HYDROXYL RADICALS-BASED DEGRADATION OF LOPERAMIDE HYDROCHLORIDE: THE EFFECT OF COMMON WATER CONSTITUENTS AND ECOTOXICOLOGY ANALYSIS

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Microcontaminants, which can be present in low concentrations in aqueous environmental matrices, are a growing problem, as they are difficult to remove using conventional wastewater treatment processes. The application of Advanced Oxidation Processes (AOPs) has been extensively studied to remove these types of organic pollutants from water. In this study, the degradation of pharmaceutical loperamide hydrochloride by artificial UV irradiation in the presence of H_2O_2 was investigated. The treatment used appears to be suitable for rapid and complete removal of loperamide (30 mg/dm^3) within 6 minutes of irradiation under optimal operating conditions. A considerable degree of loperamide mineralization was also attained at the end of the treatment. The evolution of low molecular weight organic acids, such as oxalic acid, formic acid, and acetic acid, was evidenced. The influence of typical components present in surface waters and wastewater effluents (HA, NO_3^-, HCO_3^-, Cl^-, SO_4^{2-}) on the degradation rate of loperamide was investigated. The degradation of loperamide was negatively influenced by the presence of humic acid, nitrate, and bicarbonate, whereas the influence of chloride and sulphate was neglected. The evaluation of toxicity with the marine photobacterium *Vibrio fischeri* was performed to examine whether or not the hydroxyl radical-based treatment of loperamide leads to by-products with upgraded toxicity to aquatic organisms.

Keywords: mineralization, pharmaceuticals, advance oxidation processes, *Vibrio fischeri*, water matrix

Introduction

Pharmaceuticals constitute a group of environmental pollutants that have been identified as “contaminants of emerging concern” (CECs). The main sources of environmental pollution from these compounds are industrial effluents, hospital effluents, septic tanks, and sewage treatment plants [1]. In the last 20 years, interest in studying the presence of these pollutants in the aquatic environment has increased [2-4]. Many authors reported the presence of pharmaceutical active compounds in hospital wastewaters, surface waters, and effluents from pharmaceutical manufacturers [5-7]. Despite being present in relatively low levels, which are in the order of ng/dm^3 to µg/dm^3, the continuous discharge of pharmaceuticals into the aquatic environment has led to concern about their potential impact on the environment, as well as the potential impact of their by-products on public health [8]. Adverse effects of pharmaceuticals in the environment include aquatic toxicity, development of resistance in pathogenic bacteria, genotoxicity, and endocrine disruption [9].

Since typical water and wastewater treatment processes are not able to act as a reliable barrier against some recalcitrant pharmaceuticals, it is imperative to propose additional advanced treatment technologies in areas where problem with pollution has been noted or expected. Advanced oxidation processes (AOPs) have been shown to be effective for the degradation of various organic contaminants from wastewaters and drinking waters [10-12]. Among the technologies accessible to eliminate these types of pollutants, UV light-driven advanced oxidation processes, which utilize UV light to generate highly reactive radicals, are recognized as one of the most efficient approaching technologies for environmental remediation. The degradation of organic contaminants from water with UV-activated hydrogen peroxide is primarily achieved by hydroxyl radicals, which are formed during the photolysis of hydrogen peroxide. Hydroxyl radicals are extremely strong oxidising agents capable of reacting non-selectively with many organic impurities, usually by reactions of hydrogen abstraction, electron transfer or electrophilic addition [13]. It has been reported that the second-order rate constants for the reaction of $HO^{\bullet}$ radicals with organic pollutants are in the range of 10^7

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– 10^{10} 1/Ms, relying upon on the chemical structure of the pollutant [14].

Loperamide hydrochloride (4-(p-chlorophenyl)-4-hydroxy-N,N-dimethyl-α,α-diphenyl-1-piperidine butyramide hydrochloride) is an opiate agonist that is widely used as an effective drug for the control and symptomatic relief of acute non-specific diarrhoea and chronic diarrhoea associated with inflammatory bowel disease [15]. Loperamide hydrochloride is extensively metabolised in the liver by N-demethylation to N-desmethyl loperamide, the major inactive metabolite. It is mainly excreted in the faeces as an unchanged active substance or as metabolites [16].

A comprehensive compilation of data from 2003 to 2014 on the occurrence of pharmaceuticals in the freshwater and marine environment of the Baltic Sea showed that loperamide was detected in the influents and effluents of municipal wastewater treatment plants in Denmark, Estonia, Finland, Germany, Russia, and Sweden [17]. Some authors have pointed out that loperamide can be effectively removed from water by diverse treatment processes such as biosorption [18]. In comparison with other wastewater treatment technologies, advanced oxidation could destroy pollutants without transferring them to another medium, as it is case, for example, with adsorption [19]. These processes have gained significant attention due to their strong oxidizing properties, rapid reaction kinetics, broad applicability, and minimal secondary pollution [20]. Moreover, some advantages of UV/H_2O_2 process are complete mixing of oxidant with water, ease of operation, stability of peroxide, and prevention of sludge production during the process [21]. However, in practice, complete degradation of the contaminants to the carbon dioxide, water and mineral products by these processes is not always achieved, resulting in the presence of intermediates, and the toxicity of intermediates is usually unknown [22]. Therefore, it is necessary to investigate the toxicity of degradation products.

The aim of this work was therefore to investigate whether it is possible to effectively remove loperamide hydrochloride from aqueous solutions by artificial UV irradiation in the presence of hydrogen peroxide as a source of hydroxyl radicals. The degree of mineralisation of loperamide and the development of mineralisation products was monitored by measurements of total organic carbon and ion chromatographic analyses, respectively. In addition, the possible influence of ions typically present in surface waters or wastewater on the efficiency of the process was evaluated. Finally, a toxicity assessment was performed to compare the toxicity of the degradation products formed during the treatment with the toxicity of the initial compound.

Experimental

Chemicals

Loperamide hydrochloride was purchased from Sigma-Aldrich (USA). The hydrogen peroxide solution (30%), analytical grade, was purchased from Merck (Germany). HPLC grade methanol and acetonitrile were supplied from J.T. Baker (India). Humic acid was purchased from Sigma-Aldrich (USA). All other used chemicals (NaCl, $NaHCO_3$, Na_2SO_4, $NaNO_3$) were analytical grade and supplied from Merck (Germany). All solutions were prepared with ultrapure water from the Smart2Pure system with a conductivity of 0.055 μSm^{-1} (Thermo Scientific, USA).

Irradiation system

The degradation experiments were carried out in a laboratory-scale batch photoreactor equipped with ten low-pressure mercury lamps (Philips, Holland) emitting at 253.7 nm and mounted in parallel on the top of the photoreactor. The distance between the surface of the solution and the UV lamps was kept constant at 220 mm. The light intensity on the surface of the solution was measured with a UV radiometer Solarmeter model 8.0 UVC (Solartech, USA). The schematic of the photoreactor used for the irradiation experiments can be found elsewhere [23].

