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Experimental and modeling approach to study sorption of dissolved hydrophobic organic contaminants to microbial biofilms

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ABSTRACT

A biofilm reactor was developed to investigate the sorption of polycyclic aromatic hydrocarbons (PAH) as model compounds for hydrophobic organic contaminants (HOC) to intact microbial biofilms at environmentally realistic concentrations. When operated as a differential column batch reactor, the system can be used to study the thermodynamics as well as the kinetics of the exchange of HOC between an aqueous phase and microbial biofilms. Organic carbon normalized partition coefficients (K_{oc}) for phenanthrene, fluoranthene and pyrene were at the lower end of those known for other organic sorbents. Intra-biofilm diffusion coefficients (D) were calculated from decrease in solute concentration over time using a model for diffusion through a plane sheet and ranged from 0.23 to $0.45 \times 10^{-9}\,cm^2\,s^{-1}$ for the three PAH. These diffusion coefficients are about four orders of magnitude lower than those reported in literature for free aqueous solution. These data and the experimental approach presented here are useful to assess the importance of microbial biofilms for exchange processes of HOC in heterogeneous systems such as water distribution systems, membranes and aquifers, sewer systems or surface soils.

1. Introduction

Biofilms may significantly influence the distribution of hydrophobic organic compounds (HOC) in heterogeneous aqueous systems and, hence, their residence time, mobility and fate in such systems. When the solid phase provides only poor sorption properties (sewer systems, water supply systems, sand filters, membranes, mineral particles in surface waters and aquifers), the evolution of a microbial biofilm on its surface may provide an important sink and reservoir for hydrophobic contaminants delivered by the aqueous phase. Accumulation of HOC in such biofilms and the increased contact time with bacteria residing in these biofilms may allow adaptation of microorganisms and mineralization of these trace pollutants. For these reasons, the evolution of a biofilm may be essential to retain and to remove HOC in such systems. If these biofilms are detached, however, they could turn into a source for HOC.

When the solid phase itself has good sorption properties (soil, activated carbon), a biofilm coverage may act as a barrier that slows down the mass transfer of a hydrophobic compound from the dissolved to the particulate phase. In this case, biofilm growth may hamper rather than improve the removal of HOC.

To quantitatively assess the importance of biofilm coatings on surfaces exposed to water for the fate of HOC in these systems, knowledge on the partition coefficients (K_D) as well

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as on the diffusion coefficients for the respective contaminant in biofilms is required.

Sorption of pesticides to intact biofilms of surface waters has been studied (Headley et al., 1998) and K_D values were determined. Studies using biofilm components, either the bacteria itself or their extracellular polymeric substances (EPS) are a bit more frequent. The sorption of chlorophenols (Wang et al., 2002) and of PCB (Sobek et al., 2006) to bacteria has been investigated, as well as the partitioning of phenanthrene (Brunk et al., 1997) and chlorophenol (Wang et al., 2002) to EPS.

Even less work has been directed to kinetic aspects of these processes. It has been recognized that the decrease in solute concentration due to sorption to biofilms followed a first-order kinetics (Headley et al., 1998), but only little is known about diffusion coefficients of trace pollutants inside microbial biofilms, yet. For the moderate hydrophobic compound toluene, diffusion coefficients in *Pseudomonas putida* biofilms were determined that are about two orders of magnitude lower than its aqueous diffusivity (Holden et al., 1997).

Thus, only little thermodynamic and kinetic data concerning the sorption of HOC to intact microbial biofilms are available and methods to determine these data at environmentally relevant concentrations are limited. In this study, a biofilm reactor was developed that allows to study the extent of sorption/partitioning of polycyclic aromatic hydrocarbons (PAH) as model compounds for HOC to microbial biofilms and to determine their rate of diffusion in such biofilms. Quantitative data for both aspects are provided that allow to quantitatively assess the importance of microbial biofilms on surfaces for the fate of HOC in aqueous systems.

2. Materials and methods

2.1. Materials

The standards phenanthrene (99.5+%), fluoranthene (99%) and pyrene (99%) were purchased from Aldrich (Steinheim, Germany). Solvents for the liquid chromatography were acetonitrile (gradient grade) from Promochem (Wesel, Germany), and ultrapure water (prepared with water purifier Maxima, ELGA Ltd., England).

2.2. System setup

The biofilm reactors (Fig. 1) are made of glass (length 12 cm, inner diameter 3.4 cm and volume 80 mL) with PTFE sealing and are filled with glass beads (3 mm diameter, total surface area approximately 800 cm^2). The glass beads were etched with hydrofluoric acid prior to use to roughen the surface and to improve the attachment of bacteria.

Each sorption experiment consists of two phases: a growth phase to establish the biofilm in the reactor, and a sorption phase, in which the sorption onto the biofilm was studied. A schematic view of the experimental setup for each of these phases is shown in Fig. 1a and b.

2.2.1. Biofilm production

A sterile biofilm reactor was inoculated with a pure bacterial strain (overnight culture) and allowed to stand for 6 h. Then 100% TSB medium (Tryptic Soy Broth—purchased from BD Diagnostics) is pumped from a storage bottle (5 L) by a peristaltic pump at a flow rate of 8 mL h^{-1} through the reactor into a waste bottle. A second peristaltic pump connected to an internal loop increases the flow through the reactor and is operated at 35 mL min^{-1}. All tubings are made of silicon and the whole setup was sterilized before use and operated under sterile conditions. A typical growth phase lasted 6 days.

The experiments are performed with strain 30A isolated from a slow sand filtration unit. Sequence analysis revealed strain 30A as a member of *Rhizobiaceae* with Sinorhizobium sp. TB-10-II as closest relative.

