applied sciences

MDPI

Article

Ionizing Radiation Induced Removal of Ofloxacin, Abatement of Its Toxicity and Antibacterial Activity in Various Water Matrices

László Wojnárovits [1], Renáta Homlok [1], Krisztina Kovács [1], Anikó Bezsenyi [2,3] and Erzsébet Takács [1,*]

[1] Radiation Chemistry Department, Institute for Energy Security and Environmental Safety, Centre for Energy Research, H-1121, Konkoly-Thege Miklós út 29-33, 1121 Budapest, Hungary; wojnarovits.laszo@ek-cer.hu (L.W.); homlok.renata@ek-cer.hu (R.H.); kovacs.krisztina@ek-cer.hu (K.K.)
[2] Budapest Sewage Works Pte Ltd., H-1087, Asztalos Sándor út 4, 1087 Budapest, Hungary; bezsenyia@fcsm.hu
[3] Doctoral School of Material Science and Technology, Óbuda University, H-1034, Bécsi út 96b, 1034 Budapest, Hungary
* Correspondence: takacs.erzsebet@ek-cer.hu

Abstract: The fluoroquinolone-type antibiotic ofloxacin is regularly detected in wastewaters. In this work, its hydroxyl radical ($^{\bullet}$OH) induced degradation was studied at a concentration of 0.1 mmol dm^{-3} in three water matrices, pure water, tap water, and purified wastewater (collected from a wastewater treatment plant). $^{\bullet}$OH was produced by γ-irradiation under conditions when it had a high yield. The $^{\bullet}$OH dosage dependencies of chemical (COD) and biochemical (BOD) oxygen demands, total organic carbon (TOC) content, oxygen uptake rate (OUR), Microtox® toxicity, and antibacterial activity values were evaluated. $^{\bullet}$OH effectively oxidized and mineralized ofloxacin; both processes were observed even at the lowest $^{\bullet}$OH dosage, 0.14 mmol dm^{-3} (0.5 kGy absorbed dose). When 0.28 mmol dm^{-3} $^{\bullet}$OH was introduced into pure or tap water, the antibacterial activity vanished. In purified wastewater, a higher dosage (0.56 mmol dm^{-3}) was required to achieve the same effect as in pure water, due to the organic molecules of the matrix that quenched the $^{\bullet}$OH radicals. The products did not have antibacterial activity and showed little toxicity toward the test organism *Vibrio fischeri*. $^{\bullet}$OH preferentially attacks the pharmacophore part, the 4-quinolone unit of ofloxacin. $^{\bullet}$OH-induced reactions can be safely applied to destroy the antimicrobial potency of fluoroquinolone-type antibiotics in purified wastewater matrices.

Keywords: biodegradability; antibacterial activity; chemical oxygen demand; biochemical oxygen demand; toxicity; oxygen uptake rate; hydroxyl radicals

check for updates

Citation: Wojnárovits, L.; Homlok, R.; Kovács, K.; Bezsenyi, A.; Takács, E. Ionizing Radiation Induced Removal of Ofloxacin, Abatement of Its Toxicity and Antibacterial Activity in Various Water Matrices. *Appl. Sci.* **2023**, *13*, 7211. https://doi.org/10.3390/app13127211

Academic Editor: Dino Musmarra

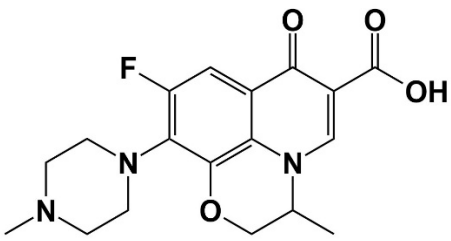
Received: 8 May 2023
Revised: 13 June 2023
Accepted: 14 June 2023
Published: 16 June 2023

1. Introduction

Ofloxacin (Scheme 1) belongs to the class of fluoroquinolone antibiotics. These broad-spectrum bactericides share a bicyclic core structure related to the substance 4-quinolone [1,2]. There is an F atom on the 4-quinolone unit; a methyl-substituted piperazine ring, a carboxyl group, and a methyl-substituted oxazine ring are also attached to the double-ring structure. Due to the methyl group on the oxazine ring, this molecule is chiral with enantiomers named levofloxacin and dextrofloxacin. Ofloxacin contains both enantiomers. The antibacterial effect of levofloxacin is higher than those of ofloxacin and dextrofloxacin [3]. The carboxyl group dissociates with a pK_a value of 5.45; at neutral pH, the molecule is an anion [4].

Due to the frequent use of ofloxacin and levofloxacin in medical practice, they are regularly found in surface waters (100–300 ng dm^{-3}, [5,6]). These medicines and many other pharmaceuticals enter surface waters predominantly through the purified wastewaters released by wastewater treatment plants, because the traditional purification techniques (biological treatment) are not effective enough in their removal. For the elimination of different pharmaceuticals from purified wastewater, new techniques, the so-called advanced

oxidation processes (AOP), are recommended. In these processes, short-lived reactive radicals (hydroxyl radicals ($^{\bullet}OH$), sulfate radical anions ($SO_4^{\bullet-}$)) induce the solute degradation (recent examples: [7,8]). $^{\bullet}OH$ is the main reactant in most AOP, and the different techniques differ mainly in the way of $^{\bullet}OH$ generation: in the Fenton, photo-Fenton, and electro-Fenton processes, the decomposition of hydrogen peroxide; in the sonochemical techniques, cavitation; in photocatalytic processes, photon–catalyst interactions; and in high-energy ionization radiation treatment, water degradation (see later) produces $^{\bullet}OH$. Here we use the latter technique. A good description of the different techniques was published in the book *Advanced Oxidation Processes for Water Treatment*, edited by M.I. Stefan [9].



Scheme 1. Ofloxacin.