Procedures

All experiments were performed with 100 cm^3 working solution, with the desired initial peroxide (5.0, 10.0, 20.0, 40.0, 60.0 and 100.0 $mmol/dm^3$) and loperamide concentration (30 mg/dm^3) and at the corresponding pH values (2.0, 3.0, 5.0, 7.0, 9.0 and 10.0) in glass Petri dishes. The set of experiments, where effect of common water constituents was investigated, were performed with the addition of calculated volumes of 1 mol/dm^3 solutions of corresponding salts (NaCl, $NaHCO_3$, Na_2SO_4, $NaNO_3$). The effect of humic acid (HA) was investigated by adding the desired amount of HA stock solution (500 mg/dm^3) to the working solution. The samples were taken at specific time intervals (0, 1, 2, 4, 6, 10, 20, 40, 60, 90, 180 and 240 minutes) and the residual concentration of loperamide or total organic carbon was measured, and ion chromatography analysis was carried out. The efficiency of degradation was defined by the following equation:

$$\text{Removal efficiency (\%)} = \left(1 - \frac{C_t}{C_o}\right) \times 100 \quad \text{.....(1)}$$

where c_0 is the initial loperamide concentration, c_t is loperamide concentration at irradiation time t.

The pseudo-first-order kinetic model, which can be described by equation (2), was used to follow the kinetics of the disappearance of loperamide.

$$\ln \frac{C_t}{C_o} = -k_{app} t \quad \text{.....(2)}$$

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where c_0 is the initial loperamide concentration, c_t is loperamide concentration at time t, k_{app} is the apparent rate constant, t is the irradiation time. From the linear plot of $\ln(c_t/c_0)$ versus time, the values of k_{app} can be calculated from experimentally obtained data. The values of R^2 are greater than 0.9 in most experiments, indicating that the model used is in good agreement with the laboratory data. All the tests were performed in triplicate and the average of the three values is shown with standard deviation error bars.

Analytical methods

The concentration of loperamide hydrochloride during the treatment was followed with an Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, USA) equipped with Zorbax Eclipse Plus C18 column (Agilent Technologies, USA) and UV-Vis detector. The mobile phase consisted of acetonitrile/water/10 mmol/dm^3 dihydrogen phosphate aqueous solution (30/60/10). The flow rate was 1 ml min^{-1}, the injection volume was 20 µL and the temperature of the column was 25 °C. Total organic carbon (TOC) was determined by means of Shimadzu TOC-V_{CPH} analyzer (Japan). Ion chromatographic measurement was done on an IC Dionex ICS-3000 (Thermo Fisher Scientific, USA). The residual hydrogen peroxide concentration was determined by a colorimetric method involving the oxidation of iodide to the triiodide anion by peroxides catalysed by ammonium molybdate [24].

Vibrio Fischeri analyses

The freeze-dried *Vibrio fischeri* bacteria (Macherey-Nagel, Germany) was used for evaluation of possible toxicity of loperamide degradation products. The analysis was performed in accordance with ISO 11348 (1998) standard protocols [25]. The freeze-dried luminescent bacteria were reconstituted with a growth medium (2% NaCl) and incubated at 15 °C for 15 minutes before use. Prior to analysis, the enzyme catalase (aqueous suspension from bovine liver, 10 000-40 000 units/mg, Sigma Aldrich, USA) was added to each sample to decompose residual peroxide. The bioluminescence was measured after 30 minutes with a Lumino m90a illuminometer at a working temperature of 15 °C. The decrease in luminescence in each sample was compared with the control and the percentage of inhibition was calculated. All measurements were performed in triplicate.

Results and Discussion

UV and UV/H_2O_2 experiments

In the first part of the study, several preliminary experiments were conducted to investigate the influence of the basic operating parameters on the degradation efficiency of loperamide hydrochloride. Experiments performed only with the addition of peroxide (20 mmol/dm^3), but without UV irradiation, showed that no degradation of loperamide hydrochloride took place (Figure 1).

On the other hand, as loperamide is able to absorb the UV light emitted by the lamp used in this study, a certain amount of loperamide could be degraded by direct UV irradiation. However, the results of the TOC measurement revealed a small drop (only 10%) during direct UV irradiation of loperamide over the 6-hour period, indicating that UV treatment alone was not as effective for the mineralization of loperamide. Otherwise, experiments carried out with both H_2O_2 and UV irradiation showed that the oxidation of loperamide was greatly enhanced. Fig. 1 shows that the concentration of loperamide decreased drastically during UV and H_2O_2 irradiation and disappeared completely after 6 minutes of irradiation.

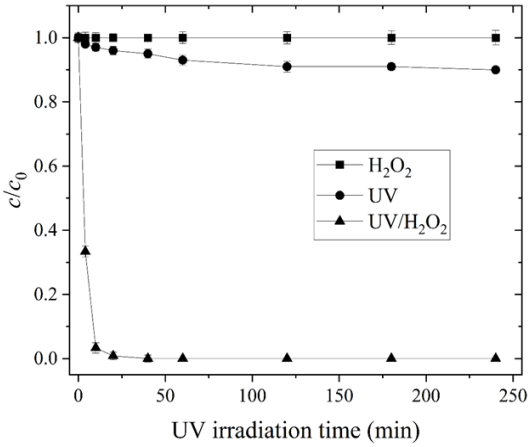


Figure 1. Decay of loperamide concentration in the presence of only H_2O_2, under UV irradiation, and during UV/H_2O_2 treatment. Experimental conditions: c_0(Lop) = 30.0 mg/dm^3, c_0(H_2O_2) = 20.0 mmol/dm^3, pH 7.0 ± 0.2, UV light intensity was 730 µW/cm^2, temperature was 25 ± 0.5 ºC

The results of the TOC analysis shown in Fig. 2 indicate that the mineralisation of loperamide during UV/H_2O_2 treatment followed a different trend than its degradation from solution. Although the loperamide almost completely disappeared within 6 minutes of irradiation, the degree of mineralisation within the same period of irradiation was only 8%. Furthermore, TOC removal gradually increased and reached 67% after 120 minutes, while 90% of TOC removal was achieved after 240 minutes of treatment. Since these experiments were performed without continuous addition of peroxide during irradiation, the absence of complete mineralisation is probably due to the decrease in H_2O_2 concentration and consequently the lack of HO$^\bullet$ radicals, as can be seen in Figure 2. Moreover, as the reaction progressed, the quantity of organic intermediates, which showed high reactivity towards HO$^\bullet$ radicals increased, so the consumption of HO$^\bullet$ radicals is higher [26].

