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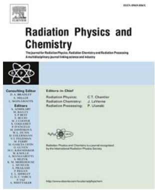

Matrix effect in the hydroxyl radical induced degradation of β-lactam and tetracycline type antibiotics

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ABSTRACT

Due to the spread of antibiotic resistant bacteria, elimination of antibiotics from purified wastewater is a highly important task. Purified wastewater contains large variety of organic/inorganic compounds that strongly influence the efficiency of advanced oxidation processes (AOP). In this work, we investigate the radiation-induced degradation of selected antibiotic contaminants (oxacillin and cloxacillin from the β-lactams; tetracycline and chlortetracycline from the tetracyclines) in four matrices: pure water, tap water, synthetic wastewater and purified wastewater received from a wastewater treatment plant. Changes in technically important parameters, such as COD, TOC, BOD, OUR, acute toxicity and antibacterial activity, were investigated at 0.1 mmol dm^{-3} (40–48 mg dm^{-3}) antibiotic concentration. None of the four antibiotics were biodegradable in any of the four matrices, however, after irradiation with relatively low doses the obtained products were biodegradable. Oxacillin and cloxacillin were not toxic in *Vibrio fischeri* test, while tetracycline and chlortetracycline showed toxicity that was strongly reduced by the irradiation treatment. Both COD and TOC decreased after irradiation, their ratio shifted towards TOC indicating an increase in the average oxidation state (AOS). The increase in AOS was lower in purified wastewater matrices: this matrix may contain various small, highly oxidized molecules in high concentration, which degrade very slowly in AOP. The antibacterial activity in most of matrices was greatly reduced or completely disappeared at around 2–4 kGy. However, in purified wastewater matrices some antibiotic activity remained even at 4 kGy. Here the degradation of antibiotic is slow (small ΔCOD/dose value) because a large fraction of the reactive radicals is scavenged presumably by small, highly oxidized molecules in the solution. Although the water radiolysis product H_2O_2 affected some of the bioassays, this phenomenon was absent in purified wastewater. The purified wastewater after ionizing radiation treatment can be safely released into the receiving lakes or rivers.

1. Introduction

In advanced oxidation processes (AOP) reactive inorganic radicals ($^{\bullet}OH$, $SO_4^{\bullet-}$, $O_2^{\bullet-}/HO_2^{\bullet}$, etc.) from photolytic, photocatalytic, radiolytic, etc., processes are used for initiating the degradation of recalcitrant organic molecules (pharmaceuticals, pesticides, personal care products, etc.) in water matrices (Wojnárovits et al., 2017). These inorganic radicals induce the oxidation of the target molecules involving the consumption of some part of the dissolved O_2 present in water. When the treatment is continued for sufficiently long time, the organic molecules in a process called mineralization completely transform to inorganic ones, H_2O, CO_2, N_2, etc.

Abbreviations: AOP, advanced oxidation processes; AOS, average oxidation state; BOD, biochemical oxygen demand; COD, chemical oxygen demand; OUR, oxygen uptake rate; TOC, total organic carbon.

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One of the most promising AOP is high-energy irradiation treatment using electron beam accelerators (Wojnárovits et al., 2017). In this technology, there is no need for additives, since the radiolysis of water supplies the reactive radicals (Buxton, 2008) and the treatment occurs in flow-through system. As compared to other AOP methods a further advantage of irradiation technology is that, as the established large-scale facilities show, the technology can be scaled up to the 10,000–100,000 m^3/day level (IAEA, 2007, 2020; CGN, 2020).

Antibiotics are especially dangerous water contaminants for human beings, due to their widely acknowledged involvement in the development and spread of antibiotic resistance in various water reservoirs (Kümmerer, 2009; Wang et al., 2020). Individual antibiotic concentrations in the final effluents of wastewater treatment plants are generally in the 50–300 ng dm^{-3} range (Rodrigez-Mozaz et al., 2020). In degradation studies, in order to ensure relatively accurate determination of the investigated parameters generally concentrations in the (0.1–1) mmol dm^{-3} range are applied. Extrapolations are used to understand processes at practical concentrations.

In this study, we selected two frequently applied β-lactam type antibiotics, oxacillin and cloxacillin and two tetracyclines, tetracycline and chlortetracycline (Scheme 1). We investigated and compared technically important parameters: chemical oxygen demand (COD), total organic carbon (TOC), biochemical oxygen demand (BOD), oxygen uptake rate (OUR), acute toxicity and antibacterial activity in 0.1 mmol dm^{-3} (40–48 mg dm^{-3}) concentration solutions during high-energy irradiation treatment in four different water matrices. The degradation reactions of these compounds have already been studied in different AOP, and a few research groups published results also on their radiolytic reaction in aqueous solutions (Yu et al., 2008; Chung et al., 2009; Jeong et al., 2010; Kim et al., 2009; Peñalver et al., 2013; Dail and Mezyk, 2010; Szabó et al., 2016a, 2016b; Nam et al., 2020). These works have shown that by irradiating β-lactam and tetracycline type antibiotics at ~0.1 mmol dm^{-3} concentration with ~1 kGy dose, practically all starting molecules undergo some transformation. However, these transformed molecules may still have an intact pharmacophore part, responsible for the antibacterial effect (Giraldo et al., 2015; Giraldo-Aguirre et al., 2015; Giraldo-Aguirre, 2015). It should be pointed out that matrix effects on the degradation of these antibiotics have not yet been studied and compared in detail for all the four compounds.

Inorganic ions, organic matter, and biological material present in real wastewater strongly influence the degradation reactions generally reducing the degradation of the target compounds either by interaction or by competition for the reactive intermediates with the substances of interest. The matrix effect on the decomposition efficiency was studied by spiking pure water, tap water, synthetic wastewater and purified wastewater (from a communal wastewater treatment plant) with the antibiotic of interest prior to irradiation. Purified water allows the investigations of degradation reactions without disturbance of impurities. In tap water there are some organic impurities and inorganic ions present, and there is a little disturbance. Synthetic wastewater has a well-defined composition and the inorganic and organic compounds may considerably influence the chemistry. The selected matrices help us to understand the ongoing processes during practical application of the method when purified wastewater is treated in advanced oxidation processes.

