Plasma Chemistry and Plasma Processing (2025) 45:1151–1164
https://doi.org/10.1007/s11090-025-10567-y

ORIGINAL PAPER

Computer Simulations to Study the Mechanisms of Cold Plasma-Induced Degradation of Amoxicillin

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Received: 11 February 2025 / Accepted: 15 April 2025 / Published online: 26 April 2025

Abstract
Due to the increasing water pollution worldwide, wastewater treatment remains one of the most important issues. Cold atmospheric plasma (CAP) has emerged as a promising and versatile technology for wastewater treatment in recent years, offering potential advantages in terms of effectiveness and cost-efficiency. Although several studies have been conducted, the mechanisms by which CAP degrades antibiotics, one of the main pollutants in pharmaceutical wastewater, remain unclear. In this study, we investigate the degradation mechanisms of the antibiotic amoxicillin using reactive molecular dynamics simulations. Specifically, we explore the interaction mechanisms between reactive oxygen and nitrogen species (i.e., O, OH, HO_2, H_2O_2, O_3, NO, NO_2, NO_2^- and NO_3^-) generated by CAP and the amoxicillin molecule. Our simulation results reveal that some of these species form weak attractive (HO_2, H_2O_2, NO_2^- and NO_3^-) and weak repulsive (NO and NO_2) interactions, whereas O_3 exhibits both weak attractive and weak repulsive interactions with the amoxicillin molecule. OH radicals exhibit the same interaction mechanisms as O atoms; in other words, O atoms react with amoxicillin in a manner similar to two OH radicals. The simulation results for O atoms show that their reactions with amoxicillin lead to the formation of hydroxyl and hydroperoxide groups, the opening or breakage of the β-lactam ring, the shortening or widening of the benzene ring, and the fragmentation of the structure. Our findings are consistent with experimental outcomes on CAP treatment of amoxicillin. This study provides a deeper understanding of the mechanisms of antibiotic degradation by CAP in wastewater treatment.

Keywords Amoxicillin · Cold atmospheric plasma · Reactive oxygen and nitrogen species · Reactive molecular dynamics

Extended author information available on the last page of the article

Introduction

Water pollution has been increasing globally in recent years [1]. The quality of water has deteriorated and become polluted due to the direct or indirect introduction of substances that alter its physicochemical and biological properties, interfering with its regular use [2]. Therefore, wastewater treatment remains one of the most important issues.

There are four main types of wastewater treatment technologies: mechanical, physical, biological, and chemical [3]. Mechanical treatment is intended to remove granular particles larger than 0.1 mm in diameter, as well as oil, grease, and larger floating and suspended solids [4]. Physical treatment includes natural phenomena such as gravity, van der Waals forces, and electric attraction in wastewater treatment. Because no chemicals are used in this process, physical treatment methods do not alter the chemical structure or biological composition of the target substances [5]. Biological treatment transforms dissolved organic matter in wastewater into flocculent or stable organic and inorganic solids [4]. The main principle of this method is the removal of pollutants through biological activity [5]. However, in some cases, its low effectiveness and application force the development of advanced technologies, especially in the wide field of advanced oxidation process (AOPs). Chemical treatment is a crucial component of hazardous water treatment and includes processes such as disinfection, neutralization, and coagulation [5]. In recent years, integration technology, which combines the advantages of traditional and advanced methods, has garnered increased attention due to its high effectiveness and cost efficiency [6, 7]. One such innovative technology is plasma processing, specifically the application of low-temperature non-equilibrium atmospheric pressure plasmas, or cold atmospheric plasmas (CAPs). CAPs have recently gained significant interest due to the availability of simpler and more cost-effective plasma sources that can operate at ambient conditions [8, 9]. One of the main advantages of CAPs is their ability to combine powerful oxidants in a single action, leading to the degradation and conversion of toxic organic compounds. This synergistic effect among various components results in highly efficient pollutant removal. Additionally, many of the reactive species produced during CAP treatment are recognized disinfectants, providing the benefits of water disinfection. Plasma treatment has gained recognition as a versatile alternative to traditional chemical methods due to its unique properties and potential applications [10, 11].

