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The effect of sorption on phenanthrene bioavailability

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Abstract

Critical parameters that need to be considered in a bioavailability study are reviewed and applied to a study on the sorption (binding) of phenanthrene to dissolved humic acid (HA). Sorption coefficients values (K_{oc}) of phenanthrene to HA were measured for HA from several sources. These values were used to calculate the amount of HA that was needed to bind different fractions of solution-phase phenanthrene. Sorption linearity and complete reversibility were assumed. A mixed culture of phenanthrene degraders, dominated by *Pseudomonas* sp. was sensitive to changes in dissolved phenanthrene concentrations in the range that was used in this study. Therefore, it was expected that if bound phenanthrene was not available, as is commonly stated in the literature, sorption would affect mineralization rate by reducing the concentration of free phenanthrene. However, it was found that mineralization of phenanthrene was not affected by sorption even when 90% of the phenanthrene was in the bound phase. It was concluded that the organisms were able to use the phenanthrene directly from the bound phase and at the same rate as from the free phase. Copyright © 1996 Elsevier Science B.V.

Keywords: Binding; Bioavailability; Polycyclic aromatic hydrocarbons; Phenanthrene; Sorption

1. Introduction

The issue of sorption and bioavailability is of interest mainly because it is believed that poor availability is one of the reasons for the limited success of bioremediation technology. Research designed to explain and overcome problems of poor availability of chemicals that are otherwise easily biodegraded would make bioremediation more widely useful (Alexander, 1991). Sorption, it is generally believed, tends to reduce the availability of the sorbed compound (Scow, 1993; Simkins and Alexander, 1984). However, it is not rare to find contradictory evidence in the literature. The addition of organic matter (sorbent) to soil has

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been reported to have a positive, negative, or no effect on the biodegradation of hydrophobic organic contaminants. This may be the result of different availabilities of the sorbed compounds to different organisms (Guerin and Boyd, 1992), or because of different combinations of inhibitory and stimulatory factors, both affected by the presence of natural organic matter. In some reports, experimental factors were not well isolated and it is hard to understand the relationships between sorption and bioavailability. For example, addition of organic matter may not only serve as a sorbent but may also stimulate microbial activity by serving as another carbon source.

In this work, some of the critical parameters that need preliminary investigation before conducting a well controlled bioavailability study are reviewed. This approach is then used to study the effect of sorption on phenanthrene bioavailability.

2. Sorption and bioavailability: critical parameters to consider

There are some key parameters that need to be investigated in order to perform a well controlled bioavailability study.

2.1. Sorbent

When dealing with hydrophobic pollutants, sorption to organic matter is mainly considered to be the dominant process. Clay minerals are hydrophilic in their nature but may become more significant in a clay-rich and organic-matter-poor environment. Natural organic matter is a heterogeneous material and includes humic materials (i.e. humic acid, fulvic acid and humin) as well as other less decomposed organic entities (mainly carbohydrates, lignins and proteins). Also, each of these entities can be found in nature in the aqueous phase (dissolved organic matter) or in the solid phase, associated with clay particles. Since sorption is affected by the nature of the organic matter and by the phase in which it is found (Murphy et al., 1990; Laor, 1995), it is critical to specify the fraction of organic matter under investigation and to distinguish between dissolved and mineral associated phases.

2.2. Sorption

Sorption intensity needs to be measured in order to calculate the amount of sorbent needed to bind different fractions of the pollutant. Assuming that sorption can be described by the Freundlich equation

$$C_s = KC_L^{1/n}, \tag{1}$$

where C_s and C_L are the equilibrium concentrations of the sorbate (the pollutant) on the sorbent and in the liquid, respectively, K and $1/n$ are constants, with $1/n$ being the slope of the isotherm on a logarithmic scale. In many cases, especially with hydrophobic pollutants, the isotherm obtained is linear with a slope $1/n = 1$. For these cases, the Freundlich expression (Eq. (1)), becomes simply a distribution expression (Eq. (2))

$$C_s = K_d C_L, \tag{2}$$

where K_d is a distribution sorption coefficient.