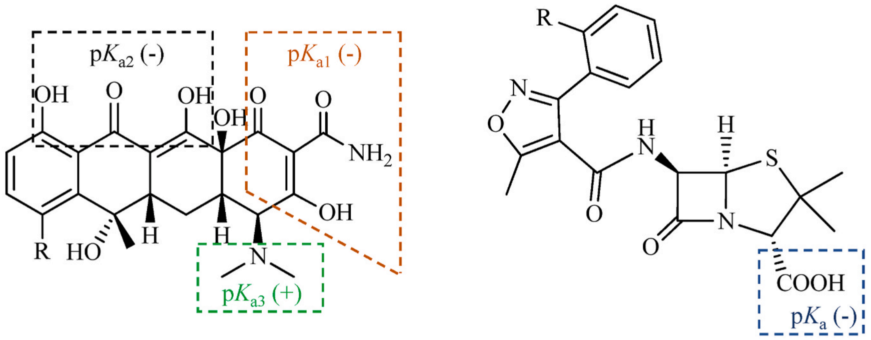
2. Experimental

2.1. Chemicals, irradiation

The pharmaceuticals investigated in this work were purchased from Sigma Aldrich. pH was adjusted using sodium hydroxide and perchloric acid (both from Reanal Laboratory Chemicals Ltd.). Irradiation was done on the selected matrices containing the corresponding antibiotic at 0.1 mmol dm^{-3} concentration. Pure water was prepared using an Adrona B30 system (Riga, Latvia), which provides high-quality water with a conductivity of 0.055 μS cm^{-1} and an organic carbon content of <2 ppb. The tap water had COD and TOC values of 2.8 mg dm^{-3} O_2 and 1.6 mg dm^{-3} C, respectively, and an inorganic carbon content of 47.7 mg dm^{-3} C, further characteristics are: Cl^- 21 mg dm^{-3}, Fe^{2+}/Fe^{3+} <5 μg dm^{-3}, pH 7.6. The synthetic wastewater had in 1 dm^3 7 mg $(NH_4)_2SO_4$, 7 mg K_2HPO_4, 7 mg humic acid, 0.7 mg $MgSO_4$ and 81 mg $NaHCO_3$ (Szabó et al., 2017). The COD value of synthetic wastewater was measured as 8.1 mg dm^{-3} O_2. The organic and inorganic carbon contents were determined as 3.2 and 10.7 mg dm^{-3} C, respectively. Purified wastewater was obtained from the South-Pest Wastewater Treatment Plant; it had no antibacterial activity but high COD, inorganic carbon (IC) and TOC values, ~45 mg dm^{-3} O_2, ~65 mg dm^{-3} C and ~20 mg dm^{-3} C, respectively. Inorganic ions: Fe^{2+}/Fe^{3+} ~0.74 mg dm^{-3}, Cl^- 112 mg dm^{-3}.

The matrices used in our study have near neutral pH (~7), thus tetracyclines are mainly present as zwitterionic (net neutral) species (-+) together with the net negatively charged molecules (- - +) (Conde-Cid et al., 2020). Penicillins are in their negatively charged form ($-COO^-$).

For research purposes instead of electron accelerators gamma sources are often used. The chemical effect of the two kind of energy source is practically the same. In the present work the samples were irradiated in a panoramic type ^{60}Co facility with 1.8 PBq activity. The doses measured with ethanol-chlorobenzene dosimetry were 0.5, 1, 2 and 4 kGy, the dose rate applied was 2 kGy h^{-1}. During irradiation gentle air bubbling was applied in order to avoid O_2 depletion.



Tetracyclines

	R =	pK_{a1}	pK_{a2}	pK_{a3}
Tetracycline	H	3.30	7.68	9.69
Chlortetracycline	Cl	3.30	7.44	9.27

Penicillins

	R =	pK_a
Oxacillin	H	2.95
Cloxacillin	Cl	2.84

Scheme 1. Structures of the antibiotics investigated, and their corresponding dissociation constants (Stephens et al., 1956; Rollo, 1972).

2.2. Chemical oxygen demand (COD)

The COD values were measured according to ISO Standard 6060:1989 using a Behrotest TRS 200 system. In our measurements, 30 cm^3 samples were heated at 148 ± 3 °C for 2 h in 8 mol dm^{-3} H_2SO_4 solution with the addition of $K_2Cr_2O_7$ as an oxidizing agent, Ag_2SO_4 as a catalyst and $HgSO_4$ for removing chloride. The non-reacted $Cr_2O_7{}^{2-}$ was removed by titration with Mohr salt, using ferroin indicator (both from Molar Chemicals Ltd.).

2.3. Total organic carbon content (TOC)

In TOC content measurements a Shimadzu TOC-L CSH/CSN analyzer was used. The method is based on catalytic combustion and non-dispersive infrared (NDIR) gas analysis. The flow rate of the carrier gas was 150 cm^3 min^{-1}, while the temperature of the furnace that contained the combustion tube filled with platinum oxidation catalyst was 680 °C.

2.4. Biochemical Oxygen demand (BOD)

During the digestion of organic compounds, the microorganisms consume O_2 and produce CO_2. The system contained NaOH (Reanal Laboratory Chemicals Ltd.) granules that reacted with the evolving CO_2. OxiTop® Control BOD Respirometer System was used for BOD experiments, which were performed according to DIN EN, 1899-1 (1998). The equipment measured the pressure drop due to the O_2 consumed by the microbial community. Dilution water was prepared and conditioned as described in the OECD Test No. 301 (1992). 20 cm^3 supernatant of activated sludge after sedimentation (taken from the aeration basin of the South-Pest municipal wastewater treatment plant, Budapest Sewage Works, Hungary) was added to 1 dm^3 of the dilution water (pH set to 7.2 ± 0.2 by NaOH or HCl). Due to these additives the 0.1 mmol dm^{-3} antibiotic solutions were diluted by a factor of 2 (the results were corrected for the dilution). Allylthiourea nitrification inhibitor (43 μmol dm^{-3}) was added to ensure that O_2 consumption derives solely from utilization of the carbon content. The abiotic reactions were separated from the biological processes by subtraction of results obtained in unseeded solutions. The results were corrected with control solutions containing only seeded dilution water.

2.5. Oxygen uptake rate (OUR)

Test mixtures consisted of 25 mg total suspended solid equivalent activated sludge and 150 cm^3 pharmaceutical solution. This amount was filled up with air saturated tap water to 300 cm^3 in a Karlsruher flask. Changes in dissolved O_2 concentration were recorded in every 30 min for 180 min at 20 ± 2 °C according to ISO 8192 (1986) standard by a WTW inoLab® Multi 9310 IDS device, equipped with FDO 925 dissolved oxygen probe. The results were corrected with the abiotic reactions and endogenous OUR.

2.6. Acute toxicity tests

Acute toxicity to *Vibrio fischeri* bioluminescent bacterium was determined by Microtox® tests according to DIN EN ISO 11348-2:1999 following 30 min incubation at 15 ± 2 °C. The method is based on evaluation of light emission changes caused by the chemicals tested. LANGE LUMIStox 300 device and freeze-dried *Vibrio fischeri* (ATCC 49387) test organisms were supplied by Hach Lange GmbH, Germany. Ready-to-use glucose/sodium chloride solution was applied to reactivate the bacteria at 15 ± 2 °C. 0.3 g solid NaCl was added to 15 cm^3 antibiotic solution (pH 7.0 ± 0.2) to obtain 2% NaCl content required for the samples during the experiment.

2.7. Agar diffusion test

Tryptone-glucose-yeast extract agar (TGYE) was prepared by mixing 5 g peptone (Art.-Nr. HP32.1, Carl Roth), 2.5 g yeast extract (Cat. No. 1.11926, Merck), 1 g glucose (Cat. No. 1.08346.9029, Merck) and 7.5 g bacteriological agar (Art.-Nr. 6494.2, Carl Roth) in 1 dm^3 pure water. *Staphylococcus aureus* strain (B.01755) for the agar diffusion tests (Collection code in the American Type Culture Collection: ATCC 6538P) was received from the National Collection of Agricultural and Industrial Microorganisms (NCAIM, Szent István University, Hungary). The microbiological work was carried out under sterile conditions.

