An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain

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The mechanism of uptake of polycyclic aromatic hydrocarbons (PAHs) was studied using a kinetic approach by electrolytic respirometry. In the case of the degradation of pyrene dissolved in a non-water-soluble non-degradable solvent (2,2,4,4,6,8,8-heptamethylnonane), by a *Rhodococcus* sp., two successive phases of exponential growth, during which over 80% of substrate degradation took place, were clearly characterized. During the second phase of biodegradation, rates of pyrene uptake were higher than those determined in abiotic conditions for the physicochemical transfer of pyrene from the solvent to the aqueous phase and no evidence for the presence of glycolipidic biosurfactants was obtained. The value of the specific growth rate for the first phase ($\mu_{\max}$) was independent of the volume of the solvent phase and of the concentration of pyrene and was, in all cases, higher than that for the second phase (μ_i). The μ_i values increased with the volume of the solvent phase but were independent of pyrene concentration, a clear indication of an interfacial uptake mechanism. The experimental kinetic data fitted well with a mathematical model incorporating PAH uptake both from the interface and from the aqueous medium by a population consisting of adsorbed cells in dynamic equilibrium with the cells in the aqueous medium, interfacial uptake being predominant in these experiments. Similar results were obtained for the degradation of fluoranthene. This newly demonstrated mechanism of PAH uptake is of great significance for the degradation of higher PAHs.

Keywords: electrolytic respirometry, interfacial uptake, polycyclic aromatic hydrocarbons, insoluble substrate, transfer mechanism

INTRODUCTION

The mechanisms involved in the uptake of hydrophobic compounds by micro-organisms are a key aspect of the degradation of these molecules and have been mainly studied in the case of long-chain alkanes. Although present knowledge of these mechanisms is in some respects still sketchy, there is a wide agreement on the existence of three models of alkane uptake (Goswami & Singh, 1991; Haferburg *et al.*, 1986; Hommel, 1994; Nakahara *et al.*, 1977): (1) after dissolution in the aqueous phase; (2) through direct contact of the micro-organisms with the hydrophobic phase–aqueous phase interface (interfacial uptake); (3) as microemulsions

promoted by the production of biosurfactants. Polycyclic aromatic hydrocarbons (PAHs) are a class of hydrophobic compounds of environmental concern because of their genotoxicity, ubiquitous presence and persistence. Their biodegradation is a subject of active research (Cerniglia, 1992, 1993; Wilson & Jones, 1993). Because the persistence of PAHs appears to be related to their low solubility and poor availability to degrading micro-organisms, the mechanisms of PAH transfer are an important point of interest (Luthy *et al.*, 1994), but knowledge of them is limited.

We devised a kinetic approach and a methodology to characterize uptake mechanisms of poorly degradable substrates and applied it to the biodegradation of PAHs. This approach (Bouchez, 1995) involves (1) the utilization of a biphasic culture medium with the PAH dissolved in a non-water-soluble, non-degradable solvent, (2) the determination of physicochemical phase-transfer rates, (3) the continuous determination of biodegradation rates by electrolytic respirometry, and

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Abbreviations: HMN, 2,2,4,4,6,8,8-heptamethylnonane; MSM, mineral salt medium; PAH, polycyclic aromatic hydrocarbon.

(4) the interpretation of the kinetic data in terms of mathematical models of uptake mechanisms. In the case of the degradation of phenanthrene [solubility 1.5 mg l^{-1} as the mean of values published by Davies & Evans (1964), May *et al.* (1978) and Stucki & Alexander (1987)] by a *Pseudomonas* sp., this approach unambiguously demonstrated the exclusive occurrence of the mechanism of substrate transfer to the aqueous phase in the conditions used (Bouchez *et al.*, 1995c). In the present study, the same approach was used to investigate the uptake of higher PAHs with a much lower solubility. The results obtained during the degradation of pyrene (solubility 0.16 mg l^{-1} according to May *et al.*, 1978) by a *Rhodococcus* sp., which show the existence of a different substrate uptake mechanism, are presented below.

METHODS

Chemicals. HPLC-grade acetonitrile was from SDS (Peypin, France), PAHs from Fluka and 2,2,4,4,6,8,8-heptamethylnonane (HMN) from Aldrich.

