Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 168 (2004) 109–115
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Photodegradation and biodegradation study of benzo(a)pyrene in different liquid media

Agata Kot-Wasik, Dagmara Dąbrowska, Jacek Namieśnik*

Department of Analytical Chemistry, Gdañsk University of Technology, 11/12 Narutowicz Str., 80-952 Gdañsk, Poland

Received 29 April 2004; accepted 25 May 2004
Available online 3 July 2004

Abstract

The rate of degradation of benzo(a)pyrene in different media has been investigated. Different parameters such as light intensity responsible for photodegradation, temperature, exposure time and the presence of naturally occurring bacterial flora blamed for biodegradation processes, influencing stability of benzo(a)pyrene in various solvents and water have been monitored. Degradation in organic solvents decreased in the following order: dichloromethane > acetonitrile > hexane ≥ cyclohexane > methanol. In the case of natural waters, the degradation rate course in the following order: pond water > river water > seawater. Benzo(a)pyrene-4,5-dihydrodiol and one of isomers of hydroxy-BaP-dione as products of photodegradation have been detected. BaP-dione-like compound formed during biodegradation was identified.

Keywords: Benzo(a)pyrene; Photodegradation; Biodegradation; Degradation products; LC-DAD-MS analysis

1. Introduction

The term polycyclic aromatic hydrocarbons (PAH) generally refers to hydrocarbons containing two or more fused benzene rings in linear, angular or clustered arrangements [1]. Generally, PAH solubility and volatility decrease and hydrophobicity increases with an increase in number of fused benzene rings [2].

Most polycyclic aromatic hydrocarbons, especially those with four or more rings and their metabolites, have a variety of mutagenic and carcinogenic effects on microorganisms, plants and animals, and are classified as compounds with significant human health risk [3]. One such high molecular weight PAH is benzo[a]pyrene (BaP), a five-ring compound, which has been classified by the US Environmental Protection Agency (USEPA) as a priority pollutant: due to its known or suspected carcinogenicity, teratogenicity or acute toxicity.

BaP has a very low water solubility ($3.8\,\mu g\,l^{-1}$) and vapour pressure (5.0×10^{-7} mm Hg at 20 °C), and a high octanol/water partitioning coefficient (6.04), which suggests its preference to non-aqueous phases [4]. BaP is one of the most potent carcinogenic PAHs, and as such, is the most studied compound of the PAH class. Several reviews have been written on the risk assessment of BaP [5], as well as the metabolism and activation of BaP in mammalian systems [6]. Numerous studies have indicated that one, two, and three-ring compounds are acutely toxic [1], while higher molecular weight PAHs, including BaP are considered to be genotoxic [7–11].

The dominant processes for the successful removal and elimination of PAHs from the environment, except volatilisation, photo-oxidation, chemical oxidation, bioaccumulation and adsorption on sediment particles, are microbiological transformation and degradation [2]. Although microbial degradation is considered the main elimination process in the environment [12], photochemical reactions are also responsible for removing PAHs from water and air [13,14]. Photolysis is an important transformation pathway for most PAHs in the environment. The photolysis rate of selected PAHs in natural water body is relatively fast [15–18]. In organic solvents, the photolysis rates of PAHs are also found to be rapid [19]. Oxidation products of PAHs, produced by photo-oxidation and microbial degradation, are more water soluble than parent PAHs [14].

PAHs and its metabolites in remote continental environments have essentially been studied in sediments [20–24];

* Corresponding author. Tel.: +48-58-347-2310; fax: +48-58-347-2694.
E-mail address: chemanal@pg.gda.pl (J. Namieśnik).

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2004.05.023

their presence in waters has received considerably less attention.

Relatively little is known about the degradation of PAHs in organic solvents.

The main purpose of this work was to recognise the rate of degradation of BaP in different media. The effect of various parameters, including light responsible for the photodegradation, temperature, time and naturally occurring bacterial flora blamed for biodegradation, on stability of BaP in various solvents and water has been investigated.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene (purity >99%) was obtained from Sigma-Aldrich (Seelze, Germany). A stock solution for the standard was prepared at the concentration of $1\,mg\,ml^{-1}$ (weight/volume). For quantitation the calibration curve was determined using working solutions of concentrations 0.1, 0.25, 0.5, 1, 2.5 and $5.0\,\mu g\,ml^{-1}$.

Methanol, acetonitryle and hexane all HPLC-gradient grade were obtained from Merck (Darmstadt, Germany), ultrapure water was provided by Milli-Q water purification system (Millipore, Bedford, MA, USA). Dichloromethane and cyclohexane (purity > 99.5%) were also obtained from Merck.

Borosilicate glassware (Merck) used in experiments was washed in chromic acid cleaning mixture for 12 h, then rinsed with deionised water, methanol, and acetone and dried at 110 °C for 3 h.