In the agar diffusion assay, 1 cm^3 of the *Staphylococcus aureus* bacterial suspension at 10^6 CFU cm^{-3} concentration and 25 cm^3 of TGYE agar were mixed and poured into Petri dish. Holes were punched in the agar (after the agar had solidified) using a glass tube with a diameter of 4 mm and the holes were filled with 0.08 cm^3 antibiotic solution of 0.1 mmol dm^{-3} concentration. The samples were allowed to diffuse into the agar for 24 h in a 37 °C incubator. The average diameter of the growth inhibition zones was calculated using three different plates (Bonev et al., 2008).

During radiolysis of aerated solutions hydrogen peroxide also forms with high yield (Illés et al., 2017; Kovács et al., 2017). Since H_2O_2 strongly disturbs the biochemical tests (Zona et al., 2003; Sági et al., 2018; Bezsenyi et al., 2021), the residual H_2O_2 was eliminated using manganese dioxide (MnO_2, 5 g dm^{-3}, Sigma-Aldrich) before the BOD and OUR measurements, and catalase enzyme (10 mg cm^{-3}, Sigma Aldrich) before the Microtox toxicity test. Before analysis all solutions were filtered. In agar diffusion tests H_2O_2 did not influence the results, so its removal was unnecessary.

The number of replicates performed in the acute toxicity test was two, while in all other tests it was three.

3. Results

3.1. COD, TOC and AOS

Oxidation and mineralization, two interrelated processes, are often characterized by chemical oxygen demand (COD) and total organic carbon (TOC) content measurements, respectively (Pisarevsky et al., 2005; Homlok et al., 2013). In COD measurements, a strong oxidant (usually dichromate) breaks down the organic molecules and the amount of O_2 (in mg) used for oxidation in 1 dm^3 solution is calculated (unit: mg dm^{-3} O_2). TOC is determined by transforming the organic carbon to CO_2 (unit: mg dm^{-3} C).

Samples containing 0.1 mmol dm^{-3} concentration of antibiotics were irradiated with 0.5, 1, 2 and 4 kGy doses in pure water (pure w.), tap water (tap w.), synthetic wastewater (synthetic ww.) and purified wastewater (purified ww.) matrices (Fig. 1). The COD values of the non-irradiated oxacillin and cloxacillin samples prepared in pure water were between 50 and 60 mg dm^{-3}, these values were close to the theoretically calculated ones based on the elemental compositions. The same values for tetracycline and chlortetracycline were 50–65 mg dm^{-3}. The starting COD values of the samples with tap water, synthetic wastewater and purified wastewater were higher than the COD values of samples prepared with pure water due to the organic content of the water matrix used. The COD decreased with the absorbed dose in the investigated 0–4 kGy range by about 40–50%, and the dose dependence in most cases was close to linear. Initial linear dependences were also reported in oxygen uptake measurements (Isildar et al., 1982; Zona et al., 2003) and also in our former COD measurements carried out on a large number of small organic molecules at ~1 mmol dm^{-3} concentration (Homlok et al., 2013). The slope values for both the COD (ΔCOD/dose) and TOC (ΔTOC/dose) are collected in Table 1. We performed several experiments with higher doses than 4 kGy, an example is shown in Fig. 1A, inset. Upon increasing the dose, further decrease in the COD and TOC values were observed, at high doses COD and TOC vanished with

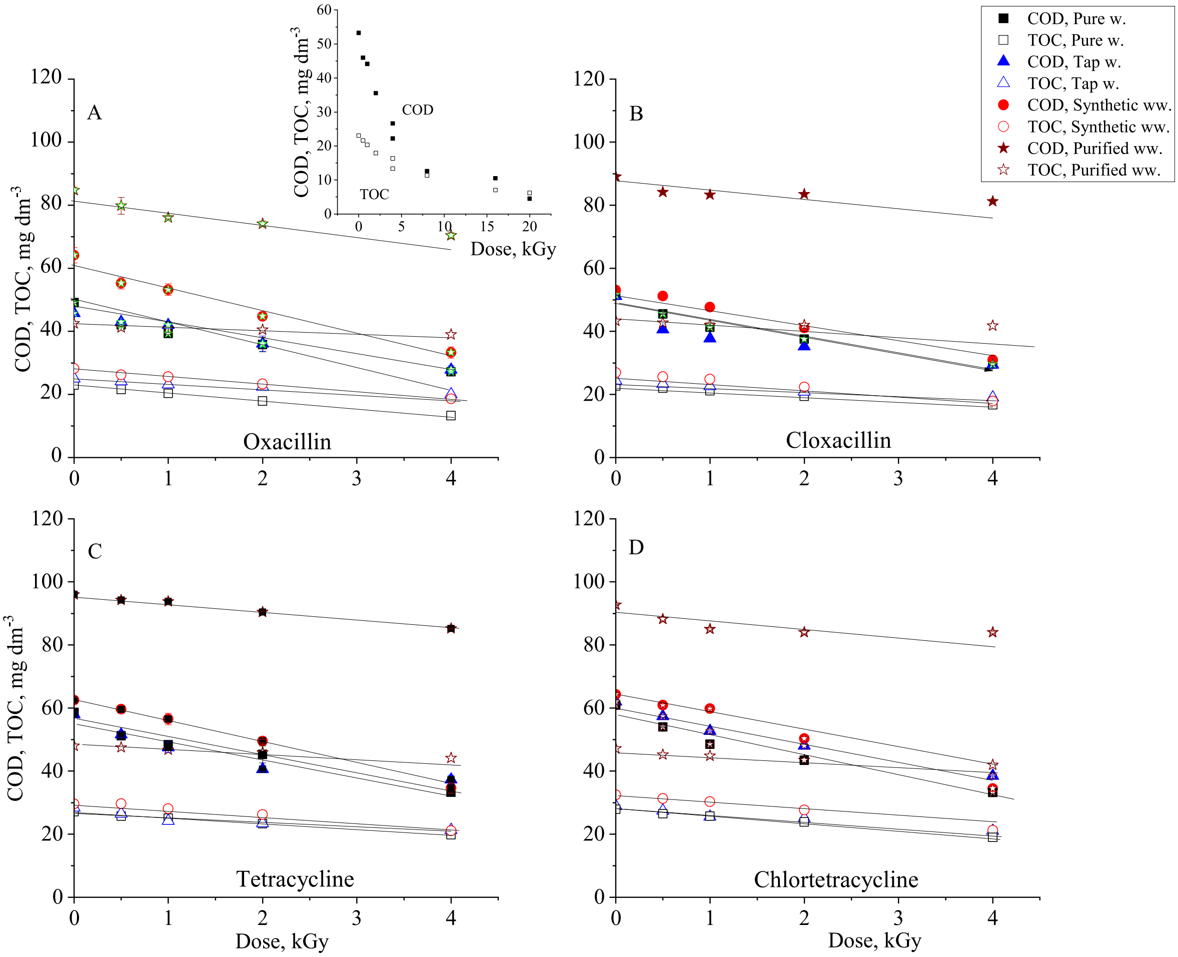


Fig. 1. Dose dependence of COD and TOC values measured in 0.1 mmol dm^{-3} oxacillin (A), cloxacillin (B), tetracycline (C) and chlortetracycline (D) solutions in pure water, tap water, synthetic wastewater and purified wastewater matrices. Inset: dose dependence in pure water in wider dose range. The standard deviation of the COD values is in the range between ±0.2 and ± 2.0 (indicated in the Fig.), that of the TOC values is in the range between ±0.01 and ± 0.4.

