

## Article

# Oxidative Degradation of Pharmaceutical Waste, Theophylline, from Natural Environment

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**Abstract:** The elimination of organic contaminants from natural resources is extremely important to ensure their (re-)usability. In this report, the degradation of a model pharmaceutical compound, theophylline, is compared between natural and laboratory-controlled environments. While the concentration of H<sub>2</sub>O<sub>2</sub> variably affected the degradation efficiency (approximately from 8 to 20 min for complete degradation) in the photo-irradiation experiments, the inorganic compounds (NaNO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and ZnSO<sub>4</sub>) present in the medium seemed to affect the degradation by scavenging hydroxyl radicals (•OH). The end-product studies using high-resolution mass spectrometry (HRMS) ruled out the involvement of secondary radicals in the degradation mechanism. The quantitative calculation with the help of authentic standards pointed out the predominant role of hydroxylation pathways, especially in the initial stages. Although a noticeable decline in the degradation efficiency was observed in river water samples (complete degradation after 25 min with an approximately 20% total organic carbon (TOC) removal), appreciable TOC removal (70%) was eventually achieved after prolonged irradiation (1 h) and in the presence of additional H<sub>2</sub>O<sub>2</sub> (5 times), revealing the potential of our technique. The results furnished in this report could be considered as a preliminary step for the construction of •OH-based wastewater treatment methodologies for the remediation of toxic pollutants from the real environment.

**Keywords:** advanced oxidation processes; emerging organic pollutants; hydroxyl radical; mass spectrometry; mineralization; oxidative degradation; pharmaceutical waste; theophylline; total organic carbon



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## 1. Introduction

Pharmaceutically active compounds become a source of concern when they are present in the environment [1–5]. These compounds, categorized as contaminants of emerging concern (CECs), are relatively new to the environment and may not be included in the regular monitoring processes of most environmental regulatory authorities. These kinds of bio-active molecules present in a given environmental resource (e.g., water) might be transported to others (e.g., air, soil), concentrated and metabolized into equally or even more toxic substances, and eventually becoming a threat to the well-being of living species [4,6–12]. This fact necessitates the development of adequate methodologies to detoxify CECs to avoid unprecedented impacts on the targeted species [13–18].

Among the degradation methods, the processes utilizing the non-selective oxidizing power of the hydroxyl radical (•OH), generally termed as advanced oxidation processes (AOPs), have been widely experimented and found effective against a wide variety of

CECs [19–23]. Dissolved organic matter (DOM) and many inorganic ions (essential constituents of natural water streams) are capable to act as  $\bullet\text{OH}$  scavengers [24–27]. For example, inorganic anions such as  $\text{Cl}^-$  and  $\text{HCO}_3^-$  convert  $\bullet\text{OH}$  into less reactive radicals and reduce the concentration of the primary oxidizing species (i.e.,  $\bullet\text{OH}$ ) available in the medium [24,28]. This fact significantly reduces the efficiency of most AOP-based degradation strategies in real water to that of the laboratory-scale model experiments, as the concentration of available  $\bullet\text{OH}$  within the reaction medium is extremely important [25,26,29]. Another important concern is the likely involvement of secondary radical species, such as  $\text{Cl}\bullet/\text{Cl}_2\bullet^-$ , generated in the case of  $\text{Cl}^-$ , resulting in chlorinated transformation products that are known carcinogens [30–32]. Moreover, due to the formation of degradation products with similar structural features, AOPs, in many cases (e.g., acetaminophen, carbamazepine, diclofenac, sulfamethoxazole, trimethoprim, etc.), may not remove the complete toxicity of the treated sample [20,33–37]. Comprehensive studies should, therefore, be conducted in a more nature-like environment (i.e., either in the real environment itself or a laboratory-controlled reaction medium offering the presence of specific environmental species such inorganic ions, dissolved organic matter, etc.) to evaluate the efficiency and suitability of AOP-based wastewater remediation methodologies prior to their real-world implementations.

Theophylline (trade name: Deriphyllin), a well-known anti-oxidant and widely used methylxanthine drug primarily used against asthma and other respiratory diseases, was selected as the model pollutant for this study [38–41]. Extensive medical use, as well as its occurrence in a number of regularly used natural products, such as cocoa beans (~3 mg/g), green tea (~0.5 g/kg), coffee and chocolate, increases the entrance of this molecule into nature [38–40]. A number of studies reporting the existence of theophylline in the aquatic environment are already available, which has eventually led to the categorization of this molecule into the group of CECs [42,43]. It is worth mentioning that the over-consumption (>20  $\mu\text{g/mL}$ ) of this bio-active molecule might result in serious health issues, such as cardiac arrest, arrhythmias, hypotension, etc. [38–40,44].

The degradation of this molecule by advanced (i.e., AOPs) [45–47] and other oxidation methods (e.g., ferrate (VI) oxidation) [48] has already been reported. An in-depth  $\bullet\text{OH}$ -mediated degradation mechanism of this molecule, along with the identities of short-lived radical species as well as stable transformation products, is also available [47]. The above-mentioned studies, which have mostly been conducted in an extremely controlled laboratory environment, may not be directly comparable to real environmental conditions, as the composition of the latter might be much more complex. In order to narrow down this knowledge gap, we devoted our main focus in this study on investigating the influence of some environmental parameters (mainly, the presence of a number of under-explored inorganic ions) on the degradation of this molecule within an oxidative environment provided by a model AOP ( $\text{H}_2\text{O}_2$ /UV photolysis). Moreover, the applicability of this protocol in a real environment was also evaluated by conducting degradation experiments on representative river water samples. It is expected that the development of these kind of protocols could be very helpful to detoxify contaminated aquatic environments and ultimately prevent the transportation of theophylline and other structurally comparable CECs to cause more environmental (such as air, soil pollution) threat.

