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MICROTOX AS A TOOL TO EVALUATE UNFAVOURABLE PHENOMENON OCCURENCES DURING MICROPOLLUTANTS DECOMPOSITIONS IN AOPS

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Abstract

Water containing bisphenol A (concentration 500 μg/dm^3) was subjected to the UV irradiation (medium-pressure immersion lamp with an electric power of 150 W) with and without the addition of hydrogen peroxide H_2O_2 (dose 9 mgH_2O_2/dm^3), ozone (1 mg/dm^3) or titanium dioxide TiO_2 (100 mg dm^3). The degree of degradation of the studied xenobiotic was assessed by the chromatographic analysis. The bioluminescence inhibition was determined by the Microtox® bioassay and was a basis for toxicological assessment of the water samples. The study demonstrated the usefulness of the bioassay in assessing the effectiveness of advanced oxidation processes (AOPs), in particular, in terms of hazardous phenomena, such as the formation of toxic by-products of the decomposition of the removed pollutants.

Streszczenie

Wodę zawierającą bisfenol A (stężenie 500 μg/dm^3) poddano napromieniowaniu UV (zanurzeniowa lampa średniociśnieniowa o mocy elektrycznej 150 W) bez i z dodatkiem nadtlenku wodoru H_2O_2 (dawka 9 mgH_2O_2/dm^3), ozonu (1 mg/dm^3) lub dwutlenku tytanu TiO_2 (100 mg/dm^3). Przy pomocy analizy chromatograficznej oceniono stopień rozkładu badanego ksenobiotyku, a przy pomocy biotestu Microtox® inhibicję bioluminescencji będącej podstawą określenia toksyczności wody. W pracy wykazano przydatność wybranego biotestu w ocenie efektywności procesów pogłębionego utleniania (AOPs), w tym w szczególności w aspekcie występowania niebezpiecznych zjawisk takich jak np. powstawanie ubocznych toksycznych produktów rozkładu usuwanych zanieczyszczeń.

Keywords: **Bisphenol A; Micropollutants decomposition; UV; UV/H_2O_2; UV/O_3; UV/TiO_2.**

1. INTRODUCTION

The bioassays are currently an important element of bioanalytics and environmental biomonitoring and they are useful in evaluating the quality of groundwater, sediments and soils [1-2]. The indicators in bioassays are plants and bacteria as well as living organisms. The fastest bioassays to perform are the bacterial tests. These assays take the advantage of the natural luminescence of e.g. marine bacteria *Vibrio fischeri*, which exhibit a high sensitivity to a wide spectrum of toxic organic and inorganic substances [3]. During the exposure of the microorganisms to the toxic substances metabolic changes occur or their population is reduced, which in turn results in a change in the intensity of the light emitted by the bacteria (inhibition of bioluminescence) [4]. The toxicity of the samples is classified based on the magnitude of the observed effect [5]. The most commonly used commercially available bacterial bioassays in Poland include: ToxAlert®10 and ToxAlert®100 (Merck), Microtox® (Azur Environmental) and LUMIStox® (Dr. Bruno Lange).

The aim of the present study was to determine the applicability of the selected bacterial bioassay Microtox® in assessing the effectiveness of selected

advanced oxidation processes (AOPS), in particular, in terms of hazardous phenomena, such as the formation of toxic by-products of the decomposition of the removed pollutants. In this study, water containing bisphenol A was subjected to the UV irradiation with and without the addition of hydrogen peroxide (H_2O_2), ozone (O_3) or titanium dioxide (TiO_2).

2. METHODS

Bisphenol A, which was selected for the study, is an organic compound belonging to a group of phenols used, among others, for the production of plastics [6]. The subject of the study was model solutions prepared using deionized water and an analytical standard of the studied xenobiotic at a concentration of 0.5 to 5.0 mg/dm^3. The analytical standard of bisphenol A was purchased from Sigma-Aldrich (Poznan, Poland). The pH of the solution was adjusted to pH 7 with 0.1 mol/dm^3 HCl solution or 0.2 mol/dm^3 NaOH. The compound was determined by solid phase extraction (SPE) method and liquid chromatography analysis (HPLC). Supelclean^{TM} ENVI-18 cartridges (volume 6 cm^3, 1.0 g solid phase) from Supelco (Poznan, Poland) were used for the extraction. The filling of the cartridges prior to the extraction was conditioned with methanol (5 cm^3) and acetonitrile (5 cm^3), and then washed with deionized water (5 cm^3). The analyte was eluted with a 1 cm^3 mixture of acetonitrile and methanol (60:40, v/v). The qualitative and quantitative analysis of the xenobiotic in the eluent was performed using HPLC with a UV detector (λ = 218 nm) from Varian (Warsaw, Poland). The eluents were previously concentrated in a gentle stream of nitrogen. The chromatographic column used for the analysis was Microsorb 100 C18 with a length of 25 cm, a diameter of 4.6 mm and a pore size of 5 μm. A mixture of acetonitrile and water (85:15, v/v) was used as the mobile phase. Organic solvents of analytical grade purchased from the Avantor Performance Materials International Company(Gliwice, Poland) were used in this study.