2.2. BaP-stock-solutions

A set of dilution of BaP solution at the concentration of $5\,mg\,l^{-1}$ was made in the following solvents: methanol, acetonitrile, hexane, cyclohexane and dichloromethane.

Benzo(a)pyrene standard solutions, prepared in the above solvents, were stored in the laboratory at the room temperature 20 ± 2 °C full exposed to sunlight (i.e. in glass containers fully transparent to light).

It is well known, that sunlight reaching the Earth has a wavelength $\lambda \geq 290$ nm. Because of their aromatic structure, PAHs strongly absorb ultraviolet part of natural sunlight in UV-A (λ = 320–400 nm) and UV-B (λ = 290–320 nm) ranges [25].

Different types of water samples were used to carry out degradation studies. All of them were collected from the Gulf of Gdañsk region and they consisted of seawater (pH 7.2, conductivity = $17\,mS\,cm^{-1}$, TOC = $2.42\,mg\,l^{-1}$) from the Southern Baltic, river water (pH 7.4, conductivity = $0.75\,mS\,cm^{-1}$, TOC = $5.50\,mg\,l^{-1}$) from the Vistula River at 30 km from Gdansk and pond water (pH 6.9, conductivity = $1\,mS\,cm^{-1}$, TOC = $7.81\,mg\,l^{-1}$). All these samples were spiked at the final concentration of $1\,\mu g\,l^{-1}$ BaP, and they were stored in amber glass flasks, at 20 ± 2 °C.

2.3. Sample preparation of BaP

Solutions prepared in methanol and acetonitrile were analysed directly. In case of solutions in hexane, cyclohexane and dichloromethane, solvent was completely evaporated under the gentle stream of nitrogen, and the remaining solids were dissolved in the volume of methanol equal to the volume of an original solvent. The solutions prepared in this way were injected onto a HPLC column.

In case of spiked natural water samples, every several days, samples of precisely 100 ml volume each were extracted with three portions of dichloromethane (30 ml each) in the extractor for liquid–liquid extractions. Each sample was vigorously shaken for 10 min each time. The extracts were pooled and dried with anhydrous sodium sulphate; excess of solvent was evaporated in a rotational evaporator to a volume of ca. 1–3 ml and then under the gentle stream of nitrogen. The solid residue was re-dissolved in 1 ml of methanol, sonicated and filtrated through 0.45 μm PTFE filters and then analysed by liquid chromatography.

The average recovery of BaP from each liquid medium with relative standard deviations R.S.D. (n = 7) are presented in Table 1.

2.4. Analytical methods

2.4.1. LC-DAD

The chromatographic experiments were performed using high performance liquid chromatograph Agilent 1100 Series equipped with diode array detector, mass spectrometric detector, a column oven, a binary pump and autosampler. A 125 mm × 4 mm LiChrospher RP-18e analytical column from Merck (Darmstad, Germany) was used for separation. The injection volume was 20 μl, flow rate $0.8\,ml\,min^{-1}$, UV detector wavelength 295 nm and column oven temperature 25 °C. The compounds were eluted using a methanol–water linear elution gradient program at: initial 80% methanol; a linear increase to 100% methanol in 11 min; hold for 4 min. Quantitation limits for benzo(a)pyrene was found to be $0.07\,\mu g\,ml^{-1}$.

Table 1
The calculated values of average recovery of BaP and relative standard deviations, R.S.D. (n = 7)

Liquid medium	Recovery (%)	R.S.D. (%)
Seawater	94	5.8
River water	90	4.7
Pond water	91	5.3
Methanol	86	3.0
Acetonitrile	86	3.0
Dichloromethane	80	8.1
Hexane	84	7.3
Cyclohexane	86	9.2

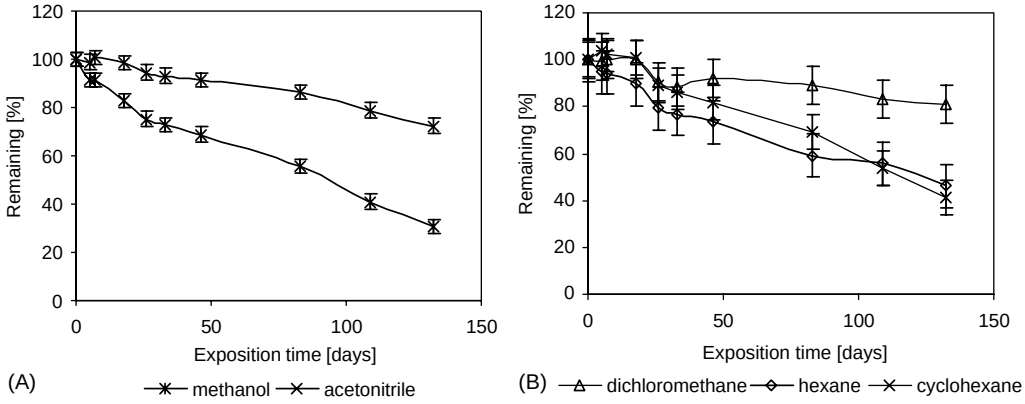

Fig. 1. Photodegradation of benzo(a)pyrene in different solvents: (A) methanol and acetonitrile; (B) dichloromethane, hexane and cyclohexane.

