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Toxicity and degradation pathways of selected micropollutants in water solutions during the O_3 and O_3/H_2O_2 process

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ABSTRACT

Ozonation is one of the most effective methods of water disinfection. This process, as a single method or with the involvement of other oxidizing agents, can be also used as an advanced decomposition process for hardly or non-biodegradable compounds. Ozone-based processes do not always lead to a complete mineralization of compounds. The oxidation of parent compounds may contribute to the formation of a significant number of new micropollutants with a high toxic potential. The paper presents the removal degrees of diclofenac sodium salt (DCF) and bisphenol A after the O_3 and O_3/H_2O_2 process. The processes were carried out at various times, that is, 10, 20, 30 and 60 min. The O_3 dose was 3 mg/L and the H_2O_2 dose was equal to 9 mg/L. The GC-MS analysis indicated the formation of different decomposition by-products during both oxidation processes. The type and concentration of generated new micropollutants depends on the pH of the water solution and the process time. It has been observed that the decomposition degree of DCF decreased with the increase in the pH of the reaction medium. An inverse relationship was observed for bisphenol A, where the largest number of oxidation by-products was observed in water solution at pH 11. The conducted toxicological analysis confirmed the toxic nature of the generated decomposition products.

Keywords: Organic micropollutants; Ozonation; Oxidation by-products; Toxicity

1. Introduction

In recent years a constantly decrease in the availability of water resources, which meet the requirements of drinking water was observed [1,2]. Climate changes, which causes with gradual drying of many aquatic ecosystems, become a major problem not only in African countries but also in many Asian regions [3]. On the other hand surface waters, which are often the only available water resources [4] are polluted with large loads of different potential toxic micropollutants. The major source of those compounds is municipal and industrial wastewater effluents. This is related to the maladjustment of conventional wastewater treatment technologies for the decomposition of hardly biodegradable compounds [5]. Advanced water treatment processes such as in-depth oxidation processes, adsorptive or membrane filtration allow to obtain high removal degrees of a large number of compounds of emerging concern [6–9].

Ozone is classified as a high reacting oxidation agent, which according to literature data is one of the most effective agents of water disinfection used in different industry sectors [10]. The process of ozonation carried out as a single method or with the involvement of other oxidizing agents and in-depth treatment processes, can be also used as an advanced decomposition process for hardly or non-biodegradable compounds [11–14]. Margot et al. [15] compared the removal degrees of 70 micropollutants, which occurring in municipal wastewater, during conventional wastewater treatment processes and processes supported by ozonation. They indicated a beneficial effect of ozone on the decomposition of micropollutants belong to the group of pharmaceuticals, endocrine disrupting compounds, pesticides and other common chemicals.

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O_3 is slightly soluble in water and very unstable in water media [16]. Ozone molecules are rapidly decomposed in water through different reactions from Eqs. (1) to (9) [17]. It leads to the formation of a large number of radicals, which are able to decompose organic pollutants. However, OH radicals are characterized by the strongest oxidation potential and they can abate compounds, which are resistant for O_3 decomposition [18].

$$O_3 + HO^- \rightarrow HO_2^\bullet + O_2^{-\bullet} \tag{1}$$

$$HO_2^\bullet \leftrightarrow O_2^{-\bullet} + H^+ \tag{2}$$

$$O_3 + O_2^{-\bullet} \rightarrow O_3^{-\bullet} + O_2 \tag{3}$$

$$O_2^{-\bullet} + H^+ \rightarrow HO_3^\bullet \tag{4}$$

$$HO_3^\bullet \rightarrow HO^\bullet + O_2 \tag{5}$$

$$HO^\bullet + O_3 \rightarrow HO_4^\bullet \tag{6}$$

$$HO_4^\bullet \rightarrow HO_2^\bullet + O_2^\bullet \tag{7}$$

$$HO_4^\bullet + HO_4^\bullet \rightarrow H_2O_2 + 2O_3 \tag{8}$$

$$HO_4^\bullet + HO_3^\bullet \rightarrow H_2O_2 + O_3 + O_2 \tag{9}$$

The main assumption of advanced oxidation processes including O_3-based process is the complete mineralization of organic compounds, but under actual process conditions pollutants degraded to different transformation products [19]. In general, the decomposition occurs as a result of reaction between O_3 and/or other reactive free radicals formed in the reactions listed above [20,21]. Yu et al. [22] appointed the bimolecular reaction rate constants for the reaction of $HO^\bullet$ with the pharmaceutical compound diclofenac at about 9.29×10^9 M^{-1} s^{-1}. The comparison of this value with several model compounds allows to determine the site of $HO^\bullet$ attack on the compound structure. Fig. 1 presents a simplified scheme of oxidation pathways of the degradation process via ozonation and ozonation supported by other oxidizing agents, that is, H_2O_2.

According to the scheme (Fig. 1) of compound oxidation pathways it can by assume, that the type of transformation products strictly depends on the oxidizing agent that mainly driven the decomposition of the micropollutant. A comprehensive review on the oxidation products from the reaction of organic micropollutants with O_3 and $^\cdot OH$ radicals was made by von Sonntag and von Gunten [21] and Hübner et al. [23].

Oxidation by-products similar to their parent compounds are still classified as biologically active. Therefore, this micropollutant can have a negative impact on water organisms. Literature [24,25] does not clearly indicate a negative impact of the ozonation process on the quality of treated water streams contaminated by organic micropollutants. However, Stalter et al. [26,27] drew attention to the toxic nature of several transformation products. Also, Li et al. [28] indicated that the micropollutant transformation products after the O_3 process can sometimes be more toxic than the parent pollutants. Whereas Bundschuh et al. [11] showed that the estimated toxicity of post-ozonated water solutions depends on the kind of used ecotoxicological test system and on the composition of the micropollutants and transformation products in the tested water.

For a full assessment of the beneficial effects of the single O_3 and other O_3-based oxidation processes of micropollutant water solutions it is necessary to evaluate the removal degree of each compound and identify all the transformation products and to carry out different toxicity tests.

Pharmaceuticals and personal care products and industrial additives belong to the most commonly identified compound groups in different environmental water samples [29,30]. The paper presents the identification of both diclofenac sodium salt (DCF) – pharmaceutical compound, and bisphenol A (BPA) – industrial additives, decomposition by-products formed during the O_3 and O_3/H_2O_2 process. The influence of the pH of the micropollutant water solutions on their oxidation and the generation of different by-products was tested. In addition, toxicological analyzes of post-treated water solutions were carried out using three biotests, basing on the analysis behavior of indicator organisms: saltwater bacteria, freshwater crustaceans and vascular plants.

2. Materials and methods

2.1. Material and reagents

The analytical standards of both, BPA and the non-steroidal anti-inflammatory drug in the form of DCF of purity grade >97% were supplied by Sigma-Aldrich (Poznań, Poland). The chemical characteristic of the tested compounds was presented in Table 1. Methanol and acetonitrile with over 99.5% of purity by Avantor Performance Materials Poland S.A. were also used in the investigations. Solid phase extraction (SPE) cartridges Supelclean™ ENVI-8 and Supelclean™ ENVI-18 were supplied by Sigma-Aldrich.

