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Mariusz DUDZIAK[1]

INFLUENCE OF THE AQUATIC ENVIRONMENT CONDITIONS ON THE DECOMPOSITION OF BISPHENOL A

WPŁYW WARUNKÓW ŚRODOWISKA WODNEGO NA ROZKŁAD BISFENOLU A

Abstract: Bisphenol A is a compound used to produce plastics. Today, it is identified in the aquatic environment. As part of the work there are performed studies to determine the effect of the aquatic environment conditions on the decomposition of bisphenol A. As the subject of research there were used different aqueous solutions prepared on the basis of deionized or surface water with addition of a bisphenol A standard at concentration of 1 mg/dm^3. To the selected solutions it was added the mineral medium or surface water, which was the source of both organic materials and inorganic compounds and microorganisms. Optionally, the selected solutions had been kept in the dark or in the light of sun, and they had been aerated. Solutions after biodegradation were also subjected of the toxicological evaluation with application of the enzymatic test using bioluminescent bacteria *Aliivibrio fischeri,* survival test using shellfish *Daphnia magna* and the growth test of aquatic plant *Lemna minor*. It was determined that the decomposition of bisphenol A in an aquatic environment is low and it is mainly under the influence of sunlight, with the participation of microorganisms. The presence of mineral salts in aquatic environment is also important. on the other hand, the toxicological assessment of solutions, which was made during testing biodegradation, showed that they have a different toxicity. Toxicity class of the solution also depended on the type of applied indicator, which proves their differences in sensitivity to bisphenol A. High toxicity was noted in the case of bioluminescent bacteria *Aliivibrio fischeri* after 14 days of the biodegradation study.

Keywords: bisphenol A, aquatic environment, decomposition, solution toxicity, biodegradation

Introduction

In aquatic environment the organic micropollutants can occur both in midwater as well as in bottom sediments. In midwater they are partially adsorbed on organic matter (molecular organic carbon). Yamamoto and Liljestrand in their study [1] have determined that approximately 15 to 50% of micropollutants, which are present in surface water, are associated with high-molecular organic substances. For example, the

[1] Institute of Water and Wastewater Engineering, Silesian University of Technology, Konarskiego 18, 44-100 Gliwice, Poland, phone: +48 32 237 16 98, fax: +48 32 237 10 47; email: mariusz.dudziak@polsl.pl

percentage of estrogen binding with each fraction, which are characteristic of surface water with typical content of total organic carbon 5.0 mg/dm^3 ranged from 23 to 33% for humic acids and 15 to 18% for fulvic acids Adsorption of five different organic micropollutants, including 17β-estradiol, 17α-ethinylestradiol, bisphenol A, 4-tert-octylphenol and 4-nonylphenol on river sediments, was described by Ying et al. at work [2]. In their comparative study the authors proved, that along with lowering the polarity of compounds, increases their adsorption on river sediments. Adsorption of the biologically active substances on river sediments depends on water salinity [3].

We should also mention about the bioaccumulation of organic micropollutants in the tissues of organisms. This phenomenon affects the levels of their concentrations in aqueous media. Bioaccumulation is estimated on the basis of bioconcentration factor or bioaccumulation factor. The first of the presented factors can be determined on the basis of coefficient value of distribution of n-octanol and water ($\log K_{ow}$), which characterizes the hydrophobic properties of chemical compounds. Bioconcentration factor (given in logarithmic form) e.g. for bisphenol A amounts 1.86, and for 17β-estradiol amounts 2.39 [4]. This confirms that the content of these micropollutants is higher in the tissues of living organisms, than in aquatic environment.

Key phenomena, which determine the occurrence of micropollutants in the aquatic environment, consist of natural degradation and biodegradation [5–7].

Degradation (decomposition) of biologically active substances in an aquatic environment can take place under the influence of various physical factors (solar radiation, temperature and others), chemical (pH, presence of mineral salts, presence of toxic substances, and others), biological (presence of microorganisms, and others), or photochemical (UV radiation) [5]. In turn, we discuss the biodegradation when compound distribution is performed by microorganisms and enzymes, regardless of environmental conditions (aerobic, anaerobic) [6]. The main end products of decomposition of organic matter are simple natural compounds i.e. CO_2 and H_2O, which are common in the natural environment. However, in the reaction environment, by-products of decomposition [8] also occur earlier. Often such by-products of decomposition of biologically active substances show a much higher biological activity against microorganisms, plants, animals and humans, than the original compounds [9].