Table 1
The slope values of the dose dependences of COD and TOC in mg dm^{-3} kGy^{-1} O_2 and mg dm^{-3} kGy^{-1} C, respectively.

Compound/water	Oxacillin		Cloxacillin		Tetracycline		Chlortetracyline	
	COD	TOC	COD	TOC	COD	TOC	COD	TOC
Pure w.	6.7	2.5	5.7	1.9	6.0	2.3	5.7	2.3
Tap w.	6.0	1.3	5.7	1.8	5.3	2.1	5.7	2.1
Synthetic ww.	6.7	2.4	5.3	1.9	5.0	2.1	5.5	1.6
Purified ww.	4.9	1.0	3.0	1.2	3.1	1.8	5.0	1.5

gradually decreasing slope. It means that with radiation technology practically complete mineralization can be achieved. This result is in contrast with the results obtained by some other techniques (e.g., photolysis), where after a certain COD conversion further removal does not take place (Serna-Galvis et al., 2016). Complete mineralization is usually expensive and unnecessary, generally it is sufficient to decrease the toxicity and the biological activity as well as increase the biodegradability of the recalcitrant compounds.

In pure water the slope values on the COD - dose plots are between 5 and 7 mg dm^{-3} kGy^{-1} O_2 (0.16–0.22 mmol dm^{-3} kGy^{-1}). Upon irradiation with 1 kGy dose 0.28 mmol dm^{-3} $^{\bullet}OH$ is introduced into the system. Since introduction of 1 O_2 molecule induces 4 electron oxidations, in our system 1 $^{\bullet}OH$ (one-electron oxidant) leads to 2–3 electron oxidations. The increased oxidation rate is certainly due to the effect of dissolved O_2 (Homlok et al., 2013). $^{\bullet}OH$ by reacting with organic molecules via possible addition, hydrogen atom abstraction and direct one-electron oxidation events generates organic radicals (first oxidation step). Then the organic radicals readily react with dissolved O_2 (second oxidation step) forming peroxy radicals. Reactions of the peroxy radical (and in general peroxides) may lead to further oxidations (Homlok et al., 2013).

The theoretical TOC value of 0.1 mmol dm^{-3} oxacillin and cloxacillin solutions is 22.8 mg dm^{-3} C, in solution of tetracyclines it is 26.4 mg dm^{-3} C and the values observed in pure water were very close to the calculated ones. In oxacillin solutions 23.0, 25.0 and 28.2 mg dm^{-3} C TOC values were measured in pure water, tap water and in the synthetic wastewater, respectively. In cloxacillin solutions, these values were found to be 22.6, 24.4 and 26.9 mg dm^{-3} C, respectively. Upon irradiation the TOC values decreased linearly with the absorbed dose (Fig. 1). Both TOC and COD started to decrease already at the lowest absorbed dose, 0.5 kGy. This suggests that oxidation (COD) and mineralization (TOC) occurs parallel. However, the percentage COD removal is higher than the percentage TOC removal. As it was mentioned in the Experimental the purified wastewater matrix had relatively high initial organic carbon content. Upon irradiation, relatively small decrease in the COD and TOC values was observed.

In Fig. 1 both COD and TOC decreased with the absorbed dose. Based on these two quantities we calculated the average oxidation state of the organic molecules (AOS) using the equation (Giannakis et al., 2015):

$$AOS = \frac{4\,(TOC - COD)}{TOC}$$

Here COD and TOC are expressed in mol dm^{-3} O_2 and mol dm^{-3} C units, respectively. AOS in agreement with its definition changes between −4 (CH_4) and +4 (CO_2). This parameter reflects the oxidation degree of the carbon atoms of organic compounds present in the solution. The more oxidized the organic molecules the higher AOS values are calculated. The theoretical values for formaldehyde and acetic acid are 0, for formic acid the value is +2 and for oxalic acid it is +3 (Al Momani et al., 2004). In non-irradiated solutions our measured AOS values in most cases were between 0.5 and 1 (Fig. 2), the values increased with the absorbed dose slowly, indicating that the chemical nature of the organic molecules did not vary significantly (Giannakis et al., 2015). At higher doses, the values were close to 1.5 suggesting that the organic contents were strongly oxidized in these solutions and small organic acids may dominate the organic molecule spectrum. These fragmented molecules degrade in AOP very slowly. The slow degradation in purified wastewater matrix is probably also due to the slow degradation of the highly oxidized fragmented molecules originally present in the matrix. A large fraction of the degradation-initiating $^{\bullet}OH$ reacts with these molecules.

3.2. Biodegradability and toxicity

The toxicity of antibiotic solutions to the mixed microbial community of the aeration tank of a wastewater treatment plant was investigated through biological oxygen demand (BOD) and oxygen uptake rate (OUR) measurements (H_2O_2 was removed by manganese dioxide). The toxicities were also investigated in Microtox® tests using *Vibrio fischeri* bioluminescent bacteria (Ren and Frymier, 2002). In BOD measurements the applied device continuously monitored the dissolved O_2 concentrations in the solutions (Bezsenyi et al., 2021). We show typical time courses in Fig. 3.

BOD at the beginning increased with incubation time, and then, after 3–4 days, showed a tendency to level off. As in usual practice, we characterize BOD with the 5 days oxygen demand results (BOD_5, Fig. 4). In the non-irradiated solutions of the four antibiotics the BOD_5 values and the BOD_5/COD ratios are low, 3–7 mg dm^{-3} O_2 and 0.05–0.1, respectively. A solution is considered easily biodegradable when the BOD_5/COD ratio is above 0.4 (Metcalf and Eddy, 2003; Takács et al., 2022). Under the experimental conditions the antibiotics are non-biodegradable. When the solutions were irradiated BOD_5 increased