The literature has reported on very few radiolytic studies with fluoroquinolones [3,10–13]. In the published works, mainly the elementary steps of $^{\bullet}OH$ reactions were investigated by using pulse radiolysis with transient kinetic techniques [10,14–16]. The rate constant of $^{\bullet}OH$ reactions with fluoroquinolones was found to be in the diffusion limited range, $k_{\bullet OH}$ = 6.2–8.1 × 10^9 mol^{-1} dm^3 s^{-1}. A similar value, 7.65 × 10^9 mol^{-1} dm^3 s^{-1}, was measured for a model compound of fluoroquinolones (6-fluoro-4-oxo-1,4-dihydro-3-quinoline carboxylic acid), which does not contain the piperazine and oxazine rings [14]. The comparable rate constant suggests that the core aromatic ring structure is the main target of $^{\bullet}OH$. In agreement with this suggestion, products revealing hydroxylation of the quinolone core form with high abundance [10–12]. Defluorination is also an important degradation pathway. $^{\bullet}OH$ addition to the carbon–fluorine position and rapid HF elimination may be the possible reaction path. By irradiating aerated 0.1 mmol dm^{-3} concentration solutions with a 1 kGy dose, ~95% of the fluoroquinolone molecules undergo chemical transformation, the majority of the original molecules disappear, and derivatives (products) appear. Using 2 kGy, most of the products formed first also undergo some form of transformation [3,12,14–17]. During irradiation in unbuffered solution, the pH decreases from 6–7 to ~4 [3,17].

As mentioned before, the basic chemical reactions between $^{\bullet}OH$ and several fluoroquinolone antibiotics were investigated in the past. However, much less is known about such technically important characteristics of pollutants' degradation as the rate of oxidation and mineralization, changes in the toxicity and antimicrobial activity of the treated solutions, and the biodegradability. In this work, the relationships between these characteristics are analyzed using the example of ofloxacin. The investigations were made in pure water, tap water, and purified wastewater matrices containing the antibiotic at 0.1 mmol dm^{-3} concentration. A pure water matrix makes it possible to study the degradation without the disturbing effects of impurities, while, for practical reasons, it is important to know the effect of these impurities in tap water and purified wastewater. The basic points were to establish the dose needed to eliminate the antimicrobial potency and the products' toxicity and to transform the solution to a biodegradable one in the mentioned matrices. With the work, we want to contribute to the spread of techniques based on AOP (and among them on irradiation technology) in water treatment.

2. Materials and Methods

2.1. Chemicals, Water Matrices, and Irradiation

Ofloxacin was purchased from Sigma Aldrich. Pure water was prepared using an Adrona B30 system (Adrona SIA, Riga, Latvia), which provides high-quality water with

a conductivity of 0.055 μS cm^{-1} and a total organic carbon content < 2 ppb. The tap water had chemical oxygen demand (COD) and total organic carbon (TOC) content values of 1.2 mg dm^{-3} O_2 and 1.1 mg dm^{-3} C, respectively, and inorganic carbon content of 44.7 mg dm^{-3} C; further characteristics are Cl^- 21 mg dm^{-3}, Fe^{2+}/Fe^{3+} < 5 μg dm^{-3}, pH 7.6. Purified wastewater was obtained from the South-Pest Wastewater Treatment Plant (SPWTP) belonging to Budapest Sewage Works; it had no antibacterial activity but high COD, inorganic carbon (IC), and TOC values, ~76 mg dm^{-3} O_2, ~48 mg dm^{-3} C and ~27 mg dm^{-3} C, respectively; inorganic ions: Fe^{2+}/Fe^{3+} ~0.74 mg dm^{-3}, Cl^- 112 mg dm^{-3}.

Irradiation was carried out in a panoramic type ^{60}Co-γ irradiator with absorbed doses in the 0–4 kGy range at a dose rate of 16 kGy h^{-1}, in open, air equilibrated glass bottles with slow air bubbling. The dose was determined using alcoholic chlorobenzene dosimetry (ISO/ASTM 51538:2009(E), [18]).

2.2. COD, TOC, OUR and BOD

The end products were featured by such sum parameters used in water analysis as COD, TOC, biochemical oxygen demand (BOD_5), and oxygen uptake rate (OUR). In COD measurements, the organic content of the sample is oxidized by potassium dichromate, and the amount of O_2 needed for oxidation (in mg dm^{-3} O_2 units) is calculated (ISO Standard 6060:1989, [19]). In the experiments, a Behrotest TRS 200 COD digestion system was used. The rate of mineralization was assessed by TOC content measurements using Shimadzu TOC-LCSH/CSN equipment. This assay is based on catalytic combustion of the organic content of samples and analysis of the formed CO_2 using nondispersive infrared detection. TOC expresses the organic carbon content in mg dm^{-3} C units.

BOD_5 is the amount of O_2 dissolved in water (in mg dm^{-3} O_2 units) required for biotransformation of organic compounds by aerobic bacteria during 5 days of incubation. BOD_5 experiments were performed using an Oxi-Top® Control BOD Respirometer System according to DIN EN 1899–1 (1998) [20]. During the test, the change in air pressure in 500 cm^3 brown bottles was measured at (20 ± 0.5) °C. In the experiments, 0.1–50 mg dm^{-3} decanted and filtered activated sludge taken from the aeration basin of the SPWTP was used. The inoculant was tested separately: 20 cm^3 of inoculant was added to 1 dm^3 standard dilution water. Three measurements were made for each concentration simultaneously, and the measurements were repeated at least four times.

Pharmaceuticals influence the biological activity of microorganisms in water. This influence was studied in oxygen uptake rate respiratory tests (OUR, mg dm^{-3} h^{-1} O_2). In OUR measurements, the dissolved O_2 uptake increases in the presence of biodegradable compounds, and it is reduced when toxic compounds are in the solution. The measurements were made according to the ISO 8192:1986 standard [21]. O_2 consumption was measured in 300 cm^3 Kalsruher flasks with an FDO® 925 O_2 sensor at 20 °C. A 150 cm^3 solution containing the pharmaceutical was poured into the flask, and an amount of sewage sludge corresponding to 25 mg of dry organic matter was added to it. Three parallel measurements were made in each case. OUR measurements for a series were made on the same day using the same activated sludge.

In the radiolysis of aerated solutions, hydrogen peroxide forms with a high yield [22,23]. Since H_2O_2 may disturb the biochemical tests [24,25], the residual H_2O_2 was eliminated using manganese dioxide (MnO_2, 5 g dm^{-3}, Sigma-Aldrich, St. Louis, MO, USA) before the BOD and OUR experiments, and catalase enzyme (1 mg cm^{-3} in 50 mmol dm^{-3} phosphate buffer, Sigma Aldrich) before the Microtox®toxicity tests.