2.4.2. LC-APCI-MS

An essential tool for the identification of the BaP metabolites were, in addition to the retention times and UV spectra, APCI-mass spectra. The parameters for the APCI interface were optimized as described elsewhere [26,27]. The measurements were performed in the positive ionization mode with 120 eV fragmentor voltage, a 450 °C vaporiser temperature, 350 °C drying gas temperatures, 50 psig (1 psi = 6894.76 Pa) drying gas pressure, 6 l min^{-1} drying gas flow rate, 6 μA corona current and 4000 V capillary voltage. Separations were performed using a 250 mm × 4 mm Nucleosil 100-5 C6H5 column (Mecherey-Nagel, Germany) with the following elution gradient programme: 50:50 (v/v) methanol–water for 2 min, to methanol–water 65:35 (v/v) in 10 min, to methanol–water 80:20 (v/v) in 11 min, hold for 12 min, then back to initial conditions and equilibration for 5 min. Using a 125 mm × 4 mm LiChrospher RP-18e column (Merck, Germany) the following elution gradient program was applied: 80:20 (v/v) methanol–water; a linear increase to 82% methanol between 4 and 6 min; linear increase to 92% between 6 and 9 min; linear increase to 100% methanol; hold for 5 min, then back to initial conditions and equilibration for 5 min. In both cases, a flow rate was 1 ml min^{-1} and temperature 25 °C.

2.5. Calculations of kinetics

Kinetics of BaP degradation were calculated using the first-order equation:

$$\ln \frac{C_0}{C} = kt \quad (1)$$

where C_0 and C are the BaP concentration at times zero and t, respectively, and k the rate constant.

The results were plotted using the natural logarithm of the BaP concentration as a function of time. First-order degradation rate constants were determined by regression analysis. Half-lives $t_{1/2}$ were calculated using Eq. (2) which was derived from Eq. (1) by replacing C with $C_0/2$:

$$t_{1/2} = \ln \frac{2}{k} \quad (2)$$

3. Results and discussion

3.1. Photodegradation of benzo(a)pyrene

Many studies have been performed on photolysis of individual PAHs in natural waters under irradiation. Zepp and Schlotzhauer [15] found that the photolysis rates of selected PAHs in natural water body were quite fast and the photolysis half-lives of PAHs ranged from several minutes to several hours. Mill [16] determined that the photolysis half-lives for benzo(a)pyrene and benzo(a)anthracene in water under sunlight irradiation were 0.69 and 5.0 h, respectively. However, relatively little is know of photodegradation of PAHs when they are not irradiated and present in pure solvents.

Degradation of BaP in selected organic solvents was monitored for more than 3 months. Fig. 1 presents the dependence of concentration of non-degraded BaP on organic solvent. For the first month, the concentration of BaP remained constant in all the solvents used. After that time degradation started: in dichloromethane and acetonitrile degradation rates seem to be the slowest. After 100 days of exposition, 60% of BaP was degraded in methanol; while closely 50% in hexane and cyclohexane.

Degradation (%) decreased in the following order: dichloromethane > acetonitrile > hexane ≥ cyclohexane > methanol. Probably this order may be strongly related to the solubility of oxygen in a solvent, and general rule and measured values [28], the content of oxygen in dichloromethane is the largest and decreases in the order of acetonitrile, methanol and water. This indirectly means that the solubility of oxygen decreases with increasing reactivity of a selected solvent. The concentration of BaP decreased with time (Fig. 1A and B). The data fitted to Eq. (1) followed well the first-order rate equation (Fig. 2).

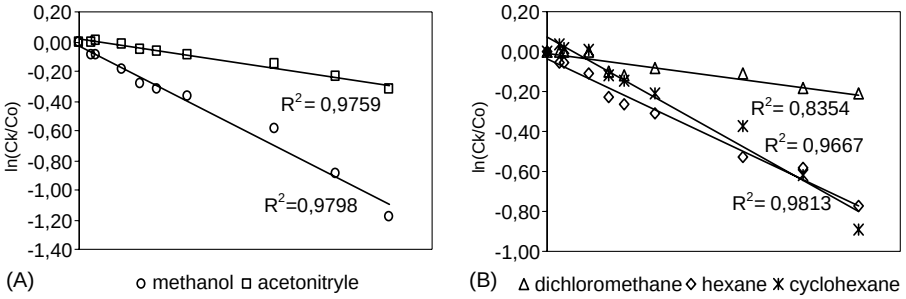

Fig. 2. The first order reaction kinetics for photodegradation of benzo(a)pyrene in different solvents.