Bisphenol A (Fig. 1) is a compound used to produce plastics. Today, it is identified in the aquatic environment. This compound is introduced to the aquatic environment,

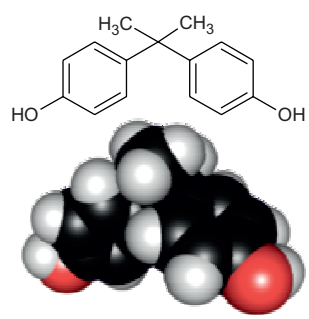

Fig. 1. Structure of bisphenol A

inter alia, as a result of inefficient removal this compound from the wastewater. It can also be secreted and washed out from plastics, which have been submerged in aquatic environment. Bisphenol A in outflows from sewage treatment plants is present in a wide concentration range from μg/dm^3 to even ng/dm^3 [10–11]. Concentration levels are lower in the surface water.

The aim of this study was to evaluate the effect of selected conditions of the aquatic environment on the decomposition of bisphenol A and the toxicity of solutions.

Materials and methods

Preparation of solutions and degradation experiments

Solutions used in the study were prepared on the basis of deionized water or surface water with addition of the standard bisphenol A of Sigma-Aldrich company. The compound concentration was 1 mg/dm^3. The composition of individual aqueous solutions and the degradation process conditions are shown in Table 1.

Table 1

The composition of aqueous solutions and the degradation process conditions

Solutions	Compositions	Degradation process conditions
I	deionized water + bisphenol A	sunlight
II	deionized water + bisphenol A	dark
III	deionized water + bisphenol A	sunlight + aeration
IV	deionized water + bisphenol A + mineral medium	sunlight
V	deionized water + bisphenol A + inoculation	sunlight
VI	deionized water + bisphenol A + mineral medium + + inoculation	sunlight
VIIs	surface water + bisphenol A	sunlight
VIIsn	surface water + bisphenol A	sunlight + aeration

The pump with a capacity of 0.25 cm^3 air per 1 hour was used for solutions aeration. Mineral medium, as a source of minerals, was made of 10 cm^3 solution of potassium dihydrogenorthophosphate (concentration of initial solution 8.50 g/dm^3), hydrogenorthophosphate potassium (21.75 g/dm^3), twelve-water sodium hydrogenorthophosphate (44.60 g/dm^3), and ammonium chloride (1.70 g/dm^3), and 1 cm^3 of solution of magnesium heptahydrate sulfate (22.50 g/dm^3), calcium chloride (27.50 g/dm^3), and hexahydrate iron chloride (III) (0.25 g/dm^3), which was then dissolved in 1 dm^3 of deionized water. Aqueous solutions V and VI were inoculated by volume of 10 cm^3 of surface water taken from the container located in Silesia Voivodship. Physico-chemical parameters of surface water, which is constituting the inoculation, are summarized in Table 2. Inoculation was the source of both organic and inorganic compounds and microorganisms potentially capable of degrading the organic substances.

Table 2

Physico-chemical parameters of surface water which is constituting the inoculation

Parameter	Unit	Value
pH	—	7.13
Temperature	°C	20
Conductivity	mS/cm	0.843
Absorbance (UV_{254})	1/cm	0.167
Total Organic Carbon	mg/dm^3	25.02

Analytical methods

For the measurement of the general parameters (pH and temperature) and the conductivity of solutions there was used laboratory multiparameter meter inoLab® 740 produced by WTW, Measurement and Analytical Equipment Technology. Absorbance was measured at 254 nm wave, using the UV-VIS Cecil 1000 of Analytik Jena AG company. Bisphenol A was marked by solid phase extraction (SPE) and the analysis of gas chromatography (GC). For extraction there were used the columns Supelclean™ ENVI-18 (volume of 6 cm^3, solid phase 1.0 g) from Supelco company. The stationary phase was conditioned with methanol (5 cm^3) and acetonitrile (5 cm^3), and then washed with deionized water (5 cm^3). The separated compound was eluted off with a mixture of acetonitrile and methanol (60 : 40, v/v) of volume of 3 cm^3. The qualitative and quantitative analysis of compound in extracts, after their previous concentration in a light stream of nitrogen, was performed using GC with FID detector of Young Lin Instrument company, equipped with a SLB™-5 ms capillary column (30 m × 0.25 mm × 0.25 μm) from Supelco company. The method included a splitless injection of 2 mm^3 of extract, an injector temperature of 270°C. Sample injections were performed manually with a micro syringe volume 10 mm^3 manufactured by Hamilton. The oven temperature program was: initial temperature of 55°C (hold 3 min), 30°C/min until 240°C and 2°C/min until 250°C (hold 0.8 min). A gaseous mixture of air (flow 300 cm^3/min), helium (20 cm^3/min) and hydrogen (30 cm^3/min) constituted a mobile phase. In the study there were used organic solvents of analytical grade of Avantor company.

