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Bioconcentration of Benzo(a)pyrene in *Chlorella BB* Cells

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Abstract

BCF_1 (bioconcentration factor after the one day of exposition) value of BaP [benzo(a)pyrene] in strain A-8, *Chlorella vulgaris* Beijerinck 1890 *(Chlorella BB)* was studied. The algae were cultured in a modified Kiihl-Lorentzen liquid medium at room temperature, under continuous illumination and stirring for 24 hours.

The final average value of BCF, equalled 2100 ± 140 for BaP initial concentrations in the range from 0.005 to 0.5 μg/cm^3 in the culture medium. Statistical analysis of the results revealed independence of the bioconcentration factor from initial concentration of BaP. The BaP amount determined in the samples after the experiment differed markedly from the initial amount introduced to the mediums.

Keywords: bioconcentration, benzo(a)pyrene, *Chlorella vulgaris,* algae.

Introduction

Benzo(a)pyrene (BaP), like other Polycyclic Aromatic Hydrocarbons (PAHs), shows the ability to accumulate in plants, animals, soil, sediments of water reservoirs and in aquatic organisms [6, 11, 17, 19, 24]. The presence of detergents or liquid hydrocarbons able to solve BaP in sewage and even in surface water increases its penetration to the plant and animal cells, which explains its comparatively large concentration in food obtained from such water reservoirs, e.g. fish meat [1, 10, 14, 22].

Because of this accumulation BaP concentration increases as organisms take higher places in the food chain [1, 16]. The process of accumulation can be accompanied by biotransformation of BaP to mutagenic and carcinogenic derivatives [8, 15, 18, 22, 23].

Some aquatic organisms such as mollusks, mussels and snails, lacking the possibilities of BaP biotransformation, are especially inclined to cumulate this hydrocarbon as well as other PAHs [2, 23].

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The accumulation of environmental pollution in living organisms is estimated by the so-called bioconcentration or bioaccumulation factor (BCF, BF) or by the biological thickening coefficient [2, 9, 10]. The BCF in the aquatic environment is calculated as the ratio of the xenobiotic concentration in the organism or population to its concentration in the medium [7, 9, 17, 19]. The presence of other organisms or other polluting substances in the medium can change the value of BCF (e.g. humus lowers BCFs) [15, 18]. BCF also depends on the contents of lipids in living cells. A greater content of lipids in the organisms causes an increase of the bioconcentration factor for hydrophobic hydrocarbons and also increases the cytotoxic activity [7, 10].

BCF can be estimated on particular days of the experiment, e.g. after the first day of exposure as BCF], after the third day as BCF_3, etc [9], or in the stationary phase by the absorption and desorption processes of xenobiotic in the organism tested [9, 15, 18, 19]. Upon the kinetics of the process investigated, the average value in a given period of time is also estimated [15]. The values of BCF in aquatic environment given in Table 1, for a longer period of exposure (in each case) can reach

Table 1. Selected average values of BaP[1] BCF[2] in aquatic environment.

Example	BCF	Literature
water-fish	1110[3]	[10]
Trout	920	[22]
Canadian water-thyme	33 125-7 730[4]	[2]
Guppy	56 850-28 875[4]	[2]
Snails	206 925-28 066[4]	[2]
Daphnia magna	1000	[18]
Daphnia pulex	13 000	[1]
Leech (Gillichthys mirabilis)	0.048[5]	[14]
Flounder (Citharichthys stigmacus)	0.02[6]	[14]

[1] BaP - benzo(a)pyrene, [2] BCF - bioconcentration factor, [3] theoretical value, [4] for initial concentration of BaP 0.04 and 0.2 μ^dm^3, [5] after 96 hours of exposure,[6] after 1 hour of exposure.

even several hundred thousand. Values smaller than one might be the result of both too short exposure time and smaller inclinations of the organism to bioaccumulation.

The aim of this work was to estimate BCF value of BaP in *Chlorella vulgaris* cells in laboratory conditions, at BaP concentration comparable to its concentrations in the wide aquatic environment (in natural water or sewage [20, 25]).

Materials and Methods

Chlorella vulgaris Beijerinck 1890, strain A-8 *(Chlorella BB)* from the synchronous culture conducted at Department of Biochemistry and Biophysics Silesian Medical University were cultured in a liquid medium of Kiihl and Lorentzen modified by Bohm [4,13]. The algae cultures were grown at room temperature in Erlenmeyer flasks of 750 cm^3 in 200 cm^3 of medium, closed with corks on cut and additionaly tightened by teflon tape. They were inoculated with a proper amount of *Chlorella BB* cells from the mother culture equivalent to approximately 20 mg of dry alga biomass, or about 2.4 x 10^9 cells [9]. BaP acetonic solution (at a concentration of 0.01 % v/v) was added to the cultures to its final concentration in the medium of 0.005, 0.05, and 0.5 μg/cm^3. The cultures were grown for 24 hours under continuous illumination with heat-mercury lamp (4800 lx) and continuous stirring (magnetic stirrer). The cultures were centrifuged at 3.5 to 4.0 thousand rpm for 25 minutes (MPW-360 centrifuge). The cell pellet was rinsed three times by suspension in redistilled water and centrifugation (as previously). After

Table 2. Bioconcentration of benzo(a)pyrene in *Chlorella BB* cells in 24 hour culture.