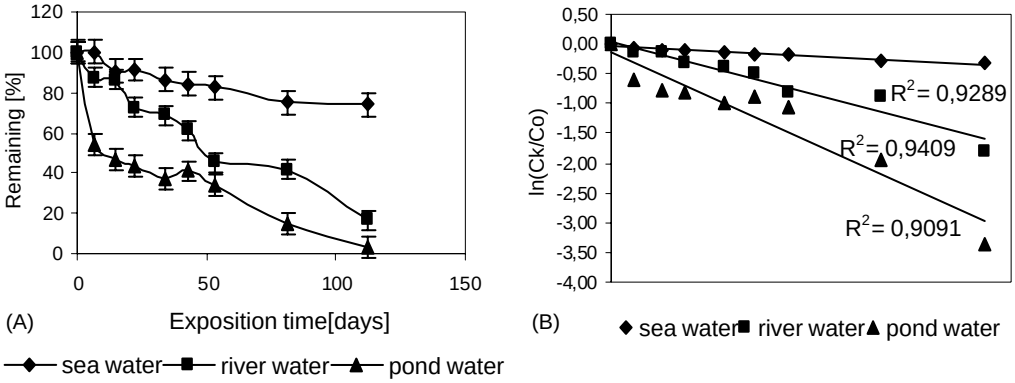

Fig. 3. Dependence of benzo(a)pyrene degradation on time for different type of water (A) and the first order reaction kinetics (B).

Rate constants and half-lives obtained for photodegradation of BaP in organic solvents are listed in Table 2.

3.2. Biodegradation of benzo(a)pyrene

The degradation of BaP is consistent with the ability of microorganisms to utilise the compound as a sole carbon source for growth. Little is known about the bacterial oxidation of BaP. To date, no bacteria have been isolated with the ability to utilise BaP as a growth substrate. However, liquid culture experiments have shown that bacteria can degrade BaP when grown on an alternative carbon source [29]. Ye et al. [30] observed a decrease in BaP concentration during incubations with *Sphingomonas paucimobilis* strain EPA 505.

BaP has also been reported to be degraded by other bacteria including *Rhodococcus* sp. strain UW1, *Burkholderia cepacia* strains, *Mycobacterium* strains, *S. maltophilia* strains, as well as a mixed culture containing *Pseudomonas* and *Flavobacterium* species [28,31–34].

Table 2
Benzo(a)pyrene photodegradation rate constants (k) and half-lives ($t_{1/2}$) for various organic solvents calculated on the basis of analytical data

Organic solvent	Polarity	k (1 per day)	$t_{1/2}$ (days)
Methanol	6.6	0.0081	85.6
Acetonitrile	6.2	0.0023	301.4
Dichloromethane	3.4	0.0016	433.2
Hexane	0.06	0.0055	126
Cyclohexane	0	0.0066	105

In natural waters, biodegradation of PAHs is enhanced by photodegradation, especially within surface and sub-surface layer (up to 2 m for transparent waters) [35]. In order to monitor BaP biodegradation naturally present in the environment for different types of natural waters were performed. The BaP concentration in sea, river and pond waters has been monitored within 4 months, and the results are shown in Fig. 3A.

The results show that the BaP concentration in pond water decreased drastically within first five days, from 1.0 $\mu g\,ml^{-1}$ (100% content) at time zero to 0.53 $\mu g\,ml^{-1}$ at 22nd day (50%). This finding can suggest that BaP was rapidly removed from water not only by biodegradation enhanced by photo-oxidation but also by accumulation on particles and glassware. According to the literature data around 30–40% of BaP can be adsorbed on the glass walls [36]. Compound concentration in seawater decreased very slowly from 1.06 (100% content) to 0.85 $\mu g\,ml^{-1}$ (80% of original content) in 90th day. This suggests, that decrease in BaP concentration in natural water bodies is mainly due to the chemical and biological processes (degradation), not physical one (accumulation, sedimentation, adsorption). This hypothesis has been supported by the results of chromatographic analysis of the degradation products, which appeared as “new” peaks on chromatograms. The degradation rate of BaP in the natural waters following in the order: pond water > river water > seawater.