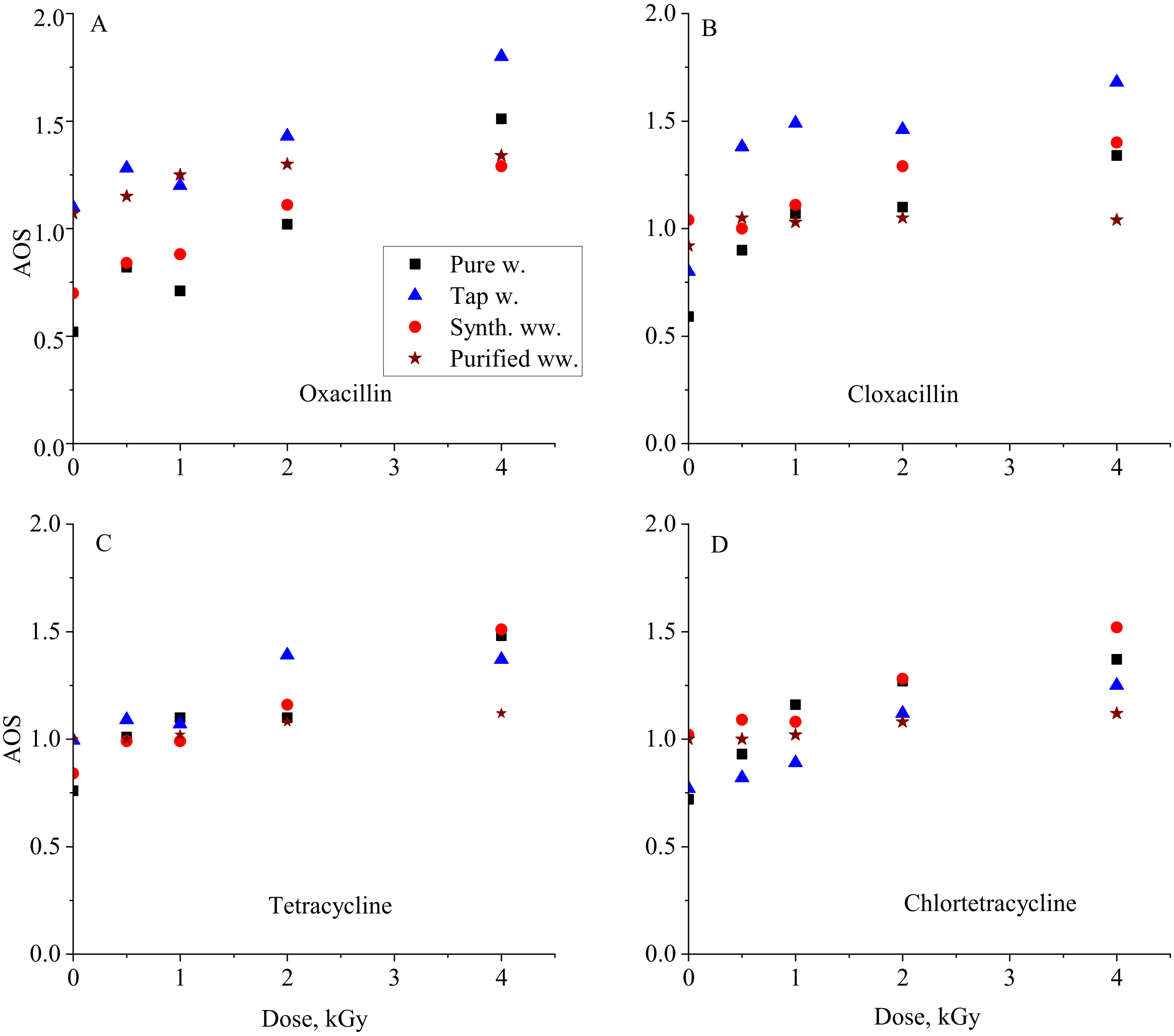


Fig. 2. Dose dependence of the average oxidation state (AOS) values measured in 0.1 mmol dm^{-3} oxacillin (A), cloxacillin (B), tetracycline (C) and chlortetracycline (D) solutions in pure water, tap water, synthetic wastewater and purified wastewater matrices.

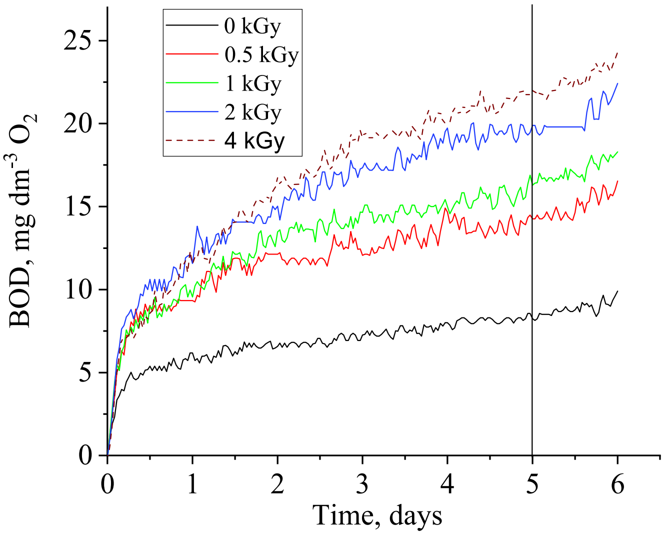


Fig. 3. Time dependence of the BOD values in 0.1 mmol dm^{-3} oxacillin solutions in purified wastewater matrices irradiated with 0–4 kGy absorbed doses.

(Fig. 4) and COD decreased (Fig. 1). The ratio of the two at around 2 kGy dose rose to ≥0.4 indicating that the solutions became biodegradable: the degradation products could be eliminated in microbiological oxidation reactions.

In OUR experiments the dissolved O_2 concentrations in the solutions were followed during 3 h period, the concentration was measured in every half an hour and the slope values on the OUR - time plots were calculated. OUR measurement requires shorter time and higher amount of inoculum than those in BOD measurement. In OUR tests at first the easily biodegradable compounds are digested, while BOD measurement needs longer time and both the easily and poorly biodegradable compounds are affected (Bezsenyi et al., 2021). When un-irradiated antibiotic solution in pure water was added to the test mixture (Section 2.5) the metabolic activity of microorganisms decreased (cloxacillin and chlortetracycline, negative OUR value) or remained practically unchanged (oxacillin and tetracycline). Fig. 5 shows the differences, increases or decreases as compared to solutions without antibiotic. In tap water the initial OUR values were also low or negative. In synthetic wastewater and purified wastewater matrices the metabolic activities were relatively high at zero dose for all the four antibiotics investigated. These matrices had higher organic molecule content in various amounts, and this content might help the biodegradation of the antibiotics by mechanism called cometabolism (co-oxidation, Bezsenyi et al., 2020). In this mechanism a non-biodegradable molecule can be degraded in the presence of an easily degradable substrate.

In all solutions irradiated with 0.5 kGy dose high increase in the OUR values were observed compared to the un-irradiated samples, indicating that the microorganisms can use the degradation products as nutrient source. The metabolic activity remained high in samples irradiated with 1 or 2 kGy doses. In some solutions at 4 kGy dose OUR decreased, e.g., in pure water solution of oxacillin. In this case, probably there was not