Strains. A series of bacterial strains, previously isolated (Bouchez *et al.*, 1995b) from soils polluted or not by coal tar for their capacities to use as sole carbon and energy source a three- or four-ring PAH, were used: *Pseudomonas* sp. SPheNa1 on phenanthrene, *Rhodococcus* sp. SFltNa1 on fluoranthene and *Rhodococcus* sp. SPyrNa1 on pyrene. They were maintained in a vitamin-supplemented mineral salt medium (MSM) with their isolation PAH.

Kinetics of oxygen consumption. Time courses of oxygen consumed during growth on PAH were conducted using a sensitive respirometric apparatus (D-12-S Sapromat, Voith, Heidenheim, Germany) consisting of twelve 500 ml Erlenmeyer flasks placed in a water bath thermostatted at 30°C . In the Sapromat culture flasks, oxygen was kept at a constant concentration by electrolytic production and its consumption quasi-continuously recorded (data acquisition every 40 s); CO_2 was trapped by soda lime pellets.

The culture flasks containing 237.5 ml MSM medium (pH 7) were inoculated with 12.5 ml of a culture grown on the PAH to be tested. PAHs were employed as sole carbon and energy sources and they were supplied dissolved in HMN. Oxygen consumption blanks (non-inoculated flasks with PAH and solvent, and inoculated flasks with solvent) were run simultaneously. No oxygen consumption was detected in these flasks.

PAH quantification. PAHs in the aqueous phase were quantified by high-performance liquid chromatography with UV detection at 254 nm. Extraction and quantification were performed as previously described (Bouchez *et al.*, 1995b).

PAHs in solvents were evaluated by direct injection (1 μl) using the gas chromatographic system previously described (Bouchez *et al.*, 1995b) and the following oven programme: 50°C for 5 min, then 2°C min^{-1} to 260°C and 20 min at 260°C .

RESULTS

Oxygen consumption during the degradation of pyrene by *Rhodococcus* sp. strain SFltNa1 was studied by electrolytic respirometry with the Sapromat apparatus.

The substrate was supplied dissolved in HMN (volume V' , in l) to form a biphasic system. The initial concentration of pyrene, S_0 , referred to the aqueous-phase volume (V , in l), was 370 mg l^{-1} . The kinetic data were obtained every 40 s but, for illustration, Fig. 1 shows the data summed over periods of 5 h and plotted versus time. As shown in this figure, the biodegradation of pyrene dissolved in HMN was reproducible and the metabolic activity was completed within 300 h. No residual PAH was detected, either in the aqueous phase or in the solvent phase. The curves obtained were analysed in order to determine the mechanism of substrate uptake and compared to the results previously obtained in the case of phenanthrene dissolved in HMN (Bouchez *et al.*, 1995c), also illustrated in Fig. 1. The curves corresponding to the phase-transfer-controlled models of biodegradation, presented in this figure, will be discussed below.

Comparison of experimental kinetics with the model of growth on the substrate dissolved in the aqueous phase

We have previously developed a model describing the kinetics of oxygen consumption when the biodegradation process implies substrate transfer to the aqueous phase before consumption (Bouchez *et al.*, 1995c). The use of oxygen consumption to follow cell growth is based on the fact that oxygen consumption, substrate consumption and biomass formation were found to be proportional during growth on PAHs (Bouchez, 1995). In this model, summarized below, oxygen consumption follows successively two relationships.

When substrate availability is not limiting, exponential growth occurs and accordingly:

$$\frac{dO_C}{dt} = \mu_{\max} \cdot t \quad (1)$$

where O_C (mg l^{-1}) is the oxygen consumed referred to V , $\mu_{\max}$ the maximal specific growth rate of bacteria on the substrate involved (h^{-1}), and t the time (h).

Then, starting at the end of the exponential phase (occurring at time t_1 , in h, and oxygen consumed O_{C1} , in mg l^{-1}), growth is controlled by the transfer of substrate from the solvent to the aqueous phase. The following equation, based on the law of transfer kinetics, is then verified:

$$O_C = O_T - (O_T - O_{C1}) \cdot e^{-k_2 \cdot (t - t_1)} \quad (2)$$

where O_T is the oxygen consumed (referred to V , in mg l^{-1}) corresponding to total substrate consumption and k_2 a constant for a given system of solvent and substrate (in h^{-1}). k_2 is expressed by the following equation based on the Raoult law (Bouchez *et al.*, 1995c):

$$k_2 = K'_L \cdot A' \cdot \frac{MW_S}{m_S \cdot MW_H} \cdot S_{\text{EQ}} \cdot \left(\frac{f_L}{f_S} \right) \quad (3)$$