Toxicity of solution and classification system

Toxicity of the tested solutions was evaluated on the base of selected tests results i.e. an enzyme Microtox® test, which is using luminescent strain of marine bacteria *Aliivibrio fischeri*, survival with shellfish *Daphnia magna* and the growth of duckweed *Lemna minor.*

The enzymatic Microtox® test is using luminescent strain of marine bacteria *Aliivibrio fischeri.* Bacteria exposure to toxic substances leads to changes in metabolic processes, that, at the same time, causes the variation of the intensity of the light emitted by the microorganisms [13]. Studies were conducted using the system MicrotoxOmni in analyzer Microtox Model 500 company Tigret acting as both an incubator and

photometer. After 5 and 15 minutes of exposure, bioluminescence inhibition percentage was determined in the relation of the control sample (2% NaCl).

In turn, the survival test of shellfish *Daphnia magna,* which was conducted in accordance with the Polish standard PN-EN ISO 6341:2013-04 [14], recording the organisms mortality after 24 and 48 hours of contact with the indicator solution. The test organisms were from own culture.

The growth test of duckweed *Lemna minor* was made according to the methodology [15] assuming the observation of its morphological changes, including an assessment of the amount of leaves before and after 7 days. In each tested solution there were placed several plants containing 2 leaves (fronde). Cultures were grown at constant light intensity of 3000 lux at 25°C. The test organisms were from own culture.

The effect of the toxicity (E) was determined according to equation:

$$E = 100 \cdot (E_b - E_t) / E_b \tag{1}$$

where: E_b – the effect observed in a blank sample;
E_t – the effect observed for a test sample.

Depending on the applied test, a measure of toxicity was the inhibition of bioluminescence *Aliivibrio fischeri* (Microtox® test), shellfish mortality *Daphnia magna* or inhibition of growth of water plants leaves *Lemna minor*.

For the classification of the toxicity it was applied the system, which is widely used by many researchers [16, 17] and which is based on the size of the observed effect induced in the body of the indicator organism (Table 3).

Table 3

Samples toxicity classification system [16, 17]

E [%]	Toxicity class
< 25	non toxic
25–50	low toxicity
50.1–75	toxic
75.1–100	high toxicity

Results and discussion

Bisphenol A biodegradation

Among the tested aqueous solutions and the applied conditions of performed biodegradation process, reduction in the concentration of bisphenol A was observed only in solution No. II, VI and VIIs (Fig. 2). However, changes in concentrations of the tested compound were very small and did not exceed 21%. The greatest reduction in the concentration was observed for the solution VI, which was consisted of the deionized water with addition of mineral medium and inoculation, which was exposed in the sunlight. The presence of inorganic salts in this solution, which are mineral medium for

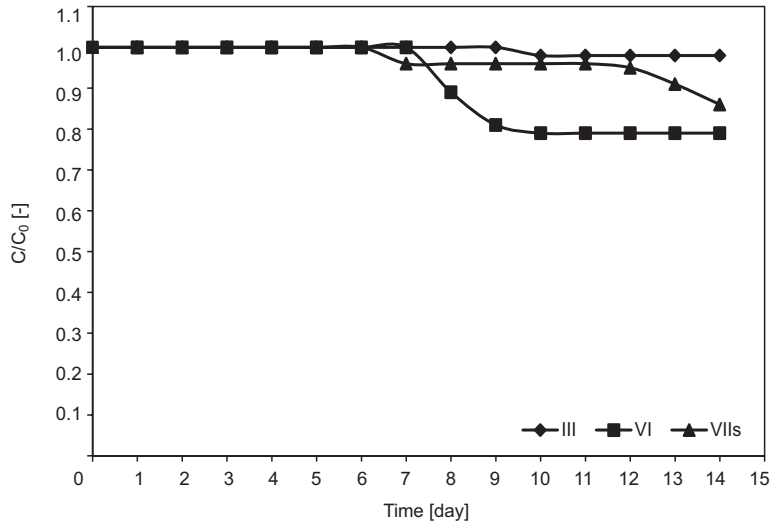

Fig. 2. The change of concentration of bisphenol A in the selected solutions during biodegradation experiments