2.2. O_3 and O_3/H_2O_2 process

Both O_3 and O_3/H_2O_2 oxidation processes were carried out in a glass reactor with the *volume of 1 L*. To eliminate the influence of light (especially UV irradiation) on the micropollutant oxidation processes the reactor was placed in a dark chamber (Fig. 2). O_3 was generated from air in the Ozoner FM500 generator by WRC Multiozone and it was introduced to the reactor through a ceramic diffuser. The O_3 dose was constant at 3 mg/L (measured at the inlet to the reactor) in both processes. However, the dose of H_2O_2 during the O_3/H_2O_2 process was equal to 9 mg/L. Both applied O_3 and H_2O_2 doses were determined during preliminary studies [32,33]. The contact time between the oxidizing reagent and the micropollutant water solutions lasted for 10, 20, 30 and 60 min. Na_2SO_3 in the concentration of 24 mmol/L was added

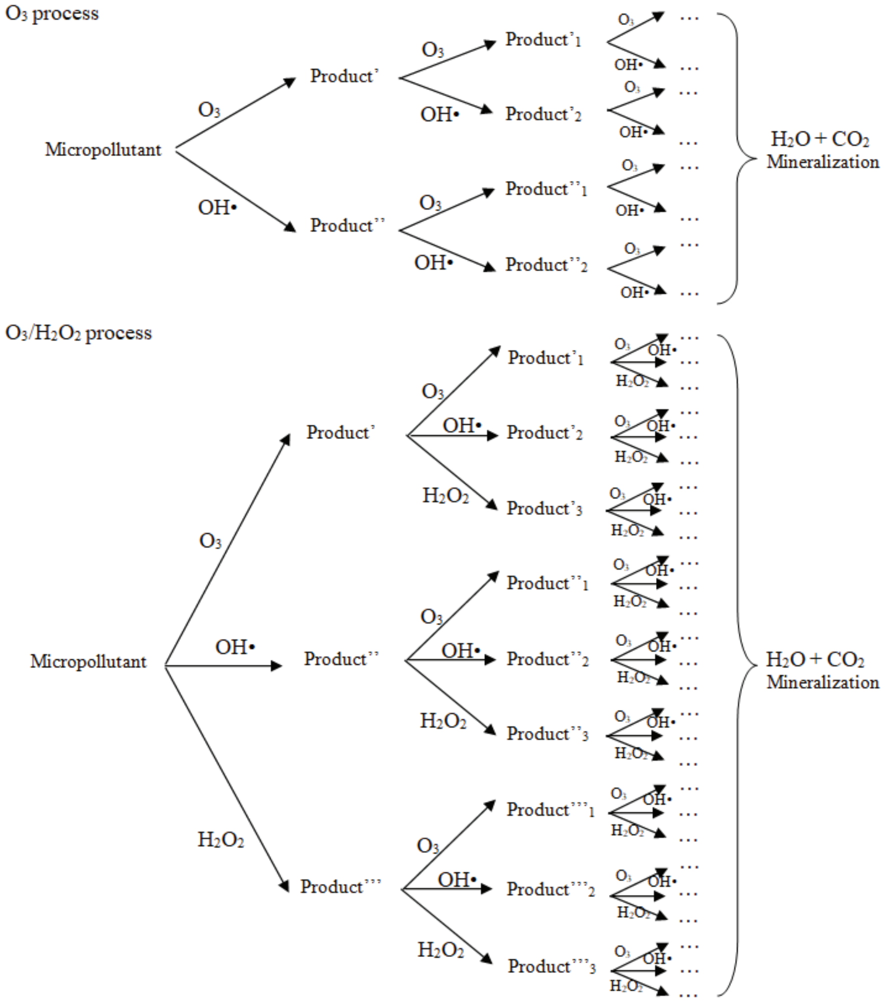
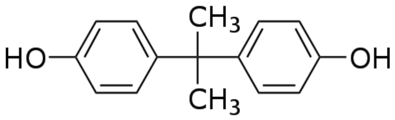
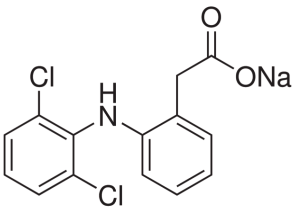


Fig. 1. Simplified scheme of micropollutant oxidation pathways during O_3 and O_3/H_2O_2 processes.

Table 1
The structural and chemical characteristics of investigated compounds [31]

Compound	Bisphenol A	Diclofenac sodium salt
Structural formula		
Molecular formula	$C_{15}H_{16}O_2$	$C_{14}H_{10}Cl_2NNaO_2$
CAS No	80-05-7	15307-79-6
Molecular mass (g/mol)	228.291	318.13
Solubility in water (mg/L)	120	50
pK_a	9.6	4.15
logK_{ow}	3.32	4.51

to the post-processed water samples in order to remove the ozone residues. The experiments for both tested compounds were carried out separately.

2.3. Water samples

The tested water solutions were prepared based on deionized water matrices with the addition of DCF or BPA standards of concentration 0.5 mg/L. The high micropollutants concentration, that is, much higher than the one occurring normally in the water environment was used in order to increase the identification precision of the generated oxidation by-products. To determine the impact of pH on the micropollutant decomposition efficiency, the pH of the prepared water solutions was adjusted to 4, 7, 9 and 11 using 0.1 mol/L HCl or 0.1 mol/L NaOH. Preliminary studies did not show any significant effect of HCl or NaOH addition on the decomposition of the tested compounds before the implementation of the oxidation processes.

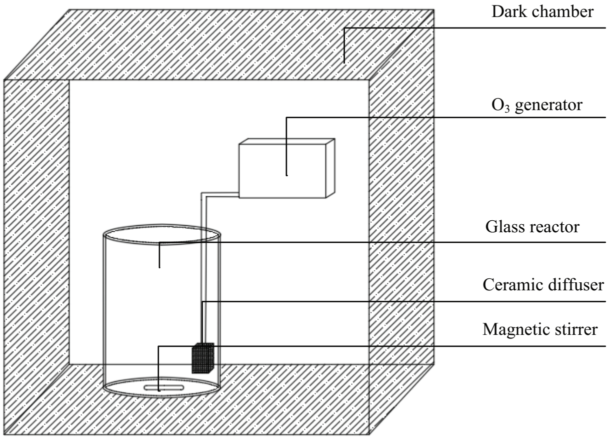


Fig. 2. Scheme of the test stand.

2.4. Analytical procedure and identification of generated oxidation by-products

The analytical procedure of tested compounds was performed by SPE of the micropollutants and their decomposition by-products and the quantitative–qualitative analysis of the obtained extracts by GC-MS(EI) chromatography.

The quantitative analysis was made by 7890B gas chromatograph (GC) coupled with mass spectrometer (MS) with electron ionization (EI) by Perlan Technologies (Warszawa, Poland). SLB™ – 5 ms 30 m × 0.25 mm capillary column of 0.25 µm film thickness by Supelco was used for the micropollutants analysis. Helium 5.0 at a constant flow rate of 1.1 mL/min was used as the carrier gas. The temperature of the ion trap was 150°C and the temperature of the ion source was set on 230°C. For the quantitative analysis, MS detector was operated in scan ion monitoring mode ranged from 50 to 400 *m*/*z*. The decreases in compounds concentrations were calculated based on the measurements of their peak areas, which were compared with data obtained during the calibration curves estimation. The injector temperature was set at 250°C.

The volume of analyzed water samples before and after the implementation of oxidation processes was equal to 20 mL. The pH of each sample was adjusted to 7. Details of the SPE method and the column oven temperature programme were given in Table 2.

Table 2
SPE details and chromatographic analysis conditions

	Analysis of parent compound and its by-products	
	Bisphenol A	Diclofenac sodium salt
Solid phase extraction		
Cartridge type	Supelclean™ ENVI-18	Supelclean™ ENVI-8
Cartridge bed	Octadecylsilane (C_{18})	Octylsilane (C_8)
Conditioning	5 mL of acetonitrile 5 mL of methanol	5 mL of methanol
Washing	5 mL of deionized water	
Sample flow (mL/min)	1	
Vacuum drying time (min)	5	
Extract elution	1.5 mL of methanol 1.5 mL of acetonitrile	3 mL of methanol
GC-MS(EI) chromatography		
Injected volume (µL)	1	2
Injector temperature (°C)	250	
Oven program	80°C (6 min)–5°C/min → 220°C (5 min)	80°C (6 min)–5°C/min → 260°C–20°C/min → 300°C (2 min)

The identification of micropollutants oxidation by-products was made based on their mass spectra, which were compared with the NIST 17 Mass Spectral Library.

The results presented in the figures are the arithmetic average of the four replicates of each experiment. For all the cases assigned error (estimated based on the standard deviation) did not exceed 5% so the results are presented without marking of the ranges of error.

2.5. Toxicity tests

The toxic potential of tested micropollutants water solution was determined by three different biotests, that is, Microtox® test, Daphtoxkit F® test and *Lemna* sp. Growth Inhibition Test. All tests were based on the observation of changes in the behavior of indicator organisms.

The Microtox® test uses saltwater bioluminescence bacteria *Aliivibrio fischeri,* which are considered to be high sensitivity for a broad range of toxic substances including organic micropollutants [34,35]. The test was carried out according to the screening test procedure of MicrotoxOmni system in Microtox analyzer Model 500 by Modern Water. The change in the intensity of bacteria light emission was measured after 5 min of the exposure to the tested water samples. 2% NaCl solution was use as a reference non-toxic sample.