where K'_L (dm h^{-1}) is the mass transfer constant for

Fig. 1. Experimental biodegradation kinetics of pyrene ($S_0 = 370 \text{ mg l}^{-1}$) by strain SFltNa1 ($\square, \blacksquare$) and of phenanthrene ($S_0 = 460 \text{ mg l}^{-1}$) by strain SPheNa1 ($\circ, \bullet$). The lines show the values predicted by the phase-transfer-controlled models for pyrene (---) and phenanthrene (—). Experiments were done in duplicate in the Sapromat apparatus, as described in Methods, with PAH dissolved in HMN ($V' = 0.022 \text{ l}$). Values of oxygen consumed (O_C), obtained every 40 s, were summed over periods of 5 h. The curves for transfer-controlled models were calculated from equations (1) and (2) using the values derived from experimental biodegradation data. For pyrene, these values are: $\mu_{\max} = 0.04 \text{ h}^{-1}$, $O_T = 660 \text{ mg l}^{-1}$, $O_{C1} = 60 \text{ mg l}^{-1}$ (from equation 4a), $t_1 = 102 \text{ h}$ (from equation 4b) and $k_2 = 0.004 \text{ h}^{-1}$. k_2 was calculated according to equation (3) as follows: the value of $K'_L A'$ was taken as equal to that determined for the system 'phenanthrene solution in HMN', i.e. $K'_L A' = 26.7 \text{ l h}^{-1}$ (Bouchez *et al.*, 1995c); f_3/f_L was obtained from the equation $f_3/f_L = \exp[6.79 \cdot (1 - T_m/T)]$ (Moretti & Neufeld, 1989), T_m being the melting point of the PAH (in K) by Bjørseth (1983) and T the temperature of the system (in K). For phenanthrene, the corresponding values are: $\mu_{\max} = 0.39 \text{ h}^{-1}$, $O_T = 800 \text{ mg l}^{-1}$, $O_{C1} = 32 \text{ mg l}^{-1}$, $t_1 = 9.5 \text{ h}$ and $k_2 = 0.014 \text{ h}^{-1}$ (as previously determined by Bouchez *et al.*, 1995c).

PAH transfer (solvent to water), A' the contact surface area between solvent and aqueous phases (dm^2), MW_H and MW_S the molar mass of PAH and of solvent respectively (g mol^{-1}), m_s the mass of solvent (in mg), S_{EQ} the saturating aqueous concentration of PAH supplied as crystals (in mg l^{-1}), and f_L/f_s the ratio of the PAH fugacities in the subcooled liquid and solid states.

The values of O_{C1} and t_1 are given by the following equations:

$$O_{C1} = \frac{k_2 \cdot O_T}{\mu_{\max} + k_2} \quad \text{and} \quad t_1 = \frac{2.3 \cdot \log O_{C1}}{\mu_{\max}} \quad (4a \text{ and } 4b)$$

Thus, according to equations (1) and (2), in a model where growth occurs at the expense of the substrate dissolved in the aqueous phase, the constants O_T , O_{C1} , t_1 , $\mu_{\max}$ and k_2 allow the kinetics of oxygen consumed during PAH biodegradation to be determined.

The experimental kinetics obtained during the degradation of pyrene dissolved in HMN by strain SFltNa1 are compared with the model presented above in Fig. 1. As discussed below, a first phase of exponential growth was observed, in agreement with the model. Later, however, experimental oxygen consumption was very different and faster than that predicted for growth on the substrate dissolved in the aqueous phase. Indeed, growth following equation (2) should be such that a portion of straight line would be obtained when plotting $(O_T - O_C)$ values versus time on a semi-logarithmic scale. How-

ever, in our experiments of pyrene biodegradation, such a plot yielded no straight line (the correlation coefficient r was below 0.957). For comparison, the degradation kinetics obtained in previous work for the degradation of phenanthrene dissolved in HMN, by a *Pseudomonas* sp., are also presented in Fig. 1. In the latter case, the experimental and model kinetics were very close, constituting clear evidence for a biodegradation process implying substrate transfer to the aqueous phase (Bouchez *et al.*, 1995c). Another mechanism of substrate uptake was then considered for the degradation of pyrene.