Pharmaceutical compounds of various types, especially antibiotics, are increasingly being detected in wastewater. Abbassi et al. demonstrated that antibiotics present in wastewater negatively impact the treatment efficiency [12]. The authors also emphasized that antibiotics inhibit the growth of microorganisms in wastewater and noted that different antibiotics have different effects on chemical oxygen demand (COD), a measure of water quality. They found that amoxicillin had the greatest effect, while ciprofloxacin had the least effect [12]. This indicates a need for more extensive research on the environmental impact of complex antibiotics in wastewater.

Antibiotics can be categorized into several classes (e.g., sulfonamides, tetracyclines, β-lactams, fluoroquinolones, macrolides, and aminoglycosides) based on their chemical structure, mechanism of action, and spectrum of activity [13]. A recent review discusses advances in the synergistic application of ultrasonic or hydrodynamic cavitation methods combined with oxidative, photocatalytic, and enzymatic strategies for the degradation of antibiotics from wastewater [14]. The article details the degradation of sulfonamide, tetracycline, and β-lactam antibiotics using a combination of these methods. It also underscores the

importance of optimizing process parameters to enhance the efficiency of these combinatorial methods in achieving a substantial reduction in COD in medical wastewater [14]. Fang et al. investigated the degradation mechanism and toxicity assessment of tetracycline using CAP [15]. The study provides insight into the safety of CAP-treated tetracycline by exploring the role of hydroxyl radicals in tetracycline degradation, the involvement of ozone in tetracycline removal, examining degradation pathways, and assessing biotoxicity. Sarangapani et al. employed CAP for the high-efficiency mitigation of two commonly used fluoroquinolone antibiotics, ofloxacin and ciprofloxacin, from water and meat effluents [16]. The results revealed that CAP successfully degrades these antibiotics and significantly reduces their activity. The authors noted that the advantages of CAP treatment include the oxidation or reduction of pollutants into biodegradable compounds without environmental risks. They concluded that this method can be an effective, eco-friendly, and economically promising technology for practical wastewater treatment applications [16].

In most countries, β-lactam antibiotics (e.g., penicillin) comprise 50–70% of the total antibiotics used in medicine [17], highlighting their frequent presence in wastewater. Several experimental studies have explored the degradation of β-lactam antibiotics in wastewater using CAP [18, 19]. For instance, Wielogorska et al. assessed the efficiency of plasma flow, a type of CAP device, in degrading β-lactam antibiotics [18]. They achieved optimal β-lactam degradation by optimizing plasma parameters such as voltage, gas composition, and exposure time. Nguyen et al. conducted experimental studies targeting ofloxacin and ciprofloxacin from the fluoroquinolone class, and amoxicillin from the β-lactam class, using CAP [19]. Their results indicated that a 15-minute treatment with 30 kV CAP led to nearly complete elimination of ciprofloxacin and over 72% degradation of both ofloxacin and amoxicillin.

Amoxicillin, a prominent member of the penicillin group within the β-lactam class, is widely used to prevent and treat bacterial infections due to its β-lactam rings [20]. The cleavage of these rings (e.g., by hydrolysis via β-lactamases) significantly reduces its activity [21]. Since amoxicillin is excreted largely unchanged (60–90%) into the environment due to the low metabolism in the human body [22, 23], it ranks among the most prevalent antibiotics found in wastewater, prompting numerous degradation studies. For instance, CAP has shown high efficiency in degrading amoxicillin under optimal conditions: pH 10.5, voltage of 36.5 kV, and a reaction time of 31 min, achieving 98.13% removal [24]. Li et al. investigated various factors influencing amoxicillin degradation by CAP treatment, including gas type (nitrogen, air, argon, and oxygen), discharge voltage, frequency, and treatment duration [25]. They found that nitrogen and air plasma treatments showed higher degradation efficiencies compared to argon and oxygen plasmas. Specifically, treatment for 7 min at 29 kV completely degraded all amoxicillin using nitrogen and air plasmas, whereas it took 10 min with argon and oxygen plasma. At 23 kV, nitrogen and air plasma achieved 100% degradation in 10 min, while argon and oxygen plasma achieved degradation efficiencies of 98% and 88%, respectively [25]. Note that the higher degradation efficiency of nitrogen and air plasma compared to argon and oxygen plasma is most likely attributed to the synergistic effects of reactive nitrogen species (RNS) and reactive oxygen species (ROS). In particular, beyond the direct reactions of RNS with amoxicillin, they can also produce secondary ROS such as OH radicals [26], which further degrade amoxicillin. Thus, increasing the concentration of these ROS (due to RNS) additionally enhances the efficacy of nitrogen and air plasmas over argon and oxygen plasmas.

