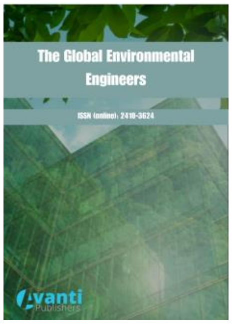
The Global Environmental Engineers, 2025, 22-36

Published by Avanti Publishers

The Global Environmental Engineers

ISSN (online): 2410-3624



Radiolytic Degradation of Sulfonamide Antibiotics: A Brief Overview of Recent Advances

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ARTICLE INFO

Article Type: Review Article

Academic Editor: Hongxing Dai

Keywords:
Overview
Radiolytic
Degradation
Sulfonamide

Timeline:
Received: April 25, 2025
Accepted: June 30, 2025
Published: August 02, 2025

Citation: Bojanowska-Czajka A, Sun Y, Wang J. Radiolytic degradation of sulfonamide antibiotics: A brief overview of recent advances. Glob Environ Eng. 2025; 12: 22-36.

DOI: https://doi.org/10.15377/2410-3624.2025.12.3

ABSTRACT

Sulfonamide antibiotics are widely used in both human and veterinary medicine. Although their use in human therapy has declined, they continue to pose a significant environmental threat due to their persistence in pharmaceutical formulations and subsequent release into the aquatic environment. The concentrations of these compounds in the influents and effluents of urban wastewater treatment plants (UWTPs) vary widely, depending on consumption patterns and the treatment technologies employed. Numerous studies have shown that conventional wastewater treatment methods are often insufficient for the complete removal of sulfonamides.

This underscores the urgent need for more effective and reliable treatment technologies capable of removing both sulfonamide antibiotics and their transformation products. Among advanced oxidation processes, electron beam (EB) irradiation has shown particular promise.

This review presents recent advances in analytical methods for detecting residues of selected sulfonamide antibiotics and explores the application of ionizing radiation—particularly gamma and EB irradiation—for their degradation in water and wastewater. The study also discusses observed changes in toxicity following treatment, offering a comprehensive perspective on the effectiveness and limitations radiation-based approaches in environmental remediation.

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1. Introduction

In recent years, increasing attention has been devoted to micropollutants, both in scientific research and by institutions responsible for water quality monitoring [1, 6]. Although micropollutants occur at relatively low concentration levels, they present a significant challenge to conventional water and wastewater treatment technologies commonly used in treatment plants. A major category of micropollutants includes pharmaceuticals of various classes, among which antibiotics are particularly noteworthy. One of the most serious public health concerns associated with the presence of antibiotics and their residues in the environment is the development of antimicrobial resistance. For example, the removal efficiency of sulfamethoxazole (SMX)—typically present at concentrations between 500 and 10,000 ng/L—can range from 65% to 96% [1, 2], with lower values (20–24%) also reported [2-4].

In the European Union, the primary legislative act governing the protection of all water types-excluding marine waters-is Directive 2000/60/EC, commonly known as the Water Framework Directive (WFD). This directive covers groundwater, inland surface waters, estuarine waters, and coastal waters. Marine waters were subsequently included in EU legislation through Directive 2008/56/EC [5, 7].

Based on criteria such as ecotoxicity, human toxicity, environmental concentrations, and production volumes, a list of priority substances posing a significant risk to ecosystems—referred to as the "Watch List"—has been established. This list is revised periodically, with new substances recommended for monitoring. SMX was added to the Watch List in 2020, and the most recent update in 2022 expanded the list to include other sulfonamide antibiotics.

According to national legislation, limits for sulfonamide antibiotics are currently defined only for residues in plants, animals, and food products of animal origin. In line with the Regulation of the Minister of Agriculture and Rural Development of 10 May 2003 (Poland) and the European Medicines Agency (EMA) Regulation (EU) No 37/2010, the maximum residue limit (MRL) for the sum of all sulfonamides in food of plant or animal origin is set at 100 µg/kg. This threshold is established based on toxicological assessments to ensure that such food products are safe for human consumption.

Sulfonamide antibiotics are among the most widely used synthetic broad-spectrum antimicrobials in agriculture, human medicine, and veterinary practice. According to available literature, more than 150 sulfonamide derivatives have been utilized to date [6, 8]. These antibiotics are effective in inhibiting the growth of most Gram-positive and some Gram-negative bacteria. Their mechanism of action involves interference with folic acid metabolism, thereby preventing bacterial growth and replication. Structurally, sulfonamide antibiotics consist of a sulfonamide group linked to a five- or six-membered heterocyclic ring (R group)—commonly pyrimidine, oxazole, isoxazole, or thiazole (Fig. **1**).

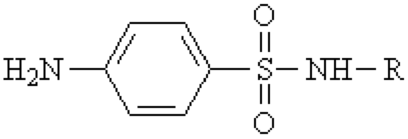

Figure 1: Generic chemical structure of sulfonamides.

The most commonly used antibiotics in this group include sulfadiazine (SDZ), sulfamethazine (SMT), SMX, and sulfathiazole (STZ). Although sulfonamide antibiotics are increasingly being replaced by newer-generation drugs with fewer side effects, their use remains relatively widespread in certain areas. Due to their well-documented environmental risks and high potential to disrupt natural bacterial communities, numerous efforts have been undertaken to degrade sulfonamide residues in both natural waters and wastewater systems.

Various treatment methods have been explored for the removal of these substances from aquatic environments. Physical methods, such as adsorption, merely transfer contaminants from one phase to another. Biological methods risk promoting the spread of antibiotic-resistant bacteria and resistance genes, while conventional chemical treatments may result in the formation of toxic by-products. All these approaches carry the risk of secondary contamination [7, 9].