2.2.2. Sorption experiments

After biofilm establishment, the reactor was transferred into the chemical laboratory and connected with the second setup (Fig. 1b). This system consisted of a piston pump Buechi B-681 (Buechi, Flawil, Switzerland) that delivers the solution from a supply bottle via PTFE tubing into the reactor, from where it flows back into the supply bottle at a flow rate of

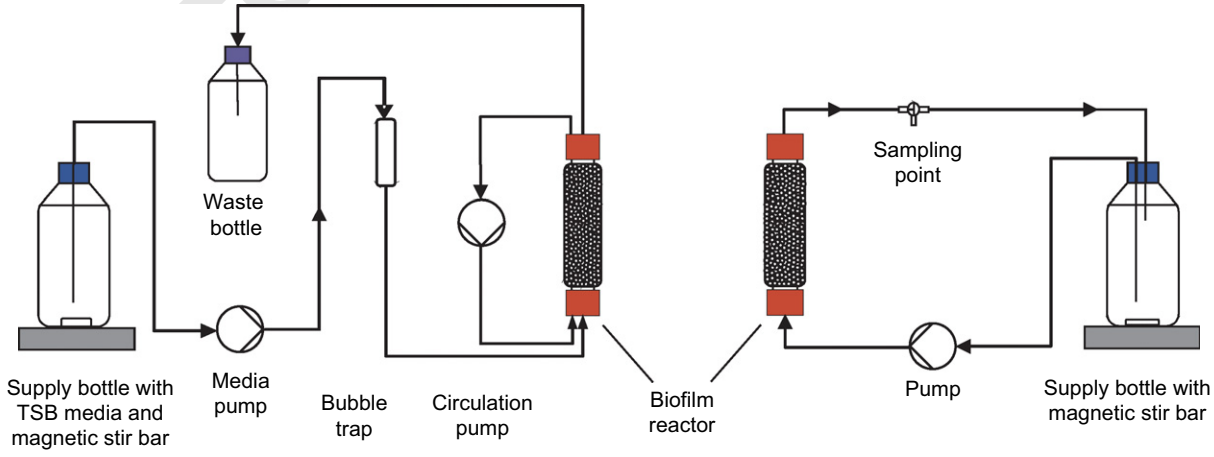

Fig. 1 – Experimental setup for biofilm growth (a) and sorption experiments (b).

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100 mL min^{-1}. The content of the supply bottle is continuously mixed by a magnetic stirrer. Experiments are performed at room temperature ($T = 21 \pm 2$ °C). After connecting the fresh biofilm reactor, the supply bottle is first filled with 1 L of tap water and the reactor is flushed to remove non-attached biomass and the growth medium.

Then, the supply bottle is exchanged, filled with 0.5 L of tap water, and spiked with phenanthrene, fluoranthene and pyrene by adding 10 μL of a stock solution (62.5 mg L^{-1} of each compound in propanol) to obtain the start concentration of 1.25 μg L^{-1}. After starting the pump, the sorption experiment was allowed to run for 2–3 h.

Tap water has been used as aqueous phase to suit the living conditions of the microorganisms (e.g. regarding ionic strength). Chemical characteristics are as following: Na^+: 28 mg L^{-1}, K^+: 5 mg L^{-1}, Ca^{2+}: 150 mg L^{-1}, Mg^{2+}: 6 mg L^{-1}, SO_4^{2-}: 125 mg L^{-1}, HCO_3^-: 218 mg L^{-1}, pH 7.6, temperature: 19 °C, conductivity: 725 μS cm^{-1} and dissolved organic carbon (DOC) concentration: 4.5 mg L^{-1}).

2.3. Sampling and analysis

Samples were taken in intervals of up to every minute from the column outlet and 800 μL of it was filled in a vial containing 200 μL of acetonitrile to prevent sorption of the PAH to the glass walls.

2.3.1. Determination of dissolved PAH

PAH analysis was performed by HPLC using a Waters 2690 liquid chromatograph equipped with a column oven (25 °C) and a Shimadzu RF-551 fluorescence detector. An Ultrasep ES PAH column (125 × 3 mm, 5 μm—Sepserv, Berlin, Germany) with a water–acetonitrile gradient at a flow rate of 0.6 mL min^{-1} was used: 0 min 60% AcCN, 14 min 95% AcCN, 15 min 60% AcCN, followed by equilibration until the end of each run (20 min). The injection volume was 100 μl. PAH were detected by time-programmed fluorescence detection (0–6.8 min λ_{EX} 260 nm, λ_{EM} 360 nm for phenanthrene, 6.8–20 min λ_{EX} 260 nm, λ_{EM} 420 nm). Retention times for phenanthrene, fluoranthene and pyrene were 5.9, 7.5 and 8.2 min, respectively.

2.3.2. Determination of biomass dry weight, biofilm volume, biofilm thickness and carbon content

After sorption experiments, the glass beads of the biofilm reactor were emptied into a flask with tap water, and the biofilm was detached off the beads by repeated washing steps and, finally, by ultrasonic treatment (10 min). The washing solutions were collected and filtered over preweighted 0.45 μm nitrocellulose membrane filters (Sartorius, Goettingen, Germany). The biomass weight was determined after drying for 24 h at 105 °C. If necessary (high biomass concentration), biomass solution was centrifuged before filtration (10 min at 1600 g). Additionally, the solution remaining in the supply bottle at the end of each sorption experiment contained small amounts of organic material that detached from the biofilm during the sorption experiment (<5%). This solution was, therefore, also filtered over preweighted 0.45 μm membrane filters, and the particulate matter remaining on the filter was added to the biofilm mass.

The water content of the biofilm was assessed from the pellet remaining from detached biofilm after centrifugation and removal of the supernatant. The mass of this pellet was determined before and after drying for 24 h at 105 °C.