The applied analytical procedure allows the determination of bisphenol A in water at low concentrations, this is, 0.3 μg/dm^3. The extraction yield exceeded 61% for the concentration of the compound in deionized water equal to 0.5 mg/dm^3 and 74% for the concentration of 5 mg/dm^3. The obtained analysis results did not differ by more than 10%.

At the preliminary stage of the study, the bioluminescence inhibition was assessed in the model solutions with varying bisphenol A concentrations. The analysis was carried out using the MICROTOX® bioassay system in the Microtox Model 500 analyser from Tigret Ltd. (Warsaw, Poland) in accordance with the *Screening Test* procedure of the MicrotoxOmni system. This analyser serves both as an incubator and a photometer. Percent bioluminescence inhibition against the control sample (bacteria not treated with the potential toxicant) was measured after 5 minutes of exposure.

The UV irradiation of the model solutions was performed at 20°C in a reactor (volume 700 cm^3) from the Heraeus Company (Warsaw, Poland) with a medium-pressure immersion lamp with the power of 150 W for 45 min (Fig. 1). The irradiation study was carried out comparatively with and without the addition of hydrogen peroxide (H_2O_2), ozone (O_3) and titanium dioxide (TiO_2). In this study a 30% analytical grade hydrogen peroxide purchased from the Stanlab (Gliwice, Poland) was used in 10-fold dilution. The dose of H_2O_2 that was used in this study was equal to 9 mg/dm^3. Ozone was produced from the air using the Ozoner FM 500 generator (purchased from WRC's Multiozon, Gdańsk, Poland) with a capacity of 0.14 mg/s. The gas was fed into the reactor through a ceramic diffuser. The ozone dose was 1 mg/dm^3. The titanium dioxide used in this study was a commercially available product purchased from Degussa marked with the symbol P25. The dose of titanium dioxide TiO_2 was 100 mg/dm^3. Both H_2O_2 and TiO_2 were added to the reactor before the lamp was switched on. The addition of O_3 was delayed by 10 min relative to the irradiation, which was designed to enable a comparison of the effectiveness of the single irradiation process and the coupled process. Samples for the analysis were collected at different times of the processes, namely 5, 10, 15, 20, 30 and 45 min. The degree of degradation of the studied xenobiotic was assessed by the chromatographic analysis, and the bioluminescence inhibition was determined by the Microtox® bioassay.

3. RESULTS

It was observed that the increasing concentration of xenobiotic in water was accompanied by simultaneous increase of the bioluminescence inhibition (Fig. 2). The presented graphical relationship between the concentration of xenobiotic and the bioluminescence inhibition shows linear correlation between the two parameters (R^2 = 0.99), confirming that the toxicity of water depends on the concentration of the xenobiotic. Based on this observation, it

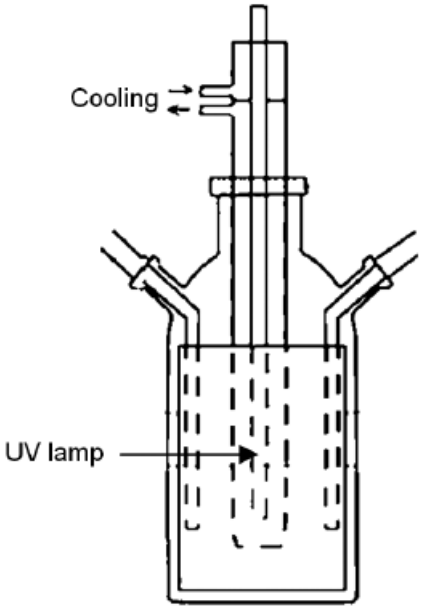

Figure 1.
The scheme of the laboratory UV reactor Heraeus

can be hypothesized that the effective elimination of the xenobiotic from water using a variety of physical and chemical processes should be accompanied with a reduction of the toxic effect. Any exception to this rule may prove the occurrence of other dangerous phenomena accompanying the implementation of these processes.