In contrast, radiation technologies have emerged as a promising alternative, largely due to their high efficiency in degrading pharmaceuticals [8, 10], including antibiotics [9-12], as well as a wide range of organic micropollutants [11-14]. These technologies-namely gamma irradiation and EB irradiation-are part of the broader class of Advanced Oxidation Processes (AOPs). Complete degradation of SMX at an initial concentration of 20 mg/L was achieved at a dose of 1.5 kGy [4, 13,]. In terms of mineralization, total organic carbon (TOC) is a critical parameter; for instance, over 40% TOC reduction was observed in a sulfapyridine (SPD) solution at an initial concentration of 50 mg/L [5, 14].

AOPs rely on the generation of highly reactive species, primarily free radicals with strong oxidizing or reducing properties. The most established AOPs include photolysis, photocatalysis, ozonation, and hydrodynamic cavitation, all of which involve the formation of hydroxyl radicals (•OH). These radicals react rapidly and non-selectively with a wide array of waterborne contaminants [15], including pesticides [16]. In contrast, for chlorinated hydrocarbons, hydrated electrons (e_{aq}^-) are generally more reactive [17].

What sets radiation-based AOPs apart is their simultaneous generation of oxidizing and reducing species—such as •OH, e_{aq}^-, and •H—making them exceptionally effective. The concept relies on the ability of ionizing radiation to deposit energy within the aqueous medium, leading to ionization and excitation of water molecules. This results in radiolysis of water, as described by Equation (1) [18], or may occur via direct energy absorption. Through successive radical-mediated reactions, pollutants are broken down into simpler compounds, and—under optimal conditions—the process can lead to complete mineralization, producing water, carbon dioxide, and inorganic salts.

$$H_2O \rightarrow {}^{\bullet}OH\ (2.7) + e_{aq}^-\ (2.6) + H^{\bullet}(0.55) + H_2\ (0.45) + H_2O_2\ (0.71) + H_3O^+\ (2.6) \quad (1)$$

The values in brackets are expressed in units of molecules/100 eV.

An aqueous solution of SMX was treated using three different AOPs—ozonation, UV irradiation, and EB irradiation—to compare their degradation efficiency [19]. In all experiments, the initial SMX concentration was maintained at 30 mg/L. The results demonstrated that each method significantly reduced the toxicity of the treated solution, as measured by the *Pseudokirchneriella subcapitata* bioassay.

Importantly, when considering only electricity consumption (excluding the costs of infrastructure development and reagent use), EB irradiation emerged as the most cost-effective method for SMX degradation. Under optimal conditions, the radiation-based process requires no chemical additives and is capable of achieving complete mineralization of the pollutant. Among the AOPs evaluated, only the EB process demonstrated the potential to serve as an effective standalone method for the treatment of industrial and municipal wastewater contaminated with various antibiotic compounds.

This work focuses exclusively on sulfonamide antibiotics, setting it apart from other published review articles. Using this specific group of organic micropollutants as a case study, the paper explores the evolution of both analytical methods used for their monitoring and the development of degradation processes. Furthermore, it highlights how, over the years, the assessment of additional parameters—such as toxicity, TOC, and identification of degradation products—has become crucial in determining the overall effectiveness and quality of the AOPs applied.

2. Methodology

2.1. Analytical Methods for Monitoring Radiolytic Degradation

In any technological process applied to water and wastewater treatment, the development of appropriate analytical methods is a crucial step for monitoring the concentration of target substances. Equally important is the ability to simultaneously identify the degradation products formed during the decomposition of the investigated pollutants [20]. The choice of analytical methods depends on the chemical and physical properties of the compounds being analyzed. A major challenge in developing suitable methods arises from the typically low concentrations of these contaminants—often in the range of a few mg/kg or ng/kg—the complexity of the sample matrix, and potential interference from other substances with similar physicochemical properties. Additionally, the lack of certified standards and reference materials for degradation products frequently complicates their identification [21].

Analytical techniques reported in the literature for sulfonamide detection include high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), gas chromatography (GC), liquid chromatography (LC), UV/Vis spectrophotometry, enzymatic assays, and electrochemical methods [22]. In cases where only the quantification of the parent compound is required and the degradation products are not of interest, spectrophotometric methods are widely used due to their low cost, accessibility of instrumentation, minimal reagent consumption, high sensitivity, and low detection limits. Fig. (**2**) shows the UV–Vis spectra of SDZ at various irradiation doses , with a characteristic absorption peak at 265 nm [23].

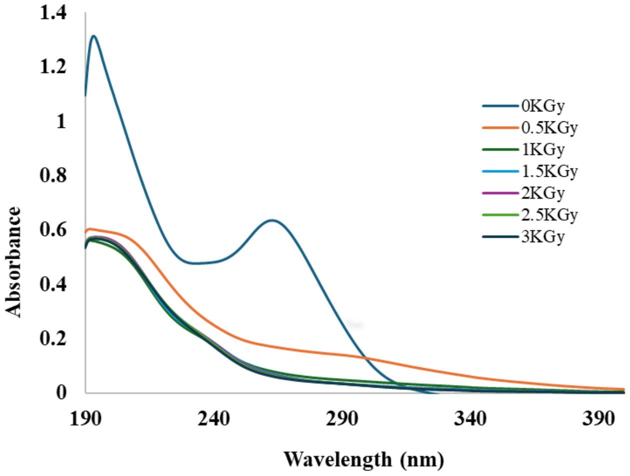

Figure 2: UV–Vis spectra of sulfadiazine (SDZ) recorded at various irradiation doses. The initial SDZ concentration was 10 mg/L at 0 kGy [23].