The second, Daphtoxkit F® assays base on immobility or mortality of freshwater crustaceans *Daphnia magna* during a 24 or 48 h period of exposition on potential toxic water samples. In the present study, the behavior of indicator organisms was tested only after 24 h. The test was performed according to OECD 202, ISO 6341 guidelines, which regulate the proper execution of tests on *Daphnias*. The toxicity effect was calculated by the use of Eq. (10).

Lemna sp. growth inhibition test (GIT) was carried out for 7 d taking into account protocols listed in the OECD 221 guidelines. Freshwater vascular plants *Lemna minor* with two fronds were used as indicator organisms. The plants came from an own plant breeding. The temperature during the test was equal to 25°C ± 1°C, while the light exposure was 6,000 lux. The toxicity effect was calculated according to the same Eq. (10) as in the test conducted on *Daphnia magna* crustaceans.

$$E = \frac{(N_C - N_T)}{N_C} \bullet 100\% \tag{10}$$

where E is the toxicity effect (%); N_C is the number of living test organisms (plant fronds) for the control sample; N_T is the number of living test organisms (plant fronds) for the test sample.

The classification of water samples after the estimation of their toxicological response was made according to the toxicity classification system presented in Table 3 [36,37].

Table 3
Toxicity classification system of micropollutant water samples [36,37]

Effect (%)	Toxicity class
<25.00	Non-toxic
25.00–50.00	Low toxic
50.01–75.00	Toxic
75.01–100	Highly toxic

3. Results and discussion

3.1. Oxidation process

The first stage of the study was focused on determination of the rates of concentration decrease of DCF (Fig. 3) and BPA (Fig. 4) in deionized water solutions of different pH during the O_3 and O_3/H_2O_2 process. It was noted that the concentration of DCF and BPA decreased with the increase of the process time in both oxidation processes. Diclofenac sodium salt was more effective decomposed in the O_3 process carried out in the presence of H_2O_2 irrespective of the time of the process, than in the O_3 process. The presence of H_2O_2 assists the formation of $OH^\bullet$ [38], so it leads to an increase in the oxidation of micropollutants. A beneficial influence of H_2O_2 on the decomposition of different pharmaceutical micropollutants was also determined by other researchers [39]. The difference in the efficiency of both processes increased with the increase of the pH of the water solutions. For example, the removal rate of the pharmaceutical compound in the solution of pH 5 after 60 min of O_3 process amounted 66% and for the O_3/H_2O_2 process exceeded 89%. While in the solutions of pH 11 the concentration of DCF after the O_3 process decreased by 35%, after the O_3/H_2O_2 supported process it decreased by 67%. Therefore, the obtained results indicate an influence of the pH of the reaction medium on the kinetic of the oxidation reaction of particular pollutants. It has been observed that decomposition degree of DCF decreased with the increase in the pH of the reaction medium. An inverse relationship was observed for the oxidation of BPA (Fig. 4). The concentration of BPA decreases with the increase of the reaction mixture pH. The value of the micropollutant removal rate in the O_3/H_2O_2 process was equal to 19% for pH 5 solutions, 22% for pH 7, 41 for pH 9 and 57% for pH 11. Unexpected results were obtained for the O_3 BPA oxidation process, which was characterized by a higher oxidation ability against that compound. After 60 min of O_3, the concentration of BPA decreased by 59% for pH 5 solution and over 81% for pH 11 solutions. A similar effectiveness of BPA decomposition in the O_3 process was observed in a previous work devoted to the comparison of BPA removal in various advanced oxidation processes [32]. von Gunten [40] and Sehested et al. [41] have demonstrated that $OH^\bullet$ radicals may lead to the destruction of O_3 in accordance with Eqs. (11)–(13), which lead to the formation of O_2 and H_2O. This phenomenon causes a decrease in the decomposition ability of the whole oxidation mixture.

$$OH^\bullet + O_3 \rightarrow HO_2^\bullet + O_2 \tag{11}$$

$$HO_2^\bullet \leftrightarrow O_2^{-\bullet} + H^+ \tag{12}$$

$$O_2^{-\bullet} + O_3 + H_2O \rightarrow 2O_2 + OH^\bullet + OH^- \tag{13}$$

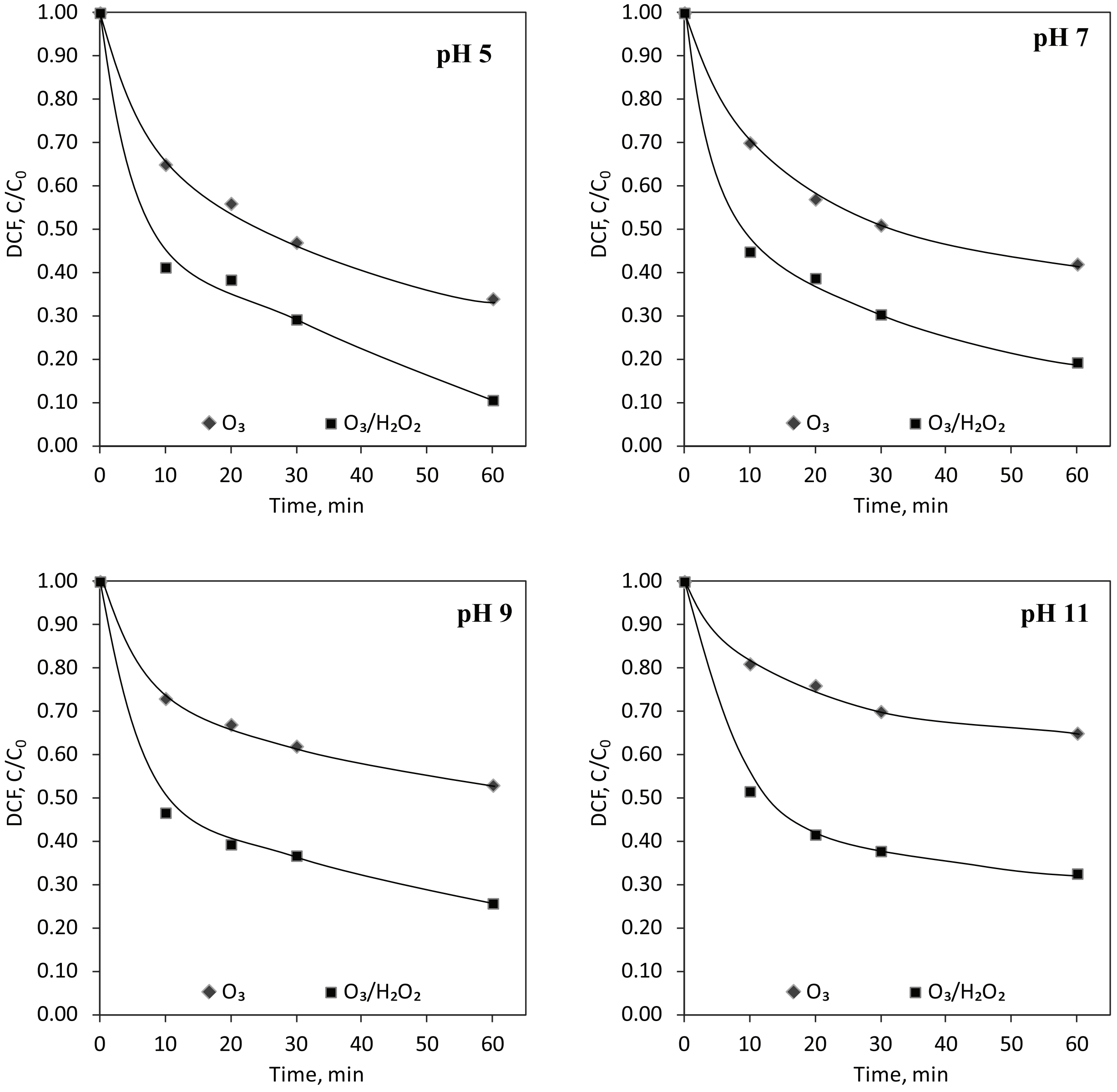


Fig. 3. Influence of the pH of the reaction medium on the degradation of diclofenac sodium salt (DCF) in the O_3 and O_3/H_2O_2 process.

It is well known that the ozone decomposition dynamic increases with the increasing pH of water solution [42]. Therefore, it can be assumed that the decomposition of DCF occurs mainly as a result of the reaction with O_3 molecules, which were more stable in the acid media. On the other hand, the oxidation of such phenolic compounds as BPA results from reactions with different radicals formed during O_3 decomposition. An increase of phenols oxidation with the increase of the pH of reaction was also reported by Poznyak et al. [43].