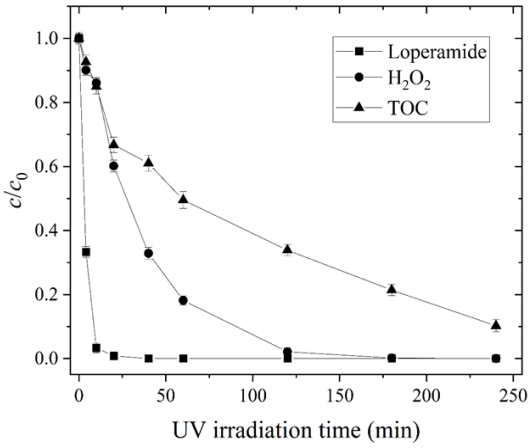


Figure 2. Decay of loperamide concentration, H_2O_2 concentration, and TOC during the UV/H_2O_2 treatment of loperamide. Experimental conditions: c_0(Lop) = 30.0 mg/dm^3, c_0(H_2O_2) = 20.0 mmol/dm^3, pH 7.0 ± 0.2, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

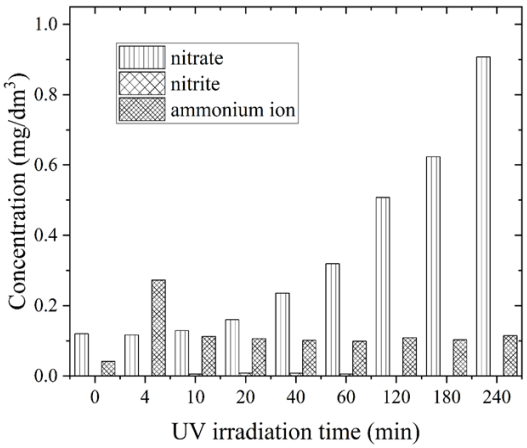


Figure 3. Evolution of mineralization products during the UV/H_2O_2 treatment of loperamide. Experimental conditions: *c0(Lop) = 30.0 mg/dm3, c0(H2O2) = 20.0 mmol/dm3,* pH 7.0 ± 0.2, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

Since loperamide is an organic molecule containing covalently bonded oxygen and nitrogen atoms, possible evolution of ions such as nitrate, nitrite, and ammonium ions as mineralization products during irradiation is expected. Considering that the oxidation process took place, the evolution of low molecular weight organic acids as oxidation products is also possible. The results of the evolution of mineralization products and organic acids during the UV/H_2O_2 treatment of loperamide are summarized in Fig. 3 and Fig. 4, where the concentrations of ions are plotted as functions of time of irradiation. As can be seen, nitrate, nitrite, and ammonium ions are the main mineralization products, whereas the carboxylic acids generated during the treatment were acetic, formic, and oxalic acids. The main N-containing mineralization product was nitrate, whose concentration significantly increased during the treatment and reached value of 0.9 mg/dm^3 by the end of the treatment. During the first 120 minutes of UV/H_2O_2 oxidation of loperamide, no significant amounts of nitrite were detected, while it decreased to undetectable levels by the end of the treatment. Otherwise, the concentration of ammonium ion stayed almost constant during the treatment. The total nitrogen content, which was determined considering the nitrate, nitrite and ammonium ion concentration after 240 minutes of irradiation, is about four times lower than the stoichiometrically expected value, indicating a possible formation of gaseous nitrogenous species.

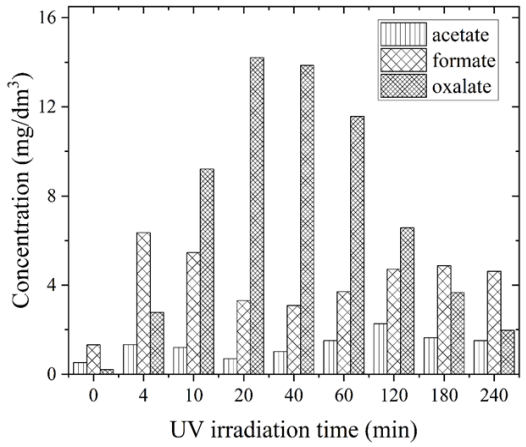


Figure 4. Evolution of low molecular weight organic acids during the UV/H_2O_2 treatment of loperamide. Experimental conditions: *c0(Lop) = 30.0 mg/dm3, c0(H2O2) = 20.0 mmol/dm3,* pH 7.0 ± 0.2, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

The evolution of formate during the UV/H_2O_2 treatment of loperamide started immediately, reached the maximum value after 4 minutes, decreased for a half up to 20 minutes, and stayed unchanged until the end of the treatment (Fig. 4). A similar pattern was obtained during the evolution of the other two detected carboxylic acids. The oxalate concentration sharply increased in the first minutes of treatment, reached the maximum up to 20 min of irradiation, after which continuous decrease of its concentration occurred. Acetate was formed at a lower concentration compared to oxalate and formate, with a similar increase in concentration in the early stages of irradiation and a decrease thereafter. From the experi-

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mental results, it is evident that some amount of formed mineralization products and oxidation products could be further degraded, while some of them were accumulated in the solution. It is reported in previous works that the formic, acetic acid and oxalic acid are the most often generated carboxylic acids during the application of AOPs processes, with a similar pattern of evolution to the degradation of loperamide [27, 28].

A series of experiments was also performed to investigate the effect of six initial peroxide concentrations (5.0, 10.0, 20.0, 40.0, 60.0 and 100.0 mmol/dm^3) on the degradation of loperamide. From the experimental results, the apparent rate constants increased with increasing H_2O_2 concentration up to 20.0 mmol/dm^3. Thereafter, a further increase in H_2O_2 concentration began to inhibit loperamide degradation (Fig. 5). Thus, the optimal initial concentration of peroxide seems to be 20.0 mmol/dm^3, which corresponds to the peroxide/loperamide molar ratio of 330. This behaviour is a consequence of the well-known scavenging effect of H_2O_2, whereby the excess peroxide reacts with HO$^\bullet$ radicals to form hydroperoxyl radicals with a much lower reduction potential than that of the HO$^\bullet$ radicals [29].