2.3. Toxicity and Agar Diffusion Tests

The acute toxicity of irradiated and nonirradiated samples was assessed with the Microtox® assay, which uses a single test organism, the bioluminescent marine bacterium *Vibrio fischeri* being sensitive to a wide range of pollutants after an incubation time of 30 min at 15 ± 2 °C (DIN EN ISO 11348-2:1999, [26]). The applied test organism was in freeze-dried form; it was reactivated with 0.5 mL ready-to-use glucose/sodium chloride reactivation

solution at 15 ± 2 °C. Prior to measurements, a solution containing 2% NaCl was prepared by the addition of 0.3 g solid NaCl to 15 mL high-purity water. The pH values of the samples were adjusted to 7.0 ± 0.2 (adding NaOH or HCl solutions). The change in light emission caused by the pharmaceuticals tested was evaluated.

The agar diffusion tests were conducted using tryptone glucose yeast extract agar (TGYE). It was prepared according to the manufacturer's instructions: 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) were mixed in 1 dm^3 pure water. The agar medium was thoroughly mixed and subjected to high-temperature sterilization (100 °C). A *Staphylococcus aureus* strain (B.01755, 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, Gödöllő, Hungary). Prior to the introduction of the bacterial suspension, the agar was allowed to cool to the appropriate temperature (45–50 °C) that was not harmful to the bacteria while still maintaining the agar in a liquid state, which allowed for the aseptic addition of the bacterial suspension and subsequent pouring into Petri dishes. The mixture was gently mixed to ensure even distribution of the bacteria throughout the agar. For each test, 1 cm^3 of 10^6 CFU cm^{-3} concentration bacterial suspension and 25 cm^3 of TGYE agar were mixed and poured into a Petri dish. The agar–bacteria mixtures in the Petri dishes were allowed to solidify at room temperature. After the agar solidified, holes were punched in it with a 4 mm diameter glass tube, and the holes were filled with 0.08 cm^3 of antibiotic solution. The samples were allowed to diffuse into the agar for 24 h in a 37 °C incubator. The average diameters of the growth inhibition zones were calculated using two different plates for each dose and three holes filled with samples on each plate [27].

3. Results and Discussion

3.1. Reaction Systems

The radiolytic degradation of water supplies hydroxyl radical ($^{\bullet}OH$, yield 0.28 μmol J^{-1}), hydrated electron (e_{aq}^-, 0.28 μmol J^{-1}), and hydrogen atom ($H^{\bullet}$, 0.06 μmol J^{-1}) reactive intermediates [28]. Under practical conditions, dissolved air is also present in water treatment, assuring an O_2 concentration of 0.25 mmol dm^{-3}. e_{aq}^- and $H^{\bullet}$ in aerated solutions mainly disappear in reaction with dissolved O_2 (Reactions (1) and (2)). In the reactions, superoxide radical anion/perhydroxyl radical pair forms ($O_2^{\bullet-}/HO_2$, pK_a = 4.8, [29]). These low-reactivity radicals mostly terminate in H_2O_2-forming reactions (Reaction (3)):

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet-} \qquad k = 1.9 \times 10^{10}\ mol^{-1}\ dm^3\ s^{-1} \tag{1}$$

$$H^{\bullet} + O_2 \rightarrow HO_2^{\bullet} \qquad k = 2.1 \times 10^{10}\ mol^{-1}\ dm^3\ s^{-1} \tag{2}$$

$$O_2^{\bullet-} + HO_2^{\bullet} + H_2O \rightarrow H_2O_2 + O_2 + OH^- \quad k = 9.7 \times 10^7\ mol^{-1}\ dm^3\ s^{-1} \tag{3}$$

Therefore, during radiolysis of aerated systems, H_2O_2 forms with a high initial yield of ~0.3 μmol J^{-1} [22]. The initially linear H_2O_2 concentration–dose dependence curve at higher absorbed doses (2–3 kGy, 1 Gy = 1 J kg^{-1}) levels off at a constant value. This leveling off is due to the competition of H_2O_2 forming and consuming reactions, e.g., Reaction (4) converts e_{aq}^- to $^{\bullet}OH$ [30].

$$e_{aq}^- + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- \quad k = 1.2 \times 10^{10}\ mol^{-1}\ dm^3\ s^{-1} \tag{4}$$

Therefore, during wastewater treatment by ionizing radiation in the presence of dissolved air, the chemical transformations are mostly initiated by $^{\bullet}OH$. Water radiolysis may provide the cleanest source of hydroxyl radicals, providing excellent potential for studying their reactions [28].

3.2. COD and TOC Removals

Figure 1A shows the COD and TOC values measured in pure water (filled symbols, irradiated and unirradiated) and also the calculated ones for the unirradiated solution (empty symbols). The calculation was based on the molecular formula and concentration using the method described in the paper of Baker et al. [31]. The measured and calculated COD values differ considerably: in pure water, 43.1 and 59.2 mg dm^{-3}, respectively. In the case of TOC, there is a reasonable agreement between the two values, 22.2 and 21.6 mg dm^{-3}, respectively. Because of the good agreement in the TOC values, it is probable that the observed difference in COD is not due to inaccuracy or impurities [32]. The disagreement between the measured and calculated COD values was observed very often in different laboratories for many chemicals. Baker et al. suggested that during the boiling process some small organic molecules may evaporate and leave the reaction vessel; this loss may cause the mentioned deviation [31].