Model of interfacial kinetics

Microscopic observation of a culture of strain SFltNa1 growing on pyrene dissolved in HMN showed that bacteria were present in the aqueous phase but also at the surface of the solvent phase (data not shown). A model where growth can occur at the interface of the aqueous and solvent phases was developed. In such a model, as observed microscopically, bacteria are present both in the aqueous phase and at the interface with the solvent phase. Two successive steps of growth take place.

During the first step, at low biomass concentrations, substrate transfer to the aqueous phase allows growth of the bacteria present in the aqueous phase at their maximal specific growth rate. Supposing that the

Fig. 2. Determination of the specific growth rates of strain SFltNa1 on pyrene dissolved in HMN. The values of oxygen consumed (O_C) for the duplicate experiments presented in Fig. 1 were summed over periods of 5 h and plotted versus time on a semi-logarithmic scale.

bacteria at the interface can also grow at a rate equal to $\mu_{\max}$, the following equation is then verified:

$$\frac{dX}{dt} = \mu_{\max} \cdot X_A + \mu_{\max} \cdot X_S = \mu_{\max} \cdot X \quad (5)$$

where X , X_A and X_S are the concentrations of total biomass, of biomass in the aqueous phase and of biomass at the interface of the aqueous and solvent phases respectively (referred to V , in mg l^{-1}). The oxygen consumed being proportional to the biomass produced, equation (5) becomes:

$$\frac{dO_C}{dt} = \mu_{\max} \cdot O_C \quad (6)$$

Growth following this relationship is exponential, with a growth rate equal to $\mu_{\max}$.

During the second step of growth, because of substrate transfer limitation, growth of the bacteria in the aqueous phase becomes negligible compared to that at the interface of aqueous and solvent phases. Then, the following equation is verified:

$$\frac{dX}{dt} = \mu_{\max} \cdot X_S \quad (7)$$

When the interfacial surface area between solvent and aqueous phases is not covered (as experimentally observed), a relation taking into account the fact that cell adsorption depends both on the amount of cells in the medium and on interfacial area available can be written according to Dunn (1968). This relation assumes that adsorption and desorption rates of bacteria from the water-solvent surface are fast compared with growth (a likely situation in a well-mixed system) and, accordingly:

$$X_A = \frac{a \cdot V}{b \cdot A'} \cdot X_S \quad (8)$$

where a is the surface area covered per mg of bacteria ($\text{dm}^2 \text{mg}^{-1}$) and b a constant of equilibrium of adsorption/desorption of bacteria for a hydrophobic surface (l mg^{-1}). So, equation (7) can be rewritten:

$$\frac{dX}{dt} = \frac{\mu_{\max} \cdot b \cdot A'}{b \cdot A' + a \cdot V} \cdot X = \mu_i \cdot X \quad (9)$$

and then:

$$\frac{dO_C}{dt} = \mu_i \cdot O_C \quad (10)$$

Thus, the model predicts the existence of an exponential phase: the specific growth rate μ_i is a constant since it incorporates a , b and A' , three constants for a given experiment, which are not individually measured.

Comparison of degradation kinetics with the interfacial uptake model

In order to test the relevance of the interfacial uptake model to the kinetics of pyrene degradation, oxygen consumption values, collected over periods of 5 h, were plotted versus time on a semi-logarithmic scale. As shown in Fig. 2, the data were in excellent agreement with the model of interfacial kinetics. In the duplicate experiments presented, two straight-line portions were obtained. The first portions ($r = 0.993$ and $r = 0.995$ respectively) corresponded to the first phase of exponential growth (growth at the interface and in the aqueous medium). The slopes yielded growth rates of 0.041 h^{-1} and 0.039 h^{-1} respectively, corresponding to the value of $\mu_{\max}$ determined for growth on crystals (Bouchez *et al.*, 1995a). The slopes of the second portions of straight line gave, with excellent correlation coefficients ($r = 0.997$ and $r = 0.999$ respectively), specific growth rates μ_i equal, in both cases, to 0.017 h^{-1} . This phase, during which around 80% of the degradation took place, was likely to correspond to the second phase

Fig. 3. Influence of the volume of the solvent phase (V') and of the initial PAH concentration (S_0) on the degradation of pyrene dissolved in HMN by strain SFltNa1. ▲, $S_0 = 530 \text{ mg l}^{-1}$ and $V' = 0.022 \text{ l}$; △, $S_0 = 510 \text{ mg l}^{-1}$ and $V' = 0.011 \text{ l}$; □, $S_0 = 370 \text{ mg l}^{-1}$ and $V' = 0.022 \text{ l}$. Experiments were done in the Sapromat apparatus, as described in Methods. Values of oxygen consumed were summed over periods of 10 h.