## 2. Materials and Methods

The high-quality standard material of the parent pharmaceutical, Theophylline (1,3-dimethyl 1,3,7-dihydro-1H-purine-2,6-dione; CAS No. 58-55-9; Quality: anhydrous;  $\geq 99\%$ ; powder), and its primary degradation product, 1, 3-Dimethyluric acid (DMU; CAS No. 944-73-0; Quality: certified reference material), purchased from Sigma Aldrich (St. Louis, MO, USA), were directly used in this study. The methanol ( $\text{CH}_3\text{OH}$ ; CAS No. 67-56-1) and formic acid ( $\text{HCOOH}$ ; CAS No. 64-18-6) used in this study were of LC-MS grade (both from Sigma Aldrich). High-quality hydrogen peroxide ( $\text{H}_2\text{O}_2$ ; CAS No. 7722-84-1; Quality: 30%, for analysis), provided by Merck Millipore, was used in the photolysis experiments. All the

other chemicals, such as sodium nitrate ( $\text{NaNO}_3$ ), monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ), zinc sulfate ( $\text{ZnSO}_4$ ), etc., used in this study were of the highest available purities. The experimental solutions throughout this study were prepared using high-purity water collected from a Millipore Milli-Q system.

Direct and hydrogen peroxide UV irradiation experiments were carried out on a photo-reactor supplied by Scientific Aids & Instruments Corporation (SAIC; Chennai, India). The reactor utilized a 125 W medium-pressure deuterium lamp kept in a quartz vessel as the light source. All the experiments were carried out at near-natural pH (~6) and room temperature.

The degradation of theophylline after the steady-state photolysis experiments was monitored by high-performance liquid chromatography (HPLC) analysis using a Shimadzu LC-20AD Prominence Liquid Chromatographic system coupled with a Diode Array Detector (at 270 nm). An isocratic elution of methanol and water (25:75) at a fixed flow rate of  $0.8 \text{ mL min}^{-1}$ , against an enable C18G column ( $250 \text{ mm} \times 4.6 \text{ mm} \times 5 \mu\text{m}$ ), was used. The concentrations of selected inorganic ions during the degradation studies were monitored on a Dionex ICS 1100 Ion chromatography (IC) system.

The degradation products of theophylline generated after the photolysis experiments were analyzed on an ultra-performance liquid chromatography (UPLC; Waters Acquity H class) system connected to a Quadrupole–Time-of-Flight (Q-TOF) high-resolution mass spectrometer (HRMS; Waters Xevo G2; together represented as LC-Q-TOF MS in this report). The UPLC used a BEH C18 column ( $50 \text{ mm} \times 2.1 \text{ mm} \times 1.7 \mu\text{m}$ ) to separate the degradation products before converting them into their protonated/deprotonated ions on the electrospray ionization (ESI) source for HRMS analysis. A gradient elution of methanol and water at a fixed flow rate of  $0.3 \text{ mL min}^{-1}$  was used as the mobile phase. All the spectra were recorded in both positive and negative ionization modes in a mass range of 50–600 Da.

The inorganic ions in river water were also characterized using the same IC analyses. The total organic carbon (TOC) of the theophylline samples in river water, before and after photo-irradiation, was measured using a HiPer TOC (Thermo Scientific, Waltham, MA, USA) using the UV-persulfate method. The UV-persulfate method oxidized the organic content of the sample (1.0 mL) into water and carbon dioxide. The above-generated carbon dioxide was, then, detected within the TOC analyzer using an infrared detector.

### 3. Results and Discussion

#### 3.1. Degradation of Theophylline by $\text{H}_2\text{O}_2$ /UV Photolysis

Organic pollutants introduced into the natural environment have been reported to undergo biological, chemical and photochemical degradation [2,49]. The reported stability of many CECs against biological and chemical degradation increases the relevance of developing photochemical (especially free-radical-based AOPs) methods. The reported concentration of most pharmaceutical compounds in natural water is between hundreds of  $\text{ng L}^{-1}$  and few  $\mu\text{g L}^{-1}$  [1,42,43,50]. However, to have a workable sensitivity for our HPLC system, the initial concentration of theophylline in the present study was fixed as  $1 \times 10^{-5} \text{ mol dm}^{-3}$  ( $\sim 1800 \mu\text{g L}^{-1}$ ). Although concentration-dependent changes may have influenced the degradation of theophylline in the present case, it was expected that the behavior of this molecule, including the reaction mechanism, in an oxidative environment was not going to be too different. The significant stability of theophylline towards direct photo-degradation has already been reported [47]. On the other hand, the photo-degradation profile of theophylline recorded in the presence of a series of  $\text{H}_2\text{O}_2$  concentrations exhibited noticeable degradation (Figure 1).

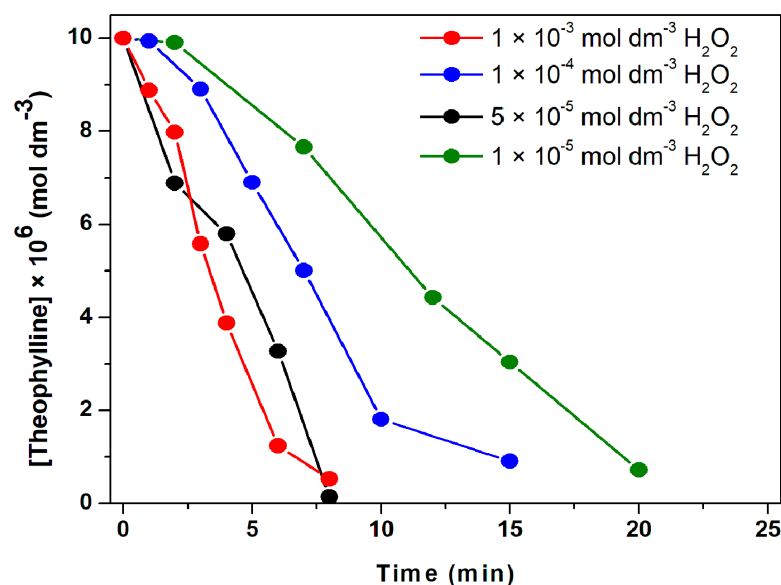
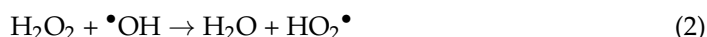


**Figure 1.** Photo-degradation profile of theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) in the presence of (red)  $1 \times 10^{-3} \text{ mol dm}^{-3}$ , (blue)  $1 \times 10^{-4} \text{ mol dm}^{-3}$ , (black)  $5 \times 10^{-5} \text{ mol dm}^{-3}$  and (green)  $1 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$ .

