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Biodegradation of selected UV-irradiated and non-irradiated polycyclic aromatic hydrocarbons (PAHs)

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Abstract

Biodegradation of UV-irradiated anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene was compared to that of the non-irradiated samples, individually and in synthetic mixtures with enrichment cultures. Combined treatment was repeated for individual anthracene and for the PAH mixture with *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae*. Enrichment culture studies were performed on the PAH mixtures in the presence of the main photoproduct of anthracene, pure 9,10-anthracenedione. Photochemically pretreated creosote solutions were also subjected to biodegradation and the results were compared to those of the non-irradiated solutions. The primary interest was on 16 polycyclic aromatic hydrocarbons (PAHs) listed as priority pollutants by European Union (EU) and the United States Environmental Protection Agency (USEPA). Irradiation accelerated the biodegradation onset for anthracene, pyrene, and benz[*a*]anthracene when they were treated individually. The biodegradation of irradiated pyrene started with no lag phase and was complete by 122 h whereas biodegradation of the non-irradiated sample had a lag of 280 h and resulted in complete degradation by 720 h. Biodegradation of PAHs was accelerated in synthetic mixtures, especially in the presence of pure 9,10-anthracenedione. In general, irradiation had no effect on the biodegradation of PAHs incubated in synthetic mixtures or with pure cultures. Under current experimental conditions, the UV-irradiation invariably reduced the biodegradation of PAHs in creosote. Based on the results of the present and previous photochemical-biological studies of PAHs, the influence of the photochemical pretreatment on the biodegradation is highly dependent on the compounds being treated and other process parameters.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants having toxic, genotoxic, mutagenic, and/or carcinogenic properties (Menzie et al. 1992). They are universal combustion products of organic matter and are prevalent in the petroleum industry, the coal-refining process, automobile exhaust fumes, forest fires, and in wood impregnation plants (Samantha et al. 1999).

The bioremediation of PAH-contaminated sites and the treatment of PAH-polluted waters are problems of great concern for environmental protection and public health (Caldini et al. 1995). Since the early 1970s, bacterial treatment of creosote contaminated waters, soils, and sludges has been developed extensively (Tremaine et al. 1994). Bioremediation of PAHs has been found to have cost and technical advantages over chemical techniques (Zeng et al. 2000a). While low molecular weight PAHs are susceptible to biodegradation, low solubility of complex PAHs strongly reduces their bioavailability and makes microbial growth and biodegradation difficult (Caldini et al. 1995; Zeng et al. 2000a). Compounds containing more than four rings, however, are biodegraded cometabolically, with other compounds as growth substrates. Mixtures of PAHs, like creosote, are often present in contaminated soils. Therefore, cometabolic degradation is important in bioremediation (Cerniglia & Heitkamp 1989; Boldrin et al. 1993; Mahro et al. 1994).

In practice, it has been observed that when benzo[*a*]pyrene is exposured to UV radiation in soil, partially oxidized products are produced. These intermediates are known to be more easily biodegradable than the parent compound (Mueller et al. 1996). Photo-initiated intermediates may also act as primary substrates enhancing the biodegradation of the parent compounds (Dyreborg 1996). The contribution of photodegradation is especially important because this process preferentially attacks the same tertiary carbon atoms that tend to block biodegradation (Atlas & Bartha 1998).

However, Dyreborg (1996) has observed an inhibitory effect of creosote substances on the degradation of many monoaromatic hydrocarbons, resulting in a residual concentration of these normally easily degradable compounds. Culture consisted of a small amount of other compounds, poorly biodegraded in enrichment, which may inhibit the biodegradation of some hydrocarbons. The toxic and inhibitory effects of the creosote substances on biodegradation are not surprising since creosote is used as a wood preservative with the intention to kill or inhibit microorganisms (Dyreborg 1996).

Developing efficient and economical degradation technologies for polycyclic aromatics is an important research need and requires new approaches. There have been several attempts to combine chemical and biological techniques in order to improve treatment efficiencies of PAH containing pollutants (Miller et al. 1988, combined photolytic and biodegradative treatment; Kornmüller & Wiesmann 1999, ozonation and biodegradation; Dutta & Harayama 2000, photooxidation and biodegradation; Nam & Kukor 2000, ozonation and biodegradation; Zeng et al. 2000a, ozonation and biodegradation; Zeng et al. 2000b, ozonation and biodegradation; Lee & Hosomi 2001, Fenton oxidation and product biodegradability).

In a previous study, the influence of a 5–30 min irradiation with a xenon lamp on the biodegradation of naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, and pyrene was investigated, individually and as the PAHs present in creosote (Lehto et al. 2000a). In the main, irradiation was observed to enhance the biodegradation onset of PAHs, especially in creosote (Lehto et al. 2000a). However, naphthalene biodegraded most efficiently without the precedent irradiation (Lehto et al. 2000a).

The purpose of the present study is the comparison of the biodegradation of irradiated and non-irradiated PAHs. Compounds were treated individually, in mixtures of four PAHs and as the PAHs present in creosote. Due to the possible differences in the substrate mixture studies between a single and diverse populations, the experiments on individual anthracene and four PAHs as a mixture were repeated with two known PAH degrading cultures, including *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae*. Four different concentrations of pure 9,10-anthracenedione, the main photoproduct of anthracene, were incubated in the mixtures of four PAHs to study the possible acceleration by anthracenedione on the biodegradation of PAHs.

Materials and methods

Chemicals and media

Naphthalene, phenanthrene, anthracene, pyrene, benz[*a*]anthracene (B[*a*]A), and dibenz[*a*,*h*]anthracene (D[*a*,*h*]A) were provided by Fluka (Buchs, Switzerland) or by Merck (Schuchardt, Germany). 9,10-anthracenedione and benz[*a*]anthracene-7,12-dione were purchased from Merck (Schuchardt, Germany) and from Sigma-Aldrich (Steinheim, Germany), respectively. Creosote used in experiments in the present study was obtained from a pole impregnation plant of Oy Valtion Rautatiet (VR)-Rata Ab, Haapamäki, Finland, which was a mixture of German and Polish creosote (qualities WEI A and B, respectively). Standard Reference Material (SRM) 1647c, consisting of 16 USEPA PAHs in acetonitrile solution, was obtained from US Department of Commerce, National Institute of Standards and Technology (NIST, Gaithersburg, USA). Internal standard 2,2′-binaphthyl was obtained from Baker (Deventer, Holland). All solvents were HPLC grade, other chemicals were reagent grade and were used without additional purification. Water was deionized with Millipore Milli-Q Plus system. A mineral salt medium (MSM) including trace elements (as Lehto et al. 2000a) was used for enrichments and batch vial experiments.

Photochemical pretreatment

Irradiation was conducted in a quartz immersion well reactor with a 16 W low pressure mercury lamp emitting >90% at 254 nm (Photochemical Reactors Ltd., UK). The incident light intensity (I_0) of the irradiation lamp was monitored by a potassium ferrioxalate actinometer solution (0.006 M) at 254 nm, and was 1.68×10^{-8} Einstein l^{-1} s^{-1} (corresponding to 1.01×10^{16} photons l^{-1} s^{-1}). A water cooling system was fitted to the reactor. The lamp was allowed to warm for 30 min prior to experiments. Sample solutions were mixed with 0.2 l min^{-1} oxygen for 30 min prior to and during irradiation.