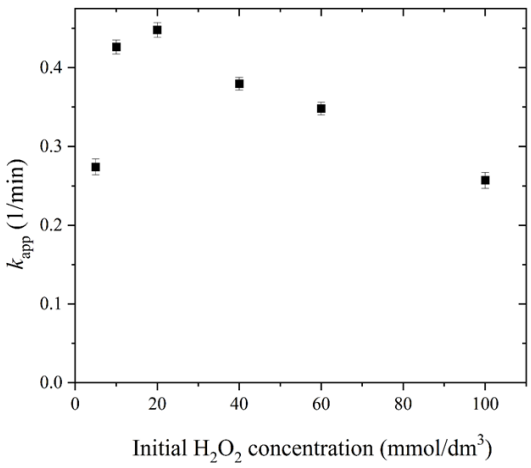


Figure 5. The effect of initial peroxide concentrations on pseudo-first rate constant of loperamide degradation during the UV/H_2O_2 treatment. Experimental conditions: c_0(Lop) = 30.0 mg/dm^3, pH 7.0 ± 0.2, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

To investigate the effect of initial pH value on the degradation of loperamide, solutions with initial loperamide concentration 30 mg/dm^3, initial peroxide concentration 20 mmol/dm^3 and at various initial pH values (2.0, 3.0, 5.0, 7.0, 9.0 and 10.0) were irradiated. The results showed that the degradation of loperamide is more efficient at pH 5.0 and pH 7.0 compared to an acidic (pH 2.0 and pH 3.0) or alkaline medium (pH 9.0 and pH 10.0) (Fig. 6). Since nitric acid was used for pH adjustment, a certain amount of nitrate ions was present in the solutions at pH 2.0 and pH 3.0, which can quench HO$^\bullet$ radicals and suppress degradation. On the other hand, several reasons can be responsible for the drastic decay of apparent rate constant in the alkaline medium. Namely, peroxide dissociate in the alkaline medium to hydroperoxyl anion (reaction 3) which can either react with a undissociated molecule of H_2O_2 (reaction 4) or consume HO$^\bullet$ radicals (reactions 5) [13, 30].

$$H_2O_2 \rightarrow HO_2^- + H^+ \quad (3)$$

$$HO_2^- + H_2O_2 \rightarrow H_2O + O_2 + OH^- \quad (4)$$

$$HO^\bullet + HO_2^- \rightarrow H_2O + O_2^{\bullet-}, k = 7.5x10^9 M^{-1}s^{-1} \quad (5)$$

In addition, the self-decomposition rate of peroxide is strongly pH-dependent and significantly higher in an alkaline medium [31]. Considering these facts, the concentration of HO$^\bullet$ radicals for reaction with loperamide in alkaline medium is lower than expected, resulting in slower apparent rate constants of loperamide degradation.

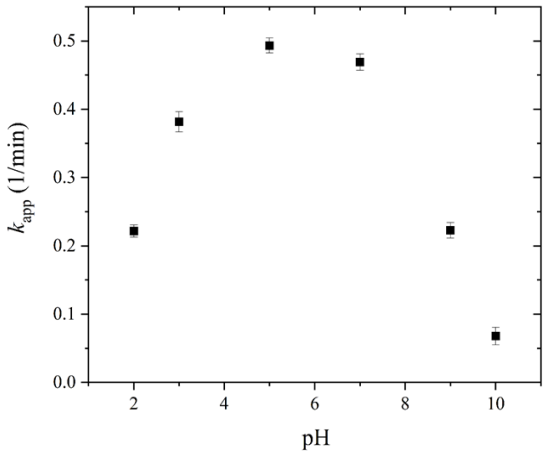


Figure 6. The effect of initial pH on pseudo-first rate constant of loperamide degradation during the UV/H_2O_2 treatment. Experimental conditions: c_0(Lop) = 30.0 mg/dm^3, c_0(H_2O_2) = 20.0 mmol/dm^3, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

The effect of water constituents

The effect of HA

Since dissolved organic matters (DOMs) can be present in different concentrations both in the effluents of wastewater treatment plants and in surface waters, it would be extremely important to investigate their influence on the efficiency of organic pollutant removal during UV/H_2O_2 treatment. The results presented in Table 1 show that as the concentration of added humic acid increased, the efficiency of loperamide removal decreased. The removal efficiency after 4 min of irradiation decreased from 93% in case of no addition of humic acid to 89% and 85% when 1 mg/dm^3 and 5 mg/dm^3 of humic acid were added, respectively. Accordingly, the apparent rate constants decreased from 0.65 1/min (without HA)

to 0.53 1/min and 0.47 1/min when solutions were spiked with 1 mg/dm^3 and 5 mg/dm^3 of humic acid, respectively. It was reported that natural organic matter can have an impact on the photochemical transformation of aqueous contaminants which may be both positive, acting as photosensitizer, and negative, competing for UV photons and HO$^\bullet$ radicals [32-34]. However, the results of this study indicated that with addition of humic acid removal, the rate of loperamide decreased, whereas this inhibitory effect is more pronounced at a higher concentration of humic acid.

Table 1. The effect of water matrix components on removal efficiency (RE) and pseudo-first rate constant (k_{app}) of loperamide degradation during the UV/H_2O_2 treatment

Anions	Concentration	k_{app} (1/min)	RE (%)
Chloride	0 mol/dm^3	0.64	97.65
	0.001 mol/dm^3	0.64	97.8
	0.01 mol/dm^3	0.59	97.03
	0.1 mol/dm^3	0.58	96.64
Bicarbonate	0 mol/dm^3	0.64	97.65
	0.001 mol/dm^3	0.55	96.18
	0.01 mol/dm^3	0.43	92.07
	0.1 mol/dm^3	0.19	69.85
Nitrate	0 mol/dm^3	0.64	97.65
	0.001 mol/dm^3	0.59	96.88
	0.01 mol/dm^3	0.45	92.38
	0.1 mol/dm^3	0.16	61.94
Sulphate	0 mol/dm^3	0.64	97.65
	0.001 mol/dm^3	0.63	98.16
	0.01 mol/dm^3	0.61	97.19
	0.1 mol/dm^3	0.57	96.43
Humic acid	0 mg/dm^3	0.64	97.65
	1 mg/dm^3	0.53	95.43
	5 mg/dm^3	0.47	93.13

The effect of inorganic anions

Many inorganic anions, such as NO_3^-, CO_3^{2-}, SO_4^{2-}, Cl^- etc. could be present in surface waters and wastewater treatment effluents and thus potentially influence the photolysis of organic pollutants during the UV/H_2O_2 process. It is known that nitrate can produce hydroxyl radicals when exposed to sunlight, and this reaction is one of the sources of HO$^\bullet$ radicals in natural waters [35-37]. On the other hand, nitrate can either absorb light in the UV range, acting as an inner filter, or efficiently scavenge HO$^\bullet$ radicals, which can result in slower elimination [37]. To examine the impact of nitrate on the efficiency of loperamide removal, three different concentrations of nitrate (0.001, 0.01 and 0.1 mol/dm^3) were added, while the other parameters were kept constant. The results shown in Table 1 indicate that loperamide was completely removed in the absence of nitrate, while in the presence of 0.001, 0.01 and 0.1 mol/dm^3 of the nitrate removal efficiency decreased, and after 10 min of irradiation reached the values of 98%, 94%, and 66%, respectively. In addition, the apparent rate constant remained almost unchanged with increasing nitrate concentration up to 0.001 mol/dm^3 (0.59 1/min), decayed to 0.44 1/min as the concentration of nitrate increased to 0.01 mol/dm^3, while the significant decay to 0.16 1/min was observed with an increase in nitrate concentration up to 0.1 mol/dm^3. Obviously, the presence of lower nitrate concentrations (0.001 mol/dm^3 and 0.01 mol/dm^3) did not have a remarkable influence on removal rate, whereas the presence of nitrate in the concentration of 0.1 mol/dm^3 diminished the removal rate threefold. Despite having the dual role, in the case of loperamide removal with UV/H_2O_2 process the negative impact of nitrate is prominent.