Upon photo-irradiation, the homolysis of  $\text{H}_2\text{O}_2$  produces  $\bullet\text{OH}$  in the medium according to reaction 1 [47,51,52]. Furthermore, it is well-understood that an excess amount of this reagent in the reaction environment may lead to a reduced degradation efficiency by an  $\bullet\text{OH}$  scavenging mechanism, as described in Reaction (2) [53,54]. It is, thus, important to optimize the most efficient concentration of  $\text{H}_2\text{O}_2$  to achieve the highest outputs of this process. Considering the extremely high oxidation potential of  $\bullet\text{OH}$  as well as its fast reaction kinetics ( $k_2 > 8 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ) reported towards the parent compound, it is reasonable to exclude the contributions from the direct oxidation of theophylline by  $\text{H}_2\text{O}_2$  and other secondary radical species from the present degradation profile [47]. Hence, the entire degradation of theophylline observed in our steady-state experiments was assigned exclusively to the reaction of  $\bullet\text{OH}$  to that molecule.



Among the four different tested concentrations of  $\text{H}_2\text{O}_2$ , the one with an initial concentration of  $5 \times 10^{-5} \text{ mol dm}^{-3}$  demonstrated the most efficient degradation capabilities toward the parent compound and, thus, was used for the rest of the studies (Figure 1). Further, the significant reduction in the degradation efficiency observed in the case of higher doses of  $\text{H}_2\text{O}_2$  was attributed to the conversion of highly reactive  $\bullet\text{OH}$  into less reactive  $\text{HO}_2\bullet$  (Reaction (2)) [53,54].

### 3.2. Effect of Inorganic Ions

The presence of inorganic anions in the natural environment is well-established [25,26]. Therefore, an idea about the effect of various inorganic ions on the degradation mechanism is very much necessary for the development of an efficient and cost-effective methodology to eliminate organic pollutants from that environment. Inorganic anions such as  $\text{Cl}^-$  and  $\text{HCO}_3^-$  have been reported to interact with  $\bullet\text{OH}$ , and their roles in the degradation efficiency have been thoroughly studied [24,28]. On the other hand, despite their widespread existence in the natural environment, detailed investigations on the role of ions such as sulfate ( $\text{SO}_4^{2-}$ ), nitrate ( $\text{NO}_3^-$ ) and dihydrogen phosphate ( $\text{H}_2\text{PO}_4^-$ ) are very limited [24,28,29]. Considering this fact, the effects of the above-mentioned anions on the degradation of theophylline were considered in this study. All the three selected

anions can be widely detected in natural water streams, as they were in the river water samples considered for this study (please see Section 3.4). The interference of these ions with  $\bullet\text{OH}$  generation/reaction chemistry was very much expected. It is worth noting that the concentrations of these ions taken in this study were very close to the reported values of each of them from different water sources [24–26].

### 3.2.1. Effect of $\text{NO}_3^-$

The effect of  $\text{NO}_3^-$ , an important contaminant affecting the quality of water bodies as a byproduct of extensive fertilizer use [55], on degradation was initially considered. The degradation profile of theophylline obtained in the presence of  $\text{NaNO}_3$  showed a significant reduction in the degradation efficiency (Figure 2). Moreover, this reduction can be directly correlated with the concentration of the added ion. For example, in the case of  $6 \times 10^{-4} \text{ mol dm}^{-3} \text{ NaNO}_3$ , an approximately 20% reduction was observed. However, upon increasing this concentration to  $1 \times 10^{-3} \text{ mol dm}^{-3}$ , a significant (~60%) reduction in the degradation efficiency was observed. Enhanced  $\bullet\text{OH}$  scavenging by inorganic ions at higher concentrations is a likely reason for this observation.

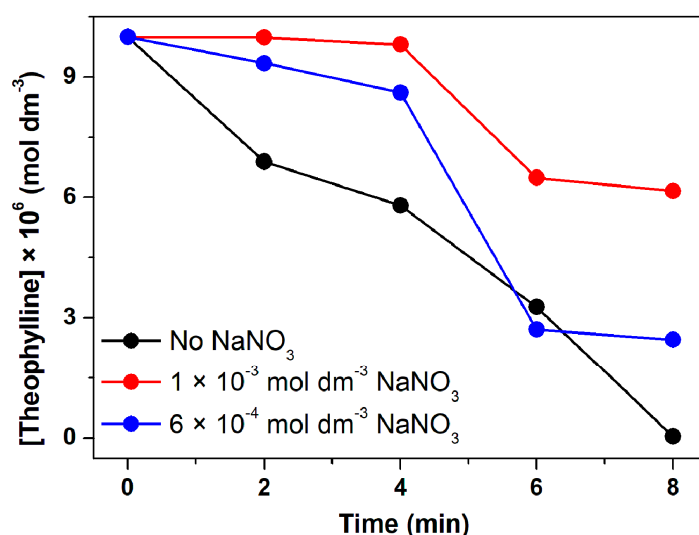


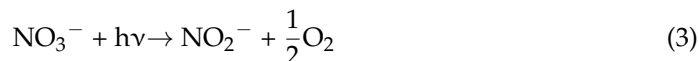
**Figure 2.** Photo-degradation profile of theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) in the presence of (black)  $5 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$  and in the presence of (red)  $1 \times 10^{-3} \text{ mol dm}^{-3}$  and (blue)  $6 \times 10^{-4} \text{ mol dm}^{-3}$  sodium nitrate.