Irradiation was performed in saturated water solutions for four PAHs individually and as a mixture. PAHs and creosote were added to brown bottles as dichloromethane solutions and the solvent was evaporated. Deionized water was then supplemented and the resulting solution was sonicated for one hour before being placed in the reactor. In the present study the synthetic PAHs were provided in crystalline form (in contrast to solubilized, e.g. by a surfactant), and thus the concentrations do not represent dissolved concentrations. The initial concentrations of synthetic PAHs were between 0.678–101.3 μM, depending on the compound. Irradiation times varied from 3.0 min to 6.0 h. The initial concentrations of creosote PAHs were between 0.305–175.4 μM, depending on the compound (Table 2). In our experimental set-up some creosote PAHs were present in concentrations above the water solubility limits, and thus all those concentrations do not represent dissolved contents. The photolysis times of creosote were 1.0, 2.5 and 4.0 h.

Microorganisms and cultivation conditions

Enrichment cultures. The source for a surface sediment sample was the creosote contaminated Lake Jämsänvesi, Central Finland. Enrichments of aerobic bacteria were performed on PAHs in two aerated fluidized-bed reactors, volume of 1 l. In reactor 1, a mixture of four PAHs, naphthalene, phenanthrene, anthracene, and pyrene (100 mg l^{-1} of each) and in reactor 2, in addition to these PAHs, benz[*a*]anthracene and dibenz[*a*,*h*]anthracene (both 20 mg l^{-1}) were used to enrich aerobic PAH degrading cultures. Feed PAHs were dissolved in dichloromethane and mixed with 20 g of 100 μm glass beads. The solvent was evaporated and the PAH-covered beads were placed in the reactors. Generally, less than 10 days were needed for the degradation of the PAHs to concentrations below the detection limit by HPLC (0.001 mg l^{-1}). When necessary the glass beads were recovered with PAHs.

Pure cultures. The culture collection deposits of strains *Sphingomonas* sp. (EPA 505) and *Sphingomonas yanoikuyae* (B1) were DSM 7526 and DSM 6900, respectively. Strain EPA 505 *Sphingomonas* sp. (formerly *Pseudomonas paucimobilis*) has utilized anthracene, fluoranthene, phenanthrene, and benzo[*b*]fluoranthene as the sole sources of carbon and energy (Mueller et al. 1990). The degradation of pyrene (Mueller et al. 1990) and creosote PAHs in a consortium has previously also been reported for this strain (Mueller et al. 1989). When the community was grown on fluoranthene and subsequently exposed to fluoranthene plus 16 other PAHs, more than 90% degradation was reported in the case of anthracene and anthraquinone (Mueller et al. 1989). It has been reported that strain EPA 505 appears to be able to act on a limited number of aromatic compounds without induction. However, the growth with fluoranthene, for example, induces the synthesis of enzymes thereby permitting a considerably expanded range of catabolic activity toward PAHs (Mueller et al. 1990). During fluoranthene utilization color changes occurred from colorless to orange and yellow, and further to brown, but in this state no attempts were made to identify the metabolites (Mueller et al. 1989, 1990). Such color changes are often associated with the transient accumulation of catechol-like compounds and their *meta*-ring fission products (Mueller et al. 1989).

PAHs known to be degraded by *Sphingomonas yanoikuyae* are anthracene, naphthalene, and phenanthrene (Kim et al. 1997). This strain has also been reported to oxidize benz[*a*]anthracene to *cis*-dihydrodiols after induction with biphenyl, *m*-xylene, and salicylate (Mahaffey et al. 1988).

In the present study, both strains were cultured on the mineral salt medium (MSM) with trace elements. Bacteria were grown on naphthalene 50 mg l^{-1}, which was dissolved in dichloromethane and the solvent evaporated. Cultures of 250 ml were incubated on a gyratory shaker table (150 rpm) at room temperature for 7 days. Sterilized (100 kPa at 121 °C for 20 min) media and apparatus were used in a laminar-flow hood when handling pure cultures.

Biodegradation experiments

Enrichment cultures. The suspensions were obtained by vortexing biofilm-covered carrier material from the bioreactors and further centrifuging the solutions. For anthracene, the suspension was obtained from reactor 1, and for pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene, reactor 2 was used as the source of the suspensions. For biodegradation experiments of four PAHs as a mixture, the suspended enrichment cultures were obtained either from reactor 1 or 2 (Table 4), and for PAHs in the presence of 9,10-anthracenedione, the suspension mixture came from both reactors 1 and 2. Suspensions for the creosote experiments were obtained either from reactor 1 (experiment III) or 2 (experiments I and II).

Pure cultures. In these experiments cultures grown on naphthalene in MSM were used without further handling.

Biological batch vial experiments for enrichment and pure cultures were conducted in 8 ml brown glass vials. The concentration of a certain non-irradiated PAH at the beginning of incubation was adjusted according to the concentration of consistent irradiated PAH after photodegradation (in creosote mixture 16 PAHs were studied; the non-irradiated creosote solution was diluted until the concentration of eight PAHs was above and the concentration of eight compounds was below compared to the concentrations of these PAHs in the irradiated solution). Irradiated PAHs with their photochemical products and non-irradiated PAHs were mixed with a concentrated mineral salt medium. Pure compounds were added to vials as dichloromethane solutions, the solvent being allowed to evaporate, and MSM was supplemented. The sample solutions (2 ml) and enrichment culture (1 ml) were then mixed in the vials.

At the beginning of incubation the measured concentrations of PAHs varied from 0.412 (dibenz[*a*,*h*]anthracene) to 41.3 μM (anthracene), depending on the compound. In order to study the ability of 9,10-anthracenedione to accelerate the biodegradation of PAHs, four different concentrations of pure anthracenedione (1.47, 2.72, 14.3, and 18.9 μM, and controls 10.9 μM) were incubated with a mixture of anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene. Mean initial concentrations were 20.6, 17.7, 1.22, and 2.43 μM, respectively. In the set of experiments where substrates are provided as crystals, a slow degradation limited by substrate availability may result (Volkering et al. 1992). Due to the crystalline form the interaction effects between PAHs are also expected to decrease due to the dissolution limitation (Tiehm & Fritzsche 1995). At the beginning of the incubation the concentrations of irradiated and non-irradiated creosote PAHs varied from 0.0130 μM to 20.67 μM, depending on the compound.

Killed controls, containing 2 v-% formaldehyde, were used to monitor possible abiotic losses during incubation and extraction. All the sample vials were incubated on a gyratory shaker table (150 rpm) in the dark at 20 °C.