Since sorption of hydrophobic organic pollutants has been shown to be well correlated with the organic carbon content of the soil or sediment and relatively independent of other sorbent properties (Hassett et al., 1980; Means et al., 1980), it is more meaningful to express K_d on the basis of organic carbon (OC):

$$K_{oc} = \frac{K_d}{f_{oc}}, \tag{3}$$

where K_{oc} is equal to the partition coefficient K_d, divided by the fraction of OC in the respective soil or sediment.

Eqs. (1) and (2) show that linearity is critical for calculations. If sorption is linear, the same concentrations of sorbent would be needed to bind different fractions of the pollutant at any initial pollutant concentration. On the other hand, for nonlinear sorption, sorbent concentrations would be dependent on the initial pollutant concentration. With polycyclic aromatic hydrocarbons (PAH) and other hydrophobic organic pollutants sorption linearity was suggested to be valid up to half of the pollutant water solubility (Karickhoff et al., 1979).

Collecting different K_{oc} values that were published for phenanthrene and plotting the amount

of organic carbon needed to bind different fractions of it (Fig. 1) emphasizes the importance of measuring K_{oc} values under experimental conditions similar to those that are later used in the bioavailability study. Fig. 1 shows that great differences in organic carbon concentrations would be needed for different K_{oc} values.

Sorption reversibility is another critical issue. If sorption is reversible, and the organisms are not able to use the pollutant from the bound phase, mineralization would be dependent on desorption rate. On the other hand, if sorption is irreversible but the bound pollutant is still consumed, this means that the organisms can utilize the substrate directly from the bound phase. Ogram et al. (1985) summarized the possibilities regarding the consumption of free and bound pollutant by free and bound microorganisms.

2.3. Microorganisms

Since the mineralization rate of hydrophobic compounds is also affected by the water solubility of the specific compound (Bossert and Bartha, 1986), the addition of organic matter may enhance degradation by increasing the apparent compound solubility. Sorption of hydrophobic chemicals to organic matter is frequently seen as partitioning where the humic material serves as a solvent (Chiou et al., 1986). This solubility enhancement may stimulate microbial degradation as the microorganisms are exposed to higher concentrations of substrates. On the other hand, it should be investigated whether the organisms utilize the substrate only when dissolved or also when suspended as solid microparticulates (Wodzinski and Coyle, 1974).

Another aspect to analyze is whether the organisms are sensitive to changes in concentration in the range of the planned study. This would indicate if the organisms are expected to be sensitive to sorption-mediated reductions in free pollutant concentration.

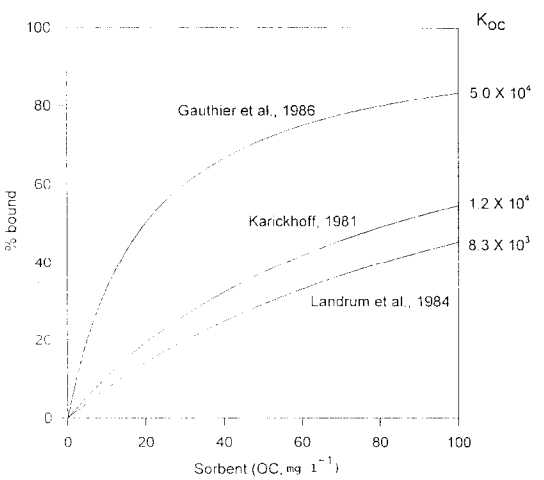

Fig. 1. Plots of percentage of bound phenanthrene vs. organic carbon concentration (sorbent) for different K_{oc} values (Gauthier et al., 1986; Karickhoff, 1981; Landrum et al., 1984). Calculations are based on linear Freundlich isotherm of the form (Carter and Suffet, 1982)

$$\% \text{ bound} = \frac{10^{-6}K_{oc}[OC]}{10^{-6}K_{oc}[OC]+1}$$

3. Materials and methods

3.1. Sorption studies

3.1.1. Chemicals and reagents

Phenanthrene at 98% purity (Aldrich, Milwaukee, WI), and phenanthrene-9-^{14}C of 98% purity, specific activity 8.3 mCi mmol^{-1} (Sigma, St. Louis, MO), were used without further purification. Radioactivity measurements were made with a liquid scintillation counter LS 5000TD (Beckman, Fullerton, CA). Values were corrected for quenching by the external standard method.