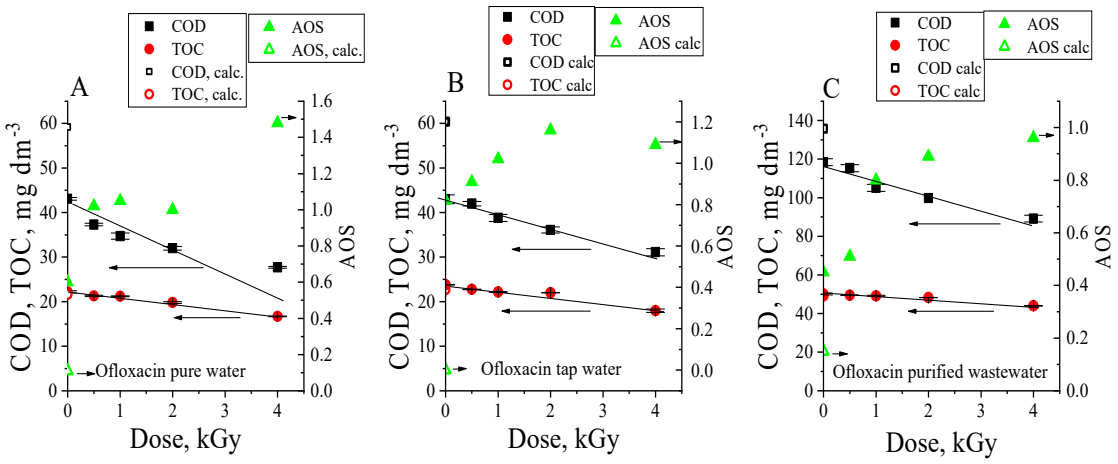


Figure 1. COD, TOC, and AOS values as a function of absorbed dose in 0.1 mmol dm^{-3} ofloxacin solutions in pure water (**A**), tap water (**B**), and purified wastewater (**C**) matrices.

In Figure 1A, both COD and TOC decrease with the absorbed dose. At doses 0.5, 1, 2, and 4 kGy, 10, 11, 15, and 40% smaller COD values, respectively, were measured than in the untreated sample; the values for TOC were 4, 5, 11, and 25%, respectively. In the experiments of Changotra et al. at 3 kGy absorbed dose, 29.3% TOC reduction was observed at the same ofloxacin concentration as we used here (0.1 mmol dm^{-3}) [12]. At the beginning of the treatment, the percentage decrease of COD, measured here, and in other AOP systems was higher than that of TOC (e.g., [13,33]. As Figure 1A shows, at higher doses the COD/TOC ratio slightly shifted toward lower values, showing that with the increase in dose, more and more oxidized products are in the solution. AOP experiments with a large number of organic molecules have shown that with the progress of degradation, small, strongly oxidized molecules, first of all organic acids (acetic, propionic, formic, oxalic), dominate the product spectrum [32,34], resulting in a decrease in pH [3,17]. These molecules have high oxygen-to-carbon ratios. They degrade very slowly in $^{\bullet}OH$ reactions [35]; this is why the COD/TOC ratio decreases with increasing dose.

The predominance of small acids in the reaction mixture at high doses is also supported by the average oxidation state (AOS) values calculated using the measured COD and TOC data (here both are expressed in mol dm^{-3} units): AOS = 4 × (1–COD_{mol}/TOC_{mol}) [33]. By definition, the AOS values vary between −4 (methane) and +4 (carbon dioxide). In our experiments, these values were around 1–1.5 in irradiated solutions (Figure 1A–C). We mention as examples some AOS values: in acetic and lactic acid solutions, they are calculated to be 0, and in malonic, formic, and oxalic acid solutions, they are 1.33, 2, and 3, respectively.

The decrease in both COD and TOC is observed even at the smallest dose, 0.5 kGy, used in our experiments, indicating that oxidation and mineralization occur simultaneously. During degradation, the antibiotic is finally mineralized, forming CO_2, H_2O, and inorganic ions. Two types of mineralization kinetics can be suggested. 1: In one of them, the molecules are oxidized step-by-step, and the degradation occurs through several stable products before final mineralization (the oxidized molecules in the last step transform into inorganic species). 2: The simultaneously observed decrease in both COD (oxidation) and TOC (mineralization) at the beginning of the treatment, i.e., building oxygen atoms into the organic substrates and also transformation of organic carbon atoms to CO_2 may reflect another mechanism. In the latter mechanism, the organic radical formed in $^{\bullet}OH$ attack undergoes several further reaction steps, e.g., peroxide formation, fragmentation, mineralization of the fragments. In the reaction sequence, the dissolved oxygen is expected to play a key role. As an example, we mention the peroxidation of hydrocarbons. A series of radical reactions was suggested for the ring opening and fragmentation reactions of aromatic molecules [32,36]. The faster decrease in the COD values compared to the TOC values and the large number of stable organic products detected in several laboratories [10,12–14,34] using low-dose irradiation suggest that the transformations basically occur with mechanism 1. However, mineralization observed at very low doses indicates that mechanism 2 cannot be neglected either.

The results of COD and TOC measurements in tap water (natural freshwater) and purified wastewater (effluent of a wastewater treatment plant) are shown in Figure 1B,C, respectively. In the calculations of the starting COD and TOC values in tap water and purified wastewater matrices, we considered both the COD and TOC values of ofloxacin added (calculated) and the contributions of the matrix (measured). Here, too, the measured and calculated COD values differ considerably: in tap water 43.0 and 60.4 mg dm^{-3}, and in purified wastewater 118.4 and 135.7 mg dm^{-3}, respectively. In tap water, the dose dependence was practically the same as observed in pure water, since the tap water matrix used had low TOC and COD values of around 1 mg dm^{-3}. However, in purified wastewater, both the COD and TOC shifted to higher values due to the high organic content of the purified wastewater used, and the decreases in both values with increasing dose were somewhat smaller than in the other two matrices. The reacting radicals not only are used for the degradation of ofloxacin, but they may also react with the organic and inorganic molecules/ions of the matrix.

3.3. BOD and OUR Measurements

Biodegradability is often characterized by the biochemical oxygen demand (BOD) values. In BOD measurements, a mixed microorganism population (activated sludge, collected from a biological degradation unit of a wastewater treatment plant) is utilized for oxidation, and usually O_2 consumption is measured over a 5-day incubation period (Figure 2).

