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Influence of Suspended Sediment on the Biodegradation of Alkyl Esters of *p*-Aminobenzoic Acid

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Sorption to suspended and settled particulate material has an important influence on the distribution and fate of many of the chemicals released into aquatic environments (Karickhoff 1974). This is especially the case for hydrophobic organic chemicals such as chlorinated aromatic compounds. The effect that sorption of such chemicals has on their uptake by aquatic organisms is receiving much attention (e.g. Knezovich et al. 1987).

The influence that sorption of hydrophobic organic chemicals to sediments and other particulates has on their biodegradation is poorly understood. We here report a study of the effect of sorption on the biodegradation of n-alkyl esters of *p*-aminobenzoic acid. These chemicals were used in this study because they cover a large range of hydrophobicity, with hexane-water partition coefficients ($K_{d,hex}$) ranging from 0.22 for the methyl ester to 6.15 x 10^3 for the octyl ester (Yalkowsky et al. 1972), and should therefore have a range of sorption coefficients. Furthermore these compounds are rapidly degraded in solution (Parsons et al. 1987). Any effect of sorption of these chemicals on their biodegradation rates should therefore be readily apparent.

MATERIALS AND METHODS

The n-propyl, n-butyl, n-hexyl, n-heptyl and n-octyl esters of *p*-aminobenzoic acid were prepared as described by Flynn and Yalkowsky (1972). The methyl ester was obtained from Aldrich Chemie (Brussels, Belgium). Sediment was taken from a freshwater lake (Oostvaardersplassen, The Netherlands) and contained 2.5% organic carbon based on dry weight. The sediment was dried in an oven at 200°C for 48 hours. A mixed culture of bacteria enriched on *p*-aminobenzoic acid was grown in a chemostat at a dilution rate of 0.04 h^{-1} on medium containing *p*-aminobenzoic acid and benzoic acid (0.5 g/l each) as carbon sources and minerals as reported previously (Parsons et al. 1987).

The hexyl, heptyl and octyl *p*-aminobenzoates were dissolved in a Na_2HPO_4/KH_2PO_4 buffer (pH 6.8) (to which $CaNO_3$ (1 g/l) had been added to prevent turbidity) using a generator column.This contained chromosorb (G-AW,

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60-80 mesh) coated with ca. 1% of each compound (Opperhuizen et al. 1988) (solution 1). The methyl, propyl and butyl esters were dissolved in the buffer by stirring for 12 h at room temperature, followed by filtration to remove undissolved residues (solution 2). The two solutions were mixed in a ratio of 10:1 (solution 1:solution 2).

Sorption coefficients were determined by shaking suspensions of 240 mg sediment (dry weight) in 200 ml of the esters solution at 24°C and 150 rpm for 48 h. The suspensions were then centrifuged at ca. 900 g for 10 minutes. 175 ml of the overlying water was extracted with 50 ml ether by stirring at room temperature for 1 h. The remaining 25 ml water and the sediment were extracted with 50 ml ether by stirring at room temperature for 2 h. The ether phases were removed and concentrated under gentle stream of nitrogen. The residue was redissolved in methanol.

Batch biodegradation experiments with and without sediment were conducted simultaneously. Solutions of the esters (175 ml) with and without sediment (240 mg) were shaken at 24°C for 48 h to allow sorption to reach equilibrium. Then 25 ml of a suspension of bacteria, taken from the stock chemostat culture, were added to each erlenmeyer. One erlenmeyer containing sediment but no bacteria suspension was used as a control for abiotic losses. At appropriate time intervals 50 ml ether were added to a suspension. Water/sediment/bacteria suspensions were extracted by stirring at room temperature for 2 h. Water/bacteria suspensions were extracted by stirring at room temperature for 1 h. The extracts were further treated as described above.

The samples were analyzed by reversed-phase HPLC, using a Zorbax BP-phenyl column (15 cm x 2.8 mm, 5-7 μm). Gradient elution started with 40/60 methanol/water changing after 2 minutes to 75/25 methanol/water over a period

Table 1. Sorption coefficients of n-alkyl *p*-aminobenzoates and ratios of residue and sorbed concentrations

Ester	K_p[a] (l/kg)	Total[b] (%)	c_r/c_s[c]
Methyl	44 (±29)	77	0.10 (±0.08)
Propyl	100 (±15)	84	0.23 (±0.12)
Butyl	69 (±7)	133	0.31 (±0.14)
Hexyl	171 (±11)	83	1.08 (±0.35)
Heptyl	325 (±20)	102	0.99 (±0.11)
Octyl	676 (±106)	107	0.65 (±0.07)

[a] Sorption coefficients determined with 1.2 g/l sediment. Means (± standard deviations) from three determinations.

[b] Total amounts of compound recovered from sediment suspensions.