Despite numerous studies on the degradation of antibiotics using CAP, the precise mechanisms through which CAP affects antibiotics, particularly amoxicillin, remain unclear. Computer modeling studies provide insights into atomic-level processes that are difficult to directly observe in experiments [27–29]. Therefore, this study employs reactive molecular dynamics (MD) simulations to investigate the underlying mechanisms of amoxicillin degradation by CAP, with a specific focus on the atomic-level interactions between amoxicillin and reactive oxygen and nitrogen species (RONS) generated by CAP.

Simulation Details

To study the interaction mechanisms between RONS (i.e., O, OH, HO_2, H_2O_2, O_3, NO, NO_2, NO_2^- and NO_3^-), and the amoxicillin molecule, we utilized reactive MD simulations based on the density functional tight-binding (DFTB) method [30]. To ensure accurate representation of interatomic interactions, we employed the "3ob-3-1" parameter set [31, 32], which was specifically developed for the DFTB3 method and optimized for organic and biological molecules composed of H, C, N, O, and S elements [33]. Given that amoxicillin contains exactly these elements, including sulfur in a chemically reactive environment, this parameter set offers the most suitable and accurate choice for our system. Alternative parameter sets, such as "mio," are designed for earlier DFTB2 models and lack reliable coverage for key bond types relevant to amoxicillin. Moreover, using non-optimal parameterizations could introduce significant inaccuracies in describing reactive events such as bond-breaking and formation. Thus, the "3ob-3-1" parameter set was selected to ensure both the robustness and physical reliability of the reactive DFTB-MD simulations performed in this study.

We focused on the non-consecutive impacts of each RONS to understand how each individual species influences the amoxicillin modification process and to compare our findings with experimental results. Moreover, we did not consider the aqueous layer surrounding the amoxicillin molecule due to the high computational cost associated with the DFTB method. Indeed, creating a water environment around the model system would require 867 water molecules, resulting in a simulation system comprising 2646 atoms (including the amoxicillin molecule and the impinging plasma particle (e.g., O atom)). Equilibrating (or thermalizing, see below) such a system would take approximately 2205 days, and each particle impact run (see below) would require 441 days. Considering that at least 100 runs are needed to obtain limited statistical data on the reaction mechanisms (see below), it is computationally not feasible to incorporate the surrounding water environment. Hence, we could not account for the water environment or its related pH parameters. Nonetheless, we believe that our approach minimally impacts the results. This belief is based on previous DFTB-MD results obtained under similar conditions with another model molecule (cellotriose), which largely aligned with mass spectrometry results [34]. Moreover, this approach appears sufficiently justified in this study, as the simulation results qualitatively agree with the experimental findings (see below).

Fig. 1 illustrates the model system representing the amoxicillin molecule used in our simulations. As is clear, amoxicillin belongs to the class of β-lactam antibiotics, characterized by the presence of a benzene group (shown in green) and a β-lactam ring (shown in blue)

The amoxicillin molecule, consisting of 44 atoms, was placed at the center of a cubic simulation box with dimensions of 30 Å × 30 Å × 30 Å. This box size is sufficiently large to

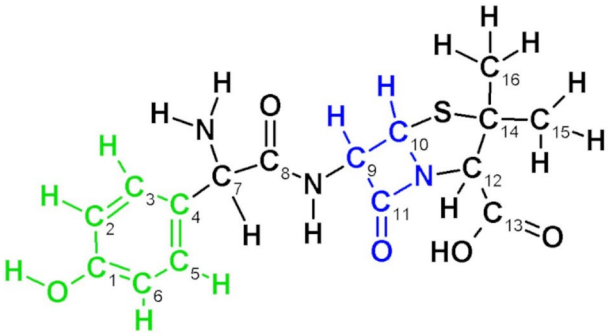

Fig. 1 Chemical structure of amoxicillin molecule. The benzene and the β-lactam rings are highlighted in green and blue, respectively. All C atoms are numbered in the structure, which might participate in the interaction of RONS with the molecule (see also Table S1 in the Supplementary Information)

place an impacting plasma particle around the molecule. Periodic boundary conditions were applied in all three directions (x, y, and z) of the simulation box. The energy of the model system (i.e., amoxicillin) was then minimized using the conjugate gradient algorithm. Following energy minimization, the amoxicillin model system underwent thermalization at room temperature (i.e., 300 K) for 750 ps in the canonical ensemble (i.e., NVT dynamics) using the Berendsen thermostat [35] with a coupling constant of 100 fs. This thermalization time was sufficient to obtain a well-equilibrated model system, see Figure S1 in the Supplementary Information (SI). In all simulations, i.e., during the thermalization, as well as during the particle impact simulations, we employed a time step of 0.5 fs.