Biofilm volume was determined by calculation of the biofilm wet weight (from dry weight and water content), taking into account an assumed biofilm density of 1 g cm^{-3}. Biofilm thickness was calculated by dividing the biofilm volume by the surface area of the glass beads.

Carbon content of detached and dried biofilm was determined using an elemental analyzer (vario EL III C/N/S, Elementar, Hanau, Germany).

2.3.3. Determination of sorbed PAH and partition coefficient K_D

The total concentration of PAH sorbed to the biofilm at equilibrium (q_∞) was determined from the decrease of solute concentration in the aqueous phase according to Eq. (1):

$$q_\infty = \frac{(c_0 - c_\infty)V}{m_s}, \quad (1)$$

where c_0 is the solute concentration at $t = 0$, c_∞ the solute concentration at equilibrium, V the volume of water and m_s the sorbent mass (biofilm dry mass).

As the duration of these sorption experiments was short (<180 min) and the microorganism forming the biofilm was not a PAH-degrading species, a concentration decrease due to biodegradation can be ruled out.

The partition coefficient K_D is the ratio between the equilibrium concentration in the biofilm (q_∞) and the corresponding equilibrium concentration in solution (c_∞):

$$K_D = \frac{q_\infty}{c_\infty}. \quad (2)$$

2.3.4. Determination of dissolved organic carbon

The DOC was determined using a TOC analyzer (highTOC, Elementar, Hanau, Germany) with catalytic high-temperature combustion. DOC was determined in the solution remaining in the supply bottle at the end of each sorption experiment and the washing solution used for detaching the biofilm mass from the glass beads after the experiment. The DOC of the latter was added to the biofilm mass because it was lost from there during the detachment process.

The quality of dissolved organic matter in solution after the sorption experiments was characterized by size exclusion chromatography (SEC) with UV and organic carbon detection (SEC-OCD) as described in Rosenberger et al. (2006).

2.4. Modeling

For the determination of intra-biofilm diffusion coefficients, the analytical solution of the diffusion equation

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad (3)$$

for the problem of diffusion from a stirred solution of limited volume into a plane sheet was used (Crank, 1975). Although these experiments were performed in column reactors filled with glass beads, this is an appropriate approach for the

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following reasons. (A) The column reactor used in these experiments was operated at high flow rates with an only incremental decrease in solute concentration during one reactor passage. This mode of operation is called differential column batch reactor (DCBR; Sontheimer et al., 1988), and the time course of concentration in these systems is equivalent to a stirred batch reactor system. (B) The glass beads in the column act as solid support for the biofilm only, without being involved in the sorption and diffusion processes. Consequently, the biofilm is seen as a plane sheet with a surface area equal to the surface area provided by the glass beads and a thickness equal to the biofilm thickness.

The solution in a form expressing the amount of solute in the sheet at time t as a fraction of the corresponding amount at equilibrium (q/q_∞) is shown in the following equation (Crank, 1975):

$$\frac{q}{q_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2\lambda_n^2} \exp(-D\lambda_n^2 t/l^2), \quad (4)$$

where the λ_n's are the non-zero positive roots of

$$\tan\lambda_n = -\alpha\lambda_n, \quad (5)$$

D the diffusion coefficient ($cm^2\,s^{-1}$), l the biofilm thickness (cm), and α a variable depending on the final fractional uptake of solute by the sheet at equilibrium (see Eq. (6)).

$$1 - \frac{c_0}{c_\infty} = \frac{1}{1+\alpha}. \quad (6)$$

This α can also be expressed as the ratio of the volumes of solution (V_L) and sheet (V_F) taking into account the partition factor K (Crank, 1975):

$$\alpha = \frac{V_L}{KV_F}. \quad (7)$$

Eq. (4) has been calculated using the first six positive roots of Eq. (5), which can be found in the literature in tabulated form (Crank, 1975; Carslaw and Jaeger, 1959). The results of Eq. (4) were transformed to the fractional loss of concentration in the solution (c/c_0) corresponding to Eq. (8) and plotted together with the experimental data.

$$\frac{c}{c_0} = 1 - \frac{q}{q_\infty} + \frac{q}{q_\infty}\frac{c_\infty}{c_0}. \quad (8)$$

Visual fitting of the modeled curve to the experimentally determined time course of the sorption process was performed by selecting an appropriate value for the diffusion coefficient D.

It has to be pointed out that the model can only be applied to partitioning processes (linear sorption isotherm). Therefore, a linear sorption isotherm for PAH partitioning into the biofilm was assumed in our experiments and verified as described below. Another general assumption for the mathematical solution of Eq. (3) is that the plane sheet is made of homogeneous (isotropic) medium, which essentially means that the diffusion coefficient is not dependent on the position in the sheet. Finally, diffusion in the boundary layer between the sorbent and the free dissolved phase is assumed to be negligible (achieved by high flow rates through the reactor).

3. Results and discussion

3.1. System design

The design of the biofilm reactors used for the sorption experiments (Fig. 1b) followed the below criteria:

- All reactor materials, namely the solid support for the biomass, should be polar to reduce hydrophobic interaction and sorption of the analytes. Therefore, glassware was used wherever possible and tubing made of PTFE. High-porosity polypropylene (Carlson and Silverstein, (1997)), silicone rubber tubes (Zhang et al., 1998) or geotextiles (Karamanev and Samson (1998)), which have been used as solid support in other investigations, were not suitable. For the same reason, no peristaltic pump but a medium-pressure liquid chromatography piston pump with adsorption-resistant materials was used to circulate the solution through the biofilm reactor. With the final setup, the control experiment without biofilm showed only marginal adsorption of the solutes with less than 5% of the initial concentration (30 ng).
- Starting concentration of the analytes should not exceed the microgram per liter range, which are concentrations not too far from those one can expect to occur in the environment.
- A decrease in the solute concentration should be detectable without the need for analyte enrichment (extraction of the aqueous phase). Flow cells (e.g. deBeer and Stoodley, 1995) would not have provided sufficient surface area.
- Despite using a column reactor for the sorption experiment, the concept of a completely mixed batch reactor system should be used for modeling (DCBR). In this system only an almost differential decrease in solute concentration during one column passage is allowable. This requires a short residence time and, thus, high flow rates to be used. The flow rate is, however, limited by the rigidity of the microbial biofilm. Vice versa, not all microorganisms form biofilms that are sufficiently stable to remain in the reactor.
- A high flow rate is also required to reduce the thickness of the boundary layer in the water surrounding the biofilm to a minimum.