As Figure 2D shows, in a pure water matrix BOD increased with the measuring time, and around 5 days it showed a tendency to level off. The microorganisms (bacteria, fungi) produce extracellular enzymes for breaking down large molecules into smaller, readily biodegradable ones, thus producing nutrients for the microorganism. In the presence of nutrients, the microorganisms multiply. This is a self-stimulating process; the more enzymes the bacteria release, the more ofloxacin will be decomposed by these enzymes, resulting in more nutrients. Because there are more nutrients, the bacteria multiply faster. The enzymes can decompose only a small part of the original molecules; therefore, the BOD value at zero dose is small. With an increasing dose, the biodegradability improves and the BOD values increase. On the fifth day, the values were 15.3 in pure water, 20 in tap water, and 41.4 mg dm^{-3} O_2 in purified wastewater at a 4 kGy absorbed dose. The high value measured in the purified wastewater is due to the biodegradable impurities in this matrix. The organic content of a solution is considered to be easily biodegradable when the BOD_5/COD ratio is above 0.4 [37]. This ratio in the untreated sample was ~0.09, showing that ofloxacin is poorly biodegradable at the concentration used in the

experiments, 0.1 mmol dm^{-3}. As a result of irradiation, the BOD_5/COD ratio increased above 0.4 at higher doses, reflecting good biodegradability in the treated solution. The ratio also increased with the absorbed dose in both the tap water and purified wastewater matrices. The nonbiodegradable organic content transformed into biodegradable upon irradiation of the solutions in these cases, too.

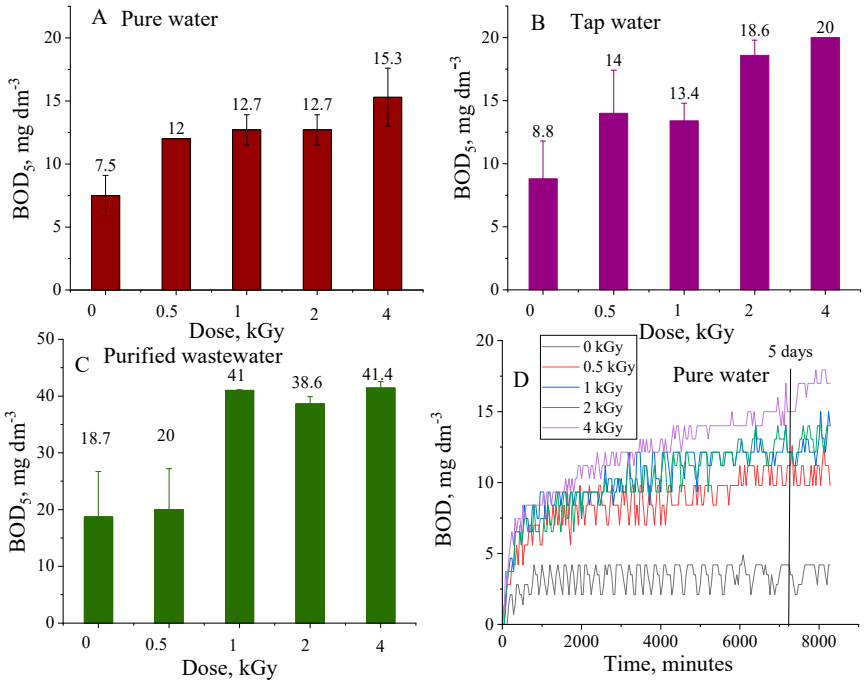


Figure 2. BOD values in 0.1 mmol dm^{-3} irradiated and nonirradiated ofloxacin solutions in pure water (**A**), tap water (**B**), and purified wastewater (**C**) matrices. Time dependence of BOD values at different doses in pure water (**D**).

The effect of degradation products on the metabolic activity of a mixed microbial population was investigated in oxygen uptake rate measurements (OUR, Figure 3). Similarly to BOD, this test also uses activated sludge with a complex mixture of bacterial species. However, OUR measurements take only three hours, whereas BOD measurement is at least five days long. The number of microorganisms used in the OUR test is around two orders of magnitude higher than the initial number of microorganisms in BOD measurement. However, this number does not change during OUR measurement; microorganisms have no time for producing extracellular enzymes, and they cannot multiply either, while at the end of the five-day measurement, the number of microorganisms in BOD measurement is two orders of magnitude higher (four orders of magnitude compared to the starting solution) than in the OUR test. Therefore, in OUR measurements, only the readily biodegradable compounds are digested. In pure water, the blank solution containing activated sludge had an OUR value of 0.70 mg dm^{-3} h^{-1}. When ofloxacin was added to the blank solution, the OUR value increased to 1.13 mg dm^{-3} h^{-1}. The value corrected with the blank is 0.43 (in Figure 3 the corrected values are shown). The corrected values increased at higher doses compared to the unirradiated one, showing more intensive metabolic activity in the treated samples in agreement with the results of the BOD_5 measurements. The values indicate that the degradation products not only are nontoxic, but they used as a source of nutrients by the microbes.

3.4. Agar Diffusion Tests

Agar diffusion tests are used to quantify the inhibition of bacterial growth exerted by antibiotics. In the experiments, we used *S. aureus* strains to test the antibacterial potency of samples. All measurements were made under the same conditions. The inhibition

zone diameters decreased with the dose, showing that irradiation decreased the antibiotic potency of the solutions (Figure 4).

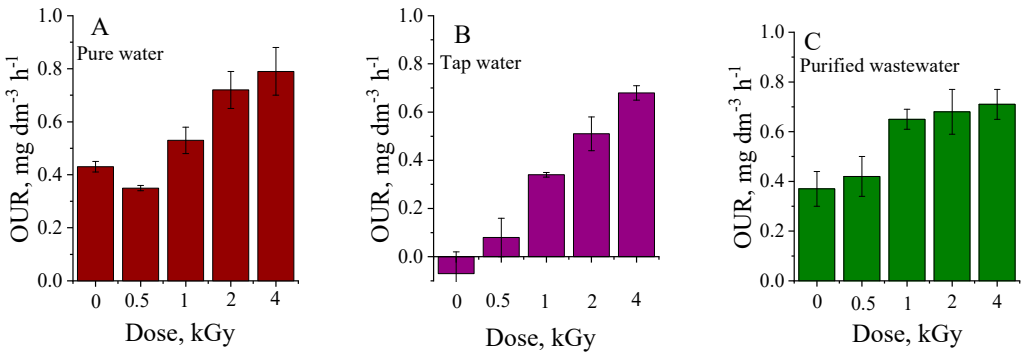


Figure 3. OUR values in 0.1 mmol dm^{-3} irradiated and nonirradiated ofloxacin solutions in pure water (**A**), tap water (**B**), and purified wastewater (**C**) matrices. The dry matter content of sewage sludge was the same for all measurements.