Table 3
Benzo(a)pyrene biodegradation rate constants (k) and half-lives ($t_{1/2}$) for different aqueous media calculated on the basis of analytical data

Sample	k (1 per day)	$t_{1/2}$ (days)
Seawater	0.0026	266.6
River water	0.0147	47.1
Pond water	0.0252	27.5

In seawater samples from the Gulf of Gdansk (an example of seawater with very low salinity) the BaP degradation started later in comparison with pond water and the rate of degradation was significantly slower. Most probably, it is so because microbial flora from the sea environment has been continuously exposed to aromatic hydrocarbons emitted from different sources, such as petroleum refinery, ship transport, ferryboat.

The decrease in the BaP concentration in waters fitted well the first order reaction kinetics (Fig. 3B) the corresponding rate constants and half-live times are summarised in Table 3.

3.3. Degradation products

Photodegradation of BaP performed in different organic solvents fully exposed to sunlight and in the presence of oxygen (conditions as close to the naturally occurring as possible) resulted in additional peaks on chromatograms of solutions exposed for 2 months. The analysis of BaP solution in organic solvent by means of HPLC-DAD and HPLC-MS revealed a number of BaP degradation products as seen in Fig. 4A and B.

In case of peak 1 maximum absorbance in UV spectrum and MS spectrum pattern suggest that BaP-4,5-dihydrodiol has been detected. MS and UV spectra of metabolite 1 were identical with the spectra of the reference BaP-4,5-dihydrodiol published elsewhere [27]. The parent ion m/z 286 undergoes immediate fragmentation [loss of H_2] giving ion m/z 283; further loss of CO [$M^+ - 28$] results in ion m/z 255. Other two further protonated fragments are ions of m/z 241 and m/z 239, both probably derived from the protonated molecule and the molecule after loss of two hydrogen atoms. Reaction scheme for fragmentation of compound 1 identified as BaP-4,5-dihydrodiol has been shown in Fig. 5A.

Peak 2 gives the base ion of m/z 299 and fragment ions at m/z 255 [$M^+ - 44$], m/z 267 [$M^+ - 32$] and m/z 241 [$M^+ - 28$], representing probable losses of CO_2, 2O and CO, respectively. Fig. 5B shows the proposed structure and MS fragmentation for metabolite 2, which can be identified as