Carbonate/Bicarbonate is the other major component usually found in real waters which can act as a scavenger of HO$^\bullet$. The scavenging phenomenon of carbonate/bicarbonate during the AOPs is well documented in the literature [38-40]. Since at pH value of surface waters the prevalent anion is HCO_3^-, various bicarbonate concentrations (0.001, 0.01 and 0.1 mol/dm^3) were added to the solution to assess whether this would influence loperamide degradation rate or not. As can be seen from the results in Table 1, retarded elimination of loperamide was obtained when bicarbonate was present. When bicarbonate was present at concentrations 0.001, 0.01, and 0.1 mol/dm^3, a decline in removal efficiency was achieved (99%, 96%, 74%, respectively) compared with the case where no bicarbonate was added (99%). Such results are expected since bicarbonate undergoing a reaction with HO$^\bullet$ radicals formed less reactive bicarbonate radicals, whereas the number of available HO$^\bullet$ radicals for interaction with loperamide was reduced. Cui *et al.* [38] has shown when the concentration of carbonate increased from 0 to 0.010 mol/dm^3, the degradation of kathon by UV/activated peroxide was slightly inhibited. Kvon *et al.* [40] reported that the removal rates of pharmaceutical ibuprofen (IBF) in UV/H_2O_2 process were decreased by increasing the initial concentration of bicarbonate from 0.001 mol/dm^3 up to 0.002 mol/dm^3.

Chloride anion is another anion with an adverse effect on AOPs process, as it has been reported by many authors [41-43]. Chloride anions can react with HO• radicals with the second-order rate constant of 4.3×10^9 1/Ms whereas hypochlorite radicals ($ClHO^{\bullet-}$) are formed [14]. Moreover, during the photochemical reaction, chloride can be transformed into another reactive species such as chlorine atom ($Cl^\bullet$) and dichloride anion radical ($Cl_2^{\bullet-}$) [44]. The formed chloride atom ($Cl^\bullet$) is capable of oxidizing organic compounds effectively, with almost the same magnitude of rate constants with organic pollutants as HO$^\bullet$ radicals, whereas the halogen radical anion ($Cl_2^{\bullet-}$) is less reactive than HO$^\bullet$ radicals [45]. However, it is obvious from experimental results that the presence of chloride did not noticeably affect the efficiency of loperamide removal. In fact, no significant changes in removal efficiency were observed when the chloride concentrations were increased from 0.001 up to 0.1 mol/dm^3 (Table 1).

The presence of sulphate changed the removal efficiency of loperamide only insignificantly within the investigated test area. Small differences in removal efficiency were noticeable after 4 min of irradiation whereby its slight decline from 93% to 92% and 90% was observed by introducing sulphate at concentrations 0.001, 0.01,

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and 0.1 mol/dm^3, respectively (Table 1). Moreover, the apparent rate constant remained almost unchanged with an increase in sulphate concentrations. It appeared that HO$^\bullet$ radicals scavenging effect of sulphate is not significant in the case of loperamide removal in UV/H_2O_2 process under applied experimental conditions. Similar results of no significant inhibitory effect of sulphate on the removal of pharmaceutical ibuprofen and disinfectant kathon in the UV/H_2O_2 process were reported by the other authors [38, 40].

Ecotoxicology study

Vibrio fischeri is a commonly used microorganism for ecotoxicity assessment during the application of various advanced oxidation processes [46-48]. The advantages of toxicity tests with *Vibrio fischeri* are the short analysis time and the simple handling [49]. The toxicity of the loperamide samples was analysed at different stages of UV/H_2O_2 treatment and the results are shown in Fig. 7 as a percentage of bacterial inhibition. The toxicity of the untreated loperamide sample was 53.58%. During the first 10 minutes of irradiation, the toxicity increased considerably and reached a value of 95.52%, remained almost unchanged during the next 60 minutes of treatment, slowly decreased to 62.58% and 50.3% after 120 and 180 minutes, respectively, and finally decreased to 44.27% by the end of treatment.

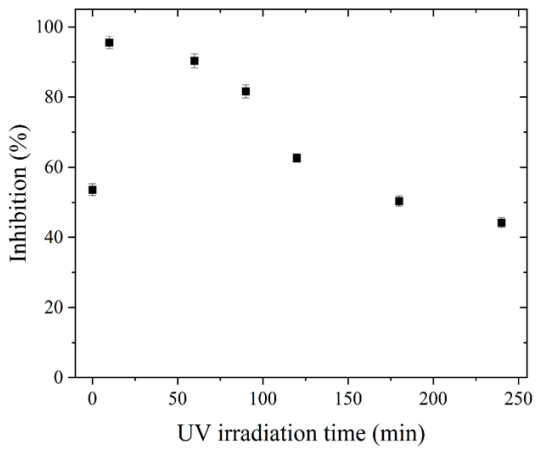


Figure 7. Toxicity assessment during the UV/H_2O_2 treatment of loperamide. Experimental conditions: c_0(Lop) = 30.0 mg/dm^3, $c_0(H_2O_2)$ = 20.0 mmol/dm^3, pH 7.0 ± 0.2, UV light intensity was 730 μW/cm^2, temperature was 25 ± 0.5 ºC

Since disappearance of loperamide to non-detectable levels was achieved in the first 6 min of irradiation and TOC was reduced for only 10% in the same period (Fig. 2), it is likely that the measured toxicity in the initial stages of the treatment originated from the degradation products present in the solution. Moreover, the evolution of low molecular weight organic acids (Fig. 3), whose formation can be correlated with toxicity, drastically increased in this period of irradiation [50]. However, the decline of toxicity below value of untreated sample was obtained after 240 min of irradiation, indicating that a longer treatment time is necessary for a more significant reduction of toxicity. This trend of increasing toxicity of treated samples in the initial stages of irradiation, due to the formation of intermediates that may cause greater toxicity than the parent compound, is consistent with reports by other authors [51-53].