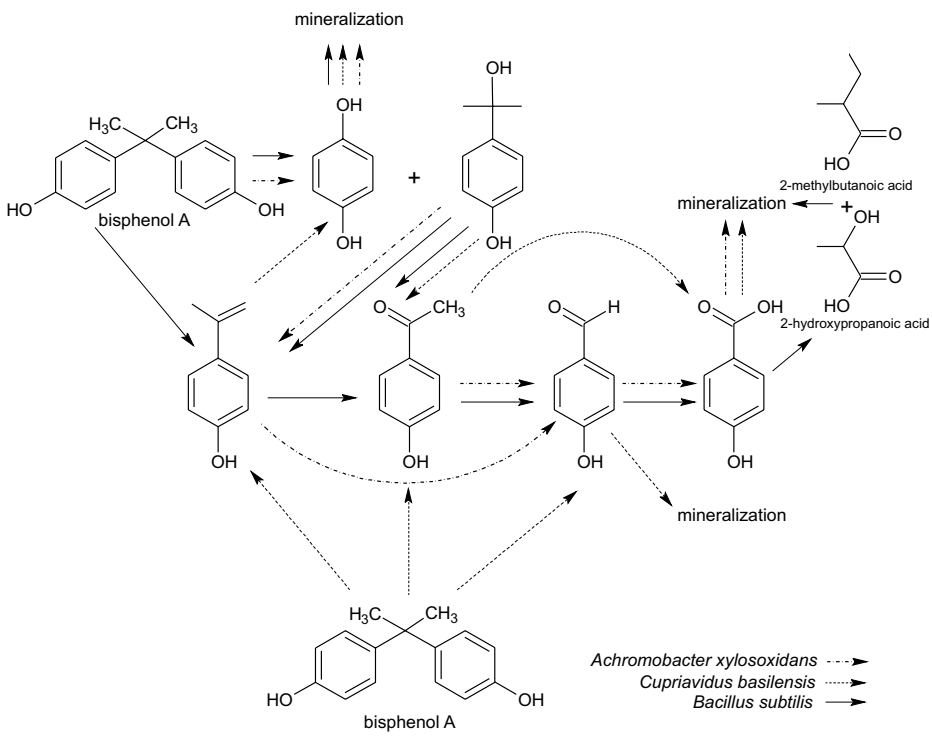

Fig. 3. The bisphenol A degradation pathways by *Achromobacter xylosoxidans*, *Cupriavidus basilensis* and *Bacillus subtilis* [19]

microorganisms introduced into the water with inoculation, influenced the intensification of metabolic processes of bacteria able to decomposite the bisphenol A.

Decomposition of micropollutants in the aquatic environment to a large extent depends on the presence of microorganisms. Herve in his work [18] documented the relationship between the distribution of pharmaceuticals selected from the group of antibiotics, and the presence in the reaction environment of each bacterial strain (*Rhodococcus rhodochrous, Aspergillus niger, Sphingomonas herbicidovorans, Bacillus subtillis, Pseudomonas fluorescens, Pseudomonas putida)*. Author stated, that for most of the decomposition of tested biologically active substances, are mainly capable bacteria *Rhodococcus rhodochrous.* In the case of the other bacterial strains, which were assessed in the author's work, the antibiotic degradation had not been observed. Bisphenol A degradation pathways proposed by Zhang et al. [19] *Achromobacter xylosoxidans, Cupriavidus basilensis and Bacillus subtilis* strains were described in Fig. 3.