3.2. Biofilm

Nine microbial strains isolated from soil samples of a public park and from a slow sand filtration unit were tested for their potential to produce firmly attached biofilms of sufficient mass that resist high flow rates. Highest biofilm production in the reactor was achieved with isolate 30A, a bacterial strain isolated from glass slides deployed in a slow sand filter test facility in Berlin, Marienfelde and identified as *Rhizobiaceae*. These are typical soil bacteria and symbiotic nitrogen fixers that can be found on the roots of plants and especially in legume plants.

The stability of the biofilm in the reactor could be improved by installing the secondary loop (see Fig. 1) that allowed to increase the flow rate (to 35 mL min^{-1}) already during the

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growth phase without increasing the consumption of growth medium. Using this modified experimental setup, between 30 and 150 mg of biofilm (dry weight) were attached to the glass beads of one reactor. Taking into account a measured water content of 89% in these biofilms of strain 30A, this corresponds to a wet biofilm mass of approximately 0.3–1.3 g per reactor and to an average biofilm thickness of about 5–20 μm.

During the experiments, a part of the biofilm was washed out and appeared in the supply bottle as particulate organic matter (POM) and DOC. Usually, between 1–5% POM and 2–10% DOC were washed out based on the mass of the biofilm at the beginning of the sorption experiment. This corresponds to POM concentrations of 1–8 mg L^{-1} and DOC concentration of 10–23 mg L^{-1} (including 4.5 mg L^{-1} background DOC of the tap water) in the supply bottle at the end of the experiment.

While the POM was added to the biofilm mass in all calculations, the DOC could be more critical for the experimental results. Elevated DOC concentrations could enhance the apparent solubility of hydrophobic contaminants in water and could, thus, lead to an experimental underestimation of their sorption tendency. However, aquatic compartments that come in contact with organic solid phases exhibit DOC concentrations in the range of 1–100 mg L^{-1}. Therefore, distribution coefficients between an aqueous phase with DOC and a biofilm are more realistic than those determined from solution without any DOC. Moreover, organic matter released from biofilms (soluble microbial products) is expected to be comparatively polar (Nielsen et al., 1997) and, thus, less critical in terms of cosolvation effects. Indeed, SEC-OCD analyses of the DOM confirmed that it primarily consisted of polysaccharides/proteins and low-molecular-weight acids. Therefore, the DOC of 10–23 mg L^{-1} is not expected to bias the sorption experiments.

3.3. *Sorption experiments*

A typical time curve of the concentration decrease of phenanthrene, fluoranthene and pyrene is shown in Fig. 2a. The partitioning equilibrium is almost reached within 2 h. These diagrams provide information on both (a) the thermodynamics of the sorption/partitioning process as visible from the final dissolved concentration at equilibrium and (b) on the kinetics of the process, indicated by the slope of the curves.

3.3.1. *Sorption equilibrium*

In case that the sorbed mass of a solute is low compared to the mass or surface of the sorbent, one can assume a linear sorption isotherm. In that case the parameter n of the Freundlich isotherm equals one. A Freundlich parameter n close to 1 (0.85–1.08) was found for sorption of phenanthrene to sediment, humin and humic acids with equilibrium concentrations between 4 and 90 μg/L (Parikh et al., 2004). Linear isotherms have also been detected for PAH sorption to estuarine colloids (Wijayaratne and Means, 1984) at equilibrium concentrations up to 30–300 μg/L.

With this assumption, a distribution/partitioning coefficient (K_D) can be approximated from the equilibrium concentration reached in the biofilm reactor experiments provided that the sorbent mass (biomass dry weight) of each