It is also clear from Figure 2 that the adverse effect of  $\text{NO}_3^-$  on the degradation of theophylline was more pronounced on short timescales (up to 4 min), even in the case of lower concentrations ( $6 \times 10^{-4} \text{ mol dm}^{-3}$ ) of that ion. The degradation efficiency on longer timescales seemed to be less affected. These behaviors suggest the fact that the reaction of  $\bullet\text{OH}$  on short timescales was mainly limited to the inorganic ions (that is,  $\text{NaNO}_3$ ). This assumption is very well supported by the markedly higher concentration (from 5 to 20 times) of  $\text{NO}_3^-$  than that of theophylline in the present reaction environment. On the other hand, the inert nature of alkali metal ions towards  $\bullet\text{OH}$  led us to exclude the contribution of the sodium ion from the declined reaction kinetics of that radical with theophylline observed in the present case.

By considering the above-mentioned facts, it is proposed that the observed reduction in the efficiency of the  $\bullet\text{OH}$ -induced degradation of theophylline is most likely due to a reduced availability of the primary oxidizing species,  $\bullet\text{OH}$ , due to the following two factors:

1. The direct photolysis of  $\text{NO}_3^-$ , which involves a series of reactions eventually resulting in the formation of the nitrite ion ( $\text{NO}_2^-$ ) and molecular oxygen ( $\text{O}_2$ ) [24], which reduces  $\bullet\text{OH}$  generation in the medium by absorbing a fraction of UV radiation;

- The reaction of  $\bullet\text{OH}$  with the peroxyxynitrite anion ( $\text{ONOO}^-$ ), a reactive photo-product generated from the excited  $\text{NO}_3^-$  possessing a nearly diffusion-controlled bimolecular rate constant ( $k_2 = 5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ) with  $\bullet\text{OH}$  (Reaction (4)), leading to the conversion of that radical into less reactive  $\text{ONOO}\bullet$  [24].



Although the reactivity of secondary reactive species is expected to be lower than that of  $\bullet\text{OH}$ , the significant oxidation redox potential of some species, such as  $\text{ONOO}\bullet$  ( $E_{1/2}^{\text{red}} = 2.50 \text{ V}$ ) [45], induces an appreciable degradation of the parent molecule on longer timescales. This fact justifies the observed behavior in the present case. A recent study investigating the effect of this ion on the persulfate-based degradation of theophylline also reported these kinds of compensations by secondary radical species [45]. It is worth mentioning that the above-mentioned persulfate-based techniques utilize a more specific radical species, the sulfate radical anion ( $\text{SO}_4^{\bullet-}$ ), as the primary oxidant. A direct comparison between the degradation efficiencies of these methods, i.e., the above-mentioned persulfate method and the  $\bullet\text{OH}$ -based AOP utilized in the present case, may not be very reliable.

### 3.2.2. Effect of $\text{H}_2\text{PO}_4^-$

The presence of  $\text{KH}_2\text{PO}_4$  ( $1 \times 10^{-3} \text{ mol dm}^{-3}$ ) did not lead to significant differences in the degradation efficiency except on the initial timescales (Figure 3). For example, in the presence of  $1 \times 10^{-3} \text{ mol dm}^{-3} \text{ KH}_2\text{PO}_4$ , an approximately 30% decline in the degradation efficiency was observed during an irradiation time of 2 min. However, further irradiation resulted in a degradation efficiency nearly comparable to the one obtained in the absence of  $\text{KH}_2\text{PO}_4$ . Although the rate constants of the  $\bullet\text{OH}$  reaction with phosphate (both hydro- and dihydro-phosphate) ions were very low ( $10^4 - 10^5 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ ), it is very likely that this species in higher concentrations might interfere with the primary degradation process [56,57]. The slight reduction in the degradation efficiency observed especially on short timescales was, therefore, very much expected. Further studies on the effect of this species were, however, excluded, considering its negligible effect upon increasing the reaction timescale even at a reasonably higher concentration (Figure 3).

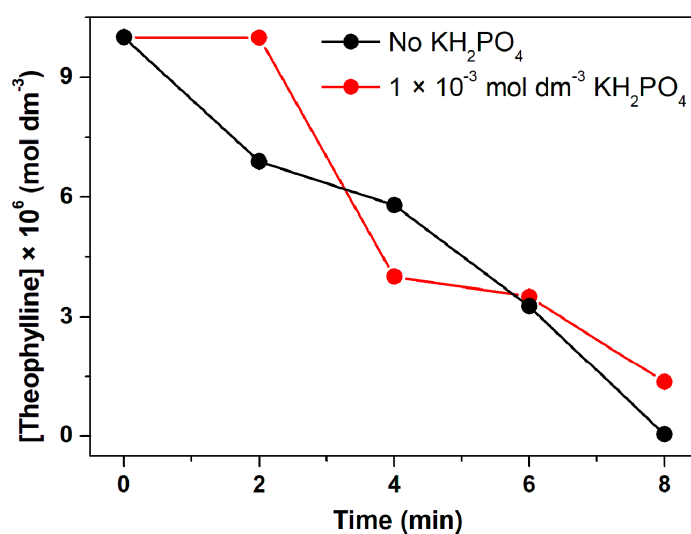


**Figure 3.** Photo-degradation profile of theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) in the presence of (black)  $5 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$  and in the presence of (red)  $1 \times 10^{-3} \text{ mol dm}^{-3}$  potassium dihydrogen phosphate.

### 3.2.3. Effect of $\text{SO}_4^{2-}$

The presence of sulfate ions in the reaction environment might result in the generation of a more selective oxidizing species,  $\text{SO}_4^{\bullet-}$  [28,29]. Despite the comparable oxidation potentials of this radical with  $\bullet\text{OH}$ , the specificity of the reaction mechanism (mainly, electron and hydrogen abstraction pathways) may restrict the applicability of  $\text{SO}_4^{\bullet-}$  with some molecules. Being non-selective in oxidation,  $\bullet\text{OH}$  is virtually efficient against any organic contaminant, as demonstrated by many researchers in the case of a vast variety of molecules [19,20,22,52,58]. Although the effect of  $\text{SO}_4^{2-}$  is compound-specific, an investigation on its effect towards  $\bullet\text{OH}$  would be very interesting. In order to study the effect of  $\text{SO}_4^{2-}$  in the present case, photo-irradiation experiments in the presence of  $\text{ZnSO}_4$  were carried out. The results as depicted in Figure 4 clearly reveal a large decline (of more than 55% over an irradiation time of 8 min) in the degradation efficiency even in the presence of a significantly smaller concentration of  $\text{ZnSO}_4$  ( $3.5 \times 10^{-4} \text{ mol dm}^{-3}$ ).