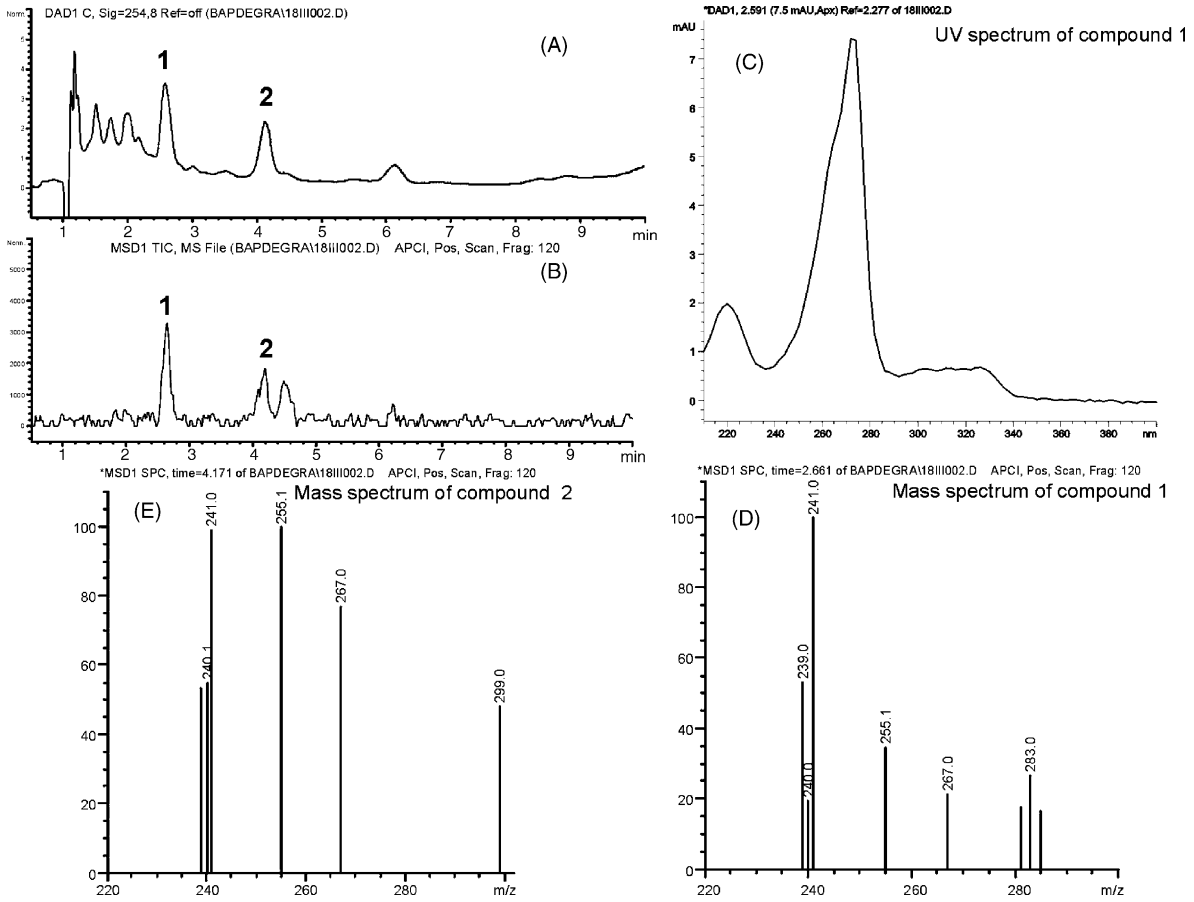

Fig. 4. HPLC chromatogram of BaP solution in cyclohexane after 2 months exposition: (A) DAD response monitored at 254 nm; (B) APCI-MS (SCAN mode); (C) UV spectrum of compound 1; (D) MS spectrum of compound 1; (E) MS spectrum of compound 2.

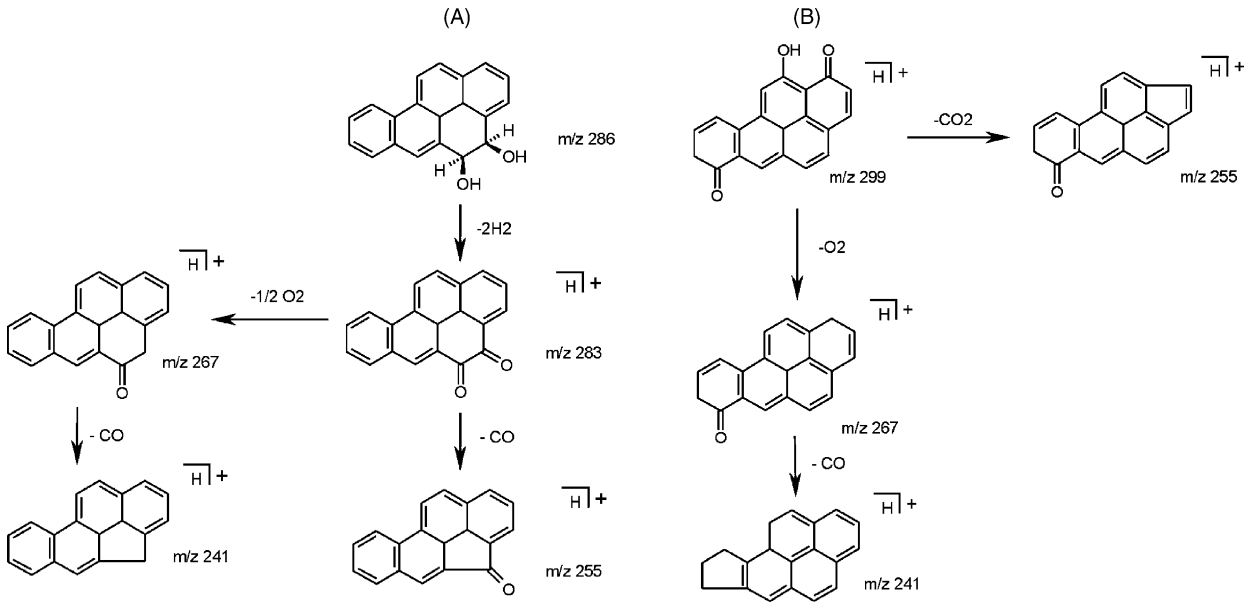

Fig. 5. Reaction scheme for fragmentation of the metabolites identified as BaP-4,5-dihydrodiol (A) and 2-hydroxy-BaP-1,6-dione (B).

2-hydroxy-BaP-1,6-dione ($C_{20}H_{10}O_3$; $M = 298\,g\,mol^{-1}$). In addition, other isomers are probable to be formed.

In case of water samples, where both processes (oxidation and biodegradation) proceed simultaneously, another compound was identified. Chromatogram obtained in HPLC-APCI-MS analysis (Fig. 6A) illustrates the fragmentation of compound 3 after 43 days of BaP degradation in river water; it is characterised by MS spectrum with a molecular ion [M + H] at *m*/*z* 283 and fragment ions at *m*/*z* 255 and *m*/*z* 253. Fragment ion at *m*/*z* 255 represents probable loss of CO [$M^+ - 28$] from the parent ion. This mass spectral fragmentation pattern (Fig. 6B) suggests that this metabolite could be BaP-dione-like compound, for example, BaP-7,10-dione, BaP-1,6-dione, BaP-3,6-dione, or BaP-6,12-dione [27]. All BaP-dione compounds have the same molecular mass ($C_{20}H_{10}O_2$; $M = 282\,g\,mol^{-1}$) and all give the same MS ions of *m*/*z* 283 (the highest intensity) and *m*/*z* 255.