A 1 mg l^{-1} phenanthrene stock solution was made by adding 0.5 ml acetone containing 1 mg of phenanthrene to 1 l double distilled water (d_2H_2O). The solution was stirred for at least 4 h in the dark and then filtered through a 0.2 μm polycarbonate filter (Poretics, Livermore, CA). The solution was later kept at 4°C in the dark. Radiolabeled solutions were prepared in the same way with the addition of [^{14}C]phenanthrene (also carried in acetone), to yield an initial activity of 3000 dpm ml^{-1}. Final phenanthrene concentration in the sorption experiments ranged between 0.5 and 0.7 mg l^{-1} (about half of its water

solubility). Solutions of phenanthrene were prepared in the mineral medium (Stanier et al., 1966) that was later used in the bioavailability study. During sorption experiments, the solution was poisoned with 0.02% NaN_3 to eliminate microbial activity.

Humic acids (HA) were extracted from a compost and from the top 5 cm of loamy (Soil 1) and clay loamy (Soil 2) soils, using the methods of Schnitzer (1982). Aldrich HA (Aldrich, humic acid, sodium salt) was used as received. Stock solutions of 300–500 mg l^{-1} were prepared by dissolving dry HA in 0.1 N NaOH and filtering it through 0.2 μm. The filtrate was kept at 4°C until used (no more than 1–2 weeks).

3.1.2. Binding of phenanthrene to dissolved HA

Analyses were performed by using the fluorescence quenching technique of Gauthier et al. (1986) with slight modifications. This method is based on the observation that PAHs fluoresce in aqueous solution but not when associated with dissolved humic material. As a consequence, the fraction of PAH associated with the humic material is determined directly from the fractional decrease in fluorescence upon addition of humic substances. Measurements were made on a fluorescent spectrophotometer LS 50 (Perkin-Elmer) at 249 and 365 nm (excitation and emission wavelengths, respectively). To correct for the 'inner filter effect', absorption measurements (Spectronic 1001, Milton Roy, Rochester, NY) were made at the same excitation and emissions wavelengths.

3.2. Biodegradation studies

3.2.1. Phenanthrene degrading mixed culture

A mixed culture of phenanthrene degraders was enriched from a coal tar-contaminated soil (a former coal tar distillation facility site). The culture was transferred several times into fresh medium (Stanier et al., 1966, containing phosphate and $(NH_4)_2SO_4$ as P and N sources) with 100 mg l^{-1} phenanthrene and then streaked on agar plates (Noble agar with mineral medium). The plates were coated with phenanthrene by spraying an ether solution with 1–2% phenanthrene, until a visible and uniform white surface layer was achieved. Growth was evident by a clearing zone surrounding the phenanthrene degrading colonies (Kiyohara et al., 1982). Plates were incubated at room temperature for about 5 days until well grown colonies appeared, then sealed with paraffin and kept at 4°C.

To identify the organisms in the enrichment culture, 10 colonies were isolated from the agar plates. Tests included Gram reaction, morphology, motility, flagella (using Ryu stain; Heimbrook et al., 1986), oxidase, catalase and fluorescence. All the isolated colonies were found to be gram-negative, short rods, motile with one polar flagellum, and oxidase, catalase and fluorescence were positive. The bacterium was tentatively identified as *Pseudomonas* sp. and it was assumed that the enrichment culture was very much dominated by this organism. However, the term 'mixed culture' is used since experiments were inoculated from the whole enrichment and not from specific isolates.

3.2.2. Mineralization assays

An apparatus to collect $^{14}CO_2$ was built with some modifications after Marinucci and Bartha (1979). Incubation flasks (250 ml) were connected to the apparatus at various time intervals for 15 min and the $^{14}CO_2$ was trapped in two traps each containing 10 ml Oxosol–^{14}C scintillation cocktail (National Diagnostics, Atlanta, GA).