Conclusions

The present study showed that the UV process alone is not completely sufficient for the effective removal of loperamide from aqueous solution; however, the addition of peroxide with UV irradiation significantly accelerated the elimination. The complete removal of loperamide (30 mg/dm^3) by UV/H_2O_2 treatment can be achieved within 6 minutes of irradiation under optimal experimental conditions. Nevertheless, 10% of the total organic carbon remained in the solution, indicating that the mineralisation of loperamide was not complete. The main N-containing mineralisation product was nitrate. Interestingly, the evolution pattern of all detected carboxylic acids was similar; their concentrations sharply increased in the initial stages of treatment and declined as the treatment progressed. Humic acid had a negative effect on the efficiency of loperamide degradation, whereas this influence was more apparent at higher humic acid concentrations. Moreover, the suppression of loperamide degradation in the presence of investigated inorganic anions assumed the following order: nitrate ≈ bicarbonate >> sulphate ≈ chloride. The ecotoxicity assessment revealed that the initial degradation products were more toxic than the parent compound; however, the inhibition decreased during irradiation.

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References

[1] Li WC. Occurrence, sources, and fate of pharmaceuticals in aquatic environment and soil. *Environmental Pollution.* 2014, 187, 193–201. https://doi.org/10.1016/j.envpol.2014.01.015

[2] Sanusi IO, Olutona GO, Wawata IG, Onohuean H. Occurrence, environmental impact and fate of pharmaceuticals in groundwater and surface water: a critical review. *Environmental Science and Pollution Research.* 2023, 30, 90595–90614. https://doi.org/10.1007/s11356-023-28802-4

[3] Hussain A, Ashique S., Hassan MZ, Afzal O, Asiri YI, Kumar P, Dua K, Webster TJ, Altamimi ASA, Altamimi MA. Pharmaceutical contaminants in aquatic systems,

conventional and green strategies, recent updates, challenges and policies, and potential outcomes. *Journal of Molecular Liquids*. 2023, 389, 122905. https://doi.org/10.1016/j.molliq.2023.122905

[4] Chen M, Cooper VI, Deng J, Amatya PL, Ambrus D, Dong S, Stalker N, Nadeau-Bonilla C, Patel J. Occurrence of Pharmaceuticals in Calgary's Wastewater and Related Surface Water. *Water Environment Research,* 2015, 87(5), 414-424. https://doi.org/10.2175/106143015X14212658614199

[5] Afonso-Olivares C, Sosa-Ferrera Z, Santana-Rodríguez JJ. Occurrence and environmental impact of pharmaceutical residues from conventional and natural wastewater treatment plants in Gran Canaria (Spain). *Science of The Total Environment.* 2017, 599–600, 934–943. https://doi.org/10.1016/j.scitotenv.2017.05.058

[6] Kleywegt S, Payne M, Ng F, Fletcher T. Environmental loadings of Active Pharmaceutical Ingredients from manufacturing facilities in Canada. *Science of The Total Environment.* 2019, 646, 257–264. https://doi.org/10.1016/j.scitotenv.2018.07.240

[7] Arvaniti OS, Arvaniti ES, Gyparakis S., Sabathianakis I., Karagiannis E., Pettas E., Gkotsis G., Nika MC, Thomaidis NS, Manios T, Fountoulakis MS, Stasinakis AS. Occurrence of pharmaceuticals in the wastewater of a Greek hospital: Combining consumption data collection and LC-QTOF-MS analysis, *Science of The Total Environment*. 2023, 858(3), 160153. https://doi.org/10.1016/j.scitotenv.2022.160153

[8] Castellano-Hinojosa A, Gallardo-Altamirano MJ, González-López J, González-Martínez A. Anticancer drugs in wastewater and natural environments: A review on their occurrence, environmental persistence, treatment, and ecological risks. *Journal of Hazardous Materials.* 2023, 447, 130818. https://doi.org/10.1016/j.jhazmat.2023.130818

[9] Kumar M, Sridharan S, Sawarkar AD, Shakeel A, Anerao P, Mannina G, Sharma P, Pandey A. Current research trends on emerging contaminants pharmaceutical and personal care products (PPCPs): A comprehensive review. *Science of The Total Environment.* 2023, 859, Part 1, 160031. https://doi.org/10.1016/j.scitotenv.2022.160031

[10] Gulde R, Clerc B, Rutsch M, Helbing J, Salhi E, McArdell CS, von Gunten U. Oxidation of 51 micropollutants during drinking water ozonation: Formation of transformation products and their fate during biological post-filtration. *Water Research.* 2021, 207, 117812. https://doi.org/10.1016/j.watres.2021.117812

[11] Pisharody L, Gopinath A, Malhotra M, Nidheesh PV, Kumar MS, Occurrence of organic micropollutants in municipal landfill leachate and its effective treatment by advanced oxidation processes. *Chemosphere.* 2022, 287, 132216. https://doi.org/10.1016/j.chemosphere.2021.132216

[12] Waris RF, Farooqi IH. Different advanced oxidation processes for the abatement of pharmaceutical compounds. *International Journal of Environmental Science and Technology.* 2024, 21, 2325–2338. https://doi.org/10.1007/s13762-023-05127-w

[13] Legrini O, Oliveros E, Braun AM. Photochemical process for water treatment. *Chemical Reviews.* 1993, 93 (2), 671-698. https://doi.org/10.1021/cr00018a003

[14] Buxton GV, Greenstock CL, Helman WP, Ross AB. Critical review of rate constants of hydrated electrons, hydrogen atoms and hydroxyl radicals ($^{\bullet}OH/^{\bullet}O^{-}$) in aqueous solution. *Journal of Physical and Chemical Reference Data.* 1988, 17 (2), 513-887. https://doi.org/10.1063/1.555805

[15] Savić IM, Nikolić GS, Savić IM, Marinković VD. Quantitative analysis of loperamide hydrochloride in the presence its acid degradation products. *Hemijska Industrija.* 2009, 63, 39–46. https://doi.org/10.2298/HEMIND0901039S.

[16] Ganßmann B, Klingmann A, Burhenn J, Tayrouz Y, Aderjan R, Mikus G. Simultaneous determination of loperamide and its desmethylated metabolites in plasma and urine by high-performance liquid chromatography - Atmospheric-pressure ionization mass spectrometry. *Chromatographia*. 2001, 53, 656–660. https://doi.org/10.1007/BF02493015.