[c] Ratios of residue concentration resistant to biodegradation in sediment suspensions (c_r) and concentration initially sorbed (c_s). Means (± standard deviations) from four experiments.

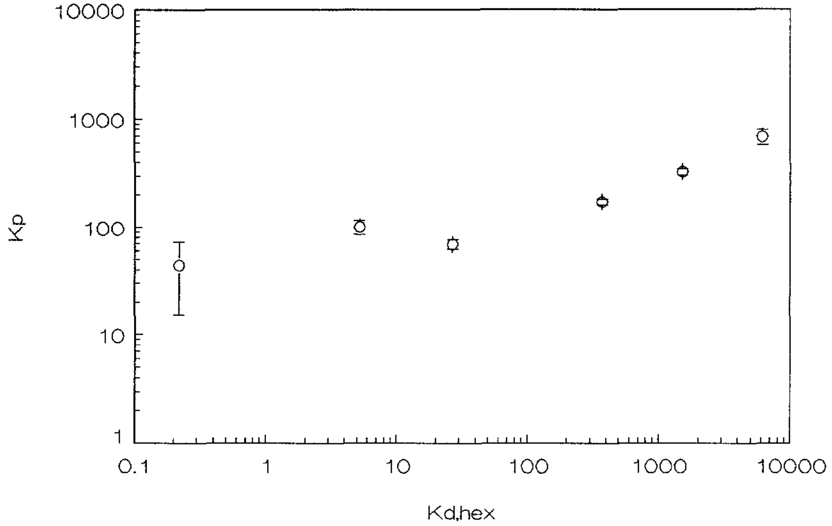

Figure 1. Relationship between sediment sorption coefficients (K_p) and hexane-water partition coefficients ($K_{d,hex}$) of n-alkyl *p*-aminobenzoates.

of 20 minutes. The detector was a Zeiss Spectral PM 2 DLC photometer operating at 280 nm connected to a Spectra-Physics 4100 computing integrator. Recoveries for the extraction of bacteria suspensions and water were between 100 and 170%. At the moment we can give no satisfactory explanation for these high values. However, since they were reproducible, all results were corrected for these recoveries. The recoveries of the water/sediment/bacteria suspensions were about 100%.

RESULTS AND DISCUSSION

Partition coefficients (K_p) for the sorption of the alkyl *p*-aminobenzoates to 1.2 g/l sediment are given in Table 1. For the butyl, hexyl, heptyl and octyl esters log K_p correlated well with log $K_{d,hex}$ (Fig. 1, $r^2 = 0.989$), indicating that for these compounds hydrophobic sorption to the organic phase of the sediment is dominant (Karickhoff, 1974). Since the methyl and propyl esters do not fit this correlation, other sorption mechanisms may be important for these less hydrophobic compounds.

Typical results of the biodegradation experiments in the presence and absence of 1.2 g/l sediment are shown for methyl *p*-aminobenzoate in Fig. 2 and for the butyl ester in Fig. 3. A comparable biodegradation behaviour was found for the other esters. It can be seen from these figures that in the experiments without sediment the esters were rapidly degraded, with half-lives in the order of minutes. After one hour the concentrations of the esters had fallen under the detection limit (ca. 0.01 μM). In the experiments with sediment comparable initial high biodegradation rates were observed. However, in contrast to the experiments without sediment, residue concentrations which resisted degradation were

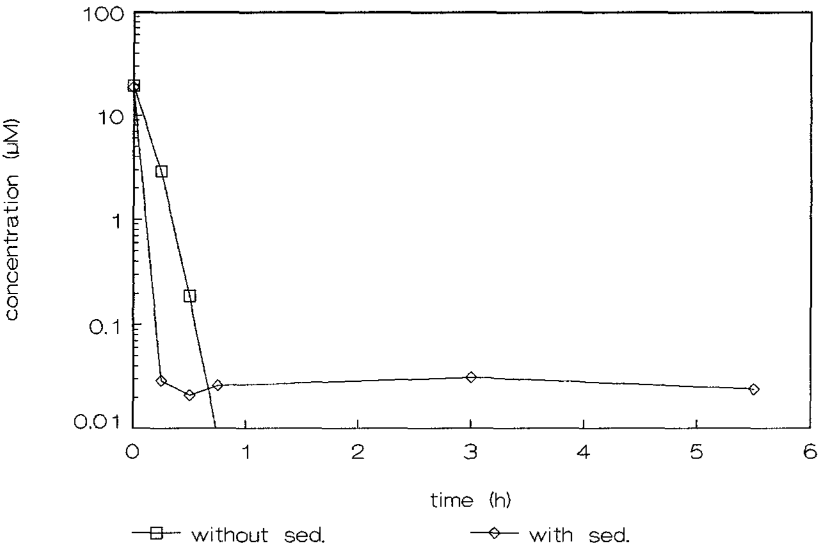

Figure 2. Degradation of methyl *p*-aminobenzoate in the presence and absence of 1.2 g/l sediment.

observed. No further biodegradation could be detected during the remainder of the experiments (up to 70 h total; data not given).