To obtain statistically valid results for bond-breaking and bond-formation processes and to explore all possible degradation mechanisms of amoxicillin, we initially conducted 10 DFTB-MD runs for each RONS (i.e., O, OH, HO_2, H_2O_2, O_3, NO, NO_2, NO_2^- and NO_3^-). After analyzing the reaction mechanisms for each RONS, we performed an additional 90 DFTB-MD simulations for the O atom (bringing the total to 100 reactive MD runs) to gather more statistically significant data. At the beginning of each run, a single plasma particle (e.g., O atom) was randomly positioned at least 7 Å away from the molecule to prevent initial interactions, including long-distance interactions such as Coulomb and van der Waals forces, with amoxicillin. The velocity direction of the incident particle was chosen randomly, and its initial energy corresponded to room temperature. DFTB-MD simulations were then performed to investigate the interaction of each RONS with amoxicillin. Each simulation trajectory lasted 200 ps (equivalent to 4×10^5 MD iterations) in the canonical ensemble (NVT dynamics). We ensured that this simulation time was sufficient to observe chemical reactions such as bond breaking or formation with the amoxicillin molecule. In fact, most reactive events occurred within the first 50–150 ps of the trajectories. Extending the simulation duration beyond 200 ps did not lead to additional chemical reactions but only reduced the effective O-atom flux per unit time without providing further mechanistic insight. Thus, 200 ps was selected as an appropriate timescale for capturing the initial reaction steps following O-atom impact.

To estimate the corresponding O-atom flux in our simulations, we considered a 30 Å × 30 Å face of the simulation box and a single O-atom interaction over 200 ps. Based on the relation $Flux = N/(A \cdot t)$, where, $N = 1$ is the number of impinging O atoms, A is the surface area, and t is the simulation time, the resulting transient flux is approximately 5.6×10^{22} $cm^{-2} \cdot s^{-1}$. While this is higher than typical experimental CAP fluxes (~10^{14}–10^{19} $cm^{-2} \cdot s^{-1}$ [26, 36, 37]), it reflects a localized, short-duration event rather than continuous exposure. Since each

simulation involved only one O-atom collision and no cumulative impacts, the overall dose per molecule remains consistent with a low-exposure framework.

All simulations were conducted using the DFTB + package [38, 39].