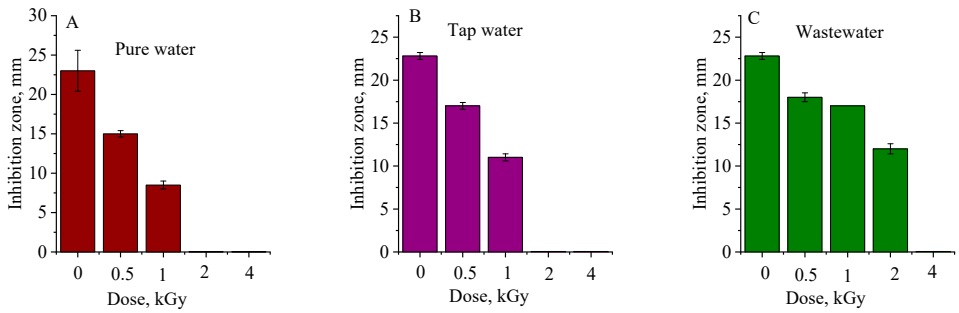


Figure 4. Inhibition zone diameters in 0.1 mmol dm^{-3} irradiated and nonirradiated ofloxacin solutions in pure water (**A**), tap water (**B**), and purified wastewater (**C**) matrices.

In pure water and tap water matrices at 1 kGy dose, the antibacterial activity was strongly reduced, whereas at 2 kGy no inhibition was observed. As we mentioned in the Introduction, at a 1 kGy dose, practically all ofloxacin molecules underwent some transformation [12]. In the transformations, the pharmacophore parts of ofloxacin molecules were modified, and, as the results show, the transformation products did not have antibacterial potency. Our results are similar to those of Changotra et al. [12]. They used an agar diffusion test also at 0.1 mmol dm^{-3} antibiotic concentration in 2 and 3 kGy irradiated solutions, and after applying *E. coli* test bacteria found complete disappearance of antibacterial effect.

In purified wastewater, we observed inhibition zones even at 2 kGy dose. This matrix has a relatively high organic molecule content (high initial COD and TOC values), and as we suppose a large part of reactive radicals formed during water radiolysis disappear in reaction with them. Therefore, a lower number of radicals are available for reacting with ofloxacin.

3.5. Toxicity Investigations

Microtox acute toxicity tests using *Vibrio fischeri* bioluminescent bacteria are often applied to characterize water samples. The method is based on the evaluation of light emission changes caused by the chemicals tested.

No toxicity to *V. fischeri* was observed in nonirradiated solutions (see in Figure 5). When the irradiated solution was introduced into the test mixture, high toxicity was observed, growing up to 100% luminescence inhibition at a 1–2 kGy dose. This high inhibition was certainly due to the effect of H_2O_2 forming during radiolysis [22–24]. However, when H_2O_2 was removed by using the enzyme catalase (recommended by Zona and Solar, [38]),

no or only minor inhibition was detected. These experiments show that the degradation products were either nontoxic or exhibited just a small toxicity to the test bacteria. The toxicity can be eliminated by properly selecting the absorbed dose [3,12].

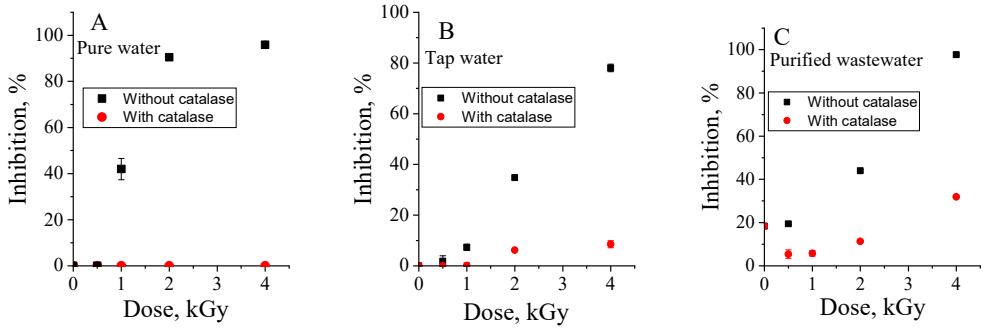


Figure 5. *Vibrio fischeri* toxicity test in 0.1 mmol dm^{-3} ofloxacin solutions in pure water (**A**), tap water (**B**), and purified wastewater (**C**) matrices.

In the experiments in tap water and purified wastewater matrices, the effect of H_2O_2 was somewhat smaller, probably due to the transition metal content of these matrices, which catalyzes peroxide decomposition in Fenton-type reactions. We have to note that under practical conditions, H_2O_2 decomposes quickly after the irradiation, since the water/wastewater to be treated always contains transition metal ions (e.g., Fe^{2+}) in a higher concentration. As was discussed before, with the increase in dose, the oxacillin molecules are fragmented and transformed into small molecular mass carboxylic acids as suggested by AOS values. The fragmented molecules exhibit very low toxicity to *V. fischeri* bacteria [39].

4. Conclusions

Ofloxacin is one of the most frequently detected fluoroquinolone antibiotics found in wastewaters [3,5,6]. As was demonstrated in this paper, antibiotics can be eliminated from purified wastewaters by AOP: $^{\bullet}OH$ efficiently degrades these molecules. When irradiation was performed in a pure water matrix, high toxicity to *Vibrio fischeri* bacteria was observed due to H_2O_2 formation. A strong decrease in toxicity was measured by removing H_2O_2 using catalase enzyme. Ofloxacin is not toxic to this bacterium, and the first formed products may show just a little toxicity. This toxicity disappears with an increase in dose. In a tap water matrix and in a purified water matrix, the effect of H_2O_2 was somewhat smaller than in pure water.

The ofloxacin solution with a concentration of 0.1 mmol dm^{-3} is not biodegradable (low BOD_5/COD ratio, low OUR value). However, after irradiation with a relatively low dose this solution becomes biodegradable. After irradiation with a 2–4 kGy dose, only highly oxidized low molecular mass fragments are present in the solution, which are readily biodegradable, not toxic, and have no antibacterial activity.

