Degradation of Streptomycin in Water by Electron Beam Irradiation - Revised Manuscript

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Abstract

Electron beam irradiation, as an advanced oxidation process, has been extensively studied for treating organic compounds in water by utilizing the highly oxidizing and reducing active radicals it generates. Currently, there is a lack of research on degrading streptomycin using advanced oxidation processes. Therefore, electron beam irradiation was employed as a novel approach to investigate the feasibility of streptomycin degradation. Experimental results demonstrated that 5 mg/L streptomycin was completely degraded under conditions of 1 MeV electron beam energy and 10 kGy irradiation dose. Through DFT prediction of reaction sites and combined analysis of degradation product structures via mass spectrometry data, four degradation products of streptomycin under electron beam irradiation were identified. Simultaneously, reasonable speculation regarding the degradation pathways was made based on the composition and content of degradation products at different irradiation doses.

Full Text

Study on the Degradation of Streptomycin in Water by Electron Beam Irradiation

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Abstract

Electron beam irradiation, as an advanced oxidation process, has been widely investigated for treating organic compounds in water through the generation

of highly oxidative and reductive reactive radicals. However, research on streptomycin degradation using advanced oxidation methods remains limited. This study therefore employs electron beam irradiation as a novel approach to explore the feasibility of degrading streptomycin. Experimental results demonstrate that complete degradation of 5 mg/L streptomycin is achieved under conditions of 1 MeV electron beam energy and 10 kGy irradiation dose. By predicting reaction sites through Density Functional Theory (DFT) and analyzing mass spectrometry data of streptomycin degradation products, four degradation products were identified. Furthermore, reasonable speculation on the degradation pathway was developed based on the composition and content of degradation products at various irradiation doses.

Keywords: electron beam irradiation; streptomycin; liquid chromatography-mass spectrometry

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Antibiotics and their metabolites are discharged into water bodies through various pathways, becoming major pollutants in aquatic environments [**?**, **?**]. Residual antibiotics in water can lead to the proliferation of antibiotic-resistant bacteria (ARB) and the emergence of antibiotic resistance genes (ARG), posing potential threats to ecological health [**?**]. Compared with conventional biological treatment methods, advanced oxidation processes have demonstrated superior performance in removing antibiotics from water and have become an effective means for antibiotic degradation [**?**].

Advanced oxidation technologies (AOPs) generate highly oxidative reactive radicals that attack and break the chemical bonds of antibiotic molecules, thereby destroying their molecular structures and effectively removing antibiotic pollution from water bodies [**?**]. As a type of AOP, ionizing radiation treatment combines both oxidation and reduction mechanisms [**?**]. This approach achieves antibiotic degradation without or with minimal addition of chemical reagents, thereby reducing the risk of secondary pollution during treatment [**?**]. The reaction process of ionizing radiation is shown in equation (1), where $\bullet$OH exhibits strong oxidizing capability with a standard oxidation potential of 2.8 eV [**?**, **?**], while e^- q and $\bullet$H possess reducing capacity [**?**].

$$H_2O \xrightarrow{\text{irradiation}} \bullet OH + e^-_{aq} + \bullet H$$

Ionizing radiation technology has been widely proven effective for degrading various organic compounds in water, including antibiotics [**?**]. Chu et al. achieved complete degradation of penicillin G through gamma irradiation [**?**], while Pearce et al. realized complete degradation of 1,4-dioxane using electron beam irradiation [**?**]. Ionizing radiation technology not only offers efficient organic degradation capability but also produces minimal secondary pollution during reaction, with simple operation. In Shiyan City, Hubei Province, China' s first electron beam irradiation facility for medical wastewater treatment has already been put into operation.

Streptomycin, as a commonly used antibiotic detected in water bodies [**?**], requires timely degradation to reduce water pollution. However, current research on streptomycin degradation in water remains limited. Based on the promising results of ionizing radiation technology for antibiotic degradation, this study aims to validate the feasibility of electron beam irradiation for streptomycin degradation in water. By systematically controlling electron beam energy and irradiation dose, we investigated the degradation efficiency under different conditions. Using mass spectrometry analysis combined with reaction sites calculated by density functional theory (DFT), we analyzed the degradation products after irradiation to infer the degradation pathways.

1.1 Reagents and Instruments

Streptomycin (CAS: 57-92-1, concentration $\geq$ 90%) was purchased from Macklin Biochemical Co., Ltd. The composition and content of samples before and after irradiation were analyzed using a Shimadzu 20A high-performance liquid chromatograph equipped with an XDB-C18 reversed-phase column and a photodiode array detector. Intermediates formed during irradiation were identified using an Ultimate 3000 UHPLC-Q Exactive LC-MS system.

1.2.1 Sample Preparation

Ultrapure water was used to prepare streptomycin solutions with initial concentrations of 5–20 mg/L. Samples were placed in polyethylene petri dishes with a diameter of 35 mm, and the liquid height was controlled according to the optimal penetration depth calculated by Geant4 for different electron beam energies.