Results and Discussion

As mentioned in the previous section, reactive DFTB-MD simulations were performed to investigate the interactions of RONS (i.e., O, OH, HO_2, H_2O_2, O_3, NO, NO_2, NO_2^- and NO_3^-) with amoxicillin. Particular attention was given to the processes of bond formation and bond breaking occurring within the amoxicillin molecule. The DFTB-MD results showed that, during the simulation time, HO_2, H_2O_2, O_3, NO, NO_2, NO_2^- and NO_3^- particles did not induce any bond-breaking or formation in the amoxicillin molecule; instead, they participated only in weak non-bonded interactions. Specifically, HO_2, H_2O_2, NO_2^- and NO_3^- species formed weak attractive interactions (see Figure S2 in the SI), while NO and NO_2 particles exhibited weak repulsive interactions with amoxicillin. Unlike these species, the O_3 molecule exhibited both weakly attractive and weakly repulsive effects, depending on which side (i.e., which oxygen atom) approached the amoxicillin molecule. Note that the fact that we did not observe chemical reactions (only weak non-bonded interactions) with the above-mentioned RONS during the simulation time used (i.e., 200 ps) do not imply that they do not react with amoxicillin at all. Certain reactions may still occur over longer timescales, as they likely involve larger energy barriers. Thus, in our simulations, we only observed the faster reactions. Specifically, bond-breaking and bond-formation processes were observed during the interactions of O atoms and OH radicals with amoxicillin. Interestingly, the reaction mechanisms of these two particles are very similar: both abstract an H atom from the amoxicillin molecule, leading to the formation of an OH radical (in the case of the O atom) or a water molecule (in the case of the OH radical). In other words, the O atom behaves like two OH radicals. Although O atoms and OH radicals displayed similar H-abstraction mechanisms in our simulations, they differ in their electronic structures, i.e., O atoms were modeled in the triplet (^{3}P) ground state, and OH radicals in the doublet ($^2\Pi$) state. These differences may contribute to subtle variations in reactivity or selectivity. However, since this study was focused on identifying statistically significant reaction mechanisms through reactive MD simulations, we did not perform explicit electronic structure analyses such as Mulliken charge population or spin-state comparisons. Such investigations, while potentially informative, fall beyond the scope of the present work. Therefore, only the interaction mechanisms of O atoms were investigated in the subsequent reactive MD simulations. Specifically, 100 interactions of randomly generated O atoms with amoxicillin were carried out to obtain some limited statistical data on these reaction mechanisms. It is worth noting that the research by Benedikt et al. demonstrated that plasma-generated O atoms can directly oxidize organic molecules in aqueous solutions without involving intermediate reactions, such as the dissociation of water molecules [40]. The study further highlighted that CAP is an efficient and effective source of reactive O atoms, eliminating the need for chemical precursors in the liquid phase. Therefore, we believe that the methodology used in this study (i.e., modeling the impact of O atoms on the amoxicillin molecule without a surrounding water environment) has minimal effect on the reliability of the MD results (see also the previous section).

Note that there are two types of impacts: consecutive and non-consecutive. In non-consecutive impacts, each MD run involves an intact antibiotic molecule, while in consecutive impacts, the O atom interacts with an antibiotic molecule that has already been modified in a previous MD run. In this study, we used non-consecutive impacts of O atoms on amoxicillin, which corresponds to a low dosage of CAP that was sufficient to degrade amoxicillin. The detailed results from the 100 DFTB-MD simulations with O atoms are listed in Table S1 of the SI. Here we highlight the key reaction mechanisms, in particular those that occurred most frequently, caused modifications of the benzene and β-lactam rings, and resulted in fragmentation of the amoxicillin structure. In general, our simulation results show that most of the possible reaction mechanisms between the O atom and the amoxicillin start with the abstraction of one or two H atoms (84% of the runs) in the molecule. Other reaction mechanisms result in the addition of the O atom to C (9% of the runs), S (4% of the runs) and N (2% of the runs) atoms in the structure. Analysis of the simulations also reveals that the most frequent outcomes are the formation of OH (63% of runs) and OOH (8% of runs) groups, liberation of H_2O (11% of runs), CO_2 (7% of runs), and CO (4% of runs), opening/breakage of the β-lactam ring (5% of runs), and shortening (2% of runs) and widening (2% of runs) of the benzene ring. Other observed events include the formation of ketone groups (6% of the runs) and epoxy groups (7% of the runs). Note that the combined contribution of these results exceeds 100%, since some simulations showed the simultaneous occurrence of the above reactions. In addition, reactions such as deamination have been reported in the literature [25]. However, this reaction was not observed in our study, likely due to the limited statistical sampling and the use of non-consecutive impacts conducted exclusively with O atoms.

As mentioned above, most of the reaction mechanisms start with H-abstraction, which can mainly lead to the formation of hydroxyl and/or hydroperoxide groups. Figure 2 shows the reaction mechanisms that result in the formation of the hydroperoxide group (reaction 1 in Table S1, see Fig. 2a) and the hydroxyl group (reaction 10 in Table S1, see Fig. 2b) in the