High-energy ionizing radiation technology can be recommended as post-treatment of wastewater treatment plant effluents. In practical applications, the radiation source is always an electron beam (EB) accelerator due to its several orders of magnitude higher dose rate than that of Co-60 gamma irradiators [40]. The advantages of electron beam (EB) treatment in comparison with other AOP techniques are: i. no need for additives; therefore, no new pollutants are produced; ii. removal of antibiotics and disinfection occurs simultaneously; iii. space requirement is small—the unit can be fitted to the end of the conventional process line; iv. the process works not only in transparent solutions (like UV processes); v. with careful planning, it is economical and cheaper than other technologies; vi. the technology was introduced on an industrial scale, e.g., treating 30,000 m^3/day dying wastewater effluent [41,42].

Author Contributions: Conceptualization, L.W. and E.T.; investigation, R.H., K.K. and A.B.; data curation, R.H., K.K. and A.B.; writing—original draft preparation, L.W. Writing—review and editing, L.W. and E.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Office for Research and Development through the Hungarian-Chinese Industrial Research and Development Cooperation Project, grant number 2017-2.3.6.-TÉT-CN-2018-00003.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The help of Petra Mandula in the COD and TOC measurements is acknowledged.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Al-Omar, M.A. Ofloxacin. In *Profiles of Drug Substances, Excipients and Related Methodology*; Brittain, H.G., Ed.; Elsevier: Amsterdam, The Netherlands, 2009; Volume 34, pp. 265–298. [CrossRef]
2. Zuccato, E.; Castiglioni, S.; Bagnati, R.; Melis, M.; Fanelli, R. Source, occurrence and fate of antibiotics in the Italian aquatic environment. *J. Hazard. Mater.* **2010**, *179*, 1042–1048. [CrossRef] [PubMed]
3. Esposito, B.R.; Capobianco, M.L.; Martelli, A.; Navacchia, M.L.; Pretali, L.; Saracino, M.; Zanellia, A.; Emmi, S.S. Advanced water remediation from ofloxacin by ionizing radiation. *Radiat. Phys. Chem.* **2017**, *141*, 118–124. [CrossRef]
4. Babić, S.; Horvat, A.J.M.; Pavlović, D.M.; Kaštelan-Macan, M. Determination of pK_a values of active pharmaceutical ingredients. *TrAC Trends Anal. Chem.* **2007**, *26*, 1043–1061. [CrossRef]
5. Sanseverino, I.; Navarro Cuenca, A.; Loos, R.; Marinov, D.; Lettieri, T. *State of the Art on the Contribution of Water to Antimicrobial Resistance*; JRC Technical Reports; European Union: Luxemburg, 2018. Available online: https://ec.europa.eu/jrc (accessed on 6 June 2023).
6. Rodriguez-Mozaz, R.; Vaz-Moreira, I.; Giustina, S.V.D.; Llorca, M.; Barceló, D.; Schubert, S.; Berendonk, T.U.; Michael-Kordatou, I.; Fatta-Kassinos, D.; Martinez, J.L.; et al. Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. *Environ. Int.* **2020**, *140*, 105733. [CrossRef]
7. Wang, J.-F.; Liu, Y.; Shao, P.; Zhu, Z.-Y.; Ji, H.-D.; Du, Z.-X.; Wang, C.-C.; Liu, W.; Gao, L.-J. Efficient ofloxacin degradation via photo-Fenton process over eco-friendly MIL-88A(Fe): Performance, degradation pathways, intermediate library establishment and toxicity evaluation. *Environ. Res.* **2022**, *210*, 112937. [CrossRef]
8. Fernández-Velayos, S.; Menéndez, N.; Herrasti, P.; Mazarío, E. Ofloxacin degradation over nanosized Fe_3O_4 catalyst via thermal activation of persulfate ions. *Catalysts* **2023**, *13*, 256. [CrossRef]
9. Stefan, M.I. *Advanced Oxidation Processes for Water Treatment*; IWA Publishing: London, UK, 2017; 710p, ISBN 97817804071280.
10. An, T.; Yang, H.; Song, W.; Li, G.; Luo, H.; Cooper, W.J. Mechanistic considerations for the advanced oxidation treatment of fluoroquinolone pharmaceutical compounds using TiO_2 heterogeneous catalysis. *J. Phys. Chem.* **2010**, *114*, 2569–2575. [CrossRef]
11. Fu, X.-M.; Luo, M.; Ma, L.-L.; Yang, G.; Xu, D.-D.; Liu, Z.-M. Studies on the degradation of ofloxacin by Electron Beam irradiation in aqueous solution. *China Environ. Sci.* **2016**, *36*, 3033–3039.
12. Changotra, R.; Guin, J.P.; Varshney, L.; Dhir, A. Assessment of reaction intermediates of gamma radiation-induced degradation of ofloxacin in aqueous solution. *Chemosphere* **2018**, *208*, 60–213. [CrossRef]
13. Changotra, R.; Guin, J.P.; Khader, S.A.; Varshney, L.; Dhir, A. Electron beam induced degradation of ofloxacin in aqueous solution: Kinetics, removal mechanism and cytotoxicity assessment. *Chem. Eng. J.* **2019**, *356*, 973–984. [CrossRef]
14. Santoke, H.; Song, W.; Cooper, W.J.; Greaves, J.; Miller, G.E. Free-radical-induced oxidative and reductive degradation of fluoroquinolone pharmaceuticals: Kinetic studies and degradation mechanism. *J. Phys. Chem. A* **2009**, *113*, 7846–7851. [CrossRef] [PubMed]
15. Tegze, A.; Sági, G.; Kovács, K.; Homlok, R.; Tóth, T.; Mohácsi-Farkas, C.; Wojnárovits, L.; Takács, E. Degradation of fluoroquinolone antibiotics during ionizing radiation treatment and assessment of antibacterial activity, toxicity and biodegradability of the products. *Radiat. Phys. Chem.* **2018**, *147*, 101–105. [CrossRef]
16. Tegze, A.; Sági, G.; Kovács, K.; Tóth, T.; Takács, E.; Wojnárovits, L. Radiation induced degradation of ciprofloxacin and norfloxacin: Kinetics and product analysis. *Radiat. Phys. Chem.* **2019**, *158*, 68–75. [CrossRef]
17. Iswarya, G.S.M.; Nirkayani, B.; Kavithakani, A.; Padmanaban, V.C. Statistical modeling of radiolytic (^{60}Co γ) degradation of ofloxacin, antibiotic: Synergetic effect, kinetic studies and assessment of its degraded metabolites. *Front. Environ. Sci. Eng.* **2019**, *13*, 42. [CrossRef]