Analytical procedures

Protein concentrations of the bacterial suspension were analyzed by the Bradford Assay (Bradford 1976) using a commercial kit (Bio-Rad, Hercules, CA) with bovine serum albumin as the protein standard. The total numbers of bacteria were determined by counting the cells with an epifluorescence microscope (Zeiss Axioskop 2) after staining the filtered samples (0.2 μm, polycarbonate, Millipore) and the slides with DAPI (4′,6′-diamidino-2-phenyl-indole).

Samples were analyzed using a Hewlett-Packard 1100 Series high performance liquid chromatograph (HPLC) with a diode array detector (Lehto et al., submitted). Duplicate samples were taken from the reactor prior to and during irradiation, and the samples were extracted and concentrated with hexane (2:1 v/v). In the biological batch vial experiments, the entire contents of the duplicate vials were used at each sampling point, and the samples were extracted and concentrated with hexane (2:1 v/v). Results are the mean values of duplicate separate units in each case ($\pm$ 0.02 mg l^{-1}, on average), and concentrations presented as if all PAHs were dissolved in the liquid phase. Absorption spectra of creosote mixtures were measured by a Shimadzu UV/visible spectrophotometer. The intention was to keep absorption $\leq$ 1.0 at the wavelength 254 nm measured in cuvettes with an optical path of 1.0 cm (data not shown). Because of this, the concentrations of some creosote PAHs in the solutions used, were by contrast below the detection limit by HPLC (0.001 mg l^{-1}).

The photodegradation products were analyzed using a Hewlett-Packard 6890 Series gas chromatograph linked to a Hewlett-Packard mass spectrometer (GC/MS) (Lehto et al. 2000b). Triplicate water samples for GC/MS analysis were taken prior to and during irradiation, the samples were extracted and concentrated with dichloromethane. Photo-initiated intermediates were identified by a comparison of their retention times and mass spectra with those of the standards.

Results and discussion

Synthetic PAHs

Progress of irradiation: formation of the photoproducts

Since the photodegradation of synthetic PAHs was performed in water solutions containing compounds as crystals, which were dissolved throughout the irradiation, the decrease in concentrations of the initial compounds and the yields of the photoproducts could not be determined accurately. The reaction was followed as the formation of photoproduct(s) or the necessary irradiation times were estimated based on the reciprocal photoreactivity of PAHs (Lehto et al. 2000b). After a 3 min irradiation of anthracene (at initial measured concentration of 28.2 μM), the main photoproduct, 9,10-anthracenedione (anthraquinone) was identified (Fox & Olive 1979; David & Boule 1993; Lehto et al., submitted). A 15 min irradiation of benz[*a*]anthracene (at concentration of 19.7 μM) produced benz[*a*]anthracene-7,12-dione (benz[*a*]anthraquinone) (Mill et al. 1981; David & Boule 1993; Lehto et al., submitted). A 10 min photolysis of anthracene and 0.5 or 1.0 h irradiation of benz[*a*]anthracene did not yield additional detectable products.

The detection of the photoproducts of pyrene and dibenz[*a*,*h*]anthracene in water was more difficult than that of anthracene and benz[*a*]anthracene (Lehto et al., submitted). After the irradiation periods of 0.5 and 1.0 h of pyrene (at concentrations of 26.4 and 72.7 μM, respectively) and 1.5 and 2.5 h of dibenz[*a*,*h*]anthracene (at concentrations of 8.55 and 6.47 μM, respectively), no products were detected. Anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene irradiated as a mixture, produced the same photoproducts as detected in the individually photolyzed samples, 9,10-anthracenedione and benz[*a*]anthracene-7,12-dione.

Creosote mixture

HPLC analysis

HPLC analysis of 16 USEPA PAHs of the creosote mixture is shown in Table 1. The major components of the sample were acenaphthene, naphthalene, phenanthrene, and fluoranthene, respectively.

Irradiation

PAHs get photodegraded efficiently in water. The concentrations prior to and after irradiation are summarized in Table 2. The shape of the PAH molecules and the availability of the reactive sites were important factors in the photodegradation of PAHs. In liquid solutions angular or bent PAHs are found to be more resistant to photodegradation than straight molecules (Low et al. 1987). Fluorene, for example, was degraded 46% by 1.0 h irradiation whereas in the case of anthracene, for example, 84% decomposition was observed with the same irradiation time. Fluorene belongs to the group, which is chemically different from other PAHs since the structures of these compounds incorporate a five-membered ring. They are less photoreactive and have a low affinity for capturing low energy electrons (Low et al. 1987).

Different initial concentrations of the compounds were used to study the possible effect on the biodegradation, which also led to a different efficiency of light absorption by a single compound. Thus, the results between different experimental set-ups are not directly comparable. Extension of the irradiation time did not enhance the decomposition of PAHs in the same ratio, though there were some differences between individual PAHs. This may be due to formed photointermediates, which make the light for absorption unavailable by a single compound (Qingrui et al. 1991; Tuhkanen 1995; Miller & Olejnik 2001).

For several high molecular weight PAHs, e.g. for chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*ghi*]perylene, the concentrations were below the HPLC detection limit before irradiation but after irradiation these compounds were detected. This is assumed to be due to the dissolution effect of these PAHs, present in very low portions in the creosote mixture. The concentration of acenaphthylene in creosote was below the detection limit by HPLC throughout the experiments.

Traces of photo-initiated products were detected after irradiation of the creosote mixtures. The products referred mainly to dione derivatives of the original compounds. 9,10-anthracenedione and benz[*a*]anthracene-7,12-dione were identified based on comparison of their mass spectra and retention times with those of the standards.

Table 1. Molecular weights, water solubilities, and relative percentages (wt) of 16 USEPA PAHs in the creosote mixture as analyzed by HPLC

Compound	Molecular weight (g mol^{-1})	Aqueous solubility[a] at 25 °C (mg l^{-1})	Retention time (min)	wt-%
Naphthalene	128	31.7	4.33	10.03
Acenaphthylene	152	–	ND	0.00
Acenaphthene	154	3.93	6.36	13.65
Fluorene	166	1.69	7.27	4.49
Phenanthrene	178	1.00	7.94	8.18
Anthracene	178	0.045	8.49	1.20
Fluoranthene	202	0.206	8.87	6.43
Pyrene	202	0.132	9.11	4.17
Benz[*a*]anthracene	228	0.0094	9.62	5.30
Chrysene	228	0.0018	9.90	0.49
Benzo[*b*]fluoranthene	252	0.0015	10.01	0.83
Benzo[*k*]fluoranthene	252	0.0008	10.63	0.66
Benzo[*a*]pyrene	252	0.0016	10.96	0.37
Dibenz[*a*,*h*]anthracene	278	0.0005	11.19	0.49
Benzo[*ghi*]perylene	276	0.0007	12.01	0.85
Indeno[1,2,3-*cd*]pyrene	276	0.0002	13.39	0.08
Total				57.24

[a](NIST 1994), ND = not detected; below the detection limit by HPLC with the solutions used.