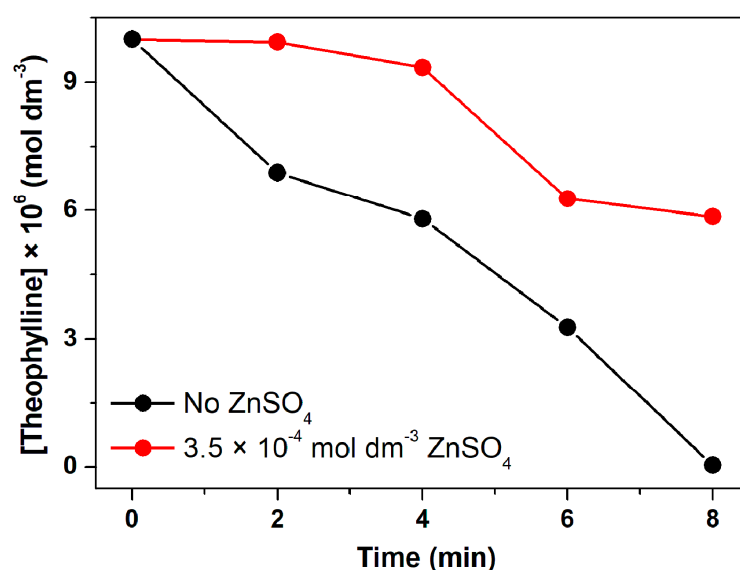
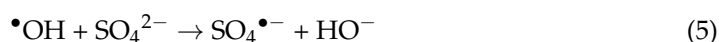


**Figure 4.** Photo-degradation profile of theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) in the presence of (black)  $5 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$  and in the presence of (red)  $3.5 \times 10^{-4} \text{ mol dm}^{-3}$  zinc sulfate.

The property of the variable oxidation state, generally exhibited by most transition elements, is not observed in the case of Zn due to the filled d orbital [28]. The hydroxyl radical scavenging mechanism exhibited by many transition metals, represented by the general mechanism  $\text{M}^{n+} + \bullet\text{OH} \rightarrow \text{M}^{(n+1)+} + \text{HO}^-$ , is very unlikely in the case of Zn [28]. The only remaining possibility to justify the above-observed decline in the degradation efficiency is the scavenging of  $\bullet\text{OH}$  by  $\text{SO}_4^{2-}$ . Thus, the reduction in the degradation efficiency as clearly seen in Figure 4 was assigned to the conversion of the non-selective  $\bullet\text{OH}$  into a more selective  $\text{SO}_4^{\bullet-}$  according to reaction 5.



### 3.3. Product Analysis by LC-Q-TOF MS

The identification of the transformation products generated upon the reaction of  $\bullet\text{OH}$  with parent molecules has effectively been utilized in many previous reports to evaluate the suitability of AOPs [37,58–63]. This argument is largely supported by the fact that the transformation products of many organic pollutants generated during the event of AOPs are reported as more toxic and persistent than their respective parent molecules [20,33–36,47,49]. An in-depth mechanistic aspect, including information on the reactive intermediates and stable transformation products, of the  $\bullet\text{OH}$ -induced degradation of theophylline has already been reported [47]. The possibility to generate secondary

reactive species in the case of photolysis experiments conducted in the presence of inorganic ions is mentioned in Section 3.2. As a result, the involvement of secondary reactive species in the degradation mechanism is very much expected. In order to account for the role of these species, a comparative study on the transformation-product profile might be very useful. LC-Q-TOF MS analyses were carried out on a number of theophylline samples after  $\text{H}_2\text{O}_2$ /UV photolysis, and the experiments were conducted in the presence of individual inorganic ions (i.e.,  $\text{NaNO}_3$ ,  $\text{KH}_2\text{PO}_4$  and  $\text{ZnSO}_4$ ). These results were compared with the transformation-product profile of theophylline taken from our previous report [47].

The initial concentration of the parent compound was taken as  $1 \times 10^{-4} \text{ mol dm}^{-3}$  for these experiments to ensure the detection of each and every transformation product generated in the reaction medium, including the ones in minor concentrations. Additionally, to avoid the loss of initially formed transformation products by the subsequent  $\bullet\text{OH}$  attack as much as possible, we selected samples with at least 60% of theophylline left for LC-Q-TOF MS analyses. Very interestingly, the results reveal that the transformation-product profiles obtained in the presence of all the three inorganic ions were more or less similar to those reported in the absence of any of them [47]. The only observed noticeable difference was the presence of intense  $m/z$  values corresponding to  $[\text{M} + \text{Na}]^+$  in the case of photo-irradiation in the presence of  $\text{NaNO}_3$ . This observation was very much anticipated, as sodium adduct formation is very common in positive ion mass spectrometry. From this result, it is understandable that the role of inorganic ions in the present case was limited to the scavenging of  $\bullet\text{OH}$  only. This fact helped us to rule out the direct contribution of various secondary radical species on the degradation of theophylline. This conclusion is supported by the expected low reactivity of secondary radicals (except  $\text{SO}_4^{\bullet-}$ ), with organic molecules, in comparison with the very high oxidation power of  $\bullet\text{OH}$  [64]. As a result, the possibilities of secondary radical reactions on theophylline are very minimum as long as the amount of  $\bullet\text{OH}$  generated is sufficient. Similar reports revealing the insignificant role of many inorganic ions have previously been reported in the case of ultrasound-based AOPs [65,66]. The proposed chemical structures of the identified transformation products, which include a number of previously reported microbial metabolites of theophylline [67] such as xanthine and uric acid derivatives, are available in Section 3.5. It is worth mentioning that, due to the limitations associated with the exact structural identification, such as the lack of authentic standards, limited sample size, etc., some of the identified masses are represented with tentative structures (e.g., two isomeric structures are utilized to represent G). The MS and MS/MS spectra of most of the transformation products were reported in our earlier article [47].