Initial concentration of BaP in culture (c_o) [μg/cm^3] (number of cultures)	Wet alga biomass [g]	Content of BaP in wet biomass of alga [μg]	Concentration of BaP in wet biomass of alga (c_g) [μg/g]	Concentration of BaP in medium[1] (c_{24}) [μg/cm^3]	Bioconcentration factor (BCF_1)	**BCF_1 ± SD** (confidence interval)
0.005 (n=7)	0.2194	0.4072	1.856	0.00068	2729.37	**2090 ± 437** (1576 – 2605)
	0.3010	0.3870	1.286	0.00070	1838.03	
	0.2543	0.5789	2.276	0.00095	2401.31	
	0.2920	0.4348	1.489	0.00077	1946.41	
	0.2849	0.4877	1.712	0.00080	2145.15	
	0.2280	0.5287	1.880	0.00085	2214.72	
	0.3033	0.4962	1.636	0.00121	1357.11	
0.05 (n=9)	0.3068	5.200	16.949	0.01355	1250.86	**1840 ± 805** (1387 – 2294)
	0.2937	3.977	13.541	0.008496	1593.81	
	0.3040	3.850	12.665	0.01025	1235.61	
	0.2568	4.125	16.063	0.00439	3659.02	
	0.3025	3.892	12.866	0.008892	1446.92	
	0.2332	4.781	20.502	0.00826	2482.04	
	0.2481	3.328	13.414	0.01099	1220.56	
	0.3121	4.082	13.080	0.00839	1558.62	
	0.2285	3.797	16.595	0.00784	2116.73	
0.5 (n=6)	0.3851	9.619	24.980	0.01140	2191.26	**2540 ± 583** (1987 – 3099)
	0.3722	11.762	31.602	0.00938	3370.86	
	0.2568	6.934	27.003	0.00844	3201.27	
	0.2899	5.874	20.264	0.00995	2037.63	
	0.3671	8.767	23.881	0.01050	2275.43	
	0.2581	7.952	30.810	0.01412	2182.76	

[1] volume of medium 200 cm^3

the last centrifugation the medium was decanted (from the cell pellet) and the tube sides (when wet) were carefully dried with paper. After that, the weight (analytical balance) of the algal biomass was estimated as the difference between the weight of the tube with and without the algae. The algal pellet was carefully transferred to a mortar. The remains were rinsed by cold phosphate buffer 100 mM, pH 7.5, out to the mortar and after refrigeration in liquid nitrogen homogenized by stirring.

BaP was extracted with cyclohexane from the mixed supernatants (medium and water after washing) and from the alga homogenates. Its concentrations were determined on the base of standardisation curve spectrofluorometrically by measuring the light emission at the wavelength of λ=426 nm with the excitation wavelength of λ =396 nm. These wavelengths were determined experimentally and did not differ from literature data [12, 21]. The BaP concentrations were recalculated to 1 gram of wet mass of algae and to 1 cm^3 of medium. The bioconcentration factor was calculated after 24 hours (cul-♦■•■ '-'ration) with the following equation [9]:

$$BCF_1 = \frac{c_g}{c_{24}} \frac{[\mu g/g]}{[\mu g/cm^3]}$$

c_g - concentration of BaP in 1 g wet biomass of alga after 24-hour duration of the culture

c_{24} - concentration of BaP in 1 cm^3 medium after 24-hour duration of the culture.

For statistical processing of the results the analysis of variance (ANOVA) was used. Bartlett's and Fisher-Snedecor's tests were used to compare the variances of the groups of the data.

Results and Discussion

The cyclohexane extracts obtained from the culture medium and biomass of *Chlorella BB* cell were used to estimate the BCF_1. The extracts were characterized by typical fluorescence spectra according to the standard spectrum presented in our earlier work [12].

The results obtained are presented in Table 2. In spite of some little differences among the averages of the series connected with initial BaP concentrations (0.005 - 0.5 μg/cm^3), it turned out to be impossible to find their dependence on the concentration. Statistical analysis shows that the three groups of data (Tab. 2) are homogenous (F_c=2.108 < F_t=3.52 at p=0.05). Thus, there are no significant differences among the groups being compared (Tab. 2). The average value of BCF, calculated for all the concentrations taken together (not the average of the averages for different concentrations) equalled 2100 ± 140 and the confidence interval at the level of 0.05 was found to be 1821-2402.

After the experiment, the total content of BaP (Tab. 2) in 200 cm^3 medium and in alga biomass of *Chlorella BB* did not balance with the initial amount of BaP introduced to the medium. This imbalance shows a considerable disappearance of BaP over the period of culture growth. The quantities recovered from each culture were about 64, 59 and even only 11 % for initial concentrations of 0.005, 0.05 and 0.5 μg BaP/cm^3, respectively. This means that a part of BaP introduced into the culture accumulates in *Chlorella BB* cells, another part remains in the medium, and that about 36, 41 and 89% of its initial quantities undergos a biotransformation with the participation of alga cells, or maybe also other processes, e.g. photodegradation. The decrease of BaP observed was greater for its higher initial concentrations.

The BCF_1 values obtained in this work, characteristic for the process (in twenty-four hours), equalls 2100, while the theoretical values calculated for BaP (depending on the computational model, on physical and chemical properties of the xenobiotic, and first of all on octanol-water partition coefficient K_{OW}) were between 20,000 and 30,000 [5, 10, 22]. This calculated value does not include many important factors connected with real processes, e.g. cell wall chemical content. The biotransformation which was ascertained in this work is another factor not included in theoretical models [5, 10] and decreasing the amount of BaP inside alga cells is the most probable explanation of this difference.

Conclusions

1. BaP penetrates *Chlorella BB* cells and accumulates in them.
2. The bioconcentration observed is probably accom panied by biotransformation of the BaP.
3. The bioconcentration factor determined (equal to about 2100) is about ten-fold lower than the ones pre dicted from the octanol/water partition coefficient for BaP. This can be explained by biotransformation of BaP in *Chlorella BB* cells.

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