Table 2. Concentrations of 16 creosote PAHs prior to and after 1.0, 2.5, and 4.0 h irradiation. Results are the mean values of duplicate samples, concentrations presented as if all PAHs were dissolved in the liquid phase

Concentration (μM) in irradiation	Prior to	After 1.0 h	Prior to	After 2.5 h	Prior to	After 4.0 h
Naphthalene	16.2	7.18	37.9	16.8	156.1	ND
Acenaphthylene	ND	ND	ND	ND	ND	ND
Acenaphthene	75.2	19.1	175.4	44.5	152.5	ND
Fluorene	10.8	5.81	25.2	6.95	22.0	ND
Phenanthrene	16.8	5.92	51.1	7.91	67.2	6.49
Anthracene	2.13	0.34	7.04	0.449	8.19	0.189
Fluoranthene	10.3	1.84	37.3	3.13	42.6	1.53
Pyrene	6.60	0.993	23.6	1.75	30.0	1.89
Benz[*a*]anthracene	6.60	ND	25.5	0.722	33.0	0.836
Chrysene	ND	5.22	2.37	0.214	3.65	ND
Benzo[*b*]fluoranthene	0.596	0.047	3.23	0.253	ND	0.563
Benzo[*k*]fluoranthene	0.569	0.040	2.25	0.142	ND	0.050
Benzo[*a*]pyrene	ND	ND	0.913	ND	0.556	ND
Dibenz[*a*,*h*]anthracene	1.37	ND	ND	ND	ND	ND
Benzo[*ghi*]perylene	1.14	1.19	ND	1.29	ND	0.844
Indeno[1,2,3-*cd*]pyrene	ND	ND	0.305	ND	ND	ND

ND = below the detection limit by HPLC with the solutions used.

Table 3. Initial protein concentrations of the bacterial suspensions analyzed by the Bradford Assay

Compound/mixture		Protein concentration (mg l^{-1})
Enrichment culture:		
Anthracene		15.1
Pyrene		18.4
Benz[*a*]anthracene		10.8
Dibenz[*a*,*h*]anthracene		27.5
The mixture of 4 PAHs:		
Experiment	I	8.70
	II	13.3
	III	35.4
	IV	37.4
	V	25.2
4 PAHs and 9,10-anthracenedione		38.9
Pure culture:		
Anthracene:	*Sphingomonas* sp.	12.1
	S. yanoikuyae	9.22
The mixture of 4 PAHs:	*Sphingomonas* sp.	6.73
	S. yanoikuyae	10.7
Enrichment culture:		
Creosote	I	34.9
	II	31.6
	III	34.0

Biodegradation of PAHs with and without preceding irradiation

Protein concentrations of the bacterial suspension in each trial are presented in Table 3. One gram (g) protein determined in the present method was equivalent to 1.3×10^{13} DAPI stained bacterial cells.

Experiments with enrichment cultures

Individual PAHs. The effect of irradiation on the biodegradation of PAHs was studied in batch vial experiments. 50.4, 72.7, 15.7, and 6.47 μM of anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene solutions were irradiated for 10 min, 1.0 h, 1.0 h, and 2.5 h, respectively (e.g. with the 3 min and 15 min irradiation of anthracene and benz[*a*]anthracene, respectively, no gain in biodegradation was achieved; data not shown). The biodegradation of the irradiated and non-irradiated solutions was followed and is illustrated in Figure 1.

The lag phase in the onset of biodegradation of PAHs was reduced by the irradiation compared to that of the non-irradiated samples. Biodegradation was complete by 300 h and 480 h for the irradiated

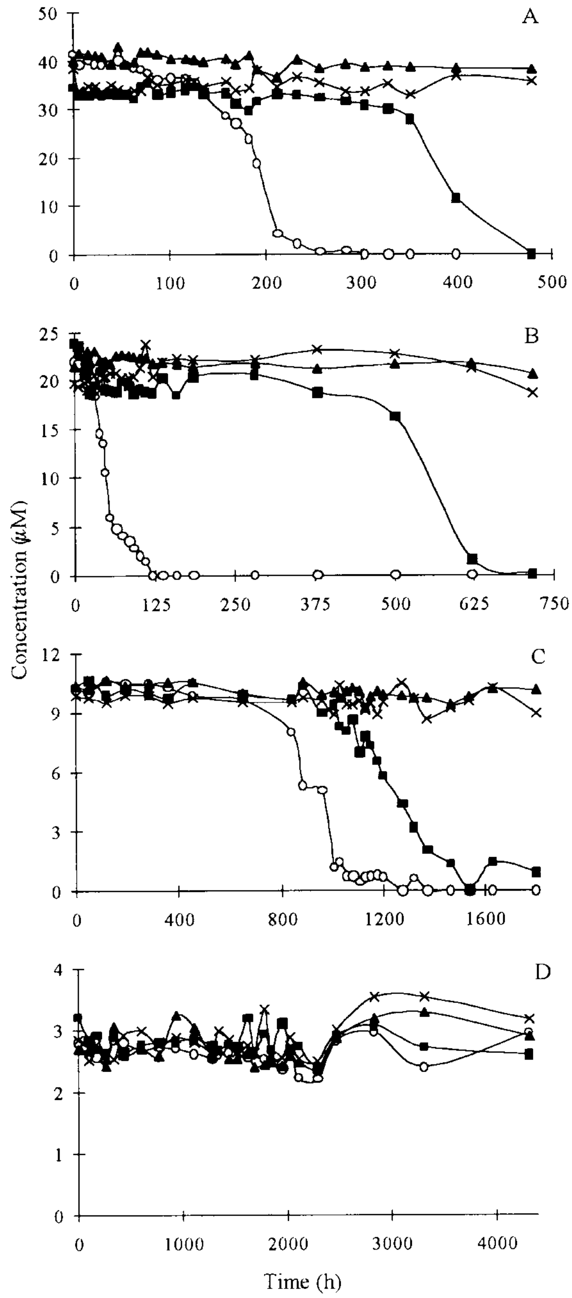

Figure 1. Biodegradation of (A) anthracene (10 min irradiated), (B) pyrene (1.0 h), (C) benz[*a*]anthracene (1.0 h), and (D) dibenz[*a*,*h*]anthracene (2.5 h) by enrichment culture: irradiated (○), non-irradiated (■), control irradiated (▲), and control non-irradiated (×). The killed controls contained 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

and non-irradiated anthracene, respectively. Irradiation enhanced the biodegradation onset of pyrene in particular. For benz[*a*]anthracene the lag phase was reduced 40% by irradiation. Reduced lag phases indicate readily degradable compounds formed under irradiation, which accelerated the biodegradation onset of the original PAHs. However, when the biodegradation of irradiated and non-irradiated dibenz[*a*,*h*]anthracene solutions was followed for 180 d, no distinct degradation of this recalcitrant 5-ring PAH was observed.