Table 1. Interfacial specific growth rates for strain SFltNa1 on pyrene dissolved in HMN

Experiments were done in the Sapromat apparatus. μ_i values were calculated from the slopes of the second portions of straight lines obtained in semi-logarithmic plots of the values of O_c summed over periods of 5 h.

Experimental conditions			μ_i (h^{-1})
V (l)	V' (l)	S_0 (mg l^{-1})	
0.250	0.022	370	0.017
0.250	0.022	530	0.017
0.250	0.011	510	0.011

of exponential growth described by the interfacial kinetics model. As indicated by this method, μ_i was lower than $\mu_{\max}$.

In the interfacial kinetics model presented, as expressed in equation (9), μ_i is independent of S_0 but increases with A' ($d\mu_i/dA' > 0$) and consequently with the volume of the solvent phase. This fact was verified experimentally by modifying S_0 and V' in experiments of degradation of pyrene dissolved in HMN, by strain SFltNa1. The kinetic data obtained are illustrated in Fig. 3 and compared to those presented before. Kinetics were found to be dependent on V' but not on S_0 . As expected, O_T was proportional to S_0 . When the values of O_c were plotted versus time on a semi-logarithmic scale, as previously, all curves showed two straight-line portions (data not shown). The slopes of the first portions gave specific growth rates equal to $\mu_{\max}$. The second straight-line portions covered around 80% of the degradation curves, with excellent correlation coefficients ($r > 0.998$ in both cases). The specific growth rates obtained from the slopes of these straight lines are presented in Table 1.

μ_i decreased with V' and consequently with A' , and did not depend on S_0 . This is in agreement with the expression of μ_i in equation (9). Moreover, the equation system (resulting directly from the expression of μ_i):

$$\begin{cases} \mu_1 = \mu_{\max} \cdot \frac{A'_1 \cdot b}{a \cdot V + A'_1 \cdot b} \\ \mu_2 = \mu_{\max} \cdot \frac{A'_2 \cdot b}{a \cdot V + A'_2 \cdot b} \end{cases}$$

can be solved only if:

$$\frac{A'_2}{A'_1} = \frac{\mu_2 \cdot \mu_{\max} - \mu_2 \cdot \mu_1}{\mu_1 \cdot \mu_{\max} - \mu_2 \cdot \mu_1}$$

In our experimental conditions, having $\mu_1 = 0.017 \text{ h}^{-1}$ and $\mu_2 = 0.011 \text{ h}^{-1}$, it results that $A'_2 = 0.57 A'_1$. This is in agreement with the volumes of solvent introduced ($V'_2 = 0.5 V'_1$).

All these results concur in showing that pyrene dissolved in HMN was consumed by strain SFltNa1 directly at the interface of aqueous and solvent phases and that it could be degraded without prior transfer to the aqueous phase.

Other examples of interfacial growth

Other experiments on the biodegradation of PAHs, when supplied dissolved in HMN, were conducted in the Sapromat apparatus: the degradation of pyrene by another *Rhodococcus* strain, SPyrNa1, and the degradation of fluoranthene by strain SFltNa1. The method described for pyrene degradation by strain SFltNa1 was employed and very similar results were obtained. The degradation kinetics were faster than those allowed by the phase-transfer model. Moreover, when the values of O_c were plotted on a semi-logarithmic scale as above, after the initial growth phase, straight-line portions

Table 2. Interfacial growth rates on four-ring PAH dissolved in HMN

Experiments were done in the Sapromat apparatus. μ_i values were calculated as described in Table 1.

Strain	Substrate	Experimental conditions			μ_i (h ⁻¹)	μ_{max} (h ⁻¹)
		V (l)	V' (l)	S_0 (mg l ⁻¹)		
SPyrNa1	Pyrene	0.250	0.022	370	0.015	ND
SFltNa1	Fluoranthene	0.250	0.022	415	0.019	0.025*

ND, Not determined.

* μ_{max} value obtained on crystals.

were obtained covering more than 50% of the degradation curves ($r > 0.998$) (data not shown). As shown in Table 2, the values of μ_i deduced from the slopes of these straight lines were lower than the measured values of μ_{max} , obtained with crystals.