The spectrophotometric method is based on the Bratton–Marshall reaction, in which sulfonamides are first diazotized with sodium nitrite under acidic conditions and subsequently coupled with a chromogenic reagent. Commonly used coupling agents include N-(1-naphthyl)ethylenediamine dihydrochloride (NEDA) and 3-methylbenzothiazoline hydrochloride (MBTH). Optimization of the coupling step may involve alternative agents such as iminodibenzyl [24] or N-(1-naphthyl)ethylenediamine dihydrochloride (NED) [25]. Refinement of reaction conditions enables the determination of sulfonamides at trace levels, with detection and quantification limits ranging from 0.019–0.05 µg/mL and 0.06–0.16 µg/mL, respectively [24, 25].

The simultaneous determination of pharmaceutical residues and their degradation products in environmental samples requires highly sensitive and selective analytical methods. In earlier studies, gas chromatography coupled with mass spectrometry (GC-MS) was used; however, the need for derivatization significantly prolonged the analysis time [26, 27]. Currently, chromatographic techniques—particularly high-performance liquid chromatography (HPLC) with spectrophotometric [28, 29] or fluorescence detection [30], as well as liquid chromatography coupled with mass spectrometry (LC-MS or LC-MS/MS) [31-32]—are the most widely employed methods for the detection of sulfonamide antibiotics and their residues in water and food samples, as well as for monitoring degradation efficiency.

HPLC spectrophotometry with fluorescence detection often provides lower detection limits for sulfonamides in water or food samples. However, the analytical procedure is more complex due to the required derivatization step, which involves converting the analytes into their fluorescent derivatives. When rapid analysis of the main components is needed—such as for evaluating degradation efficiency—HPLC with UV-Vis detection is often a suitable and practical choice.

The monitoring of sulfamethazine (SMT) concentrations in gamma-irradiated sludge was conducted using HPLC-UV-Vis with a diode array detector (DAD) and a C18 analytical column. A mobile phase consisting of water and ethanol in a 55:45 ratio was used at a flow rate of 1 mL/min. Detection was performed at a wavelength of 255 nm [33].

Additionally, a DAD-HPLC system equipped with an XDB-C18 (5 μm, 4.6 mm × 150 mm) analytical column, maintained at 30 °C, was used to monitor the degradation efficiency of 20 mg/L SMT under gamma irradiation in the presence of added hydrogen peroxide (H_2O_2). The mobile phase consisted of methanol and distilled water in a 35:65 ratio, and detection was carried out at 275 nm. Under these conditions, the retention time of SMT was 1.97 minutes [8]. Fig. (**3**) presents the HPLC chromatogram of SDZ after exposure to a 0.5 kGy dose. The mobile phase consisted of a 50:50 (v/v) mixture of acetonitrile and distilled water. The retention time for SDZ was 5.835 minutes.

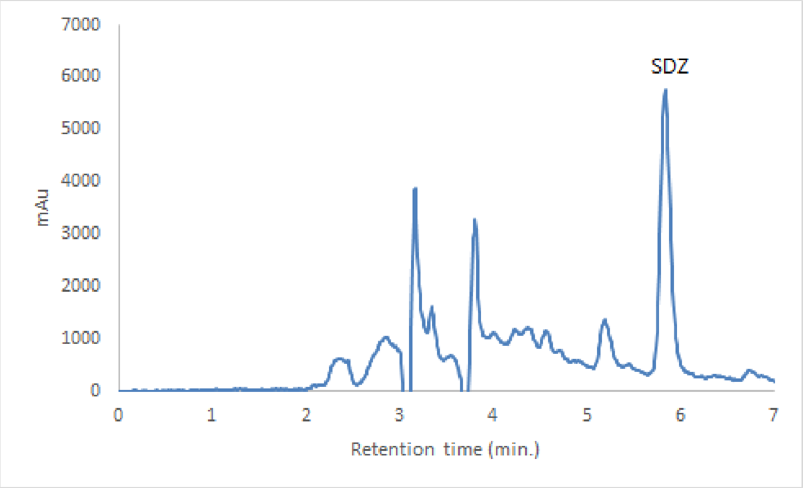

Figure 3: HPLC chromatogram of SDZ at a 0.5 kGy dose, detected at 275 nm.

The decomposition yield of 20 mg/L SMX in aqueous solution under gamma irradiation, in the presence of various yield-influencing factors, was determined using HPLC analysis. The system was equipped with a C18 column, and the mobile phase consisted of a 55:45 mixture of acetonitrile and 0.1% formic acid. Under isocratic elution at a flow rate of 1 mL/min, the retention time for SMX was approximately 2 minutes [4].

In experiments aiming not only to assess degradation efficiency but also to identify transformation products, liquid chromatography coupled with mass spectrometry (LC-MS and LC-MS/MS) offers significantly enhanced capabilities. For example, the degradation products of sulfamerazine (SMZ), sulfadiazine (SDZ), and sulfapyridine (SPD) irradiated by EB were identified using HPLC coupled with a quadrupole time-of-flight mass spectrometer (Q-TOF-MS). The analysis was performed under gradient elution conditions using a mobile phase composed of 0.1% formic acid and methanol. Ionization was conducted in positive mode, with a total run time of 15 minutes [5].

The identification of SMX degradation products in aqueous solution under both oxidative and reductive conditions was also carried out using an LC-MS system. The separation was performed under isocratic conditions on an XB-C18 capillary column, with an eluent consisting of 80% aqueous 0.1% formic acid and 20% acetonitrile [34].

2.2. Toxicity and TOC Measurements

Monitoring degradation efficiency solely by measuring the concentration of the target compound is often insufficient to fully evaluate the effectiveness of a degradation method. A critical complement to this approach is the parallel assessment of toxicity and TOC values. In fact, only a reduction in TOC provides a reliable indication of the degree of mineralization achieved.

To evaluate changes in the toxicity of solutions subjected to degradation by AOPs, including radiation-based technologies, some of the earliest and most commonly used bioindication tests were Microtox, Spirotox, and Daphnia magna assays [35]. These methods offered a relatively rapid and cost-effective means to determine whether the resulting degradation products pose a toxicological risk to the environment.