1.2.2 Irradiation Conditions

Irradiation experiments were conducted using an electron linear accelerator with electron beam energies of 1 MeV, 1.6 MeV, and 2 MeV at a beam current intensity of 2 mA. Under these operating parameters, the corresponding irradiation dose rates were 0.2 kGy/s, 0.11 kGy/s, and 0.08 kGy/s, respectively. The irradiation dose range for samples was set at 0–20 kGy.

1.2.3 Chemical Analysis

Streptomycin concentration was determined by high-performance liquid chromatography (HPLC) using a C18 column (250 mm × 4.6 mm, 5 m particle size) with a UV detector at 205 nm. The mobile phase was prepared by adding 14 g sodium sulfate, 1.5 g sodium octanesulfonate, 120 mL acetonitrile, and 50 mL of 0.2 mol/L phosphate buffer (pH 3.0) to ultrapure water, diluting to 1000 mL and mixing well. The column temperature was maintained at 40°C with an injection volume of 10 L using isocratic elution [**?**].

Degradation products of streptomycin were analyzed by liquid chromatography-mass spectrometry (LC-MS) using an Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 m). Mobile phases consisted of A (0.1% formic acid) and B (acetonitrile). The column temperature was set at 30°C with an injection volume of 10 L, and the elution program is shown in Table 1 . The mass spectrometer was a Thermo Scientific Q Exactive with the following conditions: HESI ion source, sheath gas flow rate of 40 arb, auxiliary gas flow rate of 10 arb, spray voltage of 3.8 kV for positive ions, capillary temperature of 320°C, auxiliary gas temperature of 300°C, S-lens at 50%, and Full MS scan mode. Scan parameters: primary scan resolution 70,000, range 100–1200 m/z; secondary scan resolution 17,500, starting ion 50 m/z.

2.1 Key Parameters in Electron Beam Irradiation Degradation of Streptomycin

The effectiveness of electron beam irradiation is primarily determined by irradiation dose and penetration depth [**?**]. Irradiation dose can be adjusted by changing irradiation time. Cooper et al. found that irradiation dose positively correlates with generated radical concentration [**?**]. Higher irradiation doses produce more reactive radicals, leading to more thorough degradation of organic compounds—a principle confirmed in numerous studies on organic degradation by ionizing radiation [**?**].

The penetration capability of electron beams into samples is also a critical factor determining irradiation treatment efficiency. The optimal penetration depth is defined as the depth at which energy deposition equals that at the material surface, where electron beam irradiation treatment efficiency is highest [**?**]. Using Geant4 to construct a sample model and irradiate it with electron beams of different energies, the relationship between electron beam energy and optimal penetration depth for streptomycin solution is shown in Figure 1 [Figure 1: see original paper]. The calculated optimal penetration depths for 1 MeV, 1.6 MeV, and 2 MeV electron beam energies are 0.45 cm, 0.8 cm, and 1 cm, respectively.

2.2 Degradation Efficiency of Streptomycin by Electron Beam Irradiation

The degradation effects of different electron beam energies on streptomycin aqueous solutions with various initial concentrations are shown in Figure 2 [Figure 2: see original paper]. A streptomycin sample with an initial concentration of 5 mg/L achieved complete degradation under 1 MeV electron beam energy after 50 seconds at an irradiation dose of 10 kGy. As electron beam energy increased to 1.6 MeV and 2 MeV, streptomycin samples with the same initial concentration achieved complete degradation at irradiation doses of 5 kGy and 4 kGy, requiring 45.5 seconds and 50 seconds, respectively.

Although the treatment time for complete degradation of streptomycin samples at the same concentration was approximately equivalent across different electron beam energies, comparison of degradation efficiency revealed a positive correlation between streptomycin degradation efficiency and electron beam energy. The degradation efficiency calculation formula is shown in equation (2):

$$\text{Degradation Efficiency} = \frac{(C_0 - C) \times V_E}{t}$$

where C_0 is the initial streptomycin concentration, C is the streptomycin concentration at the current irradiation dose, V_E represents the irradiated sample volume corresponding to the electron beam energy, and t is the irradiation time required to reach the current dose.

Calculations of degradation efficiency for complete degradation of 5 mg/L streptomycin solution under different electron beam energies showed that higher electron beam energy yields greater degradation efficiency. At 2 MeV electron beam energy, degradation efficiency reached 3.85×10^{-6} mg/L, while at 1 MeV it was 1.74×10^{-6} mg/L. From both experimental mechanism and accelerator irradiation process perspectives, increased electron beam energy may enhance sample penetration capability. At constant beam current intensity, higher-energy electron beams possess greater power, generating more thermal energy. Elevated temperature can promote organic degradation, suggesting that increased electron beam energy may accelerate streptomycin degradation by raising sample temperature [**?**]. For samples with the same electron beam energy and initial streptomycin concentration, degradation rate decreased with increasing irradiation dose. This phenomenon may be attributed to competition for reactive radicals generated from water irradiation between newly formed degradation products and streptomycin substrate [**?**].