18. *ISO/ASTM 51538:2009(E)*; Standard Practice for Use of the Ethanol-Chlorobenzene Dosimetry System. ISO: Geneva, Switzerland, 2009.
19. *ISO 6060:1989*; International Standard, Water-Quality: Determination of Chemical Oxygen Demand, 2nd ed.; ISO: Geneva, Switzerland, 1989.
20. *DIN EN 1899-1, 1998*; Water Quality. Determintion of the Biochemical Oxygen Demand after N Days (BOD[n]) of Water—Part 1: Dilution and Seeding Method with Allylthiourea Addition. ISO: Geneva, Switzerland, 1989.
21. *ISO 8192:1986*; Water Quality—Test for Inhibition of Oxygen Consumption by Activated Sludge. ISO: Geneva, Switzerland, 1986.
22. Illés, E.; Tegze, A.; Kovács, K.; Sági, G.; Papp, Z.; Takács, E.; Wojnárovits, L. Hydrogen peroxide formation during radiolysis of aerated aqueous solutions of organic molecules. *Radiat. Phys. Chem.* **2017**, *134*, 8–13. [CrossRef]
23. Kovács, K.; Sági, G.; Takács, E.; Wojnárovits, L. Use of bovine catalase and manganese dioxide for elimination of hydrogen peroxide from partly oxidized aqueous solutions of aromatic molecules—Unexpected complications. *Radiat. Phys. Chem.* **2017**, *139*, 147–151. [CrossRef]
24. Sági, G.; Bezsenyi, A.; Kovács, K.; Klátyik, S.; Darvas, B.; Székács, A.; Wojnárovits, L.; Takács, E. The impact of H_2O_2 and the role of mineralization in biodegradation or ecotoxicity assessment of advanced oxidation processes. *Radiat. Phys. Chem* **2018**, *144*, 361–366. [CrossRef]
25. Bezsenyi, A.; Sági, G.; Makó, M.; Wojnárovits, L.; Takács, E. The effect of hydrogen peroxide on the biochemical oxygen demand (BOD) values measured during ionizing radiation treatment of wastewater. *Radiat. Phys. Chem.* **2021**, *189*, 109773. [CrossRef]
26. *DIN EN ISO 11348-2:1999*; Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio Fischeri (Luminescent Bacteria Test) Part 2: Method Using Liquid-Dried Bacteria. ISO: Geneva, Switzerland, 1999.
27. Bonev, B.; Hooper, J.; Parisot, J. Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. *J. Antimicrob. Chemother.* **2008**, *61*, 1295–1301. [CrossRef]
28. Buxton, G.V. An overview of the radiation chemistry of liquids. In *Radiation Chemistry: From Basics to Applications in Material and Life Sciences*; Spotheim-Maurizot, M., Mostafavi, M., Douki, T., Belloni, J., Eds.; EDP Sciences: Les Ulis, France, 2008; pp. 3–16. [CrossRef]
29. Bielski, B.H.J.; Cabelli, D.E.; Ravindra, L.; Arudi, R.L. Reactivity of $HO_2/O^-{}_2$ radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **1985**, *14*, 1041–1100. [CrossRef]
30. Buxton, G.V.; Greenstock, C.L.; Helman, W.P.; Ross, A.B. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($^{\bullet}OH/^{\bullet}O^-$) in aqueous solution. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886. [CrossRef]
31. Baker, J.R.; Milke, M.W.; Michelcic, J.R. Relationship between chemical and theoretical oxygen demand for specific classes of organic chemicals. *Water Res.* **1999**, *33*, 327–334. [CrossRef]
32. Homlok, R.; Takács, E.; Wojnárovits, L. Degradation of organic molecules in advanced oxidation processes: Relation between chemical structure and degradability. *Chemosphere* **2013**, *91*, 383–389. [CrossRef]
33. Giannakis, S.; Gamarra Vives, F.A.; Grandjean, D.; Magnet, A.; De Alencastro, L.F.; Pulgarin, C. Effect of advanced oxidation processes on the micropollutants and the effluent organic matter contained in municipal wastewater previously treated by three different secondary methods. *Water Res.* **2015**, *84*, 295–306. [CrossRef]
34. Michael, I.; Hapeshi, E.; Aceña, J.; Perez, S.; Petrović, M.; Zapata, M.; Barceló, D.; Malato, S.D.; Fatta-Kassinos, D. Light-induced catalytic transformation of ofloxacin by solar Fenton in various water matrices at a pilot plant: Mineralization and characterization of major intermediate products. *Sci. Total Environ.* **2013**, *461–462*, 39–48. [CrossRef]
35. Shende, R.V.; Mahajani, V.V. Kinetics of wet air oxidation of glyoxalic acid and oxalic acid. *Ind. Eng. Chem. Res.* **1994**, *33*, 3125–3130. [CrossRef]
36. Von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, UK, 1987.
37. Metcalf, L.; Eddy, H.P. *Wastewater Engineering: Treatment and Reuse*, 4th ed.; McGraw-Hill: New York, NY, USA, 2003.
38. Zona, R.; Solar, S. Oxidation of 2,4-dichlorophenoxyacetic acid by ionizing radiation: Degradation, detoxification and mineralization. *Radiat. Phys. Chem.* **2003**, *66*, 137–143. [CrossRef]
39. Ren, S.; Frymier, P.D. Estimating the toxicities of organic chemicals to bioluminescent bacteria and activated sludge. *Water Res.* **2002**, *36*, 4406–4414. [CrossRef]
40. IAEA. *Radiation Processing, Environmental Applications*; International Atomic Energy Agency: Vienna, Austria, 2007.
41. CGN. 2020. Available online: https://www.cgndea.com/worlds-largest-industrial-wastewater-treatment-project/ (accessed on 6 June 2023).
42. IAEA. 2020. Available online: https://www.iaea.org/newscenter/news/started-with-iaea-support-chinas-electron-beam-industry-opens-worlds-largest-wastewater-treatment-facility (accessed on 6 June 2023).