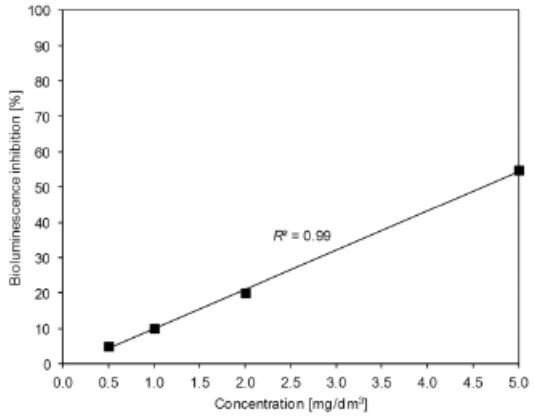

Figure. 2.
Impact of bisphenol A concentration on the bioluminescence inhibition value

The degree of decomposition of the studied xenobiotic and the change in the inhibition of bioluminescence occurring in the solutions during the UV irradiation, depending on the process time are shown in Figure 3. The degradation of the xenobiotic was occurring during the UV irradiation. The effectiveness of the decomposition of bisphenol A increased with the time of the process. For example, after 5 min of the process the degree of degradation of bisphenol A was approx. 38% and after 45 min it was approx. 72%. On the other hand, the observations regarding the bioluminescence inhibition of the solutions were surprising. In the first 15 min of the process, the decomposition of bisphenol A did not cause any reduction in the bioluminescence inhibition of the solution. It was determined, however, that the bioluminescence inhibition was greater than that specified in the solution before the process. Reduction of this parameter was observed only after 20 minutes of the process, but after 30 min the bioluminescence inhibition decreased to zero. The observed relationship indicates the formation of toxic by-products of the decomposition of bisphenol A in the first minutes of the UV irradiation of the solution. In the course of the process the resulting products are likely to be decomposed. It should also be emphasized that the results of the bacterial bioassay show also that the toxicity of the degradation products is greater than for the solution containing only bisphenol A.

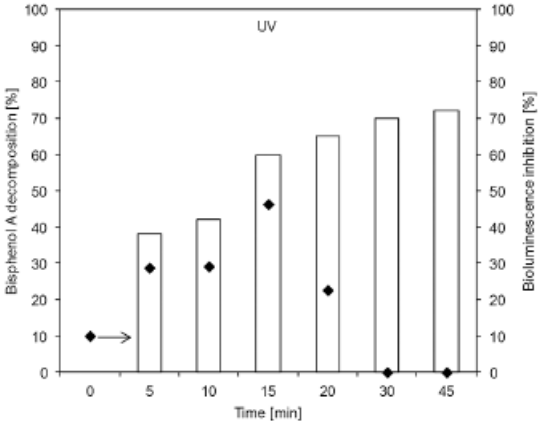

Figure 3.
Decomposition of bisphenol A and change of the inhibition of bioluminescence in solutions irradiated by UV

On the other hand, the use of H_2O_2, O_3 or TiO_2 coupled with the UV irradiation enhanced the degree of degradation of the studied xenobiotic, but also caused an increase in the inhibition of bioluminescence of the solutions (Fig. 4a-c). Obviously, there are some differences in a range of effects between the used reagents. It should be emphasized that, in the case of the UV/TiO_2 process the decomposition of