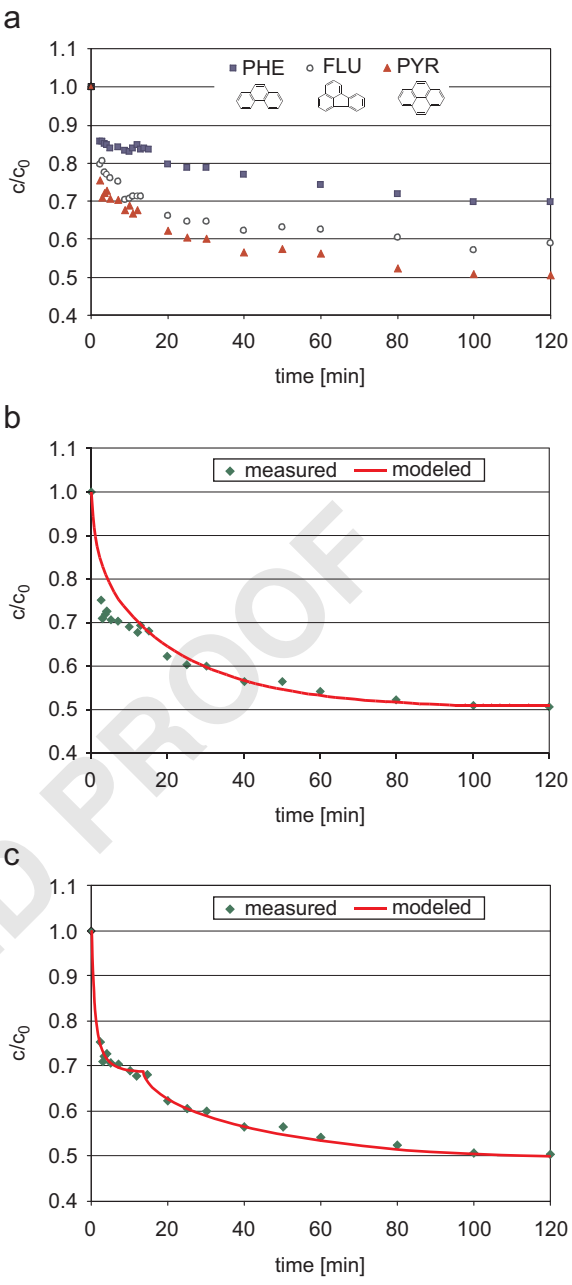

Fig. 2 – Sorption of PAH to bacterial biofilms of strain 30A (*Rhizobiaceae*). (a) Sorption curves for three PAH (initial concentration 1.25 μg L^{-1}, biofilm mass 38 mg dry weight); (b) curve fitting for sorption of pyrene with the diffusion model for a plane sheet, assuming a single-stage process; (c) curve fitting assuming a two-stage process.

experiment is determined. From these K_D values, we further calculated water/biomass organic carbon distribution coefficients (K_{OC}):

$$K_{oc} = \frac{K_D}{f_{oc}}. \quad (9)$$

An organic carbon fraction (f_{oc}) of 0.48 was measured for the biofilm (dry weight) using an elemental analyzer. In Table 1 average K_{oc} values for the bacterial strain 30A obtained from

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Table 1 – K_{oc} and log K_{oc} values for sorption of selected PAH to organic matter of a biofilm of strain 30A (*Rhizobiaceae*) compared to other organic sorbents (literature data)

	This study			Literature values (log values)			
	K_{oc} (ml g^{-1}) (mean, $n = 12$)	log K_{oc} mean $\pm S(x)$, $n = 12$	log K_{ow}	Jonassen et al. (2003)	Gauthier et al. (1986)	Krauss and Wilcke (2001)[a]	~~Parikhet~~ al. (2004)
	Biofilm		Octanol	Aldrich humic acid	Humic materials	Soils and sediments	Soils and sediments
Phenanthrene	12,400	4.1 ± 0.1	4.7 ± 0.17	4.2	4.7	4.1–6.7	4.2–4.7
Fluoranthene	28,800	4.5 ± 0.2	5.2 ± 0.17			4.6–6.7	
Pyrene	38,800	4.6 ± 0.2	5.2 ± 0.17	5.0	4.7–5.2	4.6–6.8	

[a] Including literature values of nine sources.

12 sorption experiments are compiled. The log K_{oc} values range from 4.1 for phenanthrene to 4.6 for pyrene and increase according to the K_{ow} values of these compounds. The standard deviation of experimentally determined values is in the range of 0.1–0.2 on the log scale, showing that the experimental approach is well reproducible.

These experimentally determined K_{oc} values for biofilms of strain 30A (*Rhizobiaceae*) are at the lower end of those reported in literature for other organic sorbents such as humic acids, soil and sediment (Table 1). This can be explained with the presumably higher polarity and low aromaticity of the EPS forming the biofilms like proteins and polysaccharides (Nielsen et al., 1997).

In a conceptual model a bacterial biofilm is heterogeneous, consisting of different bacterial cells with their cell membrane as potential sorbent and the EPS with its different constituents that may function as an organic phase that can take up HOC. The sorption properties of such a biofilm should, then, be a combination of the sorption properties of all its constituents. The experimental design and the modeling approach was selected to provide quantitative data for PAH sorption to intact biofilms, and it, thus, aimed at integrating rather than resolving the different biofilm components. For one of the solutes in this study, phenanthrene, partitioning coefficients towards EPS dissolved in water have been determined (log K_{oc} 3.6–4.4; Brunk et al., 1997; Dohse and Lion, 1994). Those values compare well with the average log K_{oc} of 4.1 found in these experiments (Table 1).

Sorption coefficients for any of these PAH onto bacteria were not available to us, but one would expect sorption to be stronger than to the biofilm as a whole or to pure EPS. Correspondingly, Wang et al. (2002) showed that sorption of *p*-chlorophenol was stronger to bacterial cells than to their EPS. The sorption of a series of PCB to bacteria was studied recently (Sobek et al., 2006). The experimentally determined K_{oc} values varied for bacteria of different origin but were all higher than the K_{ow} values of the respective PCB. In this study K_{oc} values for PAH sorption to biofilm are consistently lower than the K_{ow} values reported in literature (see Table 1). This indicates a generally stronger sorption tendency towards pure bacterial cells with their lipophilic cell walls as compared to a biofilm, where these bacteria are embedded into and diluted (on a carbon basis) by a more hydrophilic EPS.

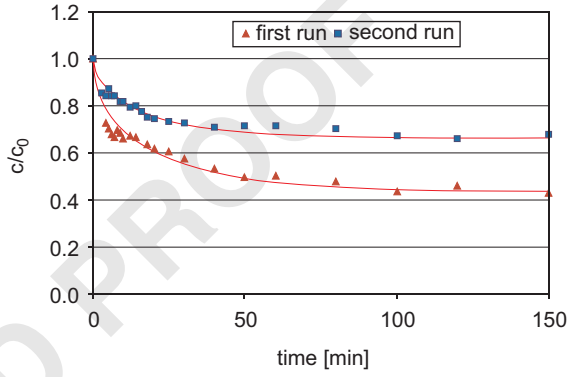

Fig. 3 – Sequential sorption experiment: concentration profiles for fluoranthene in two subsequent runs. Equilibrium concentration is higher after the second run because the biofilm was preloaded during the first run (for quantitative results refer to Table 2).