[17] UNESCO and HELCOM. Pharmaceuticals in the aquatic environment of the Baltic Sea region – A status report. UNESCO Emerging Pollutants in Water Series – No. 1, 2017, ISBN 978-92-3-100213-7, UNESCO Publishing, Paris. https://helcom.fi/wp-content/uploads/2019/08/BSEP149.pdf

[18] Velinov N, Najdanovic S, Radovic Vucic M, Mitrovic J, Kostic M, Bojic D, Bojic A. Biosorption of loperamide by lignocellulosic-Al_2O_3 hybrid: optimization, kinetics, isothermal and thermodynamic studies, *Cellulose chemistry and technology.* 2019, 53 (1-2), 175-189. https://doi.org/10.35812/CelluloseChemTechnol.2019.53.19

[19] Lojo-López M, Andrades JA, Egea-Corbacho A, Coello MD, Quiroga JM. Degradation of simazine by photolysis of hydrogen peroxide Fenton and photo-Fenton under darkness, sunlight and UV light. *Journal of Water Process Engineering.* 2021, 42, 102115. https://doi.org/10.1016/j.jwpe.2021.102115

[20] Zhao L, Hu Y, Zhao YG, Mupindu P. Removal of typical sulfonamide resistance genes by ultraviolet/hydrogen peroxide advanced oxidation process in mariculture wastewater. *Journal of Water Process Engineering.* 2023, 55, 104262. https://doi.org/10.1016/j.jwpe.2023.104262

[21] Maleki S, Ghorbani S, Monajemi P. Decolorization of azo Direct Blue 71 dye in aqueous solutions using sequential upflow anaerobic sludge blanket reactor and UV/H_2O_2 process. *Biochemical Engineering Journal.* 2023, 199, 109057. https://doi.org/10.1016/j.bej.2023.109057

[22] Tufail A, Al-Rifai J, Price WE, van de Merwe JP, Leusch FDL, Hai FI, Elucidating the performance of UV-based photochemical processes for the removal of trace organic contaminants: Degradation and toxicity evaluation. *Chemosphere.* 2024, 350, 140978. https://doi.org/10.1016/j.chemosphere.2023.140978

[23] Mitrović J, Radović M, Bojić D, Anđelković T, Purenović M, Bojić A. Decolorization of textile azo dye Reactive Orange 16 with UV/H_2O_2 process. *Journal of the Serbian Chemical Society.* 2012, 77 (4), 465-481. https://doi.org/10.2298/JSC110216187M

[24] Stylidi M, Kondarides DI, Verykios XE. Visible light-induced photocatalytic degradation of Acid Orange 7 in aqueous TiO_2 suspensions. *Applied Catalysis B: Environmental.* 2004, 47(3), 189-201. https://doi.org/10.1016/j.apcatb.2003.09.014

[25] ISO 11348-3 (1998) Water Quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test).

[26] Criquet J, Leitner NKV. Degradation of acetic acid with sulfate radical generated by persulfate ions photolysis. *Chemosphere.* 2009, 77, 194–200. https://doi.org/10.1016/j.chemosphere.2009.07.040

[27] Olmez-Hanci T, Dursun D, Aydin E, Arslan-Alaton I, Girit B,

Mita L, Diano N, Mita DG, Guida M, $S_2O_8^{2-}$/UV-C and H_2O_2/UV-C treatment of Bisphenol A: Assessment of toxicity, estrogenic activity, degradation products and results in real water. *Chemosphere.* 2015, 119, S115–S123. https://doi.org/10.1016/j.chemosphere.2014.06.020

[28] Karci A, Arslan-Alaton I, Olmez-Hanci T, Bekbolet M. Transformation of 2,4-dichlorophenol by H_2O_2/UV-C, Fenton and photo-Fenton processes: Oxidation products and toxicity evolution. *Journal of Photochemistry and Photobiology A*: Chemistry. 2012, 230(1), 65-73. https://doi.org/10.1016/j.jphotochem.2012.01.003

[29] Dhaka S, Kumar R, Lee SH, Kurade MB, Jeon BH, Degradation of ethyl paraben in aqueous medium using advanced oxidation processes: Efficiency evaluation of UV-C supported oxidants. *Journal of Cleaner Production.* 2018, 180, 505-513. https://doi.org/10.1016/j.jclepro.2018.01.197

[30] Aleboyeh A, Moussa Y, Aleboyeh H. The effect of operational parameters on UV/H_2O_2 decolourisation of Acid Blue 74. *Dyes and Pigments.* 2005, 66(2), 129-134. https://doi.org/10.1016/j.dyepig.2004.09.008

[31] Chu W. Modeling the quantum yields of herbicide 2,4-D decay in the UV/H_2O_2 process. *Chemosphere.* 2001, 44, 934-941. https://doi.org/10.1016/S0045-6535(00)00556-7

[32] Sharma J, Mishra IM, Kumar V, Degradation and mineralization of Bisphenol A (BPA) in aqueous solution using advanced oxidation processes: UV/H_2O_2 and UV/$S_2O_8^{2-}$ oxidation systems, *Journal of Environmental Management.* 2015,156, 266-275. https://doi.org/10.1016/j.jenvman.2015.03.048

[33] Yuan H, Zhou X, Zhang Y. Degradation of Acid Pharmaceuticals in the UV/H_2O_2 Process: Effects of Humic Acid and Inorganic Salts. *Clean Soil, Air, Water.* 2013, 41(1), 43-50. https://doi.org/10.1002/clen.201200107

[34] Guerard JJ, Miller PL, Trouts TD, Chin YP. The role of fulvic acid composition in the photosensitized degradation of aquatic contaminants. *Aquatic Sciences.* 2009, 71(2), 160-169. https://doi.org/10.1007/s00027-009-9192-4

[35] Zepp RG, Hoigne J, Bader H. Nitrate-induced photooxidation of trace organic chemicals in water. *Environmental Science & Technology.* 1987, 21(5), 443-450. https://doi.org/10.1021/es00159a004

[36] Brezonik PL., Fulkerson-Brekken J. Nitrate-induced photolysis in natural waters: Controls on concentration of hydroxyl radical photo-intermediates by natural scavenging agents. *Environmental Science & Technology*. 1998, 32(19), 3004-3010. https://doi.org/10.1021/es9802908

[37] Mack J, Bolton JR. Photochemistry of nitrate and nitrite in aqueous solution: a review. *Journal of Photochemistry and Photobiology A*: Chemistry, 1999, 128(1-3), 1-13. https://doi.org/10.1016/S1010-6030(99)00155-0

[38] Cui J, Wang G., Rong X., Gao W, Lu Y, Luo Y, Zhang L, Cheng Z, Gao C. Removal of kathon by UV-C activated hydrogen peroxide: Kinetics, mechanisms, and enhanced biodegradability assessment. *Chinese Journal of Chemical Engineering.* 2024, 65, 178–187. https://doi.org/10.1016/j.cjche.2023.06.023.