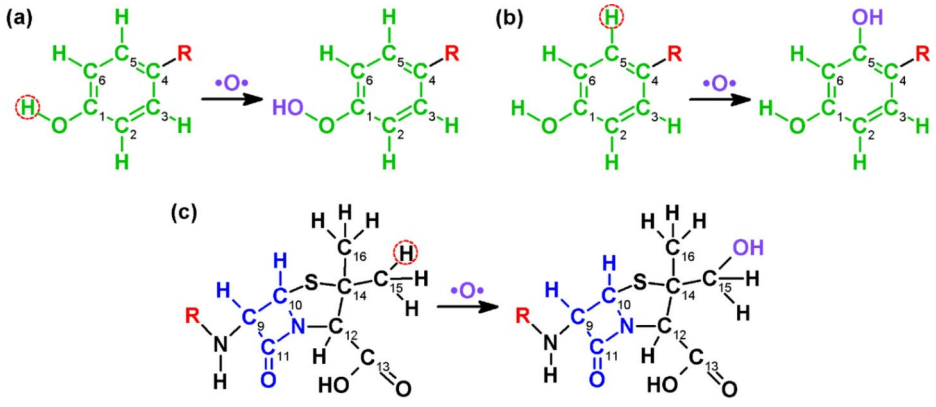

Fig. 2 Illustration of reaction mechanisms involving the O atom interaction with amoxicillin (cf. Figure 1 for the full chemical structure). Formation of a hydroperoxide group (**a**) and a hydroxyl group (**b**) in the benzene ring, as well as a hydroxyl group (**c**) in the methyl group of the structure. H atoms that are abstracted are shown within red dashed circles, while the newly formed hydroperoxide and hydroxyl groups are represented in purple

benzene ring, as well as the most frequently observed hydroxyl group formation mechanism (9% of the runs, see reaction 24 in Table S1) in the structure (Fig. 2c).

As is obvious, in all three cases, the O atom abstracts an H atom from the structure (indicated by red dashed circles), forming an OH radical and a radical site in the molecule (not depicted in the figure). Subsequently, this OH radical attaches to the radical site, resulting in the formation of a new OH group (shown in purple) in the structure, see Fig. 2. Similar OH group formation reactions were proposed in experimental studies [25, 41]. For example, in the research by Li et al., the proposed decomposition pathways begin with the formation of a new OH group and culminate in degradation [25].

Figure 3 illustrates the reaction mechanisms that lead to modifications in the benzene ring (i.e., shortening or widening, Fig. 3a, b) as well as fragmentation in the structure (Fig. 3c). In particular, as shown in Fig. 3a (reaction 4 in Table S1), the O atom abstracts H atoms from C_1O and C_6, resulting in the formation of a water molecule and radical sites in the structure (not shown in the figure). Subsequently, the benzene ring shortens to form a stable structure by breaking the C_1-C_2 bond (see light blue dashed line) and forming the C_1=O and C_2-C_6 bonds.

In Fig. 3b (reaction 33 in Table S1), H-abstraction is not observed; instead, the O atom binds to the C_1 and C_2 atoms, causing the breaking of the C_1-C_2 bond. This results in the widening of the benzene ring. In Fig. 3c (reaction 7 in Table S1), we observed the fragmentation of the amoxicillin molecule, which was also reported in [42] by photocatalytic oxidation. Specifically, H-abstraction occurs first at the C_1O site and then at the N atom (highlighted by red dashed circles), resulting in the formation of a water molecule, alterations in the benzene ring's bonding, and the creation of a C_8=N double bond. This process subsequently leads to the cleavage of the C_7-C_8 bond (indicated by the light blue dashed line), causing structural fragmentation (Fig. 3c). As a result, the amoxicillin molecule splits into two fragments with m/z=122 (Fig. 3c, left) and m/z=242 (Fig. 3c, right). The fragment with m/z=122 was also observed by Trovo et al. in their mass spectrometry experiments [43].

Incidents of the β-lactam ring opening [25, 41], which is crucial for the activity of β-lactam antibiotics, were also observed in our simulations. Since the β-lactam ring is a highly reactive component of β-lactam antibiotics, a decrease in antibiotic activity occurs