### 3.4. Degradation of Theophylline in Natural Environment

The efficiency of  $\bullet\text{OH}$  against the destruction of many organic pollutants was significantly reduced in the real environment upon comparing with the laboratory-scale experiments conducted in model water. Comprehensive photo-irradiation experiments in real water samples (river water) were conducted, and the results were compared with the ones obtained in model water to evaluate the real efficiency of this methodology. River water, collected from a local river, was initially analyzed for various water quality parameters. The results are shown in Table 1.

The degradation rate of theophylline was reduced considerably in the case of river water (Figure 5). For example, over an irradiation time of 8 min, more than 99% of the parent compound degraded in the case of model water. On the other hand, the percentage of theophylline degradation was reduced to ~34% in river water.

**Table 1.** Characteristics of river water.

| Parameter                 |           | Value |
|---------------------------|-----------|-------|
| pH                        |           | 6.75  |
| TOC (mg/L)                |           | 3.14  |
| $\lambda_{254\text{ nm}}$ |           | 0.066 |
| [Anions] in mg/L          | Chloride  | 31.96 |
|                           | Bromide   | 0.04  |
|                           | Nitrate   | 0.88  |
|                           | Phosphate | 0.83  |
|                           | Sulfate   | 13.86 |
| [Cations] in mg/L         | Sodium    | 15.44 |
|                           | Potassium | 3.66  |
|                           | Magnesium | 3.22  |
|                           | Calcium   | 7.91  |

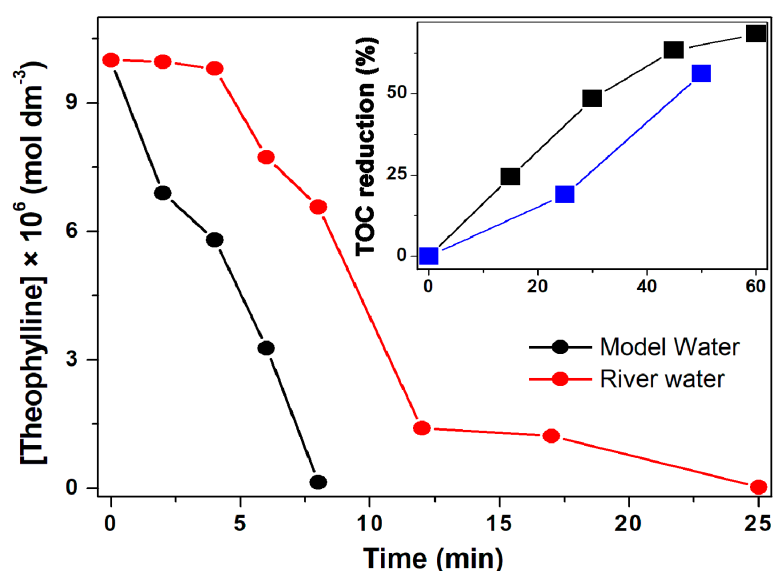


**Figure 5.** Photo-degradation profile of theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) in the presence of (black)  $5 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$  in model water and (red) in river water. (Inset) TOC reduction in the presence of (blue)  $5 \times 10^{-5} \text{ mol dm}^{-3}$  and (black)  $1 \times 10^{-4} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$ .

The above-mentioned reduction in the degradation efficiency of theophylline is most likely due to the scavenging of the primary oxidant,  $\bullet\text{OH}$ , by inorganic ions and dissolved organic matter (DOM) present in the river water. In order to assess the feasibility of this protocol in the context of real water purification purposes, the removal of TOC from river water containing theophylline ( $[\text{TOC}]_0 = 3.9 \text{ mg/L}$ ) as a function of irradiation time was also investigated (Figure 5). About 55% of the initial TOC of the theophylline ( $1 \times 10^{-5} \text{ mol dm}^{-3}$ ) solution was only reduced after 50 min of photo-irradiation in the presence of  $5 \times 10^{-5} \text{ mol dm}^{-3} \text{ H}_2\text{O}_2$ . However, a five-time increase in the  $\text{H}_2\text{O}_2$  concentration led to a TOC reduction of about 70%, clearly revealing the limited availability of oxidizing species in the former case. Since the concentration of  $\text{H}_2\text{O}_2$  used in the present study was very small, a very low yield of  $\bullet\text{OH}$  is expected on longer timescales. Another important factor is the presence of other substrates such as several inorganic ions and DOM in water that are capable to scavenge  $\bullet\text{OH}$  (Table 1). Higher concentrations of  $\text{H}_2\text{O}_2$  significantly increased the amount of available  $\bullet\text{OH}$  and enhanced TOC reduction. Thus, it

is very reasonable to expect a significant elimination of TOC by further increasing  $\text{H}_2\text{O}_2$  concentration. On the other hand, the already achieved reduction (nearly 70%) in TOC was capable of significantly reducing the toxicity of polluted water, making it available for a possible water use/reuse application. Hence, experiments in the presence of environmentally unrealistic higher concentrations of  $\text{H}_2\text{O}_2$  were not conducted.

