabutyltin were performed using GC−AAS with a gas chromatograph Perkin-Elmer 8400 (splitless 1−2 μL, carrier gas helium 0.3 bar, column DB1701, temperature program 80 °C//30 °C/min//250 °C coupled to a AAS), Perkin-Elmer 3030 with transfer line temperature of 250 °C; atomization temperature of 700 °C; flows: helium 2 mL/min, auxiliary gases: hydrogen 180 mL/min, air 60 mL/min. The recovery of tetrabutyltin from fortified water samples was 89% with a detection limit of 25 pg/μL tin.

Biofilm Analysis. Experiments were performed using procedures developed for tandem mass spectrometry characterization of biofilms (*17*). In brief, preliminary full-scan mass spectra of the biofilm were conducted using a Fisons AutospecQ mass spectrometer with EBEQ geometry, equipped with a 4100−60 VAX data system (Digital Equipment Co.) and Opus 2.1b Software. Samples were placed in shallow cups of the direct insertion probe for 30 min at room temperature (approximately 23 °C), prior to introduction to the ion source. This was necessary to reduce the moisture content of the samples and to avoid tripping the vacuum protection system (set at 5×10^{-5} Torr) of the mass spectrometer. The direct insertion probe was water-cooled, and heating was limited to the radiant heat from the ion source with no additional heat supplied by the probe heaters. The ion source was operated under electron impact conditions at 70 eV, 250 °C, and trap current 250 μA, and the mass spectrometer was operated at 1300 resolution, with a scan speed of 1 s/decade and a mass range of 50−600 Da.

For the MS/MS experiments, the precursor ions were selected manually, and the ion beam was reduced to 50% transmission using the m/z 331 ion of perfluorokerosene and xenon as the collision gas. Experiments were performed for low energy collisions in which the collision cell was held at 12 eV (laboratory frame of reference). Product ions were detected by scanning the quadrupole in the mass range 30−350 Da at unit resolution.

Results and Discussion

Representative examples of the results observed for the sorption of the contaminants in the bioreactor for the two sets of experiments are illustrated in Figure 2a−c. A summary of the calculated values of k, K_d, and the corresponding results obtained for the tandem mass spectrometry confirmations of the biofilm are given in Table 1.

TABLE 1. Pseudo-First-Order Rate Constants k, Partitioning Coefficient K_d, and Tandem Mass Spectrometry Confirmation of Contaminants in Biofilm and Elbe River Water Spiked at 1 and 10 μg/L in a Roto-Torque Bioreactor for an 8-Component Mixture of *p,p*-DDT, Diclofop-methyl, Triallate, Lindane, Atrazine, Parathion-methyl, Dimethoate, and Tetrabutyl-tin[a]

component in mixture	sorption rate constant ($k \times 10^{-4}$ min^{-1})	partitioning coeff ($K_d \times 10^3$ L/Kg)	biofilm exposed to spiked Elbe River water	biofilm exposed to Elbe River water used as control
p,p-DDT*	8	800	X	X
tetrabutyl-tin	180 (60−300)	60 (20−100)	NA	NA
diclofop-methyl and diclofop-acid	70 (60−80)	90 (80−100)	X	X
triallate	110 (90−130)	55 (40−70)	X	ND
lindane	230 (160−300)	30 (20−40)	T	ND
atrazine	370 (140−600)	25 (10−40)	?	ND
parathion-methyl*	100	60	ND	ND
dimethoate**	NA	NA	X	X

[a] Range of values for the two experiments conducted are given in parentheses; an asterisk (*) denotes contaminant determined in single experiment; two asterisks (**) denote interference from high background levels in the original biofilm prior to flushing with spiked Elbe River water. X denotes positive confirmation based on ratio of product ions within ±40% of reference standards; a question mark (?) denotes ratio of product ions greater than ±40% of reference standards; T denotes unknown transformation product; ND denotes not detected; NA denotes not analyzed.

Apart from dimethoate (where there was evidence of sporadic background releases of the pesticide originally sorbed to the biofilm), there was rapid depletion of all contaminants from the water phase within the first 5−10 min with subsequent sorption occurring according to pseudo-first-order kinetics (Figure 2a−c). This sorption was evident by the gradual increase in the concentration measured for the analytes in the effluent from the bioreactor as illustrated in Figure 2a−c. These profiles are similar to those described for PAHs and chlorophenols in sewer biofilms (*24*). For the sewer biofilms, the adsorption of PAHs was completed after a few minutes, with desorption of 10% of the load requiring more than 1 h. The desorption of the chlorophenols was much faster than for the PAHs with more than 50% desorption occurring after only 15 min (*24*).