Toxicity of solution

As it was mentioned in the work introduction, during the decomposition of the substances in the aqueous solution, there are also by-products (Fig. 4) of diverse biological activity. In order to assess this phenomenon it was compared the influence of aqueous solutions containing bisphenol A on a variety of indicator organisms. In this respect, the enzymatic tests were performed with bioluminescent bacteria *Aliivibrio fischeri,* survival with shellfish *Daphnia magna* and growth tests of water plant *Lemna minor.* The solution VI was evaluated in which the largest decrease in the concentration

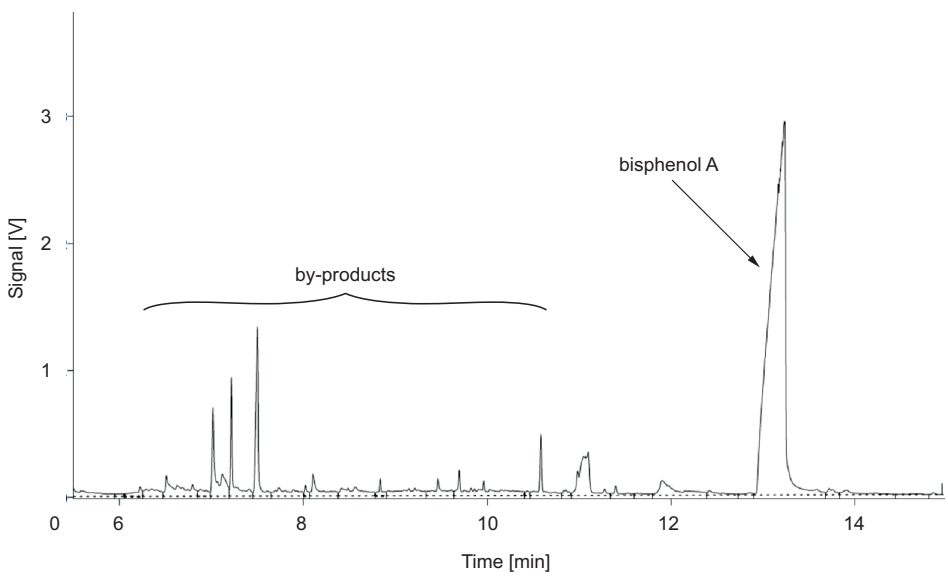

Fig. 4. Chromatogram of by-products obtained during bisphenol A biodegradation (1 mg/dm^3)

of the compound was recorded, in comparison to the other test solutions (Table 1). The obtained results are presented in Table 4.

Table 4

The influence of aqueous solutions containing bisphenol A on a variety of indicator organisms

Test/indicator organisms	Test time	Day of the biodegradation experiment*		
		0	7	14
		E [%] (toxicity class**)		
Enzymatic with *Aliivibrio fischeri*	5 min	10 (–)	51 (++)	58 (++)
	15 min	11 (–)	51 (++)	100 (+++)
Survival with *Daphnia magna*	24 h	0 (–)	40 (+)	50 (+)
	48 h	0 (–)	45 (+)	60 (++)
Growth with *Lemna minor*	7 days	0 (–)	25 (+)	50 (+)

* Aqueous solutions prepared on the basis of deionized water with addition mineral medium, inoculation and bisphenol A standard at concentration of 1 mg/dm^3; ** (–) non toxic, (+) low toxicity, (++) toxic, (+++) high toxicity.

The solution containing bisphenol A after the 7th day of the experiment was characterized by toxicity in respect to the bacteria *Aliivibrio fischeri,* wherein the sample obtained from 14th day and examined in a longer exposure time (15 min) was high toxic (Table 4). Observations related to shellfish *Daphnia magna* were similar, but