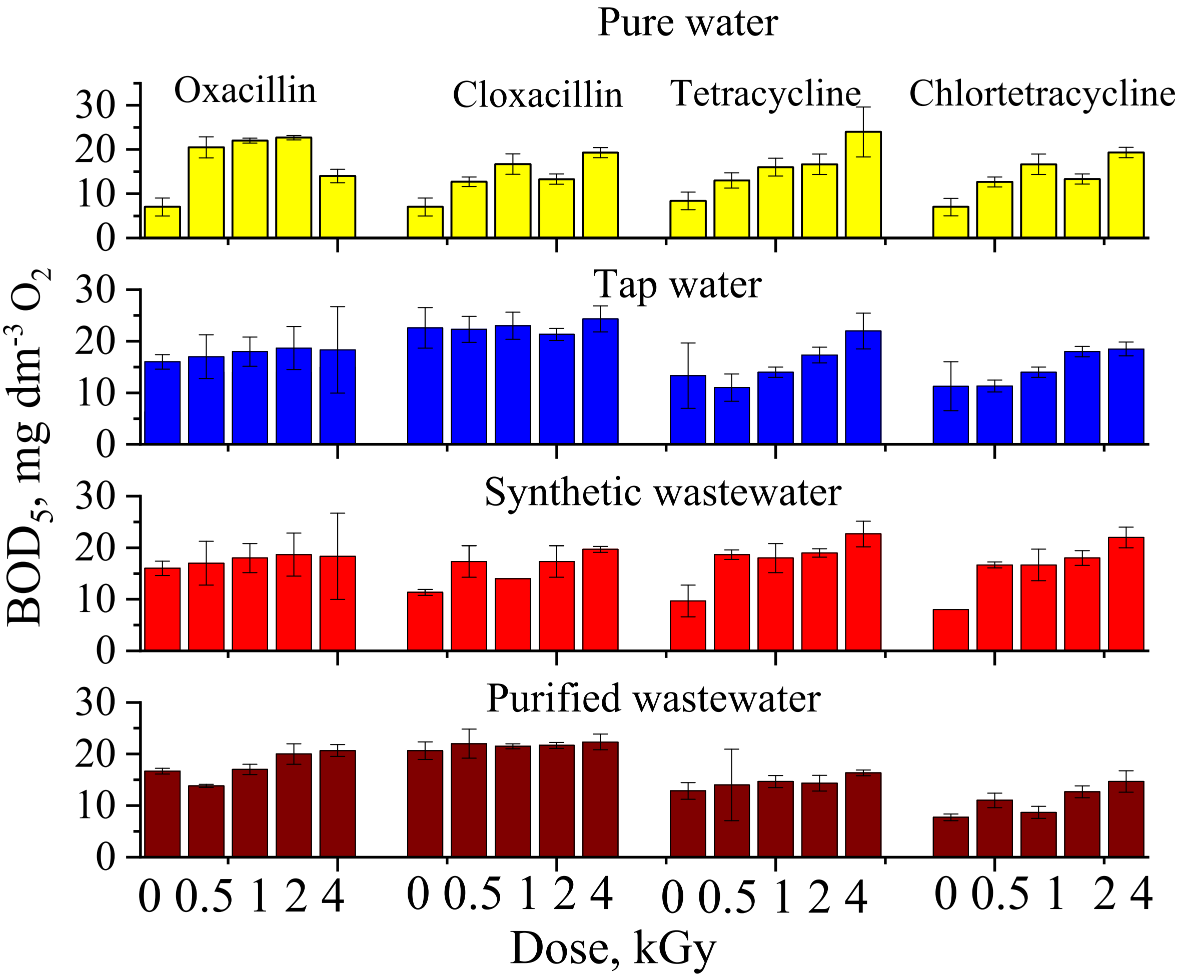


Fig. 4. BOD$_5$ values measured in 0.1 mmol dm^{-3} oxacillin, cloxacillin, tetracycline and chlortetracycline solutions in pure water, tap water, synthetic wastewater and purified wastewater matrices.

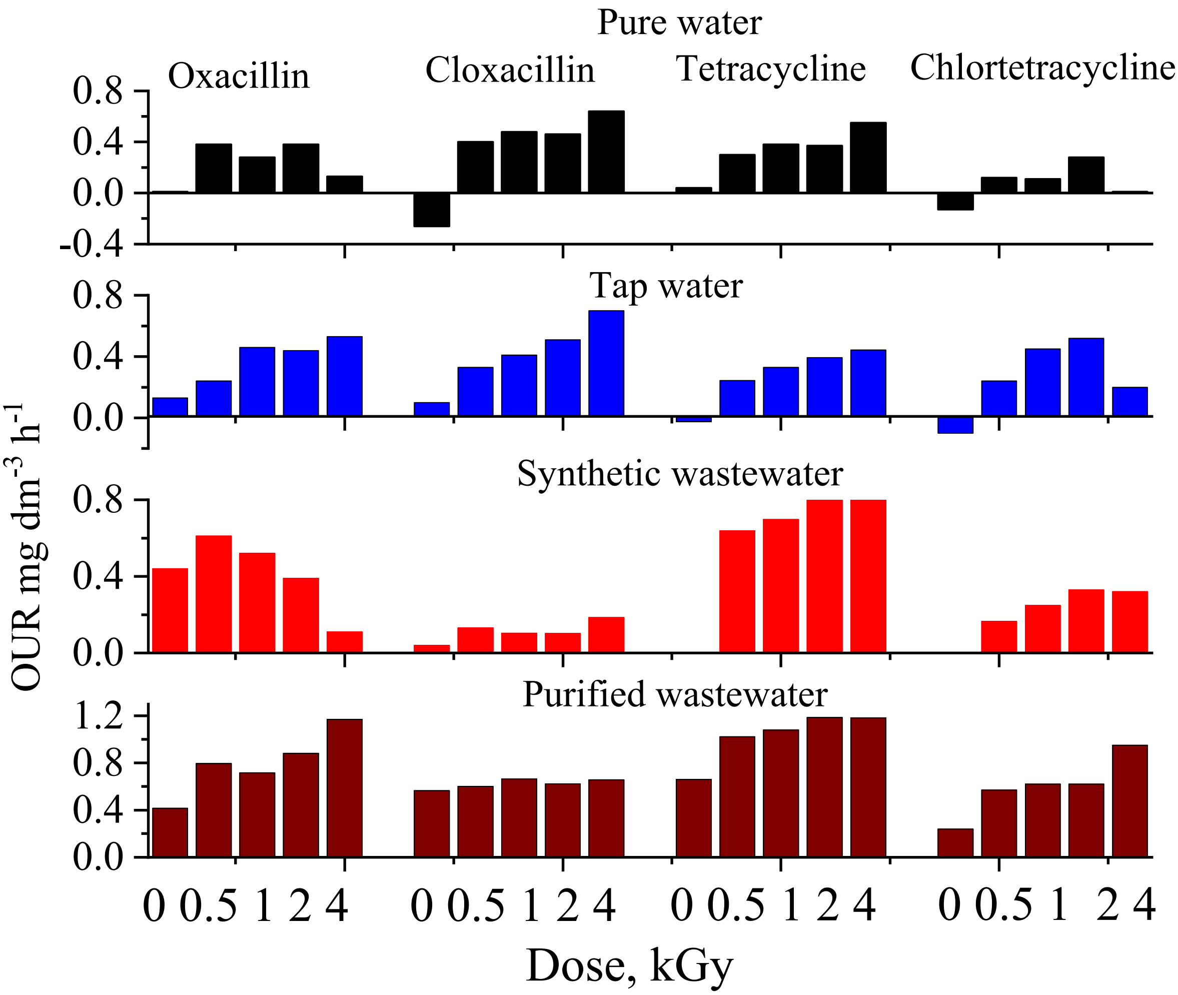


Fig. 5. OUR values measured in 0.1 mmol dm^{-3} oxacillin, cloxacillin, tetracycline and chlortetracycline solutions in pure water, tap water, synthetic wastewater and purified wastewater matrices. The standard deviation of the OUR values is in the range between ±0.01 and ± 0.1.

enough organic matter in the solution to produce high OUR value. Please note that TOC value was low in these solutions.