Miller et al. (1988) studied the effect of photolytic pretreatment on the subsequent fate of benzo[*a*]pyrene in sewage sludge and soil test systems. Photolysis was performed in methanol with and without H_2O_2 under UV (300 nm) or natural sunlight. Intact benzo[*a*]pyrene resisted biodegradation in both test systems, whereas photolytic pretreatment transformed benzo[*a*]pyrene to polar materials that were subject to increased mineralization. Photolysis decreased the mutagenicity of benzo[*a*]pyrene by two-thirds, as shown by the Ames assay, though it increased acute toxicity. However, during subsequent incubation in soil or sewage sludge, mutagenicity decreased rapidly and acute toxicity disappeared due to the mineralization and binding. They conclude that a combination of photolytic and biodegradative processes is a promising new approach for the removal and detoxification of PAHs (Miller et al. 1988). Lee & Hosomi (2001) reported microbial resistance experiments, which demonstrated that Fenton oxidation products of benzo[*a*]pyrene, benzo[*a*]pyrene-1,6-, -3,6-, and -6,12-dione, all of which were known to have lower toxicity than benzo[*a*]pyrene, were more easily degraded than the original PAH.

Mixture of four PAHs, Experiments I–V. The effect of irradiation on the biodegradation of anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene was studied as mixtures of these four PAHs. Though the initial concentrations of PAHs, the irradiation times, and the enrichment cultures were varied (Table 4), the biodegradation of PAHs, at least, was not enhanced due to the irradiation (data not shown). In these experiments the onset of biodegradation followed the same kinetics for the irradiated and non-irradiated samples. However, both in the irradiated and non-irradiated PAH samples, biodegradation was faster in the mixtures than when incubated alone. Dibenz[*a*,*h*]anthracene had a very long lag phase before showing any biodegradation, suggesting, for instance, that bacteria were able to degrade D[*a*,*h*]A only when more easy biodegradable PAHs were already degraded. The dibenz[*a*,*h*]anthracene degradation was completed only in the 1.0 h irradiated sample, which was incubated for 330 d (data not shown). In the non-irradiated sample 92% removal was observed within this incubation period. The initial protein concentrations had no distinct effect on the biodegradation of PAHs.

Similar biodegradation of irradiated and non-irradiated PAHs in the mixtures seemed initially to be due to insufficient concentrations of the photoproducts, as was the case when PAHs were treated individually in pre-experiments (data not shown). Despite the irradiation time being increased to 6.0 h, similar results were still obtained, indicating that the influence of the photoproducts was still insignificant on biodegradation compared to the impact of other PAHs. Individual pyrene in pre-experiments, for example, showed no biodegradation by 7 d, whereas in the presence of anthracene it degraded to half the concentration in 4 d (data not shown).

Bouchez et al. (1999) compared the biodegradation of a PAH mixture, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene, by defined strains to that by consortia from a PAH-contaminated soil. Specific mixed cultures of pure strains, using one or several of these PAHs as carbon sources, achieved limited degradation whereas the soil consortium readily mineralized the PAH-mixture. Thus, in soil bacterial consortia too, interactions have been claimed to promote rather than inhibit the PAH degradation (Bouchez et al. 1999).

Experiments with pure cultures

Anthracene. A 49.9 μM anthracene solution was irradiated for 10 min. The effect of the irradiation was studied with *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae* for the irradiated and non-irradiated samples. Unlike in the experiment with mixed culture, irradiation had an inhibiting (*Sphingomonas* sp.) or zero effect (*S. yanoikuyae*) on the onset of the anthracene biodegradation with pure cultures (Figure 2). Known pathways of aromatic hydrocarbon catabolism are inducible at the level of DNA transcription, and therefore the biodegradation may be repressed by the presence of a more readily utilizable carbon source for individual bacterial species. Diverse populations may contain PAH degraders, PAH cooxidizers, and bacteria that do not degrade the parent compounds but the metabolites alone. In mixed cul-

Table 4. Initial concentrations of irradiated and non-irradiated anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene biodegraded as mixtures by enrichment cultures

Experiment		I		II		III		IV		V	
Origin of the culture (reactor)		1		2		1		1		1	
Irradiation time (h)		1.0	–	1.5	–	2.5	–	4.0	–	6.0	–
Anthracene	c_{init} (μM)^{a}	26.1	24.5	26.0	26.1	19.0	17.0	32.2	30.8	26.0	23.9
Pyrene	c_{init} (μM)	14.1	14.4	14.0	13.4	11.1	9.95	19.4	18.8	18.2	16.9
B[*a*]A	c_{init} (μM)	6.18	6.14	8.18	7.59	2.50	2.49	3.20	3.17	2.35	1.88
D[*a*,*h*]A	c_{init} (μM)	1.69	1.56	2.36	2.08	1.39	1.16	0.686	0.807	0.479	0.412

– = non-irradiated.

[a] Concentration in the beginning of the incubation.

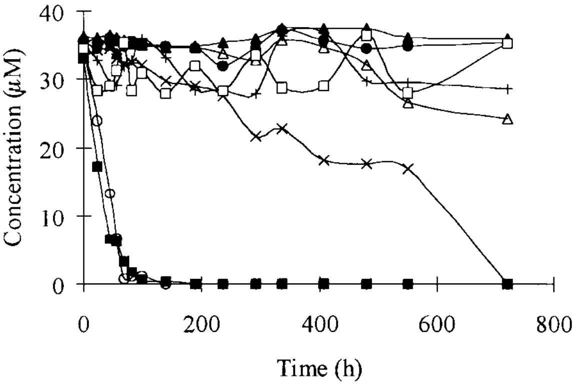

Figure 2. Biodegradation of anthracene inoculated with *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae*. *Sphingomonas* sp.: irradiated for 10 min (△), non-irradiated (×), controls irradiated (▲) and non-irradiated (+), and *Sphingomonas yanoikuyae*: irradiated for 10 min (○), non-irradiated (■), controls irradiated (●) and non-irradiated (□), containing 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

tures synergistic effects appear if cooxidation occurs to increase the microbial attack of more recalcitrant compounds (MacGillivray & Shiaris 1994).

Mixture of four PAHs. A mixture of anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene, at concentrations of 52.5, 35.0, 7.40, and 1.19 μM, respectively, was irradiated for 6.0 h. The biodegradation tests were performed with *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae* for irradiated and non-irradiated samples.

In general, irradiation had no effect on the biodegradation of PAHs in the mixture by pure strains (Figure 3). Recalcitrant dibenz[*a*,*h*]anthracene showed no significant biodegradation over a 1368-h culture period. Weissenfels et al. (1991) studied the degradation of 12 PAHs by strain *Alcaligenes denitrificans* WW1, and reported that dibenz[*a*,*h*]anthracene was neither degraded as a sole source of carbon nor cometabolized in fluoranthene-containing media.

Biodegradation of a PAH mixture in the presence of different initial concentrations of 9,10-anthracenedione with enrichment cultures

When PAHs were studied in the mixtures, photoirradiation had no notable effect on the ensuing biodegradation. Because of this, four different concentrations (1.47, 2.72, 14.3, and 18.9 μM, and control vials 10.9 μM) of pure 9,10-anthracenedione were added to the mixture of anthracene, pyrene, benz[*a*]anthracene, and dibenz[*a*,*h*]anthracene, and the sample solutions were incubated. The reason for selecting 9,10-anthracenedione was because in the previous experiments it was detected even after only short irradiation times of anthracene, irradiated individually and in the mixtures (Lehto et al., submitted). It has also been reported to biodegrade better than anthracene (Lee et al. 1998; Lehto et al., submitted). When the same initial concentrations of PAHs were used in the vials with increasing initial concentrations of 9,10-anthracenedione, higher concentrations of PAHs were also detected. This indicates an influence of 9,10-anthracenedione on the solubilities of PAHs.