According to the definition provided in [36], a bioindicator may be any living system, ranging from a single organism to entire ecosystems. Many ecotoxicologists emphasize that model ecosystems are essential for the comprehensive assessment of biologically active substances. Changes within ecosystems are not merely the sum of changes in the populations that compose them, but also the result of disruptions and/or adaptations affecting the ecosystem as a whole. Although ecosystem-level studies provide the most comprehensive and ecologically relevant information, their high cost limits such research to only a few specialized centers worldwide.

To assess toxicity changes during gamma irradiation of an aqueous solution containing 0.1 mmol/dm^3 SMX, a Microtox assay based on the bioluminescence inhibition of Vibrio fischeri was employed [34]. In the untreated solution, luminescence inhibition was measured at 10%, but as the radiation dose increased, toxicity initially increased as well. A subsequent decrease in toxicity was observed at 5 kGy, corresponding to the degradation of both SMX and its radiolytic transformation products.

Complete mineralization under these conditions was further confirmed by TOC and Chemical oxygen demand (COD) measurements. The same bioluminescence-based method was applied to monitor toxicity changes in aqueous solutions of STZ, SMX, and SMT treated with EB irradiation [37]. For all three sulfonamide antibiotics, a reduction in acute toxicity was observed after exposure to a 5 kGy dose, suggesting that electron beam treatment not only degrades the parent compounds but also reduces the toxicity of their by-products..

Another organism found to be more sensitive for evaluating antibiotic toxicity than Daphnia magna or Vibrio fischeri is the green microalga *Pseudokirchneriella subcapitata* [38]. Studies on the degradation of selected antibiotics under gamma irradiation, including SMT, showed a significant reduction in toxicity following the breakdown of the parent compounds. A comprehensive approach to evaluating the removal of antibiotic residues from water and wastewater should also consider changes in antimicrobial activity and biodegradability, in addition to toxicity. In a study involving the radiolytic degradation of four sulfonamide antibiotics in dilute aqueous solutions, complete degradation was achieved at a dose of 1.5 kGy, while a dose of 2–2.5 kGy was required to transform the parent compounds into biodegradable forms—both in river water and activated sludge environments. Importantly, it was shown that antimicrobial activity is associated exclusively with the initial compounds and declines significantly as degradation progresses [39].

In recent years, cytotoxicity assays have gained prominence in toxicity monitoring. One commonly used method is the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), which assesses the cytotoxicity of irradiated samples based on the metabolic activity of selected cell lines. Colorimetric changes are measured at 490 nm using a plate reader spectrophotometer. The MTT assay offers the advantages of rapid response, high precision, and high throughput, providing valuable insights into the cytotoxic effects of chemical compounds on human cells [39]. Depending on the specific contaminant under investigation, various cell lines can be employed, such as HepG2 (human liver cells) [10, 13] or Caco-2 (human colonic epithelial cells) [10]. Compared to conventional bioindication assays, cytotoxicity tests provide a more direct and sensitive evaluation of acute toxicity relevant to human health.

As highlighted in the introduction, a comprehensive evaluation of the performance of degradation processes—such as gamma irradiation or EB treatment—requires not only monitoring the concentration of the parent pollutant, but also identifying degradation products, assessing toxicity changes, and most importantly, tracking TOC levels. Changes in TOC provide a measure of mineralization, which is the ultimate goal of most AOPs. In many cases, even when the parent compound and major degradation products are no longer detectable, mineralization remains incomplete, highlighting the importance of TOC monitoring.

For instance, in the case of gamma-irradiated SMX solutions at initial concentrations of 5, 10, and 20 mg/L, complete degradation of SMX was observed at 1.5 kGy for the 20 mg/L sample. However, TOC reduction was limited: 21.4% and 12.4% for 5 and 10 mg/L SMX, respectively, and no significant TOC change for the 20 mg/L solution—in the presence of sulfate ions (SO_4^{2-}) and humic substances [4].

Further studies investigated the degradation of sulfamerazine (SMR), SDZ, and SPD in water under EB irradiation, with simultaneous monitoring of TOC, total nitrogen (TN), and total carbon (TC) [5]. Among the antibiotics studied, SPD showed the highest mineralization efficiency: 42.2% TOC, 45.06% TC, and 20.83% TN removal. In contrast, removal efficiencies for SMR and SDZ were below 10% for all three parameters, suggesting that under the applied conditions, these compounds were mainly converted into intermediate organic products without full mineralization.

A similar trend was observed for SMT, where 20 mg/L aqueous solutions exposed to gamma radiation up to 5 kGy achieved approximately 95% degradation. However, TOC and TN reductions were limited to 6.8% and 10.5%, respectively (Fig. **4**) [40]. These findings suggest that while SMT molecules were largely broken down, the resulting products were not fully mineralized. Under these conditions, the reactive species generated during water radiolysis (Equation 1) primarily reacted with SMT itself, with only a small portion engaging with secondary degradation products to drive mineralization—explaining the low TOC and TN reduction values.

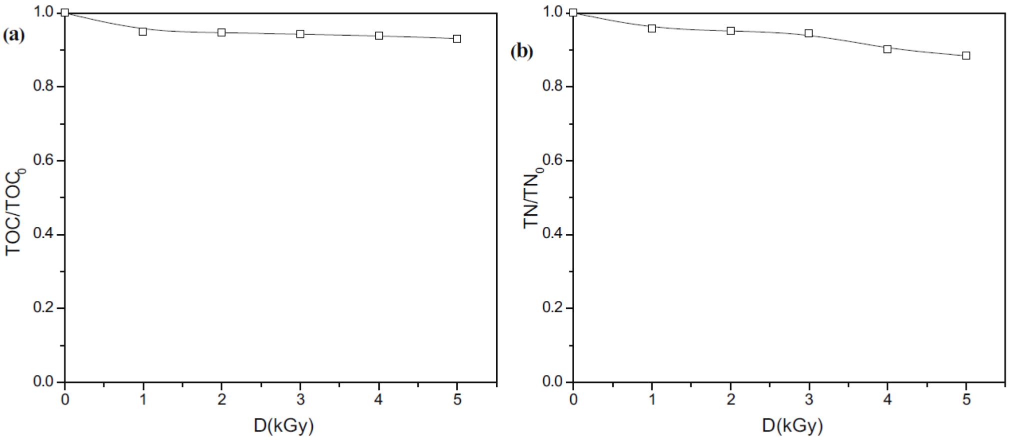

Figure 4: TOC and TN removal at different absorbed doses (SMT initial concentration: 20 mg/L; dose rate: 103 Gy/min). (**a**) TOC and (**b**) TN [40].