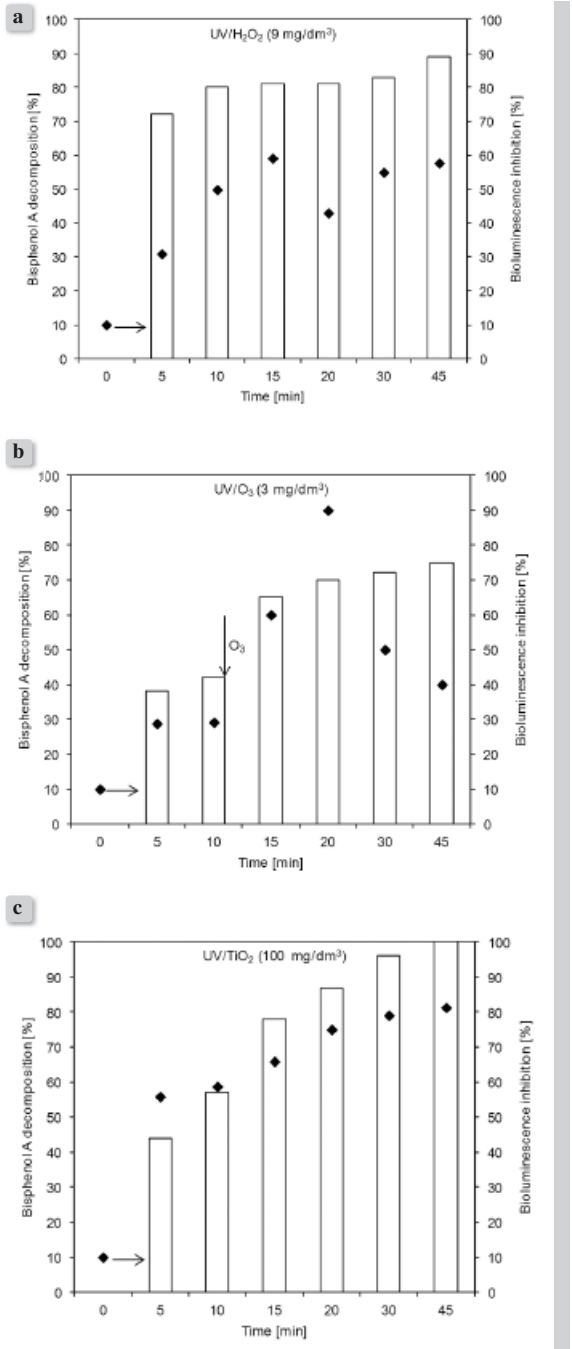

Figure 4.
Decomposition of bisphenol A and change of the inhibition of bioluminescence in solutions irradiated by UV with the addition of H_2O_2 (a), O_3 (b) and TiO_2 (c)

bisphenol A was complete after 45 min (Fig. 4. c). The increased intensity of the xenobiotic degradation was probably the result of the formation of greater amounts of hydroxyl radicals ($^{\cdot}OH$) in the presence of the oxidant or the catalyst of the process. However, the increase of the inhibition of bioluminescence of the solutions shows that toxic degradation by-products are formed to a higher extent under these particular conditions. For example, in the UV/O_3 process, the inhibition of bioluminescence increased to approx. 90% after the addition of ozone to the treatment system. Referring this value to the classes of toxicity [5] it can be concluded that the tested solution is characterized by high toxicity. In the further course of the process, the bioluminescence inhibition decreased, but its value did not reach zero as it did in the case of the single UV irradiation process (Fig. 3). Also in this case, the toxicity of the degradation products was greater than for the solution containing only bisphenol A.

The authors of [6] also discussed the complex oxidizing process of UV/H_2O_2 in terms of the degradation of selected organic micropollutants, including bisphenol A. That study was carried out to assess the effect of the type of environmental matrix on the efficiency of the process. Oxidation process was performed comparatively using a wastewater treatment plant effluent and deionized water to which the standards of the studied organic micropollutants were added. The samples of the solutions collected after the process were tested for their cytotoxicity and genotoxicity. Based on the obtained results it was found that only in the case of deionized water containing bisphenol A and ciprofloxacin (a substance from the group of chemotherapeutic agents) toxic oxidation by-products are formed causing a cytotoxic effect. This phenomenon was not observed when the water contained the other micropollutants studied, namely metoprolol (substance used in cardiac drugs) and also sulfamethoxazole (bacteriostatic antibiotic). According to the authors, the effect of the type of environmental matrix on the observed relationship resulted from the fact that the oxidation of the compounds present in the effluent from a wastewater treatment plant is accompanied by various mutually competing chemical reactions. In addition, the effluent of a waste water treatment plant apart from organic micropollutants contains various additional organic substances, which also react with hydroxyl radicals. In contrast, in another study published in this subject area [7] it was found that the oxidation of organic micropollutants occurs much faster in deionized water than in surface water. Similar results were also presented by Trovo et al. [8]. Thus, evaluation of the oxidation process of micropollutants carried out with deionized water can only give an idea of the by-products generated during the process and their bio-