2.3 Degradation Products of Streptomycin by Electron Beam Irradiation

By calculating reaction sites using DFT to assist in mass spectrometry data analysis, the structures of streptomycin degradation products can be inferred. Den-

sity Functional Theory (DFT) calculations were performed using the Gaussian09 and D01 software packages. The B3LYP functional combined with Grimme' s dispersion correction (GD3BJ) was employed in all DFT calculations. The PCM solvent model for water and the 6-31G* basis set were used. Mulliken atomic charges, Hirshfeld atomic charges, and Fukui functions were obtained through the Multiwfn program. The Mulliken atomic charge results for streptomycin are shown in Figure 3 Figure 3: see original paper, where N[16] and C[38] exhibit the highest negative charge of -0.795 and positive charge of 0.581, respectively. Since these two atoms are directly connected with the largest charge difference between them, the N[16]-C[38] bond represents the initial bond cleavage site.

The HOMO and LUMO orbitals of streptomycin were calculated to infer oxidation and reduction sites in the molecular structure. Figure 4 [Figure 4: see original paper] shows the molecular dynamics calculation results for streptomycin, with Figure 4(a) displaying bond lengths between atoms. Calculating bond lengths in the streptomycin molecular structure also aids in inferring reaction sites [**?**]. Among these, C[22]-C[26] has the longest bond length at 1.655 Å. Based on molecular structure calculations, the five-membered carbon ring in streptomycin is also susceptible to bond cleavage.

The main degradation product detected by mass spectrometry at low irradiation doses is m/z 263, with the mass spectrum shown in Figure 5 Figure 5: see original paper. Its structure and Mulliken atomic charges are displayed in Figure 3(b). N[18] and C[13] possess the highest negative charge of -0.785 and positive charge of 0.614, respectively. N[14] attached to C[13] shows a negative charge of -0.766, while C[16] attached to N[18] shows a positive charge of 0.568. The significant charge differences between N[18]-C[16] and C[13]-N[14] indicate they are prone to cleavage, leading to the formation of degradation products m/z 220 and m/z 205, with mass spectra shown in Figures 5(c) and 5(d).

Due to the complex structure of antibiotics, considering only HOMO and LUMO orbital energies is insufficient [**?**], as many similar molecular orbitals also influence reactions. Additionally, analysis of electron density and molecular electrostatic potential may yield contradictory results [**?**]. Therefore, we employed Fukui functions to predict reaction sites, as Fukui function values correspond to reactivity at specific points [**?**]. Fukui functions can be approximated by equations (3–5).

Fukui function calculations for streptomycin revealed that atomic positions N[14], N[17], and N[18] are vulnerable to radical attack and subsequent cleavage. For m/z 263, the main degradation intermediate of streptomycin, Fukui function calculations identified positions C[13] and N[15] as susceptible to reduction reactions. The reaction sites and reaction types obtained from DFT calculations both validated the structures of degradation products detected in mass spectrometry data.

2.4 Degradation Pathway of Streptomycin by Electron Beam Irradiation

By detecting degradation products and their content changes at different irradiation doses through LC-MS, the degradation pathway can be reasonably deduced. The composition and content of streptomycin solution components at various irradiation doses are shown in Figure 6 [Figure 6: see original paper]. At 0.5 kGy irradiation dose, the main degradation products were m/z 263 and m/z 337. When the irradiation dose increased to 5 kGy, streptomycin was completely degraded, with m/z 263 as the primary product, while new degradation products m/z 220 and m/z 205 were generated under the action of $\bullet$H and e^- q. As the irradiation dose rose to 10 kGy, m/z 220 and m/z 205 became the main degradation products, while the content of m/z 263 and m/z 337 decreased. At 20 kGy irradiation dose, only trace amounts of m/z 220 and m/z 205 remained in solution. This may be attributed to reactions between degradation products and reactive radicals, though further analysis was limited by the mass spectrometry detection threshold ($m/z \geq 100$). Based on the composition and content of degradation products at different irradiation doses, a reasonable speculation of the degradation pathway was developed, as shown in Figure 7 [Figure 7: see original paper].

Conclusion

This study achieved complete degradation of 5 mg/L streptomycin solution within 50 seconds at an irradiation dose of 10 kGy using electron beam irradiation. By adjusting electron beam energy and irradiation dose, we demonstrated that increasing electron beam energy enhances streptomycin degradation efficiency, while showing a negative correlation between irradiation dose and degradation rate. DFT-calculated reaction sites combined with mass spectrometry data analysis revealed the structures of streptomycin degradation products. The degradation pathway was inferred based on the composition and content of degradation products at various irradiation doses. Electron beam irradiation degradation of streptomycin represents a simple, efficient, and highly feasible treatment method with broad application prospects.

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Note: Figure translations are in progress. See original paper for figures.