[39] Sun W, Dong H, Wang Y, Duan S, Ji W, Huang H, Gu J, Qiang Z. Ultraviolet (UV)-based advanced oxidation processes for micropollutant abatement in water treatment: Gains and problems. *Journal of Environmental Chemical Engineering.* 2023, 11(5), 110425. https://doi.org/10.1016/j.jece.2023.110425

[40] Kwon M, Kim S, Yoon Y, Jung Y, Hwang TM, Lee J, Kang JW., Comparative evaluation of ibuprofen removal by UV/H_2O_2 and UV/$S_2O_8^{2-}$ processes for wastewater treatment. *Chemical Engineering Journal.* 2015, 269, 379–390. https://doi.org/10.1016/j.cej.2015.01.125

[41] Hoang NT, Manh TD, Tram NT, Nhi BD, Mwazighe FM, Van Tac D, Duyen VT, Hoang Hien Y, Thi Thy Nga N, Van Duong D, Tran Thi L, Van Doan T. Effect of the chloride ion on the degradation of PPCPs in UV/persulfate and UV/H_2O_2 and the role of radicals in these systems. *Journal of Environmental Chemical Engineering.* 2023, 12(1), 111846. https://doi.org/10.1016/j.jece.2023.111846.

[42] Lee YM, Lee G, Kim T, Zoh KD. Degradation of benzophenone-8 in UV/oxidation processes: Comparison of UV/H_2O_2, UV/persulfate, UV/chlorine processes. *Journal of Environmental Chemical Engineering.* 2024, 12(1), 111623. https://doi.org/10.1016/j.jece.2023.111623

[43] Xu Y, Lin Z, Zhang H. Mineralization of sucralose by UV-based advanced oxidation processes: UV/PDS versus UV/H_2O_2. *Chemical Engineering Journal*. 2016, 285,2016, 392–401. https://doi.org/10.1016/j.cej.2015.09.091

[44] Jayson GC, Parsons BJ, Swallow AJ. Some Simple, Highly Reactive, Inorganic Chlorine Derivatives in Aqueous Solution. *Journal of the Chemical Society, Faraday Transactions 1: Physical Chemistry in Condensed Phases,*1973, 69, 1973-1607. https://doi.org/10.1039/F19736901597

[45] Martire DO, Rosso JA, Bertolotti S, Le Roux GC, Braun AM, Gonzalez MC. Kinetic Study of the Reactions of Chlorine Atoms and $Cl_2^{\bullet-}$ Radical Anions in Aqueous Solutions. II. Toluene, Benzoic Acid, and Chlorobenzene. *The Journal of Physical Chemistry A*. 2001, 105(22), 5385-5392. https://doi.org/10.1021/jp004630z

[46] Lei S, Guan L, Liu F, Yu Q, Xu K, Ren H, Geng J. Degradation of pharmaceuticals in UV/H_2O_2 process: EEM-based kinetic model, transformation products and corresponding toxicity evaluation, *Journal of Environmental Chemical Engineering.* 2024, 12(1), 111736. https://doi.org/10.1016/j.jece.2023.111736.

[47] Fang Z, Huang R, Chelme-Ayala P, Shi Q, Xu C, Gamal El-Din M. Comparison of UV/Persulfate and UV/H_2O_2 for the removal of naphthenic acids and acute toxicity towards Vibrio fischeri from petroleum production process water. *Science of The Total Environment.* 2019, 694, 133686. https://doi.org/10.1016/j.scitotenv.2019.133686

[48] Khan JA, He X, Shah NS, Sayed M, Khan HM, Dionysiou DD. Degradation kinetics and mechanism of desethyl-atrazine and desisopropyl-atrazine in water with $^{\bullet}OH$ and $SO_4^{\bullet-}$ based-AOPs. *Chemical Engineering Journal.* 2017, 325, 485–494. https://doi.org/10.1016/j.cej.2017.05.011.

[49] Rizzo L. Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Research.* 2011, 45(15), 4311-4340. https://doi.org/10.1016/j.watres.2011.05.035

[50] Karci A, Arslan-Alaton I, Bekbolet M. Advanced oxidation of commercially important nonionic surfactant: Investigation of deghradation products and toxicity. *Journal of Hazardous Materials.* 2013, 263(2), 275-282. https://doi.org/10.1016/j.jhazmat.2013.03.052

[51] Rehman F, Sayed M, Khan JA, Shah NS, Khan HM, Dionysiou DD. Oxidative removal of brilliant green by UV/$S_2O_8^{2-}$, UV/HSO_5^- and UV/H_2O_2 processes in aqueous media: A comparative study. *Journal of Hazardous Materials.* 2018, 357, 506–514. https://doi.org/10.1016/j.

jhazmat.2018.06.012.
[52] Spina-Cruz M, Maniero MG., Guimarães JR. Advanced oxidation processes on doxycycline degradation: monitoring of antimicrobial activity and toxicity *Environmental Science and Pollution Research.* 2019, 26, 27604–27619. https://doi.org/10.1007/s11356-018-2149-1
[53] Urbano VR, Peres MS, Maniero MG, Guimarães JR. Abatement and toxicity reduction of antimicrobials by UV/H_2O_2 process. *Journal of Environmental Management.* 2017, 193, 439–447. https://doi.org/10.1016/j.jenvman.2017.02.028

Izvod

DEGRADACIJA LOPERAMIDA HIDROHLORIDA HIDROKSILNIM RADIKALIMA: UTICAJ SASTAVA VODENOG MATRIKSA I EKOTOKSIKOLOŠKA ANALIZA

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Mikropolutanti prisutni u prirodnim vodenim matriksima predstavljaju sve veći problem upravo zbog činjenice da ih je nemoguće ukloniti konvencionalnim tretmanima prečišćavanja otpadnih voda. Mogućnost primene unapređenih procesa oksidacije za uklanjanje ove vrste organskih zagađujućih materija iz vode je predmet mnogih istraživanja. U ovoj studiji je ispitivana degradacija farmaceutika loperamid hidrohlorida veštačkim UV zračenjem u prisustvu vodonik peroksida. Primenjeni postupak je pogodan za brzo i potpuno uklanjanje loperamida (30 mg/dm^3) pri vremenu tretmana od 6 minuta i optimalnim radnim uslovima. Pored degradacije, tretmanom je postignut i značajan stepen mineralizacije loperamida. Potvrđeno je formiranje organskih kiselina malih molekulskih masa, kao što su oksalna, mravlja i sirćetna. Ispitan je uticaj tipičnih sastojaka površinskih voda i efluenata otpadnih voda (HA, NO_3^-, HCO_3^-, Cl^-, SO_4^{2-}) na brzinu razgradnje loperamida. Na razgradnju loperamida negativno utiče prisustvo huminske kiseline, nitrata i bikarbonata, dok je uticaj hlorida i sulfata zanemarljiv. Test toksičnosti sa morskom fosforescentnom bakterijom *Vibrio fischeri* je sproveden da bi se utvrdila toksičnost nusproizvoda degradacije farmaceutika loperamida za živi svet u vodi.

Ključne reči: mineralizacija, farmaceutski preparati, napredni oksidacioni procesi, *Vibrio fischeri,* vodeni matriks

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