Anthracene, pyrene, and benz[*a*]anthracene biodegraded effectively in the presence of 9,10-anthracenedione, whereas dibenz[*a*,*h*]anthracene was recalcitrant within the incubation time (Figure 4). The biodegradation of benz[*a*]anthracene was higher in the presence of 9,10-anthracenedione than the biodegradation of non-irradiated benz[*a*]anthracene incubated individually and in the mixture of four PAHs. The effect of 9,10-anthracenedione concentration on the biodegradation of PAHs was not evident. The biodegradation of 9,10-anthracenedione was greater than

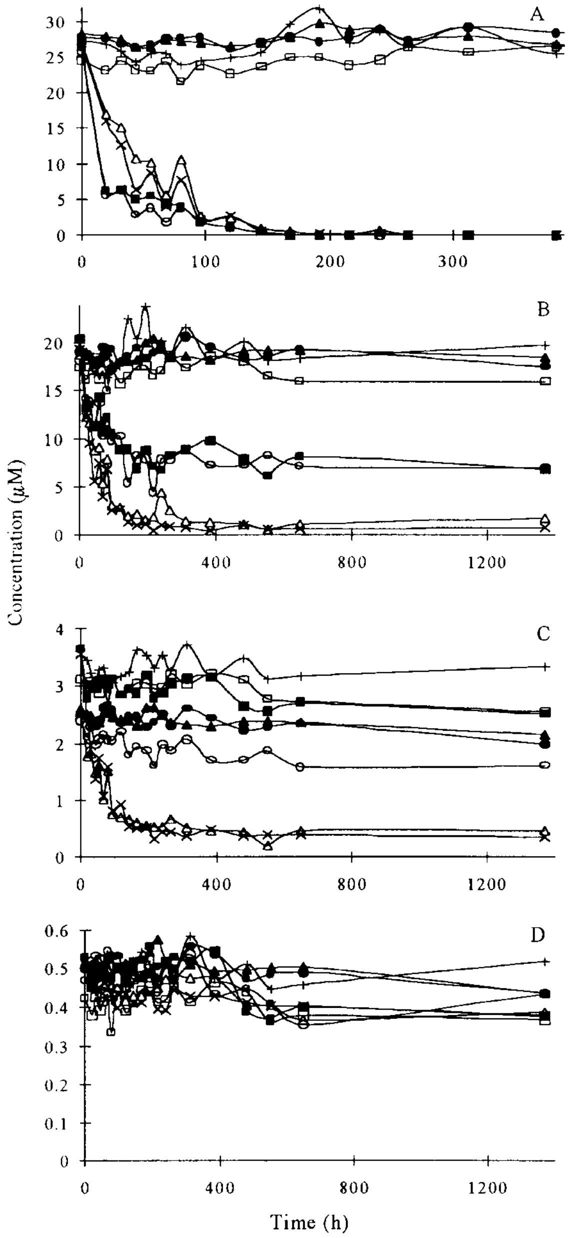

Figure 3. Biodegradation of (A) anthracene, (B) pyrene, (C) benz[*a*]anthracene, and (D) dibenz[*a*,*h*]anthracene, in a mixture of four PAHs inoculated with *Sphingomonas* sp. strain EPA 505 and *Sphingomonas yanoikuyae*. *Sphingomonas* sp.: irradiated for 6.0 h (△), non-irradiated (×), controls irradiated (▲) and non-irradiated (+), and *Sphingomonas yanoikuyae*: irradiated for 10 min (○), non-irradiated (■), controls irradiated (●) and non-irradiated (□), containing 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

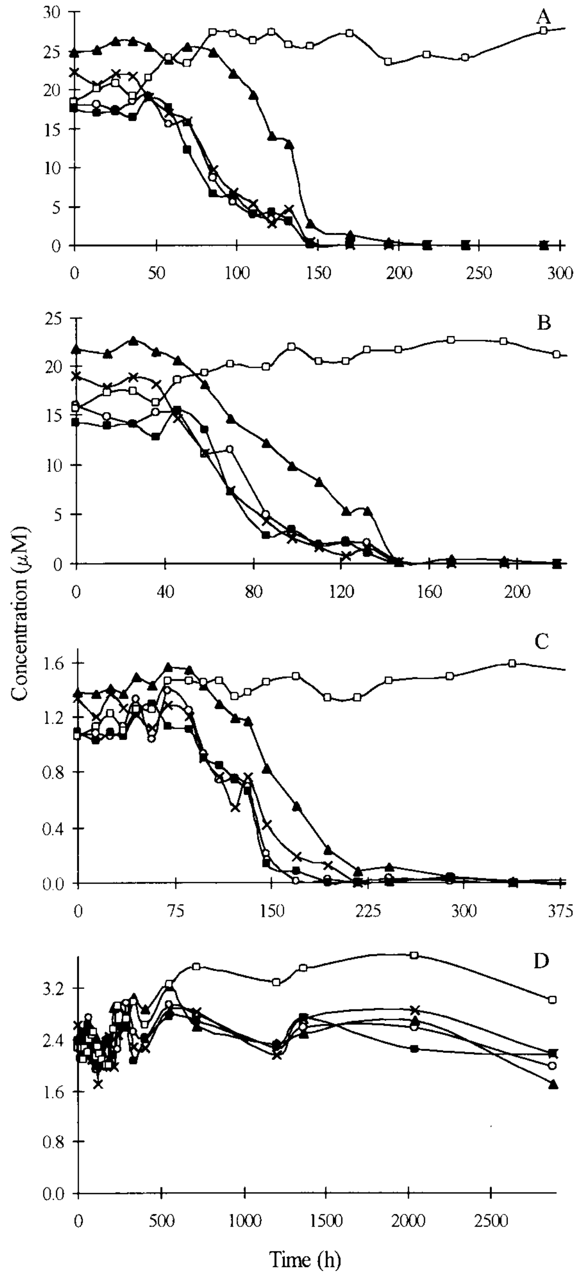

Figure 4. Biodegradation of a PAH mixture by enrichment culture in the presence of different initial concentrations of 9,10-anthracenedione: 1.47 μM (○), 2.72 μM (■), 14.3 μM (×), 18.9 μM (▲), and control vials 10.9 μM (□). (A) anthracene, (B) pyrene, (C) benz[*a*]anthracene, and (D) dibenz[*a*,*h*]anthracene. The killed controls contained 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

those of anthracene, though with different initial concentrations of the compounds (Figure 5).

Generally, in the control vials no substantial decrease in the concentrations was observed during incubation.