3. Radiation Technologies Used for the Degradation of Sulfonamide Antibiotics

In radiation-based treatment processes, the degradation of micropollutants—such as sulfonamide antibiotics—occurs primarily through reactions with radiolysis products of water, as described in Equation (1). Among these products, the hydroxyl radical (•OH) is the most reactive oxidizing species. However, because •OH is non-selective, it reacts not only with the target pollutant but also with other substances present in the solution. These competing compounds, referred to as radical scavengers, reduce the availability of •OH for pollutant degradation and thus lower the overall efficiency of the process. For this reason, degradation efficiencies are generally higher in distilled water than in complex natural matrices. Comparing treatment outcomes in both types of media provides a more realistic assessment of how the technology performs under environmental conditions.

Gamma radiation has been widely used for the degradation of SMX in both water and wastewater, with investigations focusing on the effects of solution pH and matrix composition. At an initial concentration of 20 mg/L, nearly complete degradation of SMX was achieved at a dose of 1 kGy, and the degradation process followed first-order kinetics. However, the presence of inorganic anions—such as CO_3^{2-}, HCO_3^-, NO_3^-, SO_4^{2-}, Cl^-, and HPO_4^{2-} (each at 10 mmol/L)—as well as organic matter such as 5 g/L glucose, 5 g/L peptone, and 19.25 mg C/L humic acid, led to reductions in SMX degradation efficiency. Inorganic anions and humic acid reduced efficiency by approximately 20%, whereas glucose caused a 60% decrease and peptone over 80%. These findings were confirmed in a natural matrix—wastewater treatment plant effluent—where, at a dose of 1.5 kGy, the degradation efficiency of 20 mg/L SMX dropped to 76.2% [4]. Despite these reductions, radiation-based technologies remain promising for sulfonamide removal in real environmental settings.

EB treatment has also been applied to aqueous solutions of SMR, SDZ, and SPD. In each case, the degradation followed pseudo-first-order kinetics. For SMR, complete degradation was achieved at doses of 1–2 kGy for initial concentrations of 35 and 50 mg/L, and at 8 kGy for 80 and 100 mg/L. However, in binary and ternary mixtures, degradation efficiencies decreased: to 84.21% for SMR + SDZ, 81.14% for SMR + SPD, and 66.73% for the SMR + SDZ + SPD mixture at 5 kGy [5]. It was also shown that removal efficiencies improved under acidic conditions (pH 3) and with pre-aeration of the solution. Among the antibiotics studied, SPD exhibited the highest mineralization rate (45%), likely due to its lower molecular weight compared to SMR and SDZ.

Further mechanistic studies have examined the radiolytic degradation of 0.1 mmol/L SMX under both oxidizing and reducing conditions [34]. For fundamental investigations, it is advantageous to conduct experiments under conditions that favor the formation of a single dominant radiolysis product, rather than a complex mixture. This allows for a clearer determination of the contribution of individual reactive species (e.g., •OH, e_{aq}^-, H•) to the degradation pathway.

One commonly used method to maximize •OH generation is to adjust the solution to pH 7 and saturate it with nitrous oxide (N_2O) prior to irradiation. This promotes the conversion of hydrated electrons (e_{aq}^-) into hydroxyl radicals via Reaction (2) [41]:

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^\bullet OH + OH^- + N_2 \qquad (2)$$

To study the effects of hydrated electrons or hydrogen atoms, •OH radicals must be removed from the system. In laboratory settings, this is achieved by deoxygenating the solution and adding an appropriate •OH scavenger—such as tert-butyl alcohol in concentrations ranging from 0.2 to 1 mM [42]. This scavenger reacts with •OH according to Reaction (3):

$${}^\bullet OH + (CH_3)_3\mathrm{COH} \rightarrow H_2O + {}^\bullet CH_2\,(CH_3)_2COH \qquad (3)$$

Other effective •OH scavengers include thiourea, dimethylurea, methanol, and ethanol. Experimental results have demonstrated that even trace concentrations of SMX can be efficiently removed from aqueous solutions using high-energy ionizing radiation, with degradation primarily driven by reactions with •OH radicals. This reaction produces hydroxylated SMX derivatives. Product analysis revealed that sulfur atoms in the SMX molecule were fully converted to sulfate ions (SO_4^{2-}), while nitrogen was identified as nitrate (NO_3^-) and ammonium (NH_4^+). Corresponding changes in chemical oxygen demand (COD) and TOC values confirmed a high degree of mineralization at this low concentration [34]. However, complete mineralization required doses approaching 100 kGy, indicating that further optimization is needed to reduce energy input.

A study by Wang *et al*. [43] demonstrated that the presence of inorganic anions in the solution matrix significantly influences the degradation efficiency of SMX under gamma-ray radiation. In experiments using aqueous SMX solutions at an initial concentration of 0.04 mM, degradation efficiency increased with rising radiation dose. At 800 Gy, a degradation efficiency of 97.6% was achieved, increasing to 99.6% at 1000 Gy. However, TOC reduction did not follow this trend—remaining below 12.7% at 1000 Gy—indicating limited mineralization despite high degradation of the parent compound.