These results are in agreement with the involvement of an interfacial uptake mechanism in the degradation of pyrene dissolved in HMN, by strain SPyrNa1, and in the degradation of fluoranthene dissolved in HMN, by strain SFltNa1.

DISCUSSION

We have demonstrated here that the growth kinetics of *Rhodococcus* SFltNa1 on pyrene dissolved in HMN were faster than those allowed by physicochemical transfer of the PAH from the solvent to the aqueous phase. The results fitted well with a model where the PAH was consumed both directly from the solvent phase and, after solubilization, from the aqueous phase. In these conditions, mixed growth took place at low cell densities and strictly interfacial uptake predominated later on. However, strictly interfacial uptake should occur only when high interfacial cell densities and low substrate transfer rates to the aqueous phase make negligible the contribution of bacterial growth in the aqueous phase. It is actually somewhat surprising to observe the early appearance of the strictly interfacial growth phase. However, the independence of the growth rate on substrate concentrations in the solvent phase constitutes determinant evidence for a mechanism of interfacial uptake. Indeed, as noted by Westgate *et al.* (1995), the degradation rates in both interfacial uptake and mass-transfer mechanisms are dependent on the volumes of the solvent phase. However, the degradation rates are dependent on substrate concentrations only in the mass-transfer mechanism.

An interfacial mechanism of substrate uptake implies strong interactions between the bacteria and the solvent phase. In our cultures growing on PAHs dissolved in HMN, we sometimes observed long lag phases (two or three weeks) which did not occur in cultures on pyrene supplied as crystals or in controls (growth on acetate in the presence of HMN). A possibility is that PAH could

be more toxic to the bacteria during direct contact in the solvent phase than when supplied at low concentrations via an aqueous phase. In these conditions, interfacial growth would begin after a period of adaptation.

From a general point of view, two modes of PAH uptake by degradative bacteria have now been recognized: uptake of the substrate dissolved in the aqueous phase and interfacial uptake. The first mode has been proposed by various authors for the degradation of naphthalene and phenanthrene (Stucki & Alexander, 1987; Volkerling *et al.*, 1992; Wodzinski & Bertolini, 1972; Wodzinski & Coyle, 1974) and demonstrated with *Pseudomonas* SPheNa1 in the degradation of phenanthrene (Bouchez *et al.*, 1995c). In the second mode, interfacial growth predominated, as illustrated in this work, without excluding the utilization of the substrate dissolved in the aqueous medium. The possibility of interfacial uptake in PAH degradation has been discussed by Ortega-Calvo & Alexander (1994) and Wodzinski & Larocca (1977). However, to our knowledge, this is the first time that definite evidence for such a mechanism has been presented for this type of compound. The occurrence of interfacial uptake is of considerable significance for PAH biodegradation. In particular, it dispels theoretical objections to the possibility of significant degradation of five- and six-ring PAHs on the grounds of their solubility being too low.

It is worth noting that the involvement of biosurfactants, the third mechanism proposed for the uptake of alkanes (Goswami & Singh, 1991; Haferburg *et al.*, 1986; Hommel, 1994; Nakahara *et al.*, 1977), was not observed in either case of PAH uptake examined by us. In pyrene degradation, the production of biosurfactants as a possible cause for increasing biodegradation rates (by increasing interfacial surface area) was a point to consider. However, glycolipid production by strain SFltNa1 grown on various carbon sources, including hexadecane, a quite insoluble compound, was not observed (data not shown). In addition, production of biosurfactants cannot easily explain the strictly exponential growth rate, and its characteristics, observed in the experiments presented here. Involvement of biosurfactants in other cases of PAH biodegradation remains plausible, although reports of biosurfactant production

by bacteria grown on PAH are, at present, rare (Aitken *et al.*, 1995; Déziel *et al.*, 1996).

In fact, the mode of PAH uptake should be dependent not only on the solubility of the substrate but also on the bacteria and on the hydrophobicity of their cell envelope, which determines their capacity to adhere to the hydrocarbon phase. From this point of view, it is interesting to note the lipophilic character imparted by the presence of mycolic acids to the cell envelope of rhodococci and other members of the family *Nocardiaceae* (Goodfellow, 1992). Also worth mentioning is the fact that the PAH-degrading *Rhodococcus* strains we isolated, including SFltNa1, commonly grew on hexadecane (Bouchez *et al.*, 1995a), a carbon source quite likely to be degraded via an interfacial mechanism.

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