Biodegradation of irradiated and non-irradiated creosote PAHs with enrichment cultures

The biodegradation of PAHs was related to their structure and, predictably, low molecular weight PAHs decomposed faster compared to the high molecular weight compounds (Figure 6). The biodegradation of more complex PAHs was incomplete or was slowed down, often after rapid onset, and residual concentrations were often to be found. In the control vials too, especially of anthracene and benz[*a*]anthracene (Figure 6B and C, respectively), a decrease in the concentrations was often observed, probably due to abiotic losses. Interestingly, the decrease in the control vials of higher molecular weight compounds such as benzo[*ghi*]perylene (Figure 6D) was not equally notable. Due to their small portions in creosote, the concentrations of PAHs such as benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, and indeno[1,2,3-*cd*]pyrene, were generally below the detection limit by HPLC in the creosote-water solutions used.

The purpose of this experimental setup was to compare exactly the irradiated and non-irradiated samples of similar concentrations within a single experiment. Irradiation was observed not to accelerate, but rather reduce the biodegradation of studied creosote PAHs. The effect of the irradiation period (1.0, 2.5, and 4.0 h or the origin of the culture, i.e. the chemicals used on enrichments), on the biodegradation was not evident. Due to possible variations in the initial concentrations of PAHs between different experiments, these results are not directly comparable. The biodegradation of pyrene irradiated for 1.0, 2.5, and 4.0 h is illustrated in Figure 7. The biodegradation of pyrene started efficiently in experimental set-ups, but slowed down both in the irradiated and non-irradiated samples. By day 40 the biodegradation of 1.0, 2.5, and 4.0 h irradiated and non-irradiated samples was 64% and 94%, 80% and 98%, and 46% and 85%, respectively.

Certain high molecular weight PAHs were recalcitrant under current experimental conditions. For example, dibenz[*a*,*h*]anthracene (data not shown) and benzo[*ghi*]perylene (Figure 6D) showed no distinct biodegradation within any incubation period.

When comparing microbial capacity between different cultures, it should be noticed that many factors have an influence on degradation. The nutrient composition of the media used, the growth history of microorganisms, and the growth rate or the substrate concentration in the enrichment of the culture have an effect on culture growth capacity as well as on their metabolic activities. Microbial communities grown on certain substrates become adapted, which results in selective enrichment and genetic changes (Atlas & Bartha 1998). Thus, the reason for the differences in the previous (Lehto et al. 2000a) and the present results for photochemical-biological treatment for creosote PAHs is presumably due to different experimental adjustments such as the enrichment histories of the microorganisms. In the former study, aerobic PAH degrading bacteria were newly collected from a creosote contaminated lake and enriched on creosote (Lehto et al. 2000a).

Bouchez et al. (1995) studied interactions in the biodegradation of PAHs, naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene, by various bacterial strains, and observed, in addition to cometabolism, strong inhibition effects. Inhibition was most commonly observed when the added substrate was more water-soluble. For example, naphthalene was toxic to all strains not isolated on this compound, which indicates a relationship between inhibitory efficiency and the water solubility of the substrate. In general, inhibition took place whether cometabolism occurred or not, and its high prevalence was related to the fact that, as homologous compounds, PAHs are susceptible to interactions at several levels (Bouchez et al. 1995). The substrate interactions are peculiarly complicated when mixtures contain several components, especially in creosote (Melin 1997).

Millette et al. (1995) studied the biodegradation of creosote-related compounds, phenanthrene, fluorene, *p*-cresol, pentachlorophenol, carbazole, dibenzothiophene, and dibenzofuran. They reported that, in general, the more hydrophobic and recalcitrant compounds were more greatly affected by substrate interaction. The mineralization of *p*-cresol, for example, was not affected by substrate interaction, *p*-cresol being the main inhibitor. Phenanthrene inhibited biodegradation, but to a lesser extent, whereas fluorene enhanced the mineralization of carbazole (Millette et al. 1995).

In the present study, photochemical pretreatment inhibited the biotransformation of both low and high

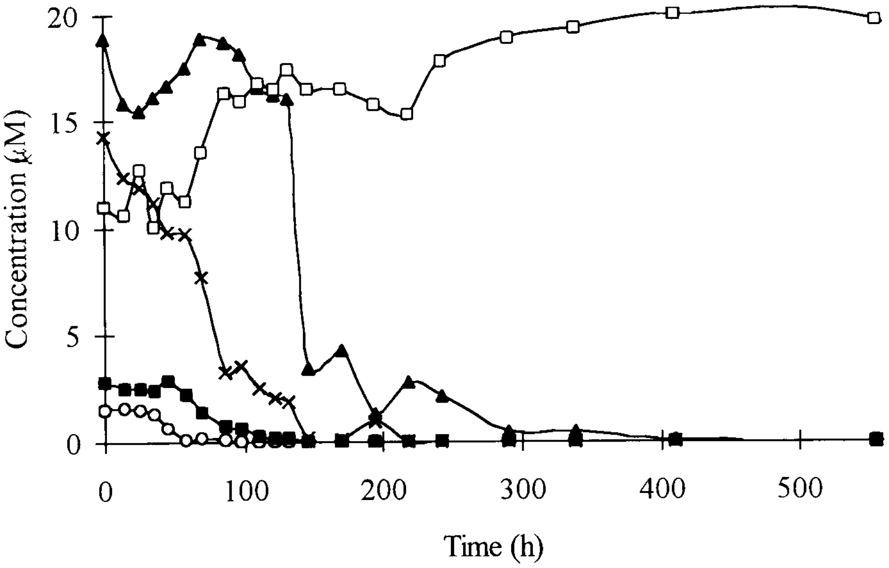

Figure 5. Biodegradation of different initial concentrations of 9,10-anthracenedione in a PAH mixture by enrichment culture: 1.47 μM (○), 2.72 μM (■), 14.3 μM (×), 18.9 μM (▲), and control vials 10.9 μM (□). The killed controls contained 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

molecular weight PAHs. Other compounds present, such as the photo-initiated products, may reduce the biodegradation of original PAHs due to metabolism of other substrates in a complex mixture or competitive substrate interactions (Lantz et al. 1997). Photo-initiated substrates are often held to be more biodegradable than the parent compound (Mueller et al. 1996), which may explain the inhibition effect observed, for the rather hydrophobic and recalcitrant creosote compounds of the present study. However, it is essential to be aware of the nature of the photoproducts due to their potential for being more toxic and recalcitrant than the original compounds (Tuhkanen 1995). Wernersson & Dave (1997) determined the difference prior to and after 2 h UV irradiation in acute toxicity to *Daphnia magna* for fluoranthene, pyrene, benzo[*a*]pyrene, acridine, 3,4-benzofluoranthene, and 1,2,5,6-dibenzoanthracene. The ratio between EC_{50} values prior to and after irradiation ranged from 4.6 (for benzo[*a*]pyrene) to > 244 (for 3,4-benzofluoranthene), demonstrating that the UV enhanced the PAH-toxicity. In mixtures, however, PAHs are not necessarily the only cause of toxicity or photoinduced toxicity, since toxicity might be caused by other hydrophobic phototoxic substances. Phototoxic strength may differ greatly between different phototoxic compounds (Wernersson & Dave 1997).