The effects of various common inorganic anions, including chloride (Cl^-), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), nitrate (NO_3^-), sulfate (SO_4^{2-}), and phosphate (HPO_4^{2-}), were also investigated. The impact on SMX degradation depended on the type and concentration of the anion. Notably, nitrate ions consistently reduced degradation efficiency, regardless of their concentration. Chloride ions had no significant effect at low concentrations, but higher concentrations led to a noticeable decline in efficiency. In contrast, the presence of 20 mM carbonate ions or 50 mM phosphate ions enhanced SMX degradation. This was attributed to the formation of carbonate and phosphate radicals, which react with the amino group of the SMX molecule, initiating further transformations and forming a variety of intermediate products.

In a comparative study involving three antibiotics from different structural classes—SMX, lincomycin, and tetracycline—complete degradation at an initial concentration of 30 mg/L was achieved at a dose of 2 kGy for SMX, whereas 1 kGy was sufficient for lincomycin and tetracycline [37].

Additional experiments involving three sulfonamide antibiotics—STZ, SMX, and SMZ—subjected to EB irradiation, showed that STZ exhibited the highest radiolysis efficiency, followed by SMX and SMZ (STZ > SMX > SMZ) [36]. These differences were attributed to variations in molecular structure, which influence reactivity with radiolysis products. Furthermore, it was observed that decreasing the solution pH enhanced decomposition efficiency across all compounds tested.

The following section will present efforts to address this challenge through the use of hybrid methods, which aim to achieve comparable degradation and mineralization levels at lower energy doses.

4. Hybrid Methods for the Degradation of Sulfonamide Antibiotics

To reduce the ionizing radiation dose required to achieve effective degradation of organic micropollutants in water and wastewater, hybrid methods are commonly employed. One of the simplest and most cost-effective approaches involves the addition of hydrogen peroxide (H_2O_2) to irradiated solutions. This strategy enhances the formation of additional hydroxyl radicals (•OH), which are key oxidizing agents in advanced oxidation processes (AOPs). The following reactions are observed [44]:

$$e_{aq}^- + H_2O_2 \rightarrow {}^{\bullet}OH + OH^- \qquad k = 1.2 \times 10^{10}\, M^{-1}s^{-1} \tag{4}$$

$$H^{\bullet} + H_2O_2 \rightarrow {}^{\bullet}OH + H_2O \qquad k = 3.5\, x\, 10^{7}\, M^{-1}\, s^{-1} \tag{5}$$

Although N_2O is more frequently used in research due to its inertness and the stability of its by-products, H_2O_2 remains a practical alternative. However, the optimal amount of H_2O_2 is difficult to estimate, and excessive concentrations can lead to undesirable side reactions. In particular, surplus H_2O_2 can react with •OH radicals, effectively scavenging them, as shown in equation (6):

$$ {}^{\bullet}OH + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \qquad k = 2.7 \times 10^{7}\, M^{-1}s^{-1} \tag{6}$$

In an experiment using sludge enriched with SMT at an initial concentration of 10 mg/L, a decomposition efficiency of 65% was observed at 1 kGy, which increased to 98% at 2.5 kGy. When 10 mg/L of H_2O_2 was added, the efficiency at 1 kGy improved to 76% [4].

Hydrogen peroxide was also applied in the treatment of three sulfonamide antibiotics—STZ, SMX, and SMZ—using EB irradiation [36]. Each compound was tested at an initial concentration of 80 mg/L, with H_2O_2 additions ranging from 2 to 100 mM. At a dose of 0.5 kGy and 0.5 mM H_2O_2, the highest degradation efficiency was observed for STZ (90%), while SMX and SMZ reached only 45% and 40%, respectively.

However, efficiency declined when the H_2O_2 concentration exceeded 5 mM, primarily due to secondary radical-scavenging reactions presented above in equation (6):

Moreover, the resulting peroxyl radicals (HO_2•) can further react with hydroxyl radicals, diminishing the overall oxidative potential. This is shown in equation (7):

$$HO_2^{\bullet} + {}^{\bullet}OH \rightarrow H_2O + O_2 \qquad k = 6.0 \times 10^{9}\, M^{-1}s^{-1} \tag{7}$$

These findings underscore the need to optimize H_2O_2 dosage to maximize the formation of reactive radicals without triggering competing side reactions that reduce degradation efficiency.

Three different concentrations of hydrogen peroxide—0 mg/L, 10 mg/L, and 30 mg/L—were tested for an aqueous SMT solution with an initial concentration of 20 mg/L. Under gamma irradiation, the SMT degradation efficiency increased with rising H_2O_2 concentrations within the tested range (Fig. **5**). However, the addition of H_2O_2 had a more pronounced effect on TOC reduction than on SMT removal efficiency. The SMT removal efficiencies were 90%, 98%, and 100% for H_2O_2 additions of 0 mg/L, 10 mg/L, and 30 mg/L, respectively. In contrast, at a 3 kGy dose, the corresponding TOC removal efficiencies were only 5%, 17%, and 48%.

This difference can be attributed to the incomplete degradation of SMT by-products within the applied dose range. The observed increase in TOC removal with higher H_2O_2 concentration is likely due to the greater production of •OH radicals from reactions involving hydrated electrons (e_{aq}^{-}) and hydrogen atoms (•H), as described in reactions (4) and (5).

Another hybrid approach involves combining radiation with ozonation. In the presence of ozone (O_3), the reactive species generated during water radiolysis—•H and e_{aq}^{-}—can be partially converted into HO_3^{-}, which subsequently decomposes to generate •OH and O_2 (reactions 8-11) [45].