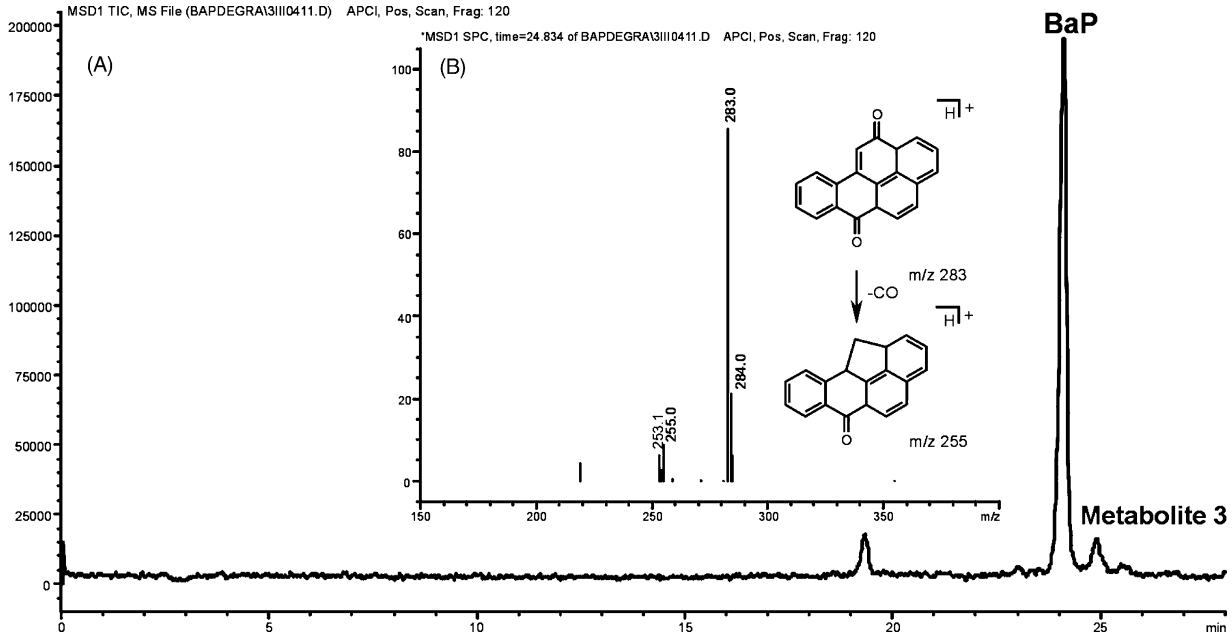

Fig. 6. HPLC-APCI-MS chromatogram an extract from river water after 43 days of exposition and MS spectrum of metabolite 3 identified as BaP-dione compound.

4. Conclusion

Photodegradation and biodegradation are major degradation processes which can naturally clean up the environment. Photodegradation of PAHs, which is assumed a preliminary process after which the microbial decomposition of compounds occurs, causes the formation of partially oxidised intermediates that are more susceptible to biodegradation than the parent compounds. In the case of natural waters, the degradation rate decreased much faster for pond water and river water than for seawater, where the degradation rate is the smallest, thus half-life times are high and remain in the range of couple of hundreds.

Quinones, ketones and alcohols are major intermediates in the environmental oxidation of PAHs. The dihydrodiols may undergo further oxidative metabolism. A number of unidentified metabolites are formed from the 4,5-dihydrodiol, and the 9,10-dihydrodiol can be oxidised to its 1- and/or 3-phenol derivatives. The principal route of oxidative metabolism of B(a)P-7,8-dihydrodiol is formation of the B(a)P-7,8-dihydrodiol-9,10 epoxide, which has been implicated regarded as the most important reactive metabolite of B(a)P for its mutagenic and carcinogenic properties.

Based on these data, monitoring of the natural waters pollution can be performed and analysis of results can help in the estimation of either the present state of the water quality (benzo(a)pyrene determination) or the history of the pollution with benzo(a)pyrene (benzo(a)pyrene's degradation products).

Acknowledgements

This study was partially financed within the framework of a research project founded by the Polish Committee for the Scientific Research in Warsaw (research project #4 T09A 083 25).