Most experiments were performed as 'short-term' experiments at an initial phenanthrene concentration of 100 μg l^{-1}, and were inoculated to achieve about 5×10^7 cells per ml. Mineralization data were fitted to a first-order production equation of the form (Larson, 1984):

$$P = P_{max}(1 - \exp(-kt)), \quad (4)$$

where P represents the mass of phenanthrene converted to $^{14}CO_2$ (μg l^{-1}) at time t (min), k is the first-order rate constant (min^{-1}), and P_{max} represents the maximal mass which can be mineralized (μg l^{-1}).

Expressing the mineralization rate as a function of time and rearranging yields,

Table 1
Humic acid OC that needs to be added in order to sorb different percentages of phenanthrene in the mineral medium solution

	Humic acid OC (sorbent) needs to be added (mg l^{-1})			
	Aldrich	Compost	Soil 1	Soil 2
K_{oc} (ml g^{-1})	1.01×10^5	5.53×10^4	6.04×10^4	4.57×10^4
Phenanthrene bound (%)				
10	1.10	2.01	1.84	2.43
25	3.30	6.03	5.52	7.30
50	9.89	18.08	16.56	21.90
75	29.67	54.25	49.69	65.70
90	89.02	162.75	149.06	197.11
100	∞	∞	∞	∞

$$\frac{dP}{dt} = P_{max}(k \exp(-kt)). \quad (5)$$

The initial mineralization rate ($t = 0$) is therefore

$$\frac{dP}{dt} = P_{max}k. \quad (6)$$

Nonlinear regression analysis (Sigma Plot, Jandel Scientific, San Rafael, CA) was used to estimate the parameters P_{max} and k, and initial mineralization rates ($\mu g\ l^{-1}\ min^{-1}$) are based on these values. At substrate concentrations below K_m (half-saturation coefficient), and with constant biomass, mineralization rates were expected to be nearly linearly proportional to substrate concentration (Larson, 1980). If bound phenanthrene was unavailable, an increase in the fraction of bound phase would result in a linear decrease in the initial mineralization rate. Therefore, a high inoculum size (negligible biomass change) was used and mineralization rates were analyzed at various phenanthrene concentrations to determine K_m. These short-term experiments lasted about 5 h.

'Long-term' experiments were conducted with initial biomass of about 10^5 cells per ml. During these experiments, biomass changed and growth curves were obtained. These experiments lasted several days.

To conduct a bioavailability study, the mineral medium containing the desired concentration of phenanthrene and spiked with [^{14}C]phenanthrene was transferred into triplicate incubation flasks. Dissolved HA was added in the amount needed to bind up to 90% of the phenanthrene (see Section 4, Table 1), and the flasks were inoculated. Flasks were incubated at room temperature, on a rotary shaker at 150 rpm ('long-term' experiments) or shaken by hand periodically ('short-term' experiments). $^{14}CO_2$ was recovered at different time intervals.

4. Results and discussion

4.1. Sorption

Table 1 summarizes the K_{oc} values obtained for the different HAs and the amount of humic acid OC needed to bind different percentages of the phenanthrene in the mineral medium solution. Binding was much higher for Aldrich HA compared with the other HA sources. It has been shown by others that HA from different sources presented different sorption properties. Among the factors affecting sorption are the aromaticity of the humic material expressed by its fraction of aromatic carbon atoms and atomic H:C ratio (Gauthier et al., 1987). Whatever the reasons for the different K_{oc} values, this emphasizes the need for such a table before conducting any bioavailability study. Much lower concentrations of Aldrich HA would be needed to bind the same fraction of phenanthrene compared with the other HA sources.

4.2. Bioavailability

Mineralization rates obtained at 1 and 5 mg l^{-1} initial phenanthrene concentration are presented in Fig. 2. This experiment was performed with a concentration above phenanthrene solubility to see if phenanthrene can be utilized both from the dissolved and the solid states. It is seen that the mass that was mineralized initially at 5 mg l^{-1} (about 5 times phenanthrene water solubility) was no higher than at 1 mg l^{-1}. It later became higher as the 1 mg l^{-1} was exhausted first. It was concluded that the organisms could utilize the phenanthrene only when dissolved. Similar conclusions were drawn by Wodzinski and Coyle (1974) for the degradation of phenanthrene by *Pseudomonas* sp. This implies that an addition of a co-solvent (as may be the case with the addition of humic substances) may enhance degradation rates by increasing phenanthrene solubility. To eliminate such an effect, in all experiments the amount of phenanthrene did not exceed its water solubility.