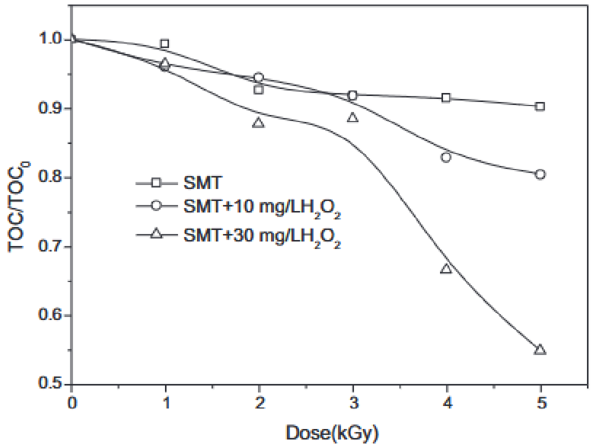

Figure 5: Effect of absorbed dose and initial hydrogen peroxide (H_2O_2) concentration on TOC removal in aqueous SMT solution [8].

$$e_{aq}^{-} + O_{3\,(aq)} \rightarrow O_{3(aq)}^{-\bullet} \qquad k = 3.6\,x\,10^{10}\,M^{-1}s^{-1} \tag{8}$$

$$O_{3(aq)}^{-\bullet} + H_{(aq)}^{+} \rightarrow HO_{3(aq)}^{\bullet} \tag{9}$$

$$H_{(aq)}^{\bullet} + O_{3(aq)} \rightarrow HO_{3(aq)}^{\bullet} \qquad k = 3.8\,x\,10^{10}\,M^{-1}s^{-1} \tag{10}$$

$$HO_{3(aq)}^{\bullet} \rightarrow {}^{\bullet}OH_{(aq)} + O_{2(aq)} \tag{11}$$

This synergy suggests that the sequence of application—ozonation followed by radiation or vice versa—plays a crucial role in pollutant elimination. For example, in a study on aqueous SMX at an initial concentration of 20 mg/L, 100% degradation was achieved either by 12 minutes of ozonation or by gamma irradiation at 1.5 kGy. However, despite full SMX degradation, mineralization levels remained low—15% for ozonation and 27% for gamma irradiation.

When 10 mM ethanol (a hydroxyl radical scavenger) was added to the solution, no significant change in SMX degradation efficiency was observed, suggesting that direct oxidation by ozone, rather than radical-mediated pathways, was the dominant mechanism. Ozone is known to selectively react with functional groups such as double bonds and aromatic rings. Additionally, indirect oxidation via •OH radicals generated from ozone is more likely at alkaline pH (pH > 9), where ozone decomposes rapidly. In contrast, ozone remains stable at acidic pH (pH < 4) [46].

To improve mineralization, a hybrid approach combining ozonation and gamma irradiation was tested. When the SMX solution was first ozonated for 30 minutes and then irradiated with a 0.5 kGy dose, TOC removal increased to 34.3%, compared to just 5.4% for irradiation alone at the same dose. However, reversing the sequence—irradiation followed by ozonation—did not result in a significant improvement in TOC reduction. This highlights the importance of process order in hybrid treatment strategies.

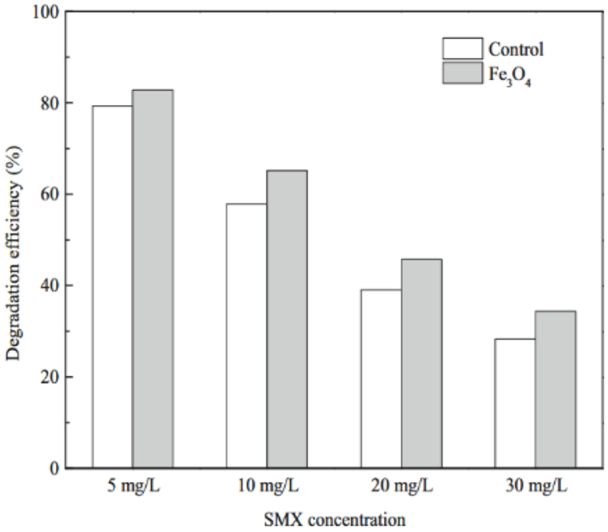

Figure 6: Degradation efficiency of sulfamethoxazole (SMX) at different initial concentrations, with and without the addition of Fe_3O_4 nanoparticles (dose = 0.2 kGy; [Fe_3O_4] = 0.1 g/L) [47].

Another approach for enhancing the degradation of sulfonamide antibiotics involved combining gamma irradiation with the addition of Fe_3O_4 nanoparticles, which served as a heterogeneous catalyst to further promote degradation reactions [47].

Magnetic Fe_3O_4 particles can react with hydrogen peroxide (H_2O_2) generated during radiolysis (Eq. 1), leading to the formation of additional hydroxyl radicals (•OH) through reactions (12) and (13) [48].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \tag{12}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^+ + HO_2^{\bullet} \tag{13}$$

At initial SMX concentrations of 5, 10, 20, and 30 mg/L, the degradation efficiencies following gamma irradiation at a dose of 0.2 kGy were 80%, 57%, 39%, and 28%, respectively. When Fe_3O_4 was added at a concentration of 0.1 g/L, the degradation efficiencies increased slightly to 83%, 64%, 45%, and 32%, respectively (Fig. **6**).

Regarding mineralization, the maximum TOC removal efficiency achieved in the 5–30 mg/L concentration range was 20% for initial SMX concentrations of 5 and 10 mg/L at a dose of 1.5 kGy. However, when 1 g/L of Fe_3O_4 was added, TOC removal for the 5 mg/L SMX solution increased significantly to approximately 50%, and ranged between 10–20% for higher concentrations (10, 20, and 30 mg/L).