3.3.2. *Linearity of isotherms*

The above calculation of K_{oc} values was based on the assumption that a linear relationship exists between the dissolved concentration and the concentration in the biofilm at equilibrium. This linearity was confirmed in two independent ways.

(i) A sequential sorption experiment was performed, in which the biofilm preloaded in a first sorption cycle was brought into contact with a fresh aqueous solution of phenanthrene, fluoranthene and pyrene under conditions identical to the first. The concentrations remaining in solution after the second cycle were higher than in the first one, because the biofilm was already loaded when the second cycle started (Fig. 3). Based on the K_D values calculated from the first sorption cycle ~~on~~ the assumption of a linear sorption isotherm, the expected equilibrium concentrations for the second cycle were predicted for each compound. The calculated equilibrium concentra-

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tions agreed well with the experimentally detected values (Table 2).

(ii) A second validation is provided by comparing the results of several independent sorption experiments. Because the biomass (and thus the amount of sorbent) growing and remaining in a biofilm reactor differs from one reactor to the next, the absolute amount of each PAH sorbed to the biofilm and the solute concentration at equilibrium also differ from one experiment to the next. By plotting the equilibrium concentrations for each of the PAH in the biofilm versus the dissolved concentrations at equilibrium for the different independent experiments (Fig. 4), linear isotherms were obtained for all three PAH. These experiments validate the assumption of a linear sorption isotherm and the whole experimental approach.

3.4. *Sorption kinetics*

As Fig. 2a illustrates, sorption of PAH to the bacterial biofilm is a comparatively fast process. During all experiments the equilibrium appears to be reached within 120–150 min. Assuming (a) a completely mixed reactor system, (b) an only incremental decrease of the solute concentration during one passage of the biofilm reactor (DBCR) and (c) a negligible boundary layer outside of the biofilm surface, the concentration decrease in solution is solely determined by the speed of diffusion of the solute in the sorbent (biofilm). The change in solute concentration over time can, then, be used to calculate its diffusion coefficient in the biofilm.

3.5. *Modeling*

Based on these time curves (Fig. 2a) and assuming a homogeneous biofilm of even thickness diffusion coefficients D for each of the PAH in bacterial biofilm were assessed using the model for diffusion through a plane sheet as described in detail in Section 2. The diffusion coefficients are obtained by visually adopting the model curve to the experimental data for each of the PAH.

The result of one such fitting process is exemplarily shown in Fig. 2b for pyrene. Mean diffusion coefficients of the three PAH obtained from a series of eight experiments are displayed in Table 3. The diffusion coefficients decrease with increasing molecular mass and size from phenanthrene to pyrene (Table 3). The standard deviation of the determined diffusion coefficients is, however, comparatively large. This may be due to the fact that the biofilm in one reactor is the product of a microbial growth process and of a complex attachment process during the growth phase of 6 days on average. Bacterial growth and biofilm formation occur in parallel and interact with each other, leading to different developments of the biofilm in each experiment. Moreover, biofilms grown in different experiments may not only differ in mass and biofilm thickness but also in their chemical composition and, thus, in their physical properties.

The diffusion coefficients calculated from these experiments for phenanthrene, fluoranthene and pyrene in microbial biofilms are between 0.23 and $0.45 \times 10^{-9}\,cm^2\,s^{-1}$ (Table 3). As these appear to be the first diffusion coefficients of PAH in biofilms, we cannot compare them with literature data. However, their plausibility can be checked in two ways:

Firstly one can compare them with diffusion coefficients of PAH and similar HOC (regarding size and hydrophobicity ($\log K_{ow}$)) in other matrices. As expected, the diffusivity of the investigated PAH is highest in water ($D_L \sim 5–7 \times 10^{-6}\,cm^2\,s^{-1}$; Schramke et al., 1999). Our values for PAH in biofilm are approximately four orders of magnitude lower, suggesting that PAH in biofilms are not transported as freely dissolved components through the aqueous phase of the biofilms. Significantly lower diffusion coefficients were found for chlorobenzenes in natural sediments and soils

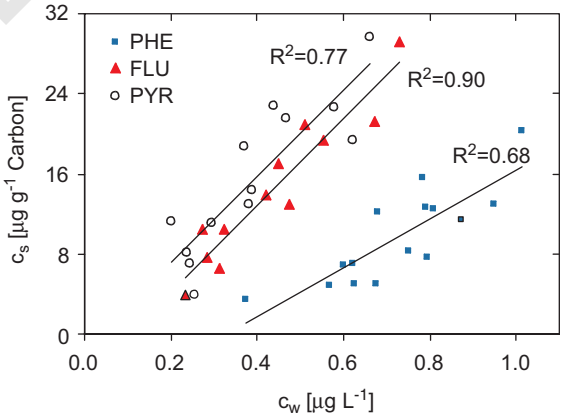

Fig. 4 – Equilibrium concentration in the biofilm (c_s) versus the equilibrium concentration in the water phase (c_w) for phenanthrene, fluoranthene and pyrene in different experiments with the same bacterial strain.