As already mentioned, the oxidation of micropollutants results from the direct reactions with O_3, but also from the reactions with reactive radicals generated during the decomposition of the O_3 molecule. Thus, the tested organic micropollutant oxidation by-products of decay can assume different forms. The tests were carried out without any scavenger of $OH^•$ radicals, therefore, it was difficult to differentiate which identified transformation products have been generated by reactions with $OH^•$ and which were induced only by ozone.

Due to high reaction rates of the oxidation process of DCF and BPA, the determination of most transformation products was possible only in samples after 10 and 20 min of the process time. Therefore, it can be assumed that the newly formed micropollutants are further decomposed in samples after 30 and 60 min of the O_3 and O_3/H_2O_2 process. The decomposition of oxidation by-products of both tested micropollutants to several compounds of a smaller molecular weight was confirmed by the increase of the phenol concentration in samples after 60 min of process duration. The identification of those small molecular weight compounds was impossible because of the adopted methodology of samples preparation.

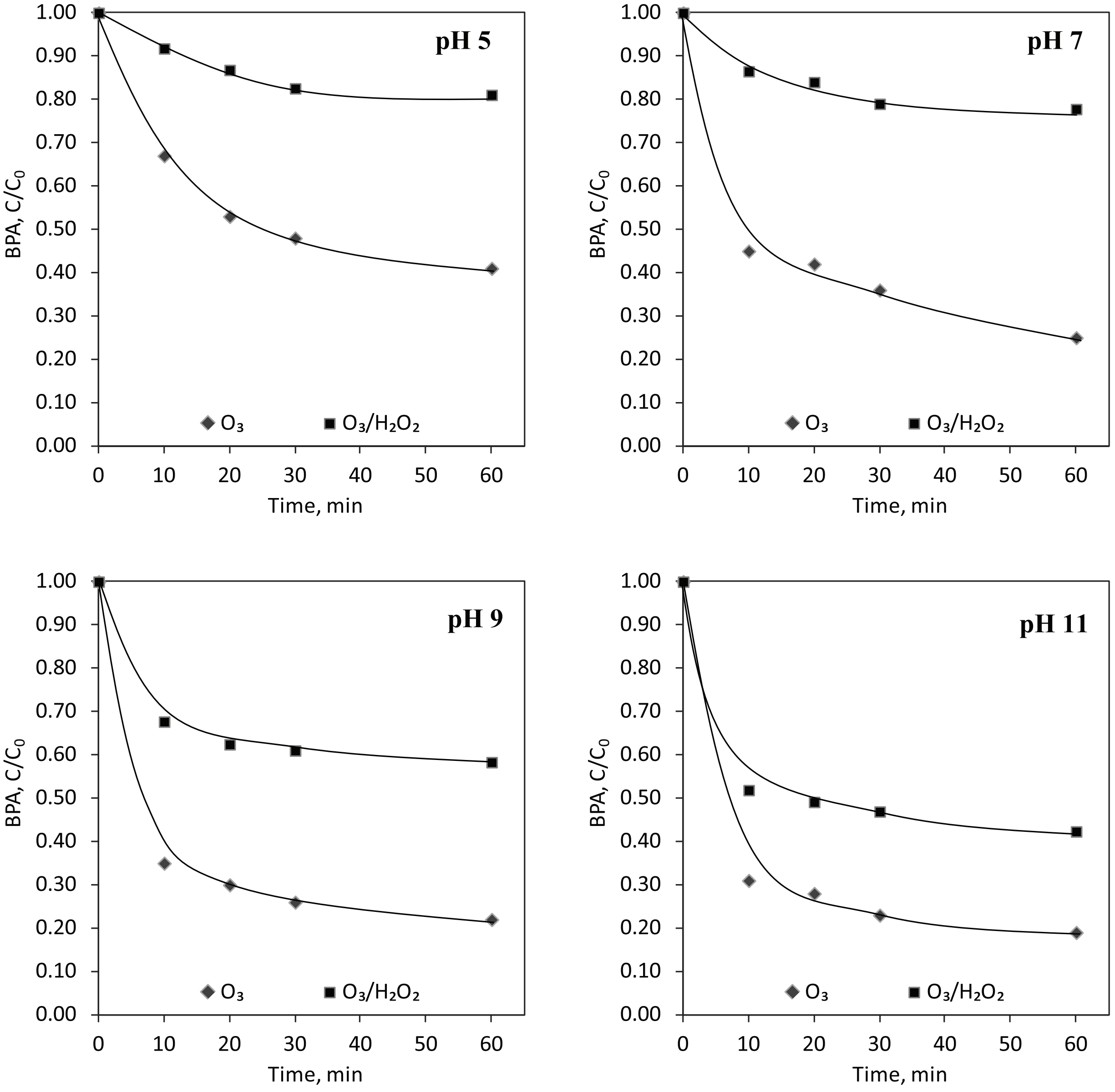


Fig. 4. Change of bisphenol A concentration during the O_3 and O_3/H_2O_2 process of micropollutant water solutions of different pH.

However, the preformed toxicity tests indirectly confirmed the presence of new generated high toxic compounds in post-process water solution, which were below the detection limit of the chromatographic analysis.

The identification of transformation products was made by the comparison of obtained MS results with mass spectra data provided by the NIST library (matching >85%). This enabled to propose possible degradation pathway of DCF (Figs. 5 and 6) and BPA (Figs. 7 and 8). The characteristics of identified micropollutants were given in Tables 4 and 5.

During the O_3 process of DCF, seven different transformation products were identified (Fig. 5). Their concentrations increased with the increase of the pharmaceutical decomposition. Thus, in the solution of pH 9 and 11 three by-product 4′,5-dihydroxydiclofenac, 5-hydroxydiclofenac and 2-aminophenylaceticacid were detected and in the solution of pH 7 and 5, all the seven diclofenac transformation products (4′,5-dihydroxydiclofenac, 5-hydroxydiclofenac, 2,6-dichlorodiphenylamine, 2,6-dichloroaniline, 2,6-dichloro-4-aminophenol, 2-aminophenylaceticacid and 2-hydroxyphenylacetic acid) were present. The O_3/H_2O_2 process, which was more effective against the decomposition of DCF, leads to the formation of only five by-products (Fig. 6), that is, 4′,5-dihydroxydiclofenac, 5-hydroxydiclofenac, 2,6-dichloro-4-aminophenol, 2-hydroxyphenylacetic acid and 2,3-dihydroxyphenylacetic acid. The presence of an additional source of $OH^{\bullet}$ radicals in form of H_2O_2 affected in a higher ability to by-products oxidizing. This relation was additionally confirmed by a smaller toxicological response of test organisms exposed to water solutions after the O_3/H_2O_2 process compared with samples after the O_3 process.

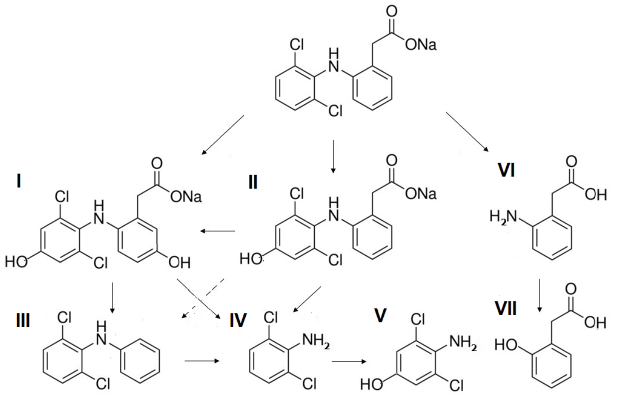


Fig. 5. Possible O_3 oxidation pathway of DCF in water solutions of different pH.

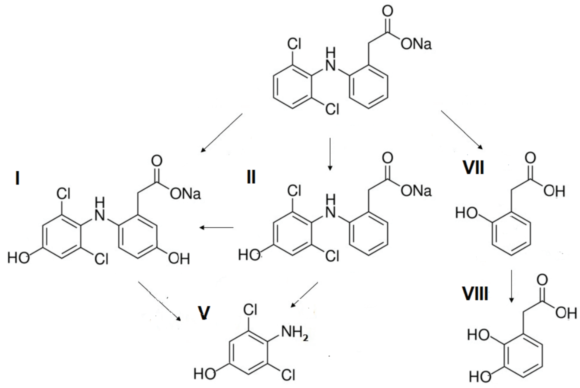


Fig. 6. Possible oxidation pathway of DCF in water solutions of different pH after the O_3/H_2O_2 process.