References

[1] R.C. Sims, M.R. Overcash, Residue Rev. 88 (1983) 1.
[2] S.C. Wilson, K.C. Jones, Environ. Pollut. 81 (1993) 229.
[3] D.F. Kalf, T. Crommentuijn, E.J. van de Plassche, Ecotoxicol. Environ. Saf. 36 (1997) 89.
[4] E. Manoli, C. Samara, Trends Anal. Chem. 18 (6) (1999) 417.
[5] J.F. Collins, J.P. Brown, S.V. Dawson, M.A. Marty, Regul. Toxicol. Pharm. 13 (1991) 170.
[6] C.S. Cooper, P.L. Grover, P. Sims, Prog. Drug Metabol. 7 (1983) 295.
[7] W. Lijinsky, Mutat. Res. 259 (1991) 251.
[8] V. Mersch-Sundermann, S. Mochayedi, S. Kevekordes, Mutat. Res. 278 (1992) 1.
[9] L. Nylund, P. Heikkila, M. Hameila, L. Pyy, K. Linnainmaa, M. Sorsa, Mutat. Res. 265 (1992) 223.
[10] D.H. Phillips, Nature 303 (1983) 472.
[11] A.L. Juhasz, R. Naidu, Int. Biodeterior. Biodegrad. 45 (2000) 57.
[12] S. Saftic, P.M. Fedorak, J.T. Andersson, Environ. Sci. Technol. 26 (1992) 1759.
[13] H.P. Hansen, Mar. Chem. 3 (1975) 183.
[14] D.E. Nicodem, M. Conceicao, Z. Fernandez, C.L.B. Guedes, R. Correa, Biogeochemistry 39 (1997) 121.
[15] R.G. Zepp, P.F. Schlotzhauer, Photoreactivity of selected aromatic hydrocarbons in water, in: P.R. Jones, P. Leber (Eds.), Polynuclear Aromatic Hydrocarbons, Ann Arbor Science Publishers, Ann Arbor, MI, 1979, pp. 141–158.
[16] T. Mill, W.R. Mabey, B.Y. Lan, A. Baraze, Chemosphere 10 (1981) 1281.
[17] K.M. Lehto, E. Vuorimaa, H. Lemmetyinen, J. Photochem. Photobiol. A Chem. 136 (2000) 53.
[18] J.S. Miller, D. Olejnik, Water Res. 35 (2001) 233.
[19] G.K. Low, G.B. Batley, C.I. Brockbank, J. Chromatogr. A 392 (1987) 199.
[20] B. McVeety, R.A. Hites, Atmos. Environ. 22 (1988) 511.
[21] E.T. Furlong, L.R. Cessar, R.A. Hites, Geochim. Cosmoch. Acta 51 (1987) 2965.
[22] W.J. Catallo, M. Schlenker, R.P. Gambrell, B.S. Shane, Environ. Sci. Technol. 29 (1995) 1436.
[23] P. Fernandez, R.M. Vilanova, J.O. Grimalt, Environ. Sci. Technol. 33 (1999) 3716.
[24] P. Fernandez, R.M. Vilanova, C. Martinez, P. Appleby, J.O. Grimalt, Environ. Sci. Technol. 34 (2000) 1906.
[25] J.L. Newsted, J.P. Giesy, Environ. Toxicol. Chem. 6 (1987) 445.
[26] T. Letzel, U. Pöschl, E. Rosenberg, M. Grasserbauer, R. Niessner, Rapid Commun. Mass Spectrom. 13 (1999) 2456.
[27] T. Letzel, E. Roseberg, R. Wissiack, M. Grasserbauer, R. Niessner, J. Chromatogr. A 855 (1999) 501.
[28] L. Moeini-Nombel, S. Matsuzawa, J. Photochem. Photobiol. A 119 (1) (1998) 15.
[29] J. Schneider, R. Grosser, K. Jayasimhulu, W. Xue, D. Warshawsky, Appl. Environ. Microbiol. 62 (1) (1996) 13.
[30] D. Ye, M.A. Siddiqi, A.E. Maccubbin, S. Kumar, H.C. Sikka, Environ. Sci. Technol. 30 (1996) 136.
[31] U. Walter, M. Beyer, J. Klein, H.J. Rehm, Appl. Microbiol. Biotechnol. 34 (1991) 671.
[32] D. Trzesicka-Mlynarz, O.P. Ward, Can. J. Microbiol. 41 (1995) 470.
[33] A.L. Juhasz, M.L. Britz, G.A. Stanley, Biotechnol. Lett. 18 (1996) 577.
[34] A.L. Juhasz, M.L. Britz, G.A. Stanley, J. Appl. Microbiol. 83 (1997) 189.
[35] S. Sinkkonen, J. Paasivirta, Chemosphere 40 (2000) 943.
[36] J.C. Bowman, J.L. Zhou, J.W. Readman, J. Environ. Monit. 4 (2002) 761.