To further enhance the degree of mineralization, a hybrid process combining gamma irradiation with the addition of H_2O_2 and Fenton's reagent was applied to an aqueous SDZ solution [49]. The effect of pH (2.6-9) on SDZ degradation was investigated for a 25 mg/L solution, considering the pKa of SDZ and its behavior under acidic, neutral, and alkaline conditions. At a dose of 1 kGy, the highest degradation efficiency (82%) was achieved at pH 5.5, followed by 75%, 60%, and 54% at pH 2.6, 7, and 9, respectively.

For the gamma/H_2O_2/Fenton hybrid process, optimal conditions for the Fenton reaction were met at pH 2.6. Under these conditions, using an H_2O_2 dose between 7.2 and 18 mmol/L led to both high SDZ degradation (≈80%) and significant mineralization (≈60%) at a radiation dose of 1 kGy. As reported in numerous studies, the Fenton process is highly effective in removing a variety of organic pollutants from water. Since the amount of H_2O_2 generated by water radiolysis (Eq. 1) may be insufficient, supplemental H_2O_2 is often added in practice.

Under optimal conditions—25 mg/L SDZ, pH 2.6, 1 kGy dose—degradation efficiencies exceeding 90% and mineralization levels of 60-70% were achieved. The applied H_2O_2 concentrations ranged from 3.6 to 36 mmol/L, while Fe^{2+} concentrations were between 0.04 and 0.36 mmol/L. Based on these findings, the authors recommend the combined use of radiation technologies with H_2O_2 and Fenton's reagent for the treatment of SDZ-contaminated wastewater.

5. Conclusion

As demonstrated, ionizing radiation is highly effective in degrading organic pollutants, including sulfonamide antibiotics, and outperforms many conventional treatment methods. At relatively low doses (1–5 kGy), nearly complete degradation—approaching 100%—can be achieved for most sulfonamides at concentrations up to 30 mg/L. However, a critical issue that remains is the relatively low degree of mineralization, even when the parent compound is no longer detectable.

To enhance treatment outcomes, the combination of radiation technologies with other AOPs is considered a promising approach, as has already been shown in pesticide degradation studies [50]. Equally important is toxicity monitoring, using both bioindication assays and cytotoxicity tests, which provides a more complete and human-relevant assessment of the safety and efficacy of the degradation process.

Some decomposition products are highly persistent and remain in solution despite the complete removal of the original compounds. This has led to increasing efforts to identify radiolysis intermediates, typically using LC-MS or LC-MS/MS.

For most sulfonamides, degradation under ionizing radiation is primarily driven by oxidation via hydroxyl radicals (•OH). Studies have shown that different sulfonamides follow similar degradation pathways, with the N–H and sulfonamide N–S bonds being particularly vulnerable to attack. Cleavage of the N–S bond leads to the formation of sulfanilic acid, which subsequently transforms into para-aminophenol and then para-nitrophenol, with the release of sulfate ions. These reactions are largely independent of the specific R-group structure, as indicated in Fig. (**7**) [51].

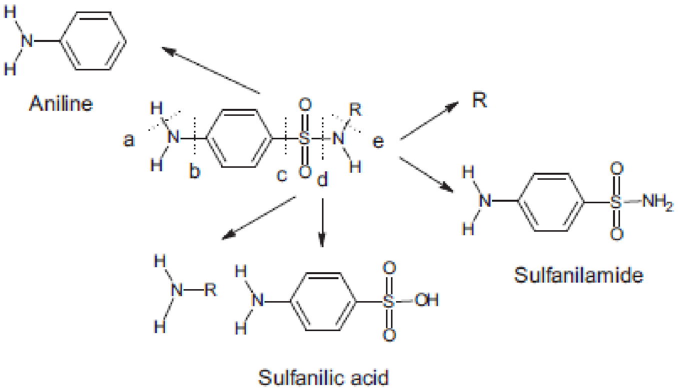

Figure 7: Potential cleavage sites of sulfonamides during advanced oxidation processes [51].

In optimal scenarios, the goal is to achieve the highest degree of mineralization with the lowest possible energy input. However, as previously highlighted, the composition of the treated matrix—including natural organic matter, inorganic ions, and other constituents—can significantly affect degradation efficiency. This underscores the need for continued research to determine the most effective treatment strategies, particularly for complex natural systems.

While EB irradiation has proven highly effective in laboratory studies for removing sulfonamides from both water and wastewater, scaling the technology for use in full-scale treatment plants poses notable challenges. These include substantial energy demands, high infrastructure costs, and stringent requirements for radiation safety. Until recently, EB applications had been limited almost entirely to the laboratory scale.

A significant advancement was achieved in Jiangmen City, Guangdong Province, China, where EB technology was successfully implemented on a full scale to treat 30,000 m^3/day of dyeing wastewater. This milestone demonstrates that, with proper engineering, EB can be effectively applied under real-world conditions. Nevertheless, several challenges remain, including incomplete mineralization, uncertainty regarding long-term operational stability, and limited data on its performance against diverse classes of contaminants.

Ongoing research is crucial to optimize system configurations, lower operational costs, and assess the potential for integrating EB with other AOPs to enhance overall treatment efficacy and safety. A deeper understanding of degradation mechanisms in environmentally relevant conditions will further support the wider adoption of radiation-based technologies, either as standalone solutions or in combination with complementary AOP methods, for the efficient and reliable removal of pharmaceutical residues from aquatic environments.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This work was funded by the National Center for Research and Development., Poland-China Cooperation Program, acronym TAPEB 'Advanced treatment of typical antibiotic pharmaceutical wastewater using EB irradiation" under contract number WPC3/2022/68/TAPEB/2024) and the Key Program for Intergovernmental S&T Innovative Cooperation Project of China (2024YFE0101700).

Additionally, this work (EU-based collaboration) was supported by the European Union's Horizon 2020 Research and Innovation programme under Grant acronym I.FAST "Innovation Fostering in Accelerator Science and Technology " Agreement No 101004730 and by the Polish ME&S under contract number 5180/H2020/2021/2.

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