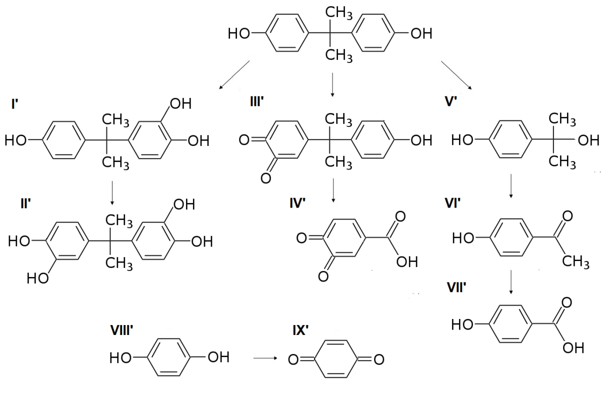


Fig. 7. Possible O_3 oxidation pathway of bisphenol A in water solutions of different pH.

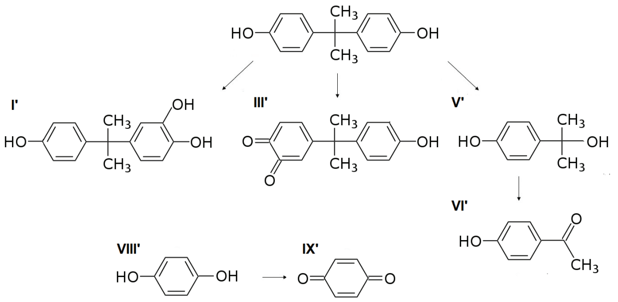


Fig. 8. Possible decomposition pathway of bisphenol A in water solutions of different pH after O_3/H_2O_2 process.

The identified compounds during the O_3 decomposition of DCF were similar to those noted by Yu et al. [22]. Sein et al. [44] estimated the dose of O_3 required for the complete decomposition of diclofenac in solution with and without an $OH^\bullet$ scavenger agent. For the elimination of 1 mol of diclofenac in the presence of *t*-BuOH scavenger 5 mol of O_3 are needed. During the performance of reaction without *t*-BuOH the mole ratio increases to 8 mol O_3 to 1 mol diclofenac. This dependency may be caused by the fact that $OH^\bullet$ radicals generated intermediates, which can react more quickly with O_3 than the diclofenac compound [44].

The largest number of nine oxidation by-products was observed in BPA water solution at pH 11 after the O_3 process. The GC-MS analysis indicated the formation of 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzenediol, 4,4′-propane-2,2-diyldibenzene-1,2-diol, 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzoquinone, 3,4-dioxo-1,5-cyclohexadiene-1-carboxylic acid, 4-(2-hydroxy-2-propanyl)phenol, 4′-hydroxyacetophenone, 4-hydroxybenzoic acid, hydroquinone and 1,4-benzochinon. The same transformation products were also present in the post-treated BPA solutions of pH 9 and 7. Only in the solution of pH 5 the parent compound oxidation leads to the formation of a smaller number of by-products among which 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzenediol, 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzoquinone, 3,4-dioxo-1,5-cyclohexadiene-1-carboxylic acid, hydroquinone and 1,4-benzochinon were listed. Deborde et al. [45] identified six BPA O_3-oxidation by-products, which included 3-formylacrylic acid derivatives. Those compounds were not detected under our experimental conditions. Garoma et al. [46] also identified other BPA ozonation reaction intermediates in the form of acetone, resorcinol, formaldehyde and the organic acids, acetic, formic, maleic and oxalic acid.

The introduction of H_2O_2 during the O_3/H_2O_2 process resulted not only in a smaller removal degree of BPA, but also contributed to the formation of only six transformation products, that is, 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzenediol, 4-[2-(4-hydroxyphenyl)-2-propanyl]-1,2-benzoquinone, 4-(2-hydroxy-2-propanyl)phenol, 4′-hydroxyacetophenone, hydroquinone and 1,4-benzochinon. Those products were identified in all micropollutant water solutions independently from the pH.

The obtained results confirmed the fact that O_3 attacks structures with high electron density such as C=C bonds, activated aromatic systems and non-protonated amines [47]. According Mvula and von Sonntag [48] ozone, due to its electrophilic nature, usually reacts with aromatic rings by electrophilic substitution or 1,3-dipolar cycloaddition, what was also observed during our investigations. Compounds with phenolic groups are in general highly reactive towards ozone and $OH^\bullet$ radicals [49,50].

Table 4
Characteristics of identified compounds during the O_3 and O_3/H_2O_2 process of diclofenac sodium salt [31]

Micropollutant		Molecular formula	CAS No.	Molecular mass (g/mol)
I	4′,5-Dihydroxydiclofenac	$C_{14}H_{11}Cl_2NO_4$	69002-86-4	328
II	5-Hydroxydiclofenac	$C_{14}H_{11}Cl_2NO_3$	69002-84-2	312
III	2,6-Dichlorodiphenylamine	$C_{12}H_9Cl_2N$	15307-93-4	238
IV	2,6-Dichloroaniline	$C_6H_5Cl_2N$	608-31-1	162
V	2,6-Dichloro-4-aminophenol	$C_6H_5Cl_2NO$	26271-75-0	178
VI	2-Aminophenylaceticacid	$C_8H_9NO_2$	3342-78-7	151
VII	2-Hydroxyphenylacetic acid	$C_8H_8O_3$	614-75-5	152
VIII	2,3-Dihydroxyphenylacetic acid	$C_8H_8O_4$	19988-45-5	168

Table 5
Characteristics of compounds identified during the O_3 and O_3/H_2O_2 process of bisphenol A [31]

Micropollutant		Molecular formula	CAS No.	Molecular mass (g/mol)
I′	4-[2-(4-Hydroxyphenyl)-2-propanyl]-1,2-benzenediol; 5-Hydroxybisphenol	$C_{15}H_{16}O_3$	79371-66-7	244
II′	4,4′-Propane-2,2-diyldibenzene-1,2-diol	$C_{15}H_{16}O_4$	18811-78-4	260
III′	4-[2-(4-Hydroxyphenyl)-2-propanyl]-1,2-benzoquinone	$C_{15}H_{14}O_3$	163405-36-5	242
IV′	3,4-Dioxo-1,5-cyclohexadiene-1-carboxylic acid	$C_7H_4O_4$	75435-17-5	152
V′	4-(2-Hydroxy-2-propanyl)phenol	$C_9H_{12}O_2$	2948-47-2	152
VI′	4′-Hydroxyacetophenone	$C_8H_8O_2$	99-93-4	136
VII′	4-Hydroxybenzoic acid	$C_7H_6O_3$	99-96-7	138
VIII′	Hydroquinone	$C_6H_6O_2$	123-31-9	110
IX′	1,4-Benzochinon	$C_6H_4O_2$	106-51-4	108

3.2. Toxicity analysis

A complete analysis of the effectiveness of advanced oxidation processes requires to estimate the toxicological influence of the post-reaction water solutions on different indicator organisms. The toxicity tests conducted on saltwater bacteria, freshwater crustaceans and vascular plants showed a continuously increase of toxicity for both micropollutants water solution after their treatment by the both O_3 and O_3/H_2O_2 process. In Figs. 9 and 10, the toxicity effect of post-processed water solutions of diclofenac was compared. All three tests confirmed that the oxidation of diclofenac in the O_3/H_2O_2 process leads to the formation of a smaller number of toxic intermediates influenced the decrease of the metabolic processes of the tested organisms than the single O_3 process. Only water samples after 10 and 20 min of ozonation were characterized by the *Lemna* sp. GIT as lower toxic than those after the O_3/H_2O_2 process. In general vascular, plants were least sensitive to diclofenac transformation products. According to the toxicity classification given in Table 3, water solutions of pH 5 after 10 min of ozonation are classified by all toxicity tests as toxic. The toxicity of post-ozonation water solution decreases with the increase of the reaction mixture pH. However, the Microtox® and the Daphtoxkit F® test indicated a high toxicity of samples after 30 min of ozonation irrespective of the solution pH. A high toxicity estimated by the use of the Microtox® biotest was also observed in solutions of pH 5 and 7 after the O_3/H_2O_2 process. The test results obtained by the Daphtoxkit F® and *Lemna* sp. GIT calcified those water samples as toxic. Whereas, the solutions of pH 11 were irrespective of the process time and the used toxicity test classified as toxic against water organisms. The oxidization of DCF by other advanced oxidation processes, that is, heterogeneous photocatalysis does not indicated a toxic nature of the post-processed solutions [51]. This is due to a significantly lower concentration of the formed transformation products of the oxidized compound.