### 3.5. Reaction Mechanism

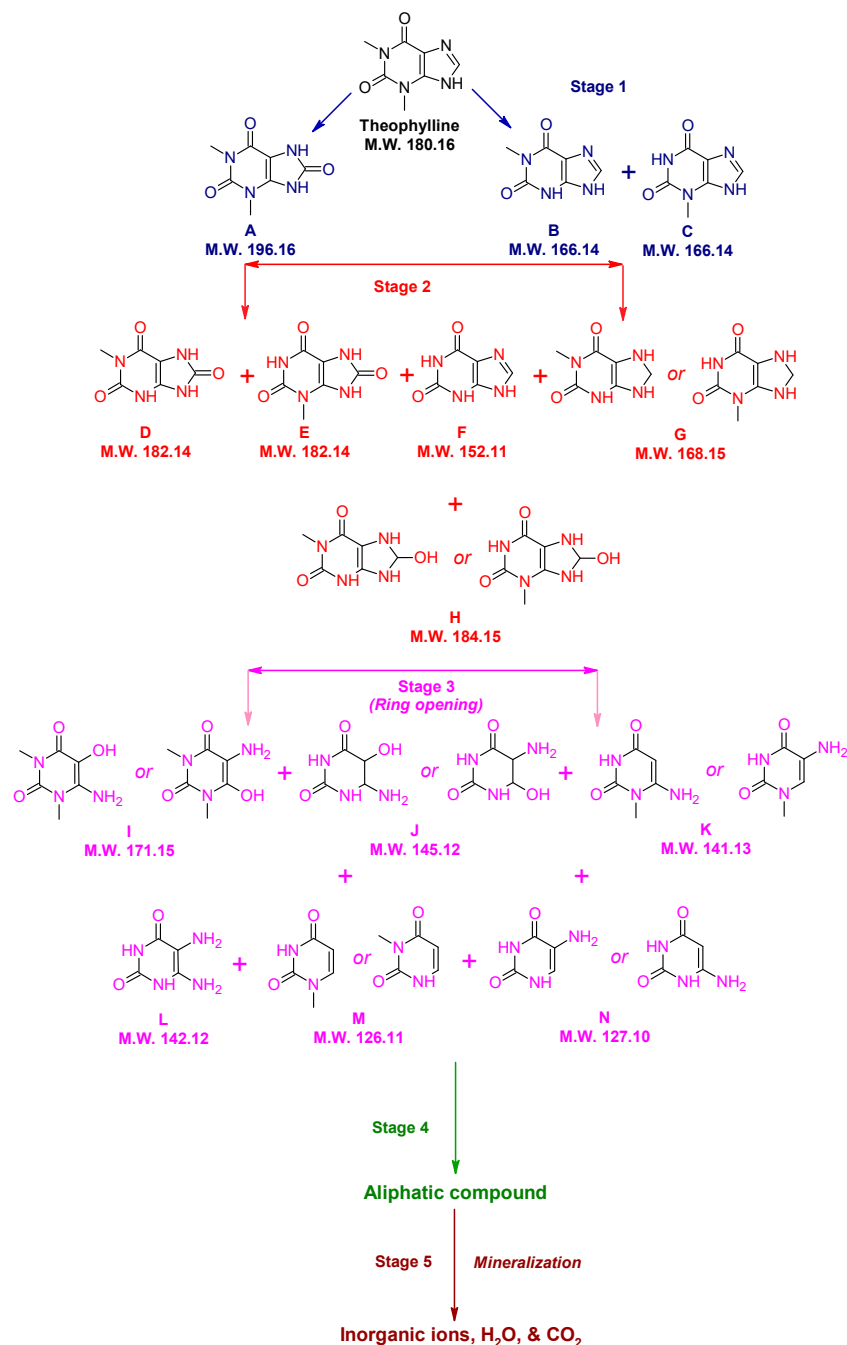
As mentioned above, the identities of the degradation products generated after the  $\text{H}_2\text{O}_2$ /UV photolysis experiments in the presence of various inorganic ions were more or less the same as those reported in the absence of any of them (please see Section 3.3). This fact clearly reveals the negligible transformation of theophylline induced by inorganic ions (either directly or via the reaction through secondary radicals). A similar behavior was also expected in the case of the  $\text{H}_2\text{O}_2$ /UV photolysis experiments conducted in river water. Based on the transformation-product profile (from LC-Q-TOF MS analyses as well as our earlier report [47]) and TOC data, the degradation mechanism leading to a nearly complete mineralization of theophylline in river water was categorized into five stages (Scheme 1).

Stage 1 (Scheme 1) explains the initial transformations of theophylline in its reaction with  $\bullet\text{OH}$ . They involved the hydroxylation (at  $\text{C}_8$ ) and demethylation (at  $\text{N}_1$  and  $\text{N}_3$ ) reactions of the parent compound, leading to the formation of 1,3-dimethyluric acid (DMU; **A**), 1-methylxanthine (**B**) and 3-methylxanthine (**C**). Among the three products (**A–C**), it was clearly seen that the intensity of the peak representing product **A** was exceptionally higher and subject to increase upon proceeding along the reaction timescale (Figure S1). This observation was not surprising to us, as hydroxylation has been reported as the most feasible reaction of  $\bullet\text{OH}$  with many organic molecules, including theophylline [47,51,68–71]. To obtain more quantitative aspects, the generation of product **A** in the present case was quantified with the help of authentic standards. The plot of [**A**] against time shows a linear increase (Figure 6). The calculations revealed that more than 85% of the total theophylline transformed due to the reaction with  $\bullet\text{OH}$  was converted into **A** at an irradiation time of 30 s. It is worth noting that the percentage of theophylline transformed on this timescale (30 s) was only ~0.6. This value was, however, decreased to 47.7%, 46.2% and 17.4% at 1, 2 and 4 min, although the concentration of **A** showed an increasing trend, as seen in Figure 6. This observation can be justified by the attack of  $\bullet\text{OH}$  on the initial products, including **A**, leading to the formation of a number of secondary oxidation products, which are also identified and presented in Scheme 1.

The enhanced anti-oxidant activities of methylxanthines, in comparison with theophylline, have been reported in the literature [41]. The transformation of theophylline into isomeric methylxanthines (**B** and **C**) is, therefore, very relevant. The characterization of position isomers such as **B** and **C** is, however, very difficult because of the very close structural similarities and the consequently comparable physical/chemical properties. On the other hand, the comparison of the high-resolution tandem mass spectra of the respective peaks (see Figure S2, Supplementary Materials) with the previously reported ones of 3-methylxanthine [72] allows a rapid characterization of these isomers (Scheme 1).