To determine K_m, mineralization was analyzed at initial phenanthrene concentrations of 20–500 μg l^{-1} (Fig. 3(a)). Initial mineralization rate values determined from the fitted curves were plotted against the initial concentration (Fig. 3(b)) and were fitted by a nonlinear regression analysis to the Michaelis–Menten equation. The fitted curve ($V_{max} = 1.46$ min^{-1}; $K_m = 579$ μg l^{-1}) shows that mineralization rates were nearly linearly proportional to substrate concentration.

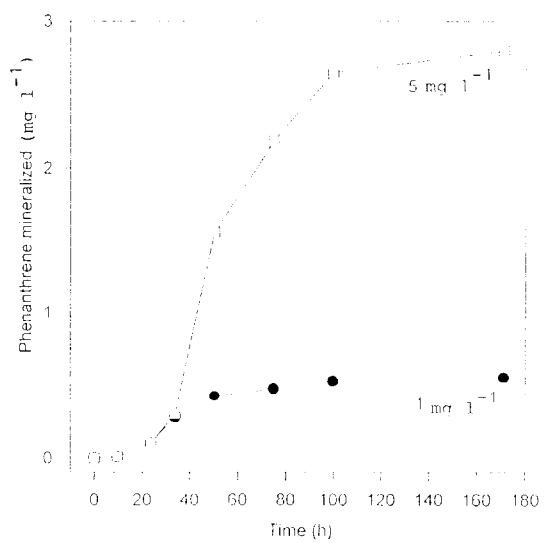

Fig. 2. Phenanthrene mineralization at 1 and 5 mg l^{-1} initial concentrations.

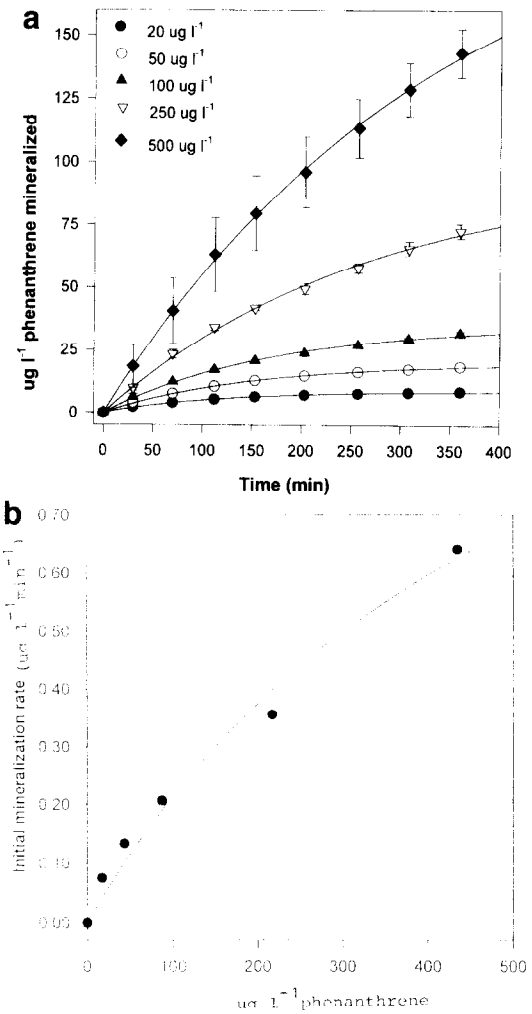

Fig. 3. Phenanthrene mineralization at 20–500 μg l^{-1} initial concentration. (a) Solid lines are fitted to Eq. (4); (b) the calculated initial mineralization rates are plotted against the initial concentration. Solid line in (b) is fitted to Michaelis–Menten kinetics.

Fig. 4(a) and (b) show the results obtained with dissolved Aldrich HA in short-term and long-term experiments. The addition of HA did not have any effect on the mineralization rate in both cases,

even when 90% of the phenanthrene was bound to the HA.