The toxicity analysis results for BPA water solutions after the process of O_3 and O_3/H_2O_2 are presented in Figs. 11 and 12. In both processes, an increase of water toxicity with the increase of the reaction mixture pH and the process time was observed. It is correlated with the continuously decrease of BPA and the formation of oxidation by-products in those samples. The highest sensitivity of those phenolic transformation products was observed for the *Lemna* sp. GIT test organisms. According to test results obtained from the vascular plant bioassay all post-processed water solution

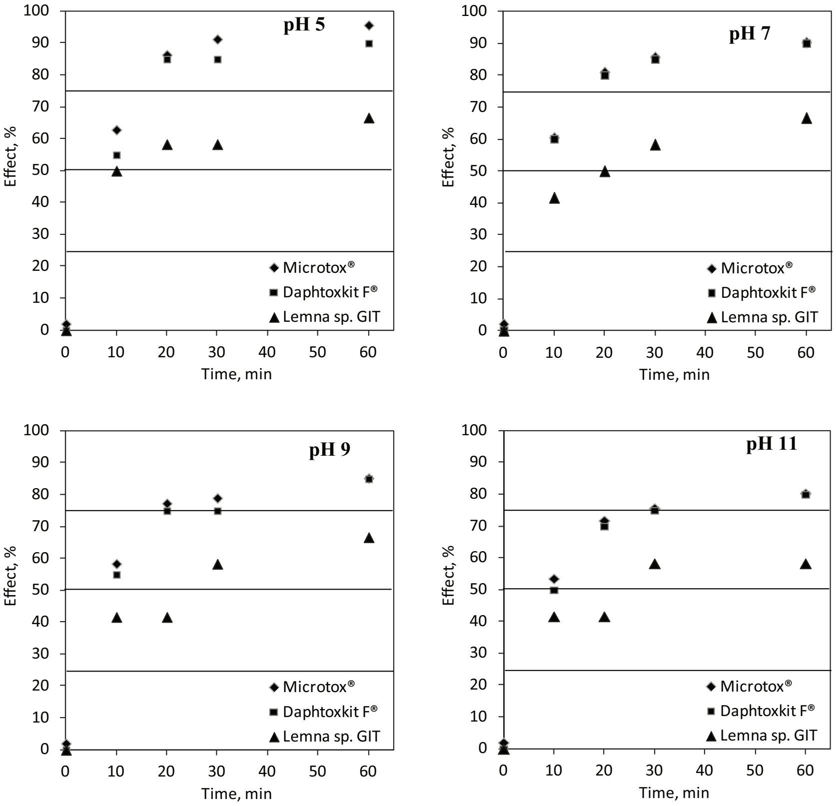


Fig. 9. Change of water toxicity during the O_3 process of DCF water solutions.

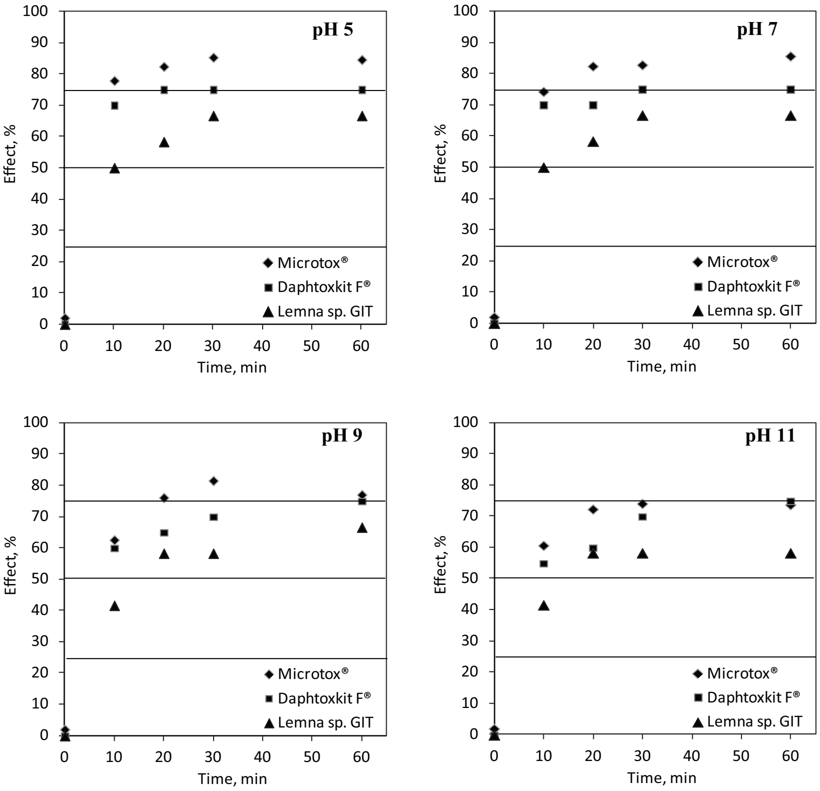


Fig. 10. Change of water toxicity during the O_3/H_2O_2 process of DCF water solutions.

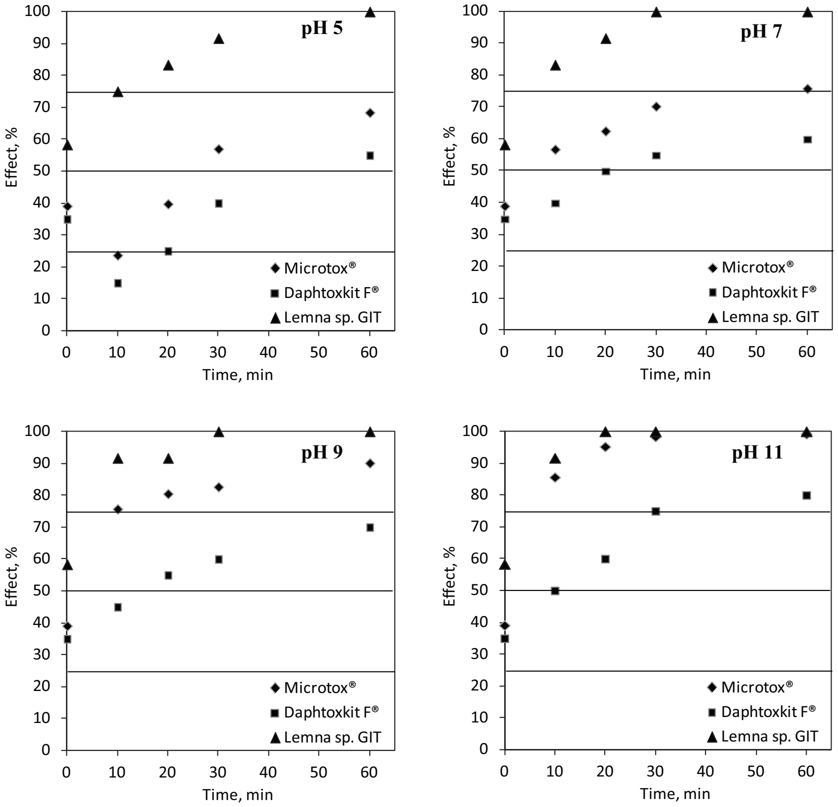


Fig. 11. Change of water toxicity during the O_3 process of bisphenol A water solutions.