The identities of five secondary transformation products (**D–H**), formed due to the subsequent  $\bullet\text{OH}$  attack on the initially formed transformation products (**A–C**), are depicted in Stage 2. Compounds **D** (1-methyluric acid) and **E** (3-methyluric acid) are also position isomers having a M.W. of 182.12. These compounds were formed by either (or both) the demethylation of **A** at  $\text{N}_1/\text{N}_3$  or the hydroxylation of **B** and **C** at  $\text{C}_8$ . The demethylation of **B** (at  $\text{N}_1$ ) and **C** (at  $\text{N}_2$ ) resulted in another important secondary oxidation product, xanthine (**F**), while the direct photo-reduction of methylxanthines (**B** and **C**) and methyluric acids (**D** and **E**) resulted in compounds such as **G** and **H**. All other products (**I–N**; Stage 3, Scheme 1) identified in the present study likely originated from the subsequent attack of  $\bullet\text{OH}$  on the primary and secondary products of theophylline (Stage 3), leading to the destruction of the five-membered imidazole ring. Due to the conversion of polycyclic compounds such as **A–H** into relatively small molecules, this stage might play an important role in

the mineralization process of this compound. These compounds are expected to undergo further oxidative breakdown into low-molecular-weight aliphatic compounds (Stage 4) and, ultimately, mineralize into  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and other inorganic ions (Stage 5). It is worth noting that the low-molecular-weight aliphatic compounds proposed to form in Stage 4 were not detected in our end-product studies, likely due to their high polarity and poor retention in a reverse-phase chromatography system.



**Scheme 1.** Proposed mineralization pathways of theophylline on its reaction with  $\bullet\text{OH}$ .

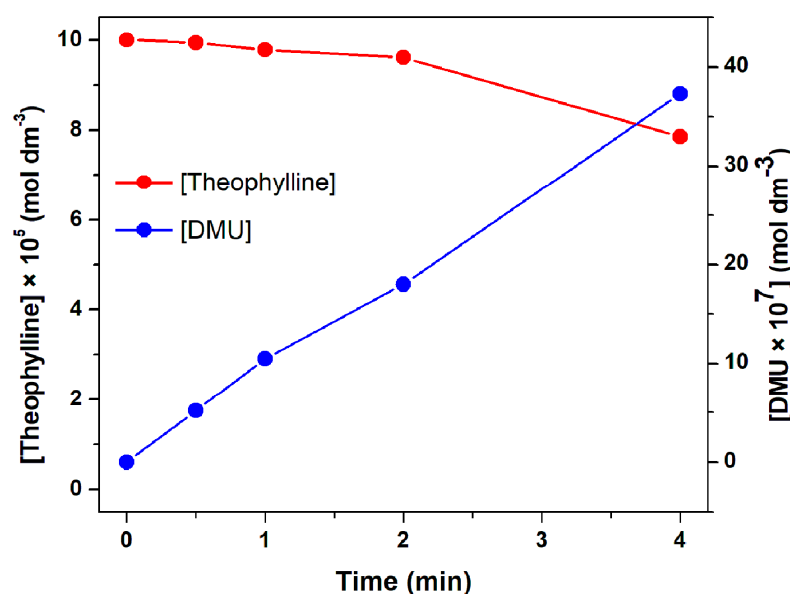


**Figure 6.** Variation in the concentration of Theophylline (red) and 1, 3-dimethyluric acid (DMU; blue) as a function of time.

#### 4. Conclusions

The widely accepted requirement of a good pollutant degradation strategy is its ability to convert the entire organic matter into non-toxic components. In our work, an ultraviolet-based advanced oxidation process ( $\text{H}_2\text{O}_2$ /UV photolysis) was thoroughly tested to evaluate their efficiency to degrade (and eventually mineralize) an organic contaminant of emerging concern (CECs), theophylline, from natural and laboratory-controlled environments. The rapid elimination of the parent molecule, as well as its primary transformation products (e.g., 1, 3-Dimethyluric acid), demonstrated in both cases is an important outcome of this work, as the previous investigations have mainly considered degradation in model water. While quantitative calculation clearly revealed the predominant role of hydroxylation pathways especially on short timescales ( $\sim 85\%$  after 30 s), high-resolution mass spectrometric analyses verified the unavoidable contribution of less common pathways (e.g., hydrogen abstraction) on the degradation process. The environmentally and economically viable concentration of  $\text{H}_2\text{O}_2$  to induce the degradation of this compound was predicted as  $5 \times 10^{-5} \text{ mol dm}^{-3}$  in the case of model water. The scavenging of the primary oxidant,  $\bullet\text{OH}$ , by inorganic ions (verified for sulfate, nitrate and phosphate ions) and dissolved organic matter (verified with the help of experiments conducted in river water) necessitated the use of a greater reagent concentration and longer irradiation time for the real samples. Despite this fact, an appreciable reduction ( $\sim 70\%$ ) in total organic carbon achieved even in highly complex river water samples demonstrated the suitability of advanced oxidation processes for the removal of these kinds of organic pollutants from real environment samples. A thorough knowledge of the degradation mechanism is expected to be beneficial for the optimization of these methods to achieve the highest mineralization efficiency, as it is, otherwise, an expensive and time-consuming process. In this context, the mineralization mechanism discussed in this report is very valuable. The ability of modern mass-spectrometric techniques to distinguish molecules with extreme structural similarities, including position isomers (e.g., 1-methylxanthine and 3-methylxanthine, 1-methyluric acid and 3-methyluric acid, etc.), is also commendable. The present ultraviolet-based degradation strategy was found to be very effective against the significantly photo-stable compounds, such as theophylline, and thereby able to hinder their transportation from aquatic medium to other valuable resources of the environment, such as air and soil. A complete elucidation of the quantitative aspects of this important environmental process still requires extensive efforts, especially related to the quantification of the remaining transformation products using authentic standards, and it is under consideration.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/atmos13050835/s1>, Figure S1: Total ion chromatogram of theophylline in the positive ionization mode recorded after 1 (A), 2 (B), and 4 (C) minutes of irradiation, Figure S2: MS/MS spectrum of 3-methylxanthine recorded in the positive ionization mode.

**Author Contributions:** Conceptualization, methodology and validation, S.P.M.M. and C.T.A.; formal analysis, investigation, data curation, S.P.M.M.; writing—original draft preparation, S.P.M.M.; writing—review and editing, U.K.A. and C.T.A.; visualization, supervision, project administration and funding acquisition, C.T.A. All authors have read and agreed to the published version of the manuscript.

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