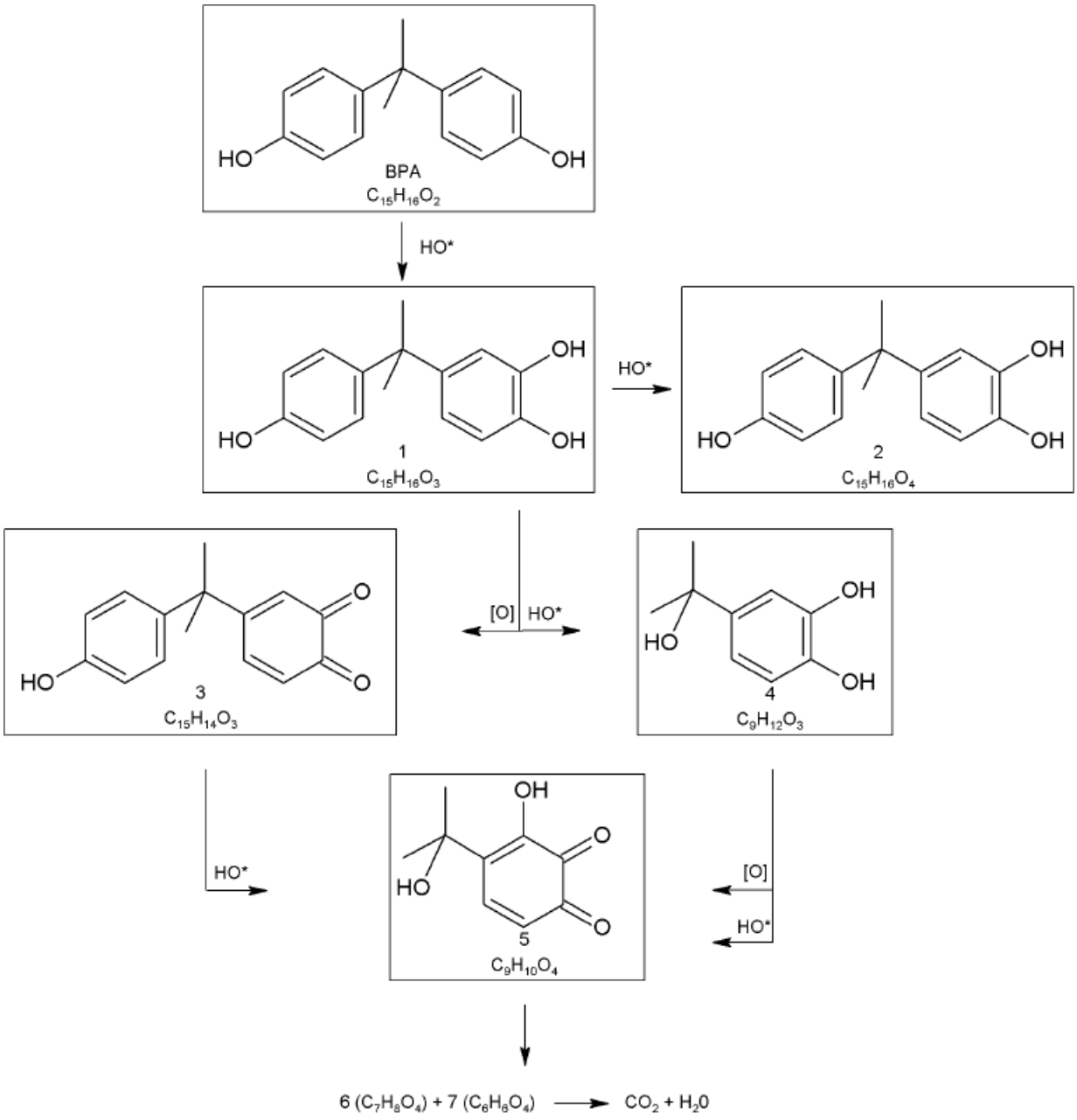

Figure 5.
Proposed route for the degradation of bisphenol A in water as induced by the photodegradation systems [9]

logical effect (including e.g. toxicological effect), but the research addressing real environmental matrices is indispensable.

Furthermore, the identification of oxidation by-products of bisphenol A was reported by the authors of [9] who determined that the decomposition of this compound yields several major by-products, some of which contain additional hydroxyl groups (Fig. 5). Additional oxidation products are formed as a result of the opening of the aromatic ring [10]. It should be emphasized that the identification of the by-products of oxidation requires time-consuming chromatographic analytical procedures. The authors of [9] state that under actual process conditions, e.g. during the treatment of typical surface water, the formation of the by-products of bisphenol A decomposition is negligible due to the low environmental concentrations of this micropollutant. Additionally, the resulting by-products are characterized by low persistence.

4. CONCLUSION

This study demonstrated the usefulness of the Microtox® bioassay in assessing the effectiveness of advanced oxidation processes (AOPs), in particular, in terms of hazardous phenomena, such as the formation of toxic by-products of the decomposition of the removed pollutants. It was determined that the most favorable conditions for the selection of the oxidizing process cannot be merely based on the effectiveness of the degradation efficiency, but the toxicological assessment of the solution after the process should be also considered. The advantage of the applied bioassay compared to the instrumental chromatographic analysis used to identify each of the oxidation by-products is duration of the analysis. The results presented in the literature (available worldwide) concerning the elimination of various organic micropollutants in advanced oxidation processes indicate the complexity of the phenomena occurring in these processes. Many mechanisms of the degradation of micropollutants have not been fully understood.

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