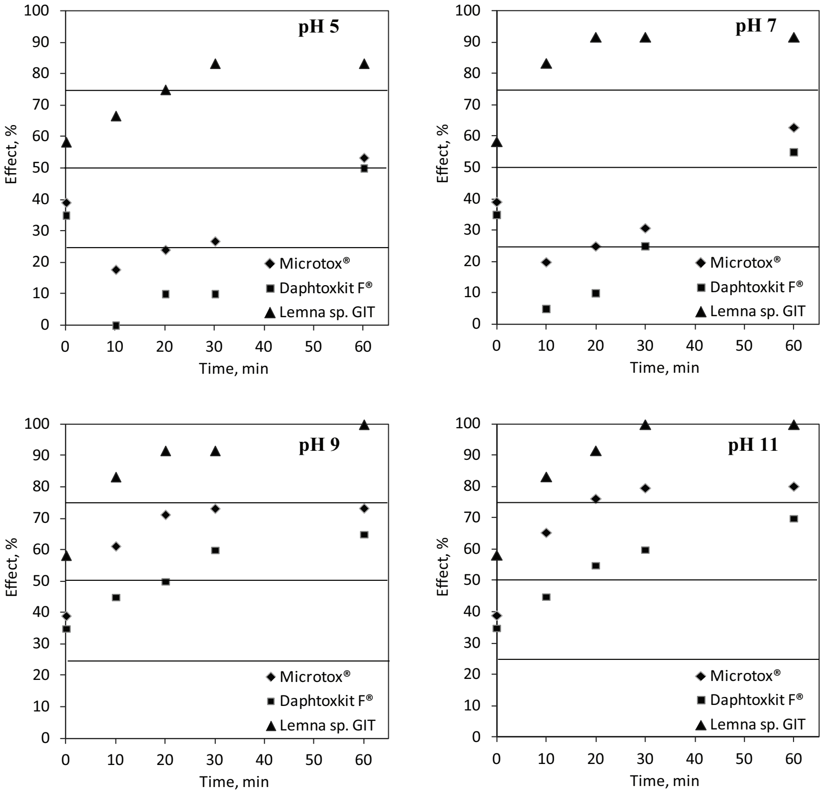


Fig. 12. Change of water toxicity during the O_3/H_2O_2 process of bisphenol A water solutions.

are high toxic. While the Microtox® and the Daphtoxkit F® tests indicated the decrease in water toxicity of solutions of pH 5 and 7 after 5 min of the O_3/H_2O_2 process and the solution of pH 5 after 5 min of the O_3 process. Those solutions were not toxic for saltwater bacteria and freshwater crustaceans. The Daphtoxkit F® test also classified all solutions after 60 min of process duration as toxic. While the Microtox® and *Lemna* sp. GIT pointed a high toxicity of samples of pH 7, 9 and 11 after the O_3 process and the sample of pH 11 after 60 min of O_3/H_2O_2 oxidation. Kim et al. [52] and Bertanza et al. [53] reported a reduction in both estrogenicity and acute aquatic toxicity in BPA water samples containing other organic matter such as wastewater or wastewater effluents, after the O_3 process. Therefore, it can be assumed, that the presence of organic matter contributes to the improvement of the removal efficiency of toxic transformation products.

4. Conclusions

The results obtained during the conducted investigations proved that during the oxidation of organic micropollutants several transformation products were formed. The decomposition of DCF coursed more effectively in the ozonation process supported by the addition of H_2O_2 than during single O_3 oxidation. The removal rate of this pharmaceutical compound after 60 min of O_3/H_2O_2 process ranged from 67% to 89%, while for the O_3 process, the compound removal rate did not exceed 66%. An inverse relationship was observed for the oxidation of BPA. The highest removal rate of that compound was noted for the single O_3 process. Additionally, the pH of the reaction mixtures affected the effectiveness of both O_3 and O_3/H_2O_2 processes. The removal rate of DCF increases with the decrease of the water pH. On the other hand, the oxidation of BPA decreases with the decrease of the reaction mixture pH. The micropollutant concentration in solutions of pH 5 was reduced in the O_3/H_2O_2 process by 19%, while in solutions of pH 11 the removal rate of BPA was over 57%.

The GC-MS analysis of post-processed water solution indicated the formation of several decomposition by-products. During the ozonation of DCF seven transformation products were identified. However, the O_3/H_2O_2 process leads to the formation of only five by-products. The largest number of nine oxidation by-products was observed in BPA water solution at pH 11 after the O_3 process. The negative impact of the formed by-products on water organisms was confirmed by the performed toxicity tests. Saltwater bacteria were more sensitive to diclofenac transformation products than other tested organisms and classified all water solution after 60 min of both O_3 and O_3/H_2O_2 processes as high toxic. The obtained toxicity results for water solutions containing BPA transformation products indicated the highest toxic influence of those compounds on vascular plans. The post-processed water solution was also classified as high toxic.

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References

[1] R.P. Schwarzenbach, T. Egli, T.B. Hofstetter, U. von Gunten, B. Wehrli, Global water pollution and human health, Annu. Rev. Environ. Resour., 35 (2010) 109–136.

[2] H.-P. Kaiser, O. Köster, M. Gresch, P.M.J. Périsset, P. Jäggi, E. Salhi, U. von Gunten, Process control for ozonation systems: a novel real-time approach, Ozone Sci. Eng., 35 (2013) 168–185.

[3] J. Xia, Q.-Y. Duan, Y. Luo, Z.-H. Xie, Z.-Y. Liu, X.-G. Mo, Climate change and water resources: case study of Eastern Monsoon Region of China, Adv. Clim. Change Res., 8 (2017) 63–67.

[4] World Water Assessment Programme, The United Nations World Water Development Report 3: Water in a Changing World, UNESCO Publishing, and London; Earthscan, Paris, 2009.

[5] P. Falås, A. Wick, S. Castronovo, J. Habermacher, T.A. Ternes, A. Joss, Tracing the limits of organic micropollutant removal in biological wastewater treatment, Water Res., 95 (2016) 240–249.

[6] H. Shi, X. Cheng, Q. Wu, R. Mu, Y. Ma, Assessment and removal of emerging water contaminants, J. Environ. Anal. Toxicol., S2:003 (2012) 1–14.

[7] A.R. Ribeiro, O.C. Nunes, M.F.R. Pereira, A.M.T. Silva, An overview on the advanced oxidation processes applied for the treatment of water pollutants defined in the recently launched Directive 2013/39/EU, Environ. Int., 75 (2015) 33–51.

[8] M.T. Amin, A.A. Alazba, U. Manzoor, A review of removal of pollutants from water/wastewater using different types of nanomaterials, Adv. Mater. Sci. Eng., 2014 (2014) 1–24.

[9] P.S. Nasirabadi, E. Saljoughi, S.M. Mousavi, Membrane processes used for removal of pharmaceuticals, hormones, endocrine disruptors and their metabolites from wastewaters: a review, Desal. Wat. Treat., 57 (2016) 24146–24175.

[10] M.V. Selma, A. Allende, F. López-Gálvez, M.A. Conesa, M.I. Gil, Disinfection potential of ozone, ultraviolet-C and their combination in wash water for the fresh-cut vegetable industry, Food Microbiol., 25 (2008) 809–814.

[11] M. Bundschuh, M.O. Gessner, G. Fink, T.A. Ternes, C. Sögding, R. Schulz, Ecotoxicological evaluation of wastewater ozonation based on detritus–detritivore interactions, Chemosphere, 82 (2011) 355–361.

[12] S. Sudhakaran, J. Calvin, G.L. Amy, QSAR models for the removal of organic micropollutants in four different river water matrices, Chemosphere, 87 (2012) 144–150.

[13] J. Derco, J. Dudáš, M. Valičková, K. Šimovičová, J. Kecskés, Removal of micropollutants by ozone based processes, Chem. Eng. Process., 94 (2015) 78–84.

[14] J. Hollender, S.G. Zimmermann, S. Koepke, M. Krauss, C.S. McArdell, C. Ort, H. Singer, U. von Gunten, H. Siegrist, Elimination of organic micropollutants in a municipal wastewater treatment plant upgraded with a full-scale post-ozonation followed by sand filtration environ, Sci. Technol., 43 (2009) 7862–7869.

[15] J. Margot, C. Kienle, A. Magnet, M. Weil, L. Rossi, L.F. de Alencastro, C. Abegglen, D. Thonney, N. Chèvre, M. Schärer, D.A. Barry, Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? Sci. Total Environ., 461–462 (2013) 480–498.

[16] A.K. Biń, Ozone solubility in liquids, Ozone Sci. Eng., 28 (2006) 67–75.

[17] L.M. da Silva, J.F. Wilson, Trends and strategies of ozone application in environmental problems, Quim. Nova, 29 (2006) 310–317.