Conclusions

UV-irradiation accelerated biodegradation when anthracene, pyrene, and benz[*a*]anthracene were treated individually with enrichment cultures. In particular, irradiation facilitated the microbial degradation of pyrene. The gain achieved with irradiation was mainly the result of reducing the lag phase of biodegradation. This is assumed to be due to more easily degradative compounds produced under irradiation, which increased the biodegradation of the original PAHs. Dibenz[*a*,*h*]anthracene was rather recalcitrant to biodegradation under conditions tested.

Biodegradation of anthracene, pyrene, and benz[*a*]anthracene was more efficient in the mixtures, especially in the presence of pure 9,10-anthracenedione, than in the individually incubated samples. The stimulating effect may be a result of an increase in biomass since other compounds serve as better growth substrates and, thereby, increasing biomass, or accelerated enzyme induction. Generally, there were no difference between irradiated and non-irradiated PAHs when samples were incubated as mixtures or with pure cultures. The impact of other PAHs on their biodegradation was more significant compared to the influence of the photo-initiated products. The enrichment cultures may contain PAH degraders as well as degraders of

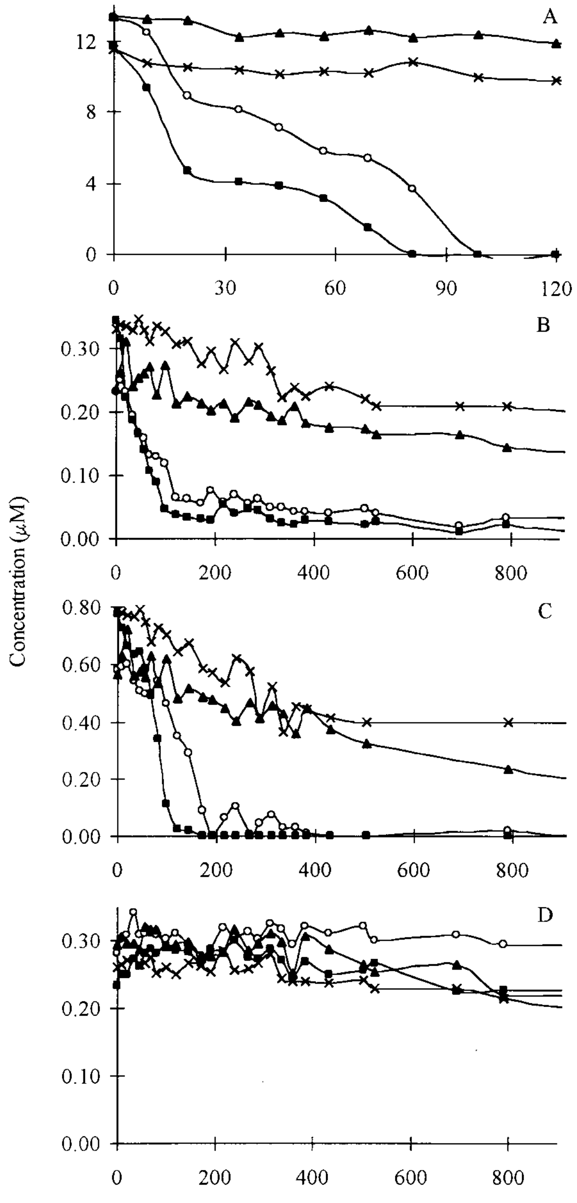

Figure 6. Biodegradation of (A) naphthalene, (B) anthracene, (C) benz[*a*]anthracene, and (D) benzo[*ghi*]perylene as these PAHs present in creosote by enrichment culture: irradiated for 1.0 h (○), non-irradiated (■), controls irradiated (▲) and non-irradiated (×), containing 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

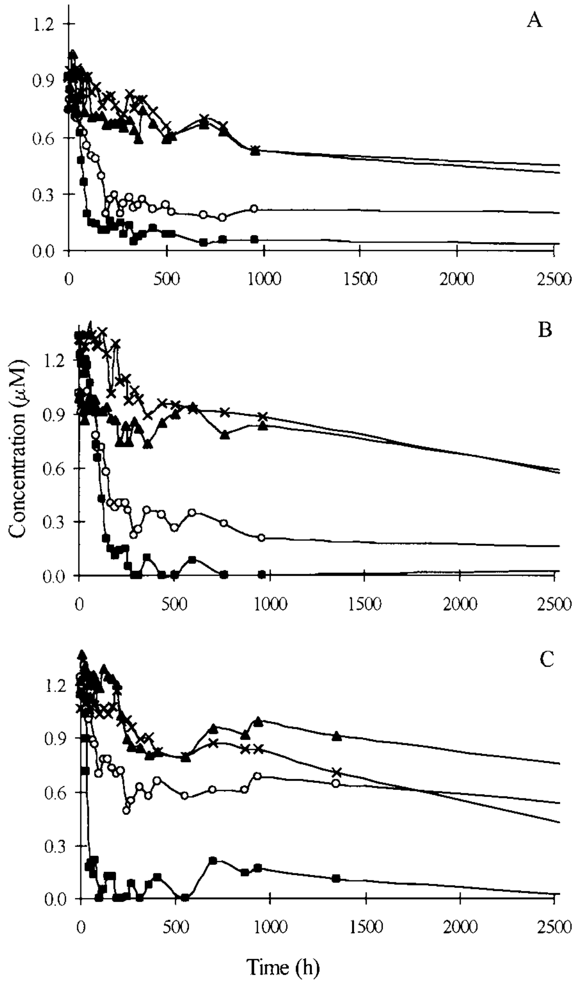

Figure 7. Biodegradation of pyrene in creosote by enrichment culture: irradiated for (A) 1.0 h, (B) 2.5 h, and (C) 4.0 h (○), non-irradiated (■), controls irradiated (▲) and non-irradiated (×), containing 2 v-% formaldehyde. Each point represents the mean of two separate experiments.

their photoproducts, increasing the interactions among microbial populations.

UV-irradiation invariably decreased the biodegradation of the creosote PAHs studied here. In general, inhibition is due to toxicity or competitive inhibition. Many organic contaminants acting as substrates for microorganisms start to inhibit growth at higher concentrations. In creosote mixtures, the substrate interactions are extremely complicated. There was no clear influence of the irradiation time on biodegradation.

The gain achieved by photochemical treatment was highly dependent on the solutions being treated and on the microbial cultures present. The interactions between the substrates are very important in biodegradation and these should be investigated for the culture(s) used. This is particularly important when trying to combine photochemical and biological treatment processes. Since the effectiveness of photochemical pretreatment on the enhancement of the biodegradability is highly dependent on the PAHs being treated and other process parameters, laboratory- or pilot-scale studies need to be conducted in order to integrate photochemical-biological treatment processes for aromatic compounds. Accordingly, the effect of the irradiation dose, the initial concentrations of the compound(s) treated, and also the influence of the present compounds on the bacterial culture used all need to be examined carefully. The effect of photolysis on acute toxicity and mutagenicity also needs to be examined.

Acknowledgment

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