The ratios of these residue concentrations (c_r) and the concentrations of the esters initially present in the sorbed state (c_s, calculated from K_p) are given in Table 1. For the hexyl and heptyl esters this ratio is about 1, indicating that it is the sorbed fraction of these compounds that is resistent to degradation. Suprisingly, the octyl ester has a somewhat lower value (0.65). The much lower values of c_r/c_s for the methyl, propyl and butyl esters suggest that a, presumably rapidly desorbed, part of the sorbed fraction of these compounds is susceptible to biodegradation.

If it is indeed only the dissolved fraction of a chemical that is degraded, the effect that sorption to sediment will have on biodegradation kinetics in sediment suspensions will depend not only on the fraction sorbed, but also on the relative rates of desorption and biodegradation. The desorption rate constants (k_{des}) of hydrophobic compounds are inversely proportional to K_p (Brusseau et al. 1990).

If the rate of desorption is much higher than that of degradation, the dissolved and sorbed fractions of compound will be in equilibrium. In this case the apparent degradation rate (the rate of decrease of the total concentration) in a sediment suspension will depend on the fraction of the compound that is sorbed. Since the fraction sorbed is dependent on both the sediment/water ratio and the sorption coefficient, the decrease of the apparent biodegradation rate depends on properties of both the compound and of the test system. If, however, the rate of desorption is much lower than that of degradation, one would expect rapid initial decrease of the total concentration of compound due to degradation of the

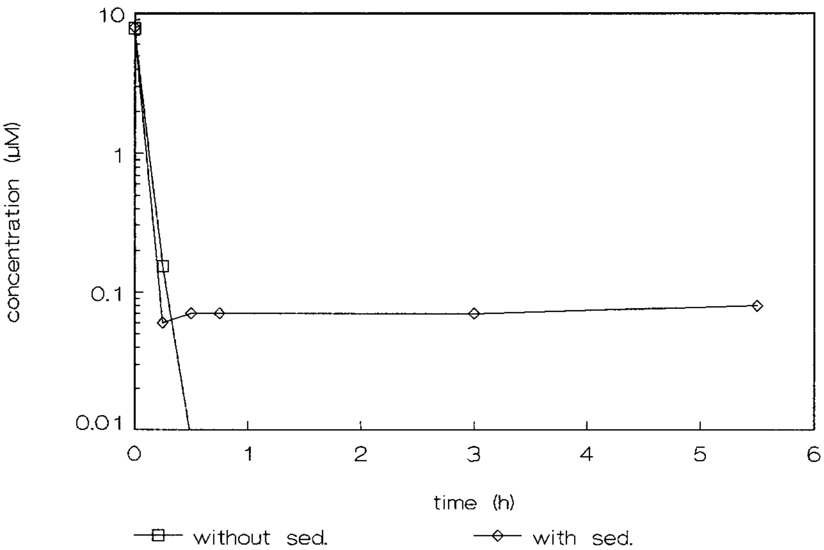

Figure 3. Biodegradation of butyl *p*-aminobenzoate in the presence and absence of 1.2 g/l sediment.

dissolved fraction. This would then be followed by degradation of the initially sorbed fraction at a much lower apparent rate, determined by the desorption rate.

The results of this study indicate that for the n-alkyl *p*-aminobenzoates the effect of sediment is a reduction of the overall biodegradation rate, with rapid initial biodegradation being followed by very slow degradation of the initially sorbed fractions. This is not suprising considering the extremely high biodegradation rates and moderately high partition coefficients of these compounds. For less strongly sorbing compounds, such as toluene (K_p = 1.42) (Robinson et al. 1990), 2,4-D (K_p = 0.09 to 9.05 for different sediments) (Ogram et al. 1985), chlorpropham and di-n-butyl phthalate (K_p values not given) (Steen et al. 1980) and naphthalene (K_p = 12) (Mihelcic and Luthy 1991), the biodegradation kinetics in sediment suspensions indicate that the desorption rates are higher than the biodegradation rates and that equilibrium partitioning applies. The presence of 10 to 1000 mg/l of different sediments was reported to have little effect on the initial rate of biodegradation of anthracene (K_p = 104 to 306), but there was some evidence for much slower degradation of a fraction of the compound in 1000 mg/l suspensions (Leslie et al. 1987).

Little is known of the influence of sorption on the biodegradation kinetics of more hydrophobic and more strongly sorbing compounds. Such compounds, such as chlorinated biphenyls and dibenzo-*p*-dioxins and other chloroaromatics, often have very low biodegradation rates as well as low desorption rates. Since these compounds are largely present in natural aquatic systems sorbed to sediments and other particulates, more work on the influence of sorption on the biodegradation kinetics of such compounds is therefore of great importance.

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