[18] M. Bourgin, E. Borowska, J. Helbing, J. Hollender, H.-P. Kaiser, C. Kienle, C.S. McArdell, E. Simon, U. von Gunten, Effect of operational and water quality parameters on conventional ozonation and the advanced oxidation process O_3/H_2O_2: kinetics of micropollutant abatement, transformation product and bromate formation in a surface water, Water Res., 122 (2017) 234–245.

[19] Y. Lee, U. von Gunten, Advances in predicting organic contaminant abatement during ozonation of municipal wastewater effluent: reaction kinetics, transformation products, and changes of biological effects, Environ. Sci. Water Res., 2 (2016) 421–442.

[20] X. Jin, S. Peldszus, P.M. Huck, Reaction kinetics of selected micropollutants in ozonation and advanced oxidation processes, Water Res., 46 (2012) 6519–6530.
[21] C. von Sonntag, U. von Gunten, Chemistry of Ozone in Water and Wastewater Treatment, IWA Publishing, London, 2012.
[22] H. Yu, E. Nie, J. Xu, S. Yan, W.J. Cooper, W. Song, Degradation of diclofenac by advanced oxidation and reduction processes: kinetic studies, degradation pathways and toxicity assessments, Water Res., 47 (2013) 1909–1918.
[23] U. Hübner, U. von Gunten, M. Jekel, Evaluation of the persistence of transformation products from ozonation of trace organic compounds – a critical review, Water Res., 68 (2015) 150–170.
[24] M. Bourgin, G. Gervais, E. Bichon, J.P. Antignac, F. Monteau, G. Leroy, L. Barritaud, M. Chachignon, V. Ingrand, P. Roche, B. Le Bizec, Differential chemical profiling to identify ozonation by-products of estrone-sulfate and first characterization of estrogenicity in generated drinking water, Water Res., 47 (2013) 3791–3802.
[25] H. Mestankova, K. Schirmer, B.I. Escher, U. von Gunten, S. Canonica, Removal of the antiviral agent oseltamivir and its biological activity by oxidative processes, Environ. Pollut., 161 (2012) 30–35.
[26] D. Stalter, A. Magdeburg, J. Oehlmann, Comparative toxicity assessment of ozone and activated carbon treated sewage effluents using an in vivo test battery, Water Res., 44 (2010) 2610–2620.
[27] D. Stalter, A. Magdeburg, M. Weil, T. Knacker, J. Oehlmann, Toxication or detoxication? In vivo toxicity assessment of ozonation as advanced wastewater treatment with the rainbow trout, Water Res., 44 (2010) 439–448.
[28] K. Li, A. Yediler, M. Yang, S. Schulte-Hostede, M.H. Wong, Ozonation of oxytetracycline and toxicological assessment of its oxidation by-products, Chemosphere, 72 (2008) 473–478.
[29] A.J. Ebele, M. Abou-Elwafa Abdallah, S. Harrad, Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment, Emerg. Contam., 3 (2017) 1–16.
[30] M. Česen, K. Lenarčič, V. Mislej, M. Levstek, A. Kovačič, B. Cimrmančič, N. Uranjek, T. Kosjek, D. Heath, M. Sollner Dolenc, E. Heath, The occurrence and source identification of bisphenol compounds in wastewaters, Sci. Total Environ., 616–617 (2018) 744–752.
[31] S. Kim, P.A. Thiessen, E.E. Bolton, J. Chen, G. Fu, A. Gindulyte, L. Han, J. He, S. He, B.A. Shoemaker, J. Wang, B. Yu, J. Zhang, S.H. Bryant, PubChem substance and compound databases, Nucleic Acids Res., 4 (2016) D1202–D1213.
[32] M. Dudziak, E. Burdzik, Oxidation of bisphenol A from simulated and real urban wastewater effluents by UV, O_3 and UV/O_3, Desal. Wat. Treat., 57 (2016) 1075–1083.
[33] M. Dudziak, E. Kudlek, Application of Microtox® biotest for controlling quality of xenobiotics-containing water during its UV irradiation with or without addition of H_2O_2, Przem. Chem., 94 (2015) 1916–1918.
[34] S. Parvez, C. Venkataraman, S. Mukherji, A review on advantages of implementing luminescence inhibition test (*Vibrio fischeri*) for acute toxicity prediction of chemicals, Environ. Int., 32 (2006) 265–268.
[35] J. Menz, M. Schneider, K. Kümmerer, Toxicity testing with luminescent bacteria – characterization of an automated method for the combined assessment of acute and chronic effects, Chemosphere, 93 (2013) 990–996.
[36] C. Mahugo Santana, Z. Sosa Ferrera, M.E. Torres Padrón, J.J. Santana Rodríguez, Methodologies for the extraction of phenolic compounds from environmental samples: new approaches, Molecules, 14 (2009) 298–320.
[37] J. Bohdziewicz, M. Dudziak, G. Kamińska, E. Kudlek, Chromatographic determination and toxicological potential evaluation of selected micropollutants in aquatic environment – analytical problems, Desal. Wat. Treat., 57 (2016) 1361–1369.
[38] P. Somasundaran, Encyclopedia of Surface and Colloid Science, Vol. 6, CRC Press, London, 2006.
[39] G. Li, J. He, D. Wang, P. Meng, M. Zeng, Optimization and interpretation of O_3 and O_3/H_2O_2 oxidation processes to pretreat hydrocortisone pharmaceutical wastewater, Environ. Technol., 36 (2015) 1026–1034.
[40] U. von Gunten, Ozonation of drinking water: Part I. Oxidation kinetics and product formation, Water Res., 37 (2003) 1443–1467.
[41] K. Sehested, J. Holcman, E. Bjergbakke, E.J. Hart, A pulse radiolytic study of the reaction OH + O_3 in aqueous medium, J. Phys. Chem., 88 (1984) 4144–4147.
[42] B.G. Ershov, P.A. Morozov, The kinetics of ozone decomposition in water, the influence of pH and temperature, Russ. J. Phys. Chem. A, 83 (2009) 1295–1299.
[43] T. Poznyak, R. Tapia, J. Vivero, I. Chairez, Effect of pH to the decomposition of aqueous phenols mixture by ozone, J. Mex. Chem. Soc., 50 (2006) 28–35.
[44] M.M. Sein, M. Zedda, J. Tuerk, T.C. Schmidt, A. Golloch, C.von Sonntag, Oxidation of diclofenac with ozone in aqueous solution, Environ. Sci. Technol., 42 (2008) 6656–6662.
[45] M. Deborde, S. Rabouan, P. Mazellier, J.-P. Duguet, B. Legube, Oxidation of bisphenol A by ozone in aqueous solution, Water Res., 42 (2008) 4299–4308.
[46] T. Garoma, S.A. Matsumoto, Y. Wu, R. Klinger, Removal of bisphenol A and its reaction-intermediates from aqueous solution by ozonation, Ozone Sci. Eng., 32 (2010) 338–343.
[47] M. Deborde, S. Rabouan, J.P. Duguet, B. Legube, Kinetics of aqueous ozone-induced oxidation of some endocrine disruptors, Environ. Sci. Technol., 39 (2005) 6086–6092.
[48] E. Mvula, C. von Sonntag, Ozonolysis of phenols in aqueous solution, Org. Biomol. Chem., 1 (2003) 1749–1756.
[49] H.-S. Kim, H. Yamada, H. Tsuno, The removal of estrogenic activity and control of brominated by-products during ozonation of secondary effluents, Water Res., 41 (2007) 1441–1446.
[50] W. PengZhen, L. Jian, L. ShuJin, T. Fu-Ming, Theoretical study of mechanism and kinetics for the addition of hydroxyl radical to phenol, Sci. China Chem., 55 (2012) 270–276.
[51] E. Kudlek, M. Dudziak, J. Bohdziewicz, Influence of inorganic ions and organic substances on the degradation of pharmaceutical compound in water matrix, *Water*, 8 (2016) 532.
[52] S.-E. Kim, N.-S. Park, H. Yamada, H. Tsuno, Modeling of decomposition characteristics of estrogenic chemicals during ozonation, Environ. Technol., 29 (2008) 287–297.
[53] G. Bertanza, R. Pedrazzani, M. Dal Grande, M. Papa, V. Zambarda, C. Montani, N. Steimberg, G. Mazzoleni, D. Di Lorenzo, Effect of biological and chemical oxidation on the removal of estrogenic compounds (NP and BPA) from wastewater: an integrated assessment procedure, Water Res., 45 (2011) 2473–2484.