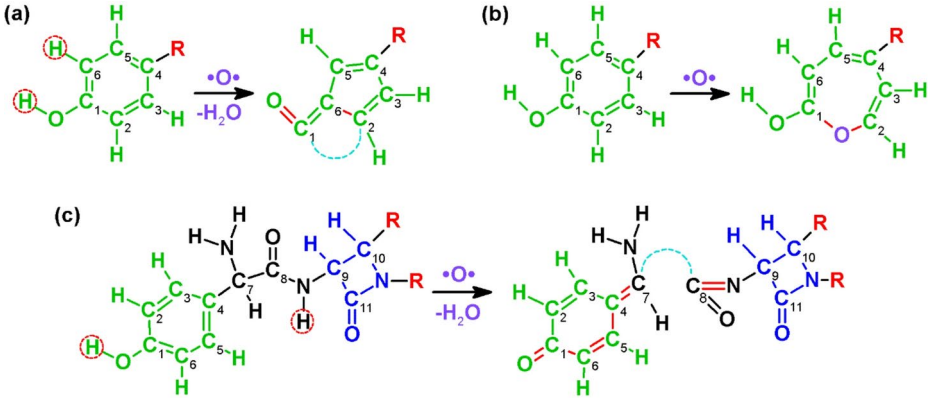

Fig. 3 Illustration of reaction mechanisms showing the shortening (**a**) and widening (**b**) of the benzene ring, as well as fragmentation of the structure (**c**). Red dashed circles indicate H-abstraction, light blue lines represent bond dissociation, and modified bonds are highlighted in red

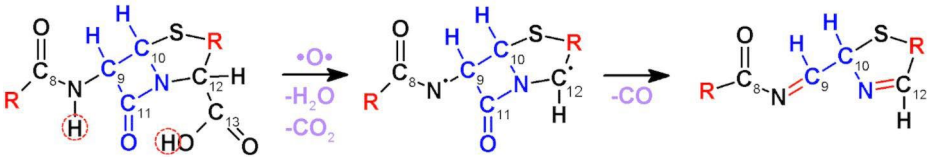

Fig. 4 Illustration of the reaction mechanism that leads to the opening/breakage of the β-lactam ring. Red dashed circles indicate H-abstraction, while modified bonds are highlighted in red

when this ring is opened [21, 44]. Figure 4 illustrates one of the reaction mechanisms observed in our simulations that leads to the opening or breakage of the β-lactam ring (reaction 21 in Table S1).

As is clear, the O atom abstracts H atoms from $C_{13}O$ and N (indicated by the red dashed circles), resulting in the formation of a water molecule and the cleavage of the C_{12}-C_{13} bond (not shown in the figure). This process forms a carbon dioxide molecule and radical sites in the structure (middle reaction path in Fig. 4). Subsequently, double bonds C_{12}=N and C_9=N are formed to stabilize the structure, leading to the dissociation of the C_9-C_{11} and C_{11}-N bonds. This process results in the release of CO, ultimately causing the opening or breakage of the β-lactam ring (as shown in the final structure in Fig. 4). The release of H_2O and CO_2 was also shown to contribute to the opening of the β-lactam ring in the decomposition pathways proposed by Magureanu et al. [41].

It should be noted that among the 100 O-atom impacts, β-lactam ring opening was observed in 5% of the trajectories. Although this represents a relatively low occurrence rate, the reaction was reproduced in multiple independent runs and followed a chemically consistent sequence of events, including H-abstraction, CO_2 release, and bond cleavage, supporting its mechanistic plausibility. DFTB, while computationally efficient for large-scale reactive MD simulations, exhibits typical energy errors in the range of 0.1–0.2 eV, and therefore, energy barriers and reaction frequencies should be interpreted qualitatively. More accurate energy estimates using static DFT or NEB-based calculations would be valuable for further quantifying these low-frequency processes. Nonetheless, the consistent observation of this degradation route, even as a rare event, aligns with experimental findings (see below) and reinforces its potential relevance in CAP-induced amoxicillin degradation.

As mentioned above, the OH group formation [25, 41], structural fragmentation [43], and β-lactam ring opening [25, 41] reactions were observed in experiments. Thus, our simulation results are qualitatively in line with experimental outcomes. These observations support the validity of our simulation approach, despite the absence of an explicit solvent environment. Although solvent effects, particularly localized hydrogen bonding, can influence reaction energetics, especially for H-abstraction pathways, our simulations were conducted in the gas phase due to methodological and computational limitations associated with reactive MD at the DFTB level. While implicit solvent models such as COSMO and Generalized Born exist, they are primarily developed for static calculations and are not yet fully validated for simulations involving frequent bond-breaking and formation events. Including even a few explicit water molecules would considerably increase the computational cost of statistically meaningful sampling. Nevertheless, as mentioned above, the degradation mechanisms identified in our study, including hydroxylation, β-lactam ring opening, and fragmentation, are qualitatively consistent with experimental results on amoxicillin degradation in aqueous CAP environments. This indicates that the primary reaction pathways are robust and

preserved even in the gas-phase approximation, providing mechanistic insights that remain relevant under more realistic conditions.