In the present work, it was essential to precondition the walls of the 40-L reservoir prior to commencing the kinetic runs and to further check the inlet concentration during the experiments. The initial decrease in the measured inlet concentrations was significant as compared to actual concentrations added to the reservoir (Figure 2a−c). Once the reservoir was preconditioned, monitoring of the inlet concentration at the start and subsequently at 180 min indicated that these concentrations were constant during the experiments. Possible adsorption and remobilization from the walls of the apparatus were not therefore contributing factors to the observed sorption rates.

The first-order kinetics observed for the sorption of the contaminants in Elbe River biofilm are similar to the kinetics reported for the sorption and transformation of other pollutants on biofilm in natural waters (*26, 27*). For example, some biofilms have been shown to adapt to the degradation of mixtures of aromatic pollutants at relatively high concentrations in the <20−100 mg/L concentration range, according to first-order kinetics with similar removal rate constants for different aromatic compounds (*28*). Relative pseudo-first-order rate coefficients have also been observed for microbial transformation in biofilms containing 2,4-dichlorophenoxyacetic acid methyl ester (2,4-DME), the butoxyethyl ester of 2,4-dichlorophenoxyacetic acid (2,4-DBE), parathion-methyl, and methyl-3-chlorobenozoate (*29*). Likewise, apparent first-order rate coefficients have been observed for the processes of disinfection and detachment of biofilms (*30−32*) and the aerobic biological degradation of chlorinated aliphatic hydrocarbons using a completely mixed laboratory biofilm reactor (*33*). One exception to this trend, however, was the second-order kinetics observed for the biotransformation of the herbicides atrazine and alachlor under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions (*34*).

As illustrated in Figure 3, there is a trend for the values of log k to increase linearly with the log of the aqueous

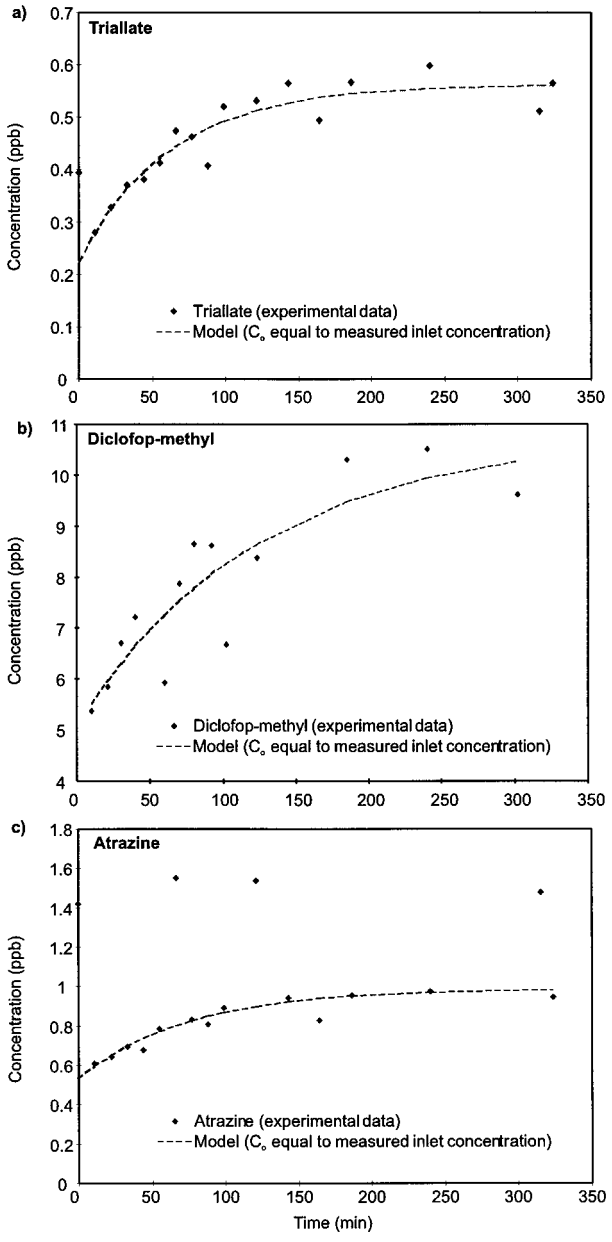

FIGURE 2. Uptake of pesticides from Elbe River water to biofilm in a roto-torque bioreactor, showing experimental data and results calculated using first-order kinetics for (a) triallate, (b) diclofop-methyl, and (c) atrazine.