Table 2 – Normalized concentration derived from sequential sorption experiment (two stages, Fig. 3)

	Sorption 1		Sorption 2	
	c/c_0 measured	K_D (~~µg~~ l)	c/c_0 calculated	c/c_0 measured
Phenanthrene	0.63	8.0	0.87	0.84
Fluoranthene	0.44	17.5	0.69	0.68
Pyrene	0.37	23.5	0.60	0.57

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Table 3 – Diffusion coefficients (*D*) in biofilm of strain 30A (*Rhizobiaceae*) assuming a single-stage and a two-stage process ($n = 8$)

	Single-stage sorption	Two-stage sorption	
	(0–150 min) D (10^{-9} cm^2 s^{-1}) mean ± $S(x)$	Stage 1 (0–15 min) D (10^{-9} cm^2 s^{-1}) mean ± $S(x)$	Stage 2 (15–150 min) D (10^{-9} cm^2 s^{-1}) mean ± $S(x)$
Phenanthrene	0.45 ± 0.16	3.1 ± 0.73	0.35 ± 0.16
Fluoranthene	0.24 ± 0.09	2.1 ± 0.59	0.30 ± 0.12
Pyrene	0.23 ± 0.07	1.8 ± 0.72	0.30 ± 0.13

(4×10^{-11} cm^2 s^{-1} for tetrachlorobenzene (log $K_{ow} = 4.2$) to 8×10^{-12} cm^2 s^{-1} for pentachlorobenzene, log $K_{ow} = 4.6$) (Wu and Gschwend, 1986). Diffusion coefficients for three-and four-ring-PAH in condensed-phase organic matter of sediments were found to be six orders of magnitude lower than those we have determined in biofilm (8.5×10^{-16} cm^2 s^{-1}; Chai et al., 2006).

Secondly, the diffusivities of PAH in biofilms can be compared to that of other solutes in biofilm. For highly soluble substrates (e.g. O_2, glucose, NO_3^- and acetate), values between 110% and 8% of the aqueous diffusivity were reported in a review paper (Stewart, 1998). For the more hydrophobic compound toluene (log $K_{ow} = 2.7$) in biofilm of *Pseudomonas putida*, a diffusion coefficient of 1.3×10^{-7} cm^2 s^{-1} was determined, which was about two orders of magnitude slower than in water (Holden et al., 1997). For the slightly more hydrophobic dye fluorescein (log $K_{ow} = 3.0$), a diffusion coefficient of 7.7×10^{-8} cm^2 s^{-1} was experimentally detected using confocal laser microscopy (Lawrence et al., 1994). It is, then, reasonable that the PAH with log K_{ow} values between 4.7 and 5.2 exhibit diffusion coefficients of 0.23–0.45 $\times 10^{-9}$ cm^2 s^{-1}. This further agrees to a conclusion of Wu and Gschwend (1986) that compounds with higher K_{ow} values show slower sorption.

3.5.1. *Two-stage process*

In many experiments, the single-stage sorption curves do not seem to adequately describe the initial phase of PAH sorption (Fig. 2b). This is partly due to a discontinuity in the concentration gradient occurring after 10–15 min in this and most other experiments. A better adaptation of the modeled curve to the measured data is obtained by assuming two sorption phases, a rapid initial process to an 'equilibrium' concentration corresponding to the point of discontinuity and a second phase of slower sorption continuing until the final equilibrium is reached (Fig. 2c). The two-stage sorption may reflect the heterogeneity of microbial biofilms with more easily accessible and more rigid and, thus, less easily accessible zones.

Therefore, a two-stage model was adopted to the data and the diffusion coefficients obtained for the initial diffusion phase (1.8–3.1 $\times 10^{-9}$ cm^2 s^{-1}) are about one order of magnitude larger than those obtained for the second phase (around 0.3×10^{-9} cm^2 s^{-1}) (Table 3). For the whole sorption process, however, the second phase appears to be more decisive, as the diffusion coefficients during this phase are similar to those obtained with the single-stage approach for the whole sorption process (Table 3).

Several studies have shown previously that mass transport through a biofilm may be influenced by its heterogeneity with voids and cell clusters (DeBeer and Stoodley, 1995). In a study using NMR techniques, the authors found indications for a more rapid diffusion of glycerol in biofilm pores and a significantly slower diffusion through the EPS network (Vogt et al., 2000). Similar mechanisms may also explain the two speeds of diffusion visible for the PAH in these experiments.

Two-stage processes have been repeatedly observed for the sorption and desorption of PAH and other HOC from soil and sediment (Sun et al., 2003; Shor et al., 2003).

4. Conclusions

The developed biofilm reactor operated as a DCBR allows to study the sorption of PAH to intact microbial biofilms at environmentally relevant solute concentrations. An integrated view is provided on the thermodynamics as well as the kinetics of the exchange of HOC between an aqueous phase and microbial biofilms. The log K_{oc} values of the PAH for bacterial biofilm are in the range of 4.1–4.6, showing that biofilms can be a significant sink for HOC.

Using the analytical solution of diffusion from a stirred solution of limited volume into a plane sheet, diffusion coefficients for the intra-biofilm diffusion were calculated from the experimental data. With values between 0.23 and 0.45×10^{-9} cm^2 s^{-1}, diffusion coefficients of the investigated HOC were about four orders of magnitude lower than those found for diffusion in water, but higher than those found in soil organic matter. Most of the sorption processes showed two phases, a rapid initial sorption followed by a second phase of slower diffusion into the biofilm.

These thermodynamic and kinetic data can be used to quantitatively assess the importance of microbial biofilms for the fate of HOC in different systems comprising an aqueous and a biofilm phase. Biofilm reactors are useful means to study the influence of various factors on transport processes in biofilms, whether they are biological (kind of microorganisms and physiological status), chemical (composition of the water phase) or physical (temperature).

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REFERENCES

Brunk, B.K., Jirka, G.H., Lion, L.W., 1997. Effects of salinity changes and the formation of dissolved organic matter coatings on the sorption of phenanthrene: Implications for pollutant trapping in estuaries. Environ. Sci. Technol. 31, 119–125.