It should also be noted that the reaction mechanisms illustrated in Figs. 2, 3 and 4 were obtained from unbiased reactive MD simulations using the DFTB method. Since these mechanisms emerge dynamically along the simulation trajectories, transition states and energy barriers were not explicitly characterized. A full mapping of these processes, including the optimization of reactant, transition-state, and product geometries, as well as associated energy profiles, would require additional static quantum chemical calculations. While such an analysis lies beyond the scope of this MD-focused study, we consider it an important future direction and plan to investigate selected reaction channels in follow-up work.

It is also important to note that the current simulations were limited to single RONS species interacting independently with the amoxicillin molecule (i.e., non-consecutive single-particle impacts). This design was chosen to isolate and characterize the intrinsic reactivity of each species. However, experimental evidence suggests that synergistic effects of RONS can significantly enhance degradation efficiency. Capturing such cooperative or cascade-type behavior would require temporally or spatially coupled RONS simulations, which are computationally demanding and fall beyond the scope of this work. Nevertheless, understanding these synergistic effects remains an important direction for future research in plasma-assisted degradation chemistry.

Finally, it should be acknowledged that the present simulations do not account for radical chain reactions or degradation cascades involving pre-modified amoxicillin molecules. While such processes are likely to contribute significantly to overall degradation in real plasma environments (e.g., through OH radical formation from H_2O_2 or reactions with partially oxidized intermediates), they are not captured in our single-impact, low-dose simulation framework. As a result, the degradation rates observed in this study may therefore underestimate the cumulative degradation observed under prolonged CAP treatment.

Conclusions

In this study, the atomic-level interaction mechanisms of RONS with the antibiotic amoxicillin were investigated using reactive DFTB-MD simulations. The simulation results reveal that RONS such as HO_2, H_2O_2, NO_2^- and NO_3^- form weak attractive interactions with amoxicillin, while NO and NO_2 species exhibit weak repulsive interactions with the amoxicillin molecule. In contrast, the O_3 molecule demonstrates both weakly attractive and weakly repulsive effects, depending on which oxygen atom approaches the amoxicillin molecule. On the other hand, O atoms and OH radicals were found to induce bond breaking and bond formation in amoxicillin. Remarkably, the reaction mechanisms of these two species are very similar, with the O atom behaving as if it were two OH radicals. Therefore, the interaction mechanisms of O atoms were prioritized in subsequent reactive MD simulations.

Our findings demonstrate that non-consecutive impacts of O atoms, corresponding to low-dosage plasma, are sufficient to degrade amoxicillin. Specifically, our MD simulations reveal the formation of hydroxyl and hydroperoxide groups, the opening or breakage of the β-lactam ring, alterations in the benzene ring (shortening or widening), and structural fragmentation. Additionally, some reactions lead to the release of molecules such as water,

carbon dioxide, and carbon monoxide. Overall, our simulation results are qualitatively consistent with experimental findings.

Our study highlights the efficacy of short-term plasma exposure in breaking down β-lactam antibiotics, with amoxicillin serving as a representative example. Our findings emphasize the critical role of reactive species, such as O and OH radicals, in driving the degradation process. These insights advance our understanding of the atomic-level mechanisms underlying CAP-induced antibiotic degradation, indirectly facilitating the development of sustainable and cost-effective strategies for the removal of antibiotics from the environment using CAP technology.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11090-025-10567-y.

Acknowledgements O.R. acknowledges the financial support from the Institute of Ion-Plasma and Laser Technologies of the Academy of Sciences of Uzbekistan, where research was carried out using the basic funding allocated to the institute. The computational resources used in this work were partially provided by the MatthUA cluster of the PLASMANT research group at the University of Antwerp, Belgium.

Author Contributions Conceptualization: O.R. and M.Y.; methodology: O.R.; software: Q.Z. and N.M.; validation: O.R. and M.Y.; formal analysis: O.R., N.M. and Y.Z.; investigation: O.R.; resources: O.R. and M.Y.; data curation: A.B. and M.Y.; writing–original draft preparation: O.R. and M.Y.; writing–review and editing: Q.Z., N.M., Y.Z. and A.B.; visualization: O.R., N.M. and Y.Z.; supervision: M.Y. and A.B.; project administration: M.Y.; funding acquisition: M.Y. All authors have read and agreed to the published version of the manuscript.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval Not applicable.

Competing Interests The authors declare no competing interests.

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