Microtox® test with *Vibrio fischeri* bioluminescent bacteria is often used to characterize the acute toxicity of water samples. During their metabolic activity these bacteria emit luminescence, decrease or absence of this luminescence reflects the level of toxicity of the solution. In the solution of non-irradiated oxacillin samples, we measured the same luminescence intensity as in the test solution without this antibiotic in all the four water matrices (Fig. 6) indicating that oxacillin does not exert toxicity on this bacterium. In cloxacillin solutions at zero dose slight fluorescence inhibition was observed. When irradiated oxacillin or cloxacillin solutions were introduced into the test mixture very high toxicity (inhibition of bioluminescence) was detected, with up to 100% inhibition at 2 kGy dose in pure water and synthetic wastewater matrix. In tap water, the inhibition was increasing with absorbed dose more slowly and it did not reach 100% at the highest absorbed dose, 4 kGy. The high inhibition is certainly due to the effect of H_2O_2 forming during the radiolysis (Zona et al., 2003; Illés et al., 2017; Sági et al., 2018). However, when H_2O_2 was eliminated by using catalase enzyme much smaller inhibition was obtained. This inhibition was in the 10–30% range and it hardly depended on the type of matrix. Cloxacillin in synthetic wastewater matrix showed high luminescence inhibition (83%) even at 0.5 kGy absorbed dose. This value decreased slightly in the presence of catalase enzyme.

The luminescence intensity in tetracycline and chlortetracycline solutions showed different trend. In untreated 0.1 mmol dm^{-3} samples, 50–60% inhibitions were observed compared to the reference samples without the antibiotics. Without catalase the inhibition went up to 90–100% in irradiated samples. However, when catalase was added to the samples at low doses only a small increase was observed in the inhibition in tetracycline solutions, after the maximum at 0.5 kGy dose the inhibition decreased showing that the toxicity can be eliminated by irradiation. A similar conclusion has been reported in a study by Kim et al. (2009). The small initial increase is certainly due to the forming products, e.g., to the phenol type molecules that form in the initial period of degradation and that are more toxic than the intact compounds (Peñalver et al., 2013).

3.3. Antimicrobial activity

The antibacterial potency of untreated and irradiated samples was investigated in agar diffusion tests. The antibiotic-containing solution was placed into the punched hole on the agar plate seeded with the test bacteria (*Staphylococcus aureus* strains). When the solution diffusing into the agar layer has antibacterial activity, a visible inhibition zone appears around the hole. The antibacterial activity is characterized by the diameter of the zone, absence of this indicates the absence of antibacterial effect. When the non-irradiated antibiotic solutions were tested inhibition zones with 17–31 mm diameters appeared around the punched holes (Table 2). In irradiated solutions, the diameters were smaller and in most cases, they disappeared when the dose was higher than 1 kGy. This was not true for oxacillin, cloxacillin and

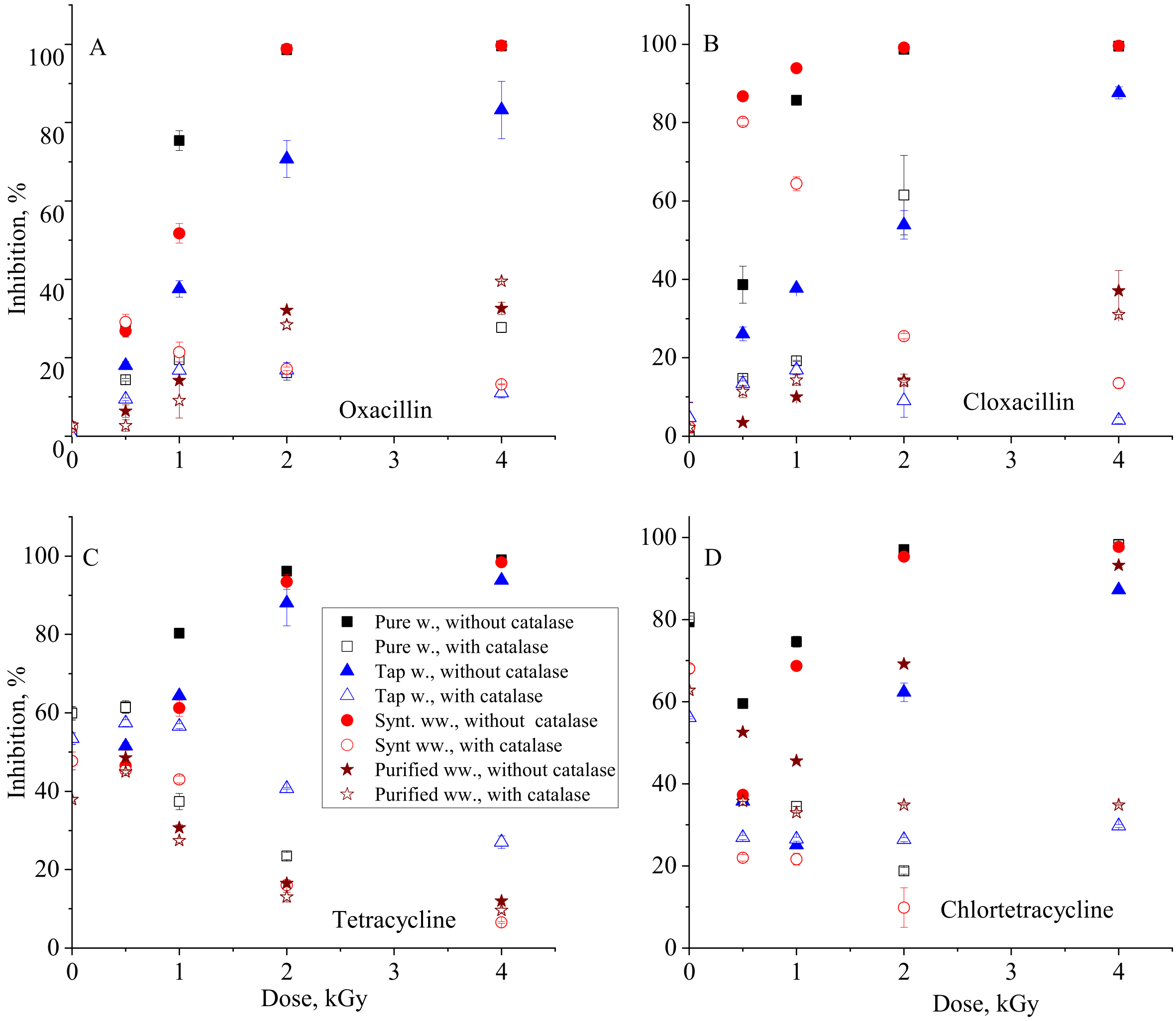


Fig. 6. *Vibrio fischeri* toxicity test measured in 0.1 mmol dm^{-3} oxacillin, cloxacillin, tetracycline and chlortetracycline solutions in pure water, tap water, synthetic wastewater and purified wastewater matrices.

Table 2
Inhibition zones in agar diffusion tests (mm) measured in 0.1 mmol dm^{-3} antibiotic solutions using *Staphylococcus aureus* strain (B.01755).