Carlson, G., Silverstein, J., 1997. Effect of ozonation on sorption of natural organic matter by biofilm. Water Res. 31, 2467–2478.

Carslaw, H.S., Jaeger, J.C., 1959. Conduction of Heat in Solids, 2nd ed. Clarendon Press, Oxford.

Chai, Y., Kochetkov, A., Reible, D.D., 2006. Modeling biphasic sorption and desorption of hydrophobic organic contaminants in sediments. Environ. Toxicol. Chem. 25, 3133–3140.

Crank, J., 1975. The Mathematics of Diffusion, second ed. Clarendon Press, Oxford.

deBeer, D., Stoodley, P., 1995. Relation between the structure of an aerobic biofilm and transport phenomena? Water Sci. Technol. 32 (8), 11–18.

Dohse, D.M., Lion, L.W., 1994. Effect of microbial polymers on the sorption and transport of phenanthrene in a low-carbon sand. Environ. Sci. Technol. 28, 541–548.

Gauthier, T.D., Shane, E.C., Guerin, W.F., Seitz, W.R., Grant, C.L., 1986. Fluorescence quenching method for determining equilibrium-constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials. Environ. Sci. Technol. 20, 1162–1166.

Headley, J.V., Gandrass, J., Kuballa, J., 1998. Rates of sorption and partitioning of contaminants in river biofilm. Environ. Sci. Technol. 32, 3968–3973.

Holden, P.A., Hunt, J.R., Firestone, M.K., 1997. Toluene diffusion and reaction in unsaturated pseudomonas putida biofilms. Biotechnol. Bioeng. 56, 656–670.

Jonassen, K.E.N., Nielsen, T., Hansen, P.E., 2003. The application of high-performance liquid chromatography humic acid columns in determination of K_{oc} of polycyclic aromatic compounds. Environ. Toxicol. Chem. 22, 741–745.

Karamanev, D.G., Samson, R., 1998. High-rate biodegradation of pentachlorophenol by biofilm developed in the immobilized soil bioreactor. Environ. Sci. Technol. 32, 994–999.

Krauss, M., Wilcke, W., 2001. Predicting soil–water partitioning of polycyclic aromatic hydrocarbons and polychlorinated biphenyls by desorption with methanol–water mixtures at different temperatures. Environ. Sci. Technol. 35, 2319–2325.

Lawrence, J.R., Wolfaardt, G.M., Korber, D.R., 1994. Determination of diffusion-coefficients in biofilms by confocal laser microscopy. Appl. Environ. Microbiol. 60, 1166–1173.

Nielsen, P.H., Jahn, A., Palmgren, R., 1997. Conceptual model for production and composition of exopolymers in biofilms. Water Sci. Technol. 36 (1), 11–19.

Parikh, S.J., Chorover, J., Burgos, W.D., 2004. Interaction of phenanthrene and its primary metabolite (1-hydroxy-2-naphthoic acid) with estuarine sediments and humic fractions. J. Contam. Hydrol. 72, 1–22.

Rosenberger, S., Laabs, C., Lesjean, B., Gnirss, R., Amy, G., Jekel, M., Schrotter, J.C., 2006. Impact of colloidal and soluble organic material on membrane performance in membrane bioreactors for municipal wastewater treatment. Water Res 40, 710–720.

Schramke, J.A., Murphy, S.F., Doucette, W.J., Hintze, W.D., 1999. Prediction of aqueous diffusion coefficients for organic compounds at 25 degrees C. Chemosphere 38, 2381–2406.

Shor, L.M., Rockne, K.J., Taghon, G.L., Young, L.Y., Kosson, D.S., 2003. Desorption kinetics for field-aged polycyclic aromatic hydrocarbons from sediments. Environ. Sci. Technol. 37, 1535–1544.

Sobek, A., Olli, K., Gustafsson, O., 2006. On the relative significance of bacteria for the distribution of polychlorinated biphenyls in arctic ocean surface waters. Environ. Sci. Technol. 40, 2586–2593.

Sontheimer, H., Crittenden, J.C., Summers, R.S., 1988. Activated carbon for water treatment. DVGW Forschungsstelle am Engler-Bunte-Institut der Universität Karlsruhe (TH), AWWA Research Foundation, Karlsruhe, Denver.

Stewart, P.S., 1998. A review of experimental measurements of effective diffusive permeabilities and effective diffusion coefficients in biofilms. Biotechnol. Bioeng. 59, 261–272.

Sun, H., Tateda, M., Iki, M., Fujita, M., 2003. Short- and long-term sorption/desorption of polycyclic aromatic hydrocarbons onto artificial solids: effects of particle and pore sizes and organic matters. Water Res. 37, 2960–2968.

Vogt, M., Flemming, H.C., Veeman, W.S., 2000. Diffusion in Pseudomonas aeruginosa biofilms: a pulsed field gradient NMR study. J. Biotechnol. 77, 137–146.

Wang, W.J., Wang, W.H., Zhang, X.L., Wang, D.H., 2002. Adsorption of *p*-chlorophenol by biofilm components. Water Res. 36, 551–560.

Wijayaratne, R.D., Means, J.C., 1984. Sorption of polycyclic aromatic hydrocarbons by natural estuarine colloids. Mar. Environ. Res. 11, 77–89.

Wu, S., Gschwend, P.M., 1986. Sorption kinetics of hydrophobic organic contaminants to natural sediments and soils. Environ. Sci. Technol. 20, 717–725.

Zhang, S.-F., Splendiani, A., Freitas dos Santos, L.M., Livingston, A.G., 1998. Determination of pollutant diffusion coefficients in naturally formed biofilms using a single tube extractive membrane bioreactor. Biotechnol. Bioeng. 59, 80–89.

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