Assuming that the mineralization rate is solely or mainly determined by the concentration of free phenanthrene (i.e. bound phenanthrene is completely unavailable or has slower kinetics), even if sorption is completely reversible (as was shown by McCarthy and Jimenez, 1985) and desorption is immediate, it is still expected that binding would lower the mineralization rate. This is because the organisms are instantaneously exposed to lower concentrations of free phenanthrene. Since no change in mineralization rate was observed (Fig. 4), it is suggested that the microorganisms were able to use the phenanthrene directly from the bound phase and at the same rate as from the free phase.

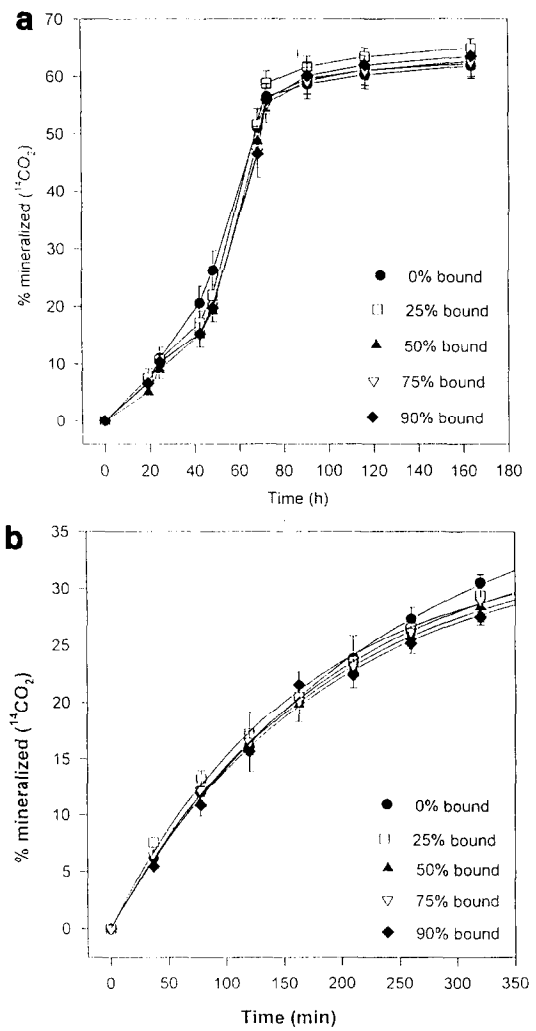

Fig. 4. Phenanthrene mineralization at 1 mg l^{-1} (a) and 100 $\mu g\ l^{-1}$ (b) initial concentration; 0–90% of the phenanthrene was bound to dissolved Aldrich HA. Solid lines in (a) are fitted to Eq. (4).

This conclusion is in contradiction to what is generally believed regarding the effect of sorption on bioavailability. However, it should be noted that it is one of the first times that bioavailability was determined for pollutants that are bound to dissolved humic material. Also, the results may be different with different organisms. However, it should be emphasized that these experiments were performed with a mixed culture that was enriched from a contaminated site and not with an isolated exotic species.

In evaluating the applicability of these results, we note that since a well controlled sorption and bioavailability study was the aim of this work, natural factors have been isolated; however, the system as a whole may still not represent what happens in a natural environment. Since all experiments were conducted in liquid, it seems to be more suitable to apply this study to processes occurring in aquatic systems or in the soil solution. Biodegradation rates of chemicals in soil are frequently far lower than rates measured in solution, and also aggregation may affect bioavailability in a completely different way. Soil intra-aggregate pores may be too small to be accessible to bacteria and the substrate sorbed within organic matter would be accessible only after desorption (Scow and Alexander, 1992). However, soil slurries which have been used in many studies are not much more useful to represent the soil environment, since those systems also eliminate aggregation effects. Moreover, the use of soil slurries may confound biodegradation measurement by making soil organic matter another carbon source available for metabolism.

5. Summary and conclusions

Sorption of phenanthrene to dissolved HA was found to be dependent on the HA source and was

substantially higher for Aldrich HA than for HA extracted from compost and loamy soils. Accurate measurements of K_{oc} values were shown to be critical to conduct a well controlled bioavailability study. Binding of phenanthrene to dissolved HA did not reduce phenanthrene bioavailability. It is suggested that the organisms were able to use phenanthrene directly from the HA-bound phase and at the same rate as from the free phase.

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