Dose, kGy	0	0.5	1	2	4
Oxacillin					
Pure water	30 ± 0.5	25 ± 0.4	14 ± 0	–	–
Tap water	26 ± 0.6	20 ± 0.6	12 ± 0	–	–
Synthetic wastewater	25 ± 0.7	18 ± 0.5	12 ± 0	–	–
Purified wastewater	28 ± 0	26 ± 0	25 ± 0	20 ± 0	7 ± 0
Cloxacillin					
Pure water	29 ± 0.6	19 ± 1.2	8 ± 0	–	–
Tap water	24 ± 0.6	18 ± 0	11 ± 0	–	–
Synthetic wastewater	24 ± 0.6	20 ± 0.4	12 ± 0	–	–
Purified wastewater	28 ± 0.5	26 ± 0.4	24 ± 0.4	20±	6 ± 0
Tetracycline					
Pure water	17 ± 0	14 ± 0.5	1 ± 0,2	–	–
Tap water	27 ± 0	24 ± 0	19 ± 0	6 ± 0	–
Synthetic wastewater	31 ± 0	22 ± 0	12 ± 0.6	–	–
Purified wastewater	28 ± 0.5	16 ± 0.5	10 ± 0.4	–	–
Chlortetracycline					
Pure water	30 ± 0.4	25 ± 0	17 ± 0.5	–	–
Tap water	29 ± 0.4	24 ± 0.5	24 ± 0.4	24±	13 ± 0.4
Synthetic wastewater	29 ± 0.5	19 ± 0.8	13 ± 0.4	6±	–
Purified wastewater	28 ± 0.5	24 ± 0.5	24 ± 0.5	20 ± 0.4	7 ± 0

chlortetracycline solutions prepared in purified wastewater matrices, halo around the punched hole was present even for solutions irradiated with 4 kGy dose. In these solutions, due to the higher and (in AOP) badly degradable organic molecule content of the matrix, the degradation of the antibiotic was slower. Interestingly, we did not observe this phenomenon in tetracycline solution prepared in purified wastewater. In chlortetracycline tap water solution, the antibacterial activity also disappeared very slowly with the absorbed dose. This phenomenon needs further clarification in the future.

As it was mentioned in the Introduction, in pure water around 1 kGy dose the intact antibiotic molecules disappear from the solution. The results of diffusion tests indicate that the degradation products may not have antibacterial potency, although a more accurate method, such as a broth microdilution assay would be necessary to confirm this. By using broth microdilution assays, it has been shown that in the hydroxyl radical induced degradation of β-lactam antibiotics products that retain the antibacterial potency can form (Dodd et al., 2009, 2010; Szabó et al., 2016a, 2016b), while this phenomenon is absent for tetracycline (Dodd et al., 2009). It should be pointed out that the microorganisms in purified wastewater could considerably interfere with this type of assay, and therefore, we have not included this method in our experiments. Serna-Calvis et al. (2017) obtained similar result using UV/persulfate advanced oxidation process in oxacillin degradation. These authors published for oxacillin a minimum inhibitory concentration (MIC) value of 2 μmol dm^{-3} using *S. aureus* (ATCC 6538).

4. Discussion

The results of the COD and BOD measurements reflected that the four antibiotics at 0.1 mmol dm^{-3} concentration in neither of the four matrices were biodegradable (low BOD/COD ratio). Oxacillin and cloxacillin showed no toxicity in *Vibrio fischeri* test, and in line with this, in un-irradiated solutions cloxacillin just slightly retarded the biological activity of the microbial community of a wastewater treatment plant (negative OUR value), while oxacillin did not influence it at all. Tetracycline and chlortetracycline showed toxicity in the *Vibrio fischeri* tests in agreement with suggestions in the literature (Peñalver et al., 2013). Interestingly, they influenced just slightly the metabolic activity of the microbial population of the activated sludge in BOD and OUR measurements in pure water and tap water matrices. When the solutions were irradiated, both COD and TOC decreased, their ratio shifted towards TOC indicating an increase in the average oxidation state (AOS). The increase in AOS was very low in purified wastewater matrices. This matrix may contain in high concentration different small, highly oxidized molecules, which degrade very slowly in AOP. For instance, the rate constants of $^{\bullet}OH$ reaction with oxalate and acetate ions are only 8×10^6 and $\sim 8 \times 10^7$ mol^{-1} dm^3 s^{-1} (Buxton et al., 1988), respectively, while with the starting antibiotic molecules the rate constants are in the 6×10^9–9×10^9 mol^{-1} dm^3 s^{-1} range (Wojnárovits et al., 2018). The antibacterial activities in most of matrices decreased strongly with the absorbed dose, they disappeared at around 2–4 kGy. Since the original antibiotic molecules at ~1 kGy absorbed dose disappear from the solutions, one can conclude that the product molecules may not have antimicrobial potency. However, in purified wastewater matrices some activity remained even at 4 kGy dose. Here the degradation of antibiotic is slower (small ΔCOD/dose value, Table 1) due to the competing reactions with the molecules and ions present in the matrix.

In purified water and synthetic wastewater matrices hydrogen peroxide forming during water irradiation strongly influences the bioassays (BOD, OUR, Microtox®). In tap water, which always contains in low concentration some transition metal ions, the influence of H_2O_2 is smaller. In purified wastewater matrices no H_2O_2 effect was observed in the biological measurements. Since these measurements are highly sensitive to H_2O_2, we assume that peroxide decomposes during the treatment practically completely. This degradation is expected to proceed with a Fenton or Fenton-like mechanism, in which hydroxyl radicals form that may also contribute to the degradation. H_2O_2 does not influence the antimicrobial activity measurements in agar diffusion tests using *S. aureus* stains. The purified wastewater after ionizing radiation treatment can be safely released into the receiving lakes or rivers.

5. Conclusions

Antimicrobial agents that are recalcitrant against conventional wastewater treatment procedures may end up in receiving water reservoirs, where they can facilitate the development and spread of antibiotic resistant bacteria. Although, advanced oxidation processes provide promising ways to eliminate these contaminants, the effectiveness of the process is greatly compromised by the complex nature of the wastewater matrix harbouring the pollutants at trace concentrations. Here we investigated the radiation-induced degradation of four antibiotics (two tetracyclines and two penicillin derivatives) in selected matrices including pure water, tap water, synthetic wastewater and purified wastewater received from a wastewater treatment plant. We found that radiation treatment can render these non-biodegradable antibiotic-containing matrices biodegradable, reduce their toxicity and eliminate their antimicrobial activity. Reduction in other technical parameters (COD, TOC) indicated the applicability of radiation technology to degrade the recalcitrant compounds even in highly complex water matrices. Therefore, radiation treatment can be recommended to treat antibiotic containing purified wastewater matrices, as a measure to mitigate the development and spread of antibiotic resistant bacteria in receiving water reservoirs. The purified wastewater after ionizing radiation treatment by 2–4 kGy can be safely released into the receiving lakes or rivers.

Author contributions

L. Wojnárovits: Conceptualization, Data curation, Writing - original draft, J. Wang: Writing - review & editing, L. Chu: Writing - review & editing, T. Tóth: Supervision, Project administration, Funding acquisition, K. Kovács: Investigation, Methodology, Writing - review & editing, A. Bezsenyi: Investigation, Methodology, Writing - review & editing, L. Szabó: Writing - review & editing R. Homlok: Investigation, Methodology E. Takács: Conceptualization, Data curation, Writing - review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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