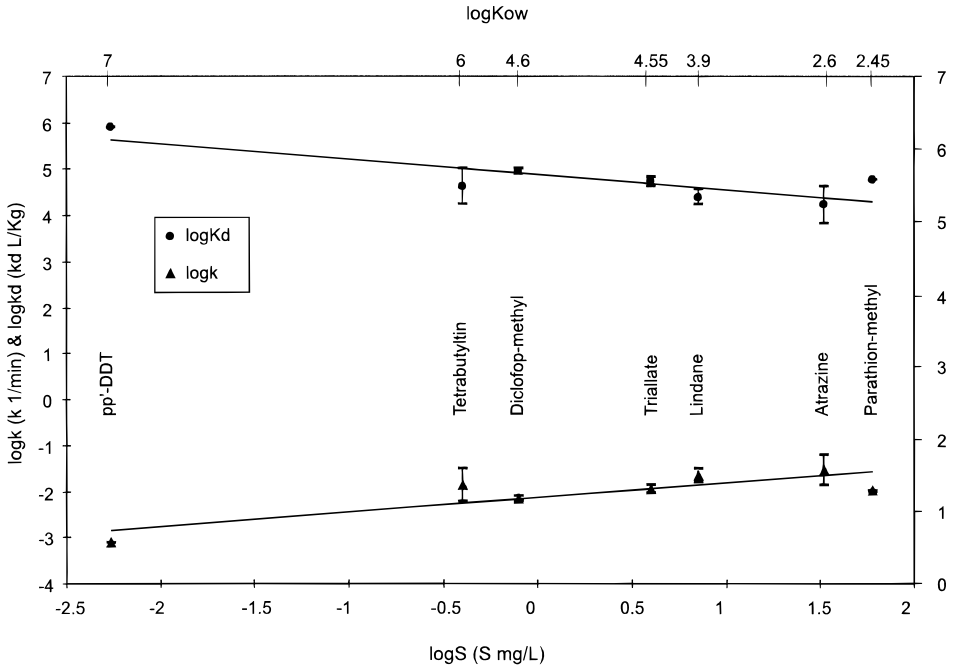

FIGURE 3. Comparison of the sorption rate constants (log *k*) and partitioning coefficients (log K_d) with the aqueous solutility (log *S*) and octanol−water partitioning coefficient (log K_{ow}) of contaminants investigated for two series of experiments.

solubility. Likewise, there is a general trend for log K_d to increase with increase in log K_{ow} (*35*). However this increase is limited to a relatively small range of a factor of 50, in comparison to the much wider range of K_{ow} (1×10^{-1}−1×10^7) studied. In general, these overall trends indicate that the net sorption in the biofilm was weakly dependent on the organophilic nature and the solubility of the contaminants. This sorption to the biofilm was however significant and detectable using tandem mass spectrometry, as evidenced from the results summarized in Table 1.

Although little is known about the mechanisms involved for the sorption of pesticides in biofilms, processes similar to those described for metals have been postulated for the competitive sorption of organic contaminants (*36, 37*). However, since the EPS can contain significant amounts of proteins, there may be other sites for interaction with nonpolar or lipophilic regions of pesticides. Consequently, in addition to the solubility of the pesticides being a key parameter for sorption of dissolved organics to biofilms, the lipophilic nature of the contaminants is expected to be an important factor, as evidenced from the results of this investigation.

The difficulties associated with measurements to quantify biofilm dynamics is illustrated in Figure 2c, in which it is evident that the model employed provides a reasonable fit for 13 of the 17 data points. After the initial sample at time zero, there are three experimental points that differ significantly from that predicted by the model. The experimental profile thus appears to contain three sharp pulses of analytes released to the water. These pulses appear to be real since the levels are orders of magnitude greater than the precision of the overall analytical method (<10% RSD). It is possible that the pulses may be due to the observed periodic detachment of biofilm from the reactor. However, the time required for collection of a given sample was approximately 10 min, a period greater than the time required for detachment of the biofilm. The observations are thus considered to be preliminary, since it was not possible to measure the repeatability of such detachments from run to run in this investigation. To better resolve and understand this possible biofilm dynamics, including toxic effects attributed to periodic detachment of biofilm material, further study is warranted employing shorter sampling times and smaller sample volumes.

Although factors influencing the detachment of biofilm and the pulsed releases of contaminants are not fully understood, some insights are gradually emerging. The mechanism has been reported to be a dynamic process involving detachment of portions of the biofilm and reentrainment in the bulk fluid (*38*) under turbulent flow conditions. Combined transport and adsorption processes appear to be important in only the very early stages of biofilm accumulation with detachment rates increasing continuously with biofilm accumulation (*38*). The distribution of pollutants in aquatic environments may thus be affected to a significant extent by the heterogeneity of biofilms in which contaminants exhibit different rates of sorption. Detachment of such biofilms from surfaces may lead to possible periodic releases of contaminants in natural waters, further study of which is warranted.

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