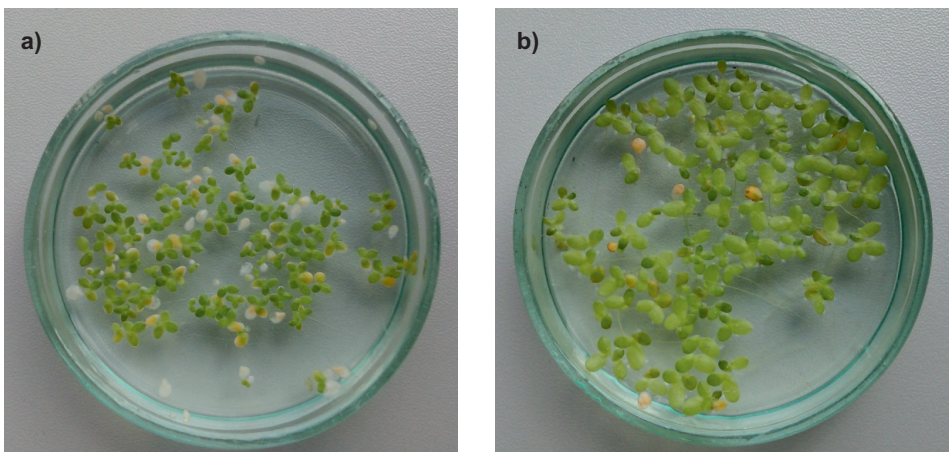

Fig. 5. The images of duckweed which had been contacting the solution containing bisphenol A: a) after 14 days of biodegradation and b) control sample

in this case the toxicity class was lower, wherein the duration of the test did not have much effect on the observed relationship. In the case of *Daphnia magna* test, in most the test sample solutions had been characterized by low toxicity. Low toxicity was also observed for water plant *Lemna minor* after 7^{th} and 14^{th} day of biodegradation research. However, analyzing the images showing duckweed (Fig. 5), which had been contacting the solution containing bisphenol A and a control sample, it can be seen, that the tested compound inhibited the growth of plants. In this case the leaves of plants were very small. It was also the phenomenon of mycorrhiza.

Conclusions

1. It was determined, that the decomposition of bisphenol A in an aqueous environment depends on the water environment conditions. In most of tested solutions in the relevant biodegradable time (14 days), decomposition of the compound was not observed. Low decomposition of bisphenol A (below 21%) was observed in a solution exposed to sunlight, to which the mineral medium, as a source of minerals, was added, and which was inoculed by microorganisms.
2. The toxicological assessment of solutions, which was made during testing biodegradation, showed that they have a different toxicity. Toxicity class of the solution also depended on the type of applied indicator organism, (bioluminescent bacteria *Aliivibrio fischeri.* shellfish *Daphnia magna,* water plant *Lemna minor)* which proves their differences in sensitivity to bisphenol A. High toxicity was noted in the case of bioluminescent bacteria after 14 days of the biodegradation study. In turn, the sensitivity of shellfish and water plants on bisphenol A was comparable.

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WPŁYW WARUNKÓW ŚRODOWISKA WODNEGO NA ROZKŁAD BISFENOLU A

[1] Instytut Inżynierii Wody i Ścieków
Politechnika Śląska, Gliwice

Abstrakt: Bisfenol A to związek chemiczny stosowany do produkcji tworzyw sztucznych. Współcześnie identyfikowany jest on w środowisku wodnym. W ramach pracy podjęto badania dotyczące oceny wpływu warunków środowiska wodnego na rozkład bisfenolu A. Przedmiot badań stanowiły różne roztwory wodne sporządzone na bazie wody zdejonizowanej lub powierzchniowej z dodatkiem wzorca bisfenolu A w stężeniu 1 mg/dm^3. Do wybranych roztworów dodawano pożywkę mineralną lub wodę powierzchniową, która to stanowiła źródło zarówno substancji organicznych, jak i nieorganicznych oraz mikroorganizmów. Opcjonalnie wybrane roztwory były przetrzymywane w ciemni lub w świetle słonecznym oraz napowietrzane. Roztwory po biodegradacji poddano również ocenie toksykologicznej z użyciem testu enzymatycznego z bakteriami bioluminescencyjnymi *Aliivibrio fischeri,* testu przeżywalności ze skorupiakami *Daphnia magna* oraz testu wzrostowego z rośliną wodną *Lemna minor*. Określono, że rozkład bisfenolu A w środowisku wodnym jest niewielki i zachodzi głównie pod wpływem światła słonecznego przy udziale mikroorganizmów. Istotna jest również obecność w środowisku wodnym soli mineralnych. Natomiast dokonana ocena toksykologiczna roztworów podczas badań biodegradacyjnych wykazała, że charakteryzują się one różną toksycznością. Klasa toksyczności roztworu zależała także od rodzaju użytego organizmu wskaźnikowego, co dowodzi o ich różnej wrażliwości na działanie bisfenolu A. Wysoką toksyczność odnotowano w przypadku bakterii bioluminescencyjnymi *Aliivibrio fischeri* po 14 dobach trwania badań biodegradacyjnych.

Słowa kluczowe: bisfenol A, środowisko wodne, rozkład, toksyczność roztworu, biodegradacja