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# Carbamazepine in water: persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity

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**Abstract**

The presence of carbamazepine (CBZ), an antiepilectic drug, has been reported in sewage treatment plant (STP) effluents as a result of its low biodegradability. In the present work, the persistence of CBZ in aquatic environment with respect to abiotic transformation processes along with its toxicity and capability of accumulating in single aquatic organisms (algae) are evaluated. The possibility of removing CBZ from STP effluents is studied by characterizing its ozonation process through the assessment of kinetics and the distribution of oxidation products. 

*Keywords:* CBZ; Photodegradation; Ozonation; Drugs; Surface water; Algal bioassay

## 1. Introduction

The presence of pharmaceutical compounds in river and surface waters has been frequently reported in the last years [1–5]. Veterinary and human use are generally recognized as the most relevant sources for this new class of environmental pollutants [6]. In fact many of the numerous active substances used to treat illnesses are excreted unmetabolised or as active metabolites. Moreover, improper disposal of expired medications can contribute to the contamination of the environment. Particularly, it has been reported in Germany the presence of up to 32 drugs in the influents to Municipal Sewage Treatment Plants [7]. Some of these compounds are only partly removed in conventional biological treatments since they are found at significant concentrations in STP effluents and therefore in surface waters. Whether the presence of these compounds in the environment may be harmful for man and living organisms is still under debate. In fact, although these substances have the capability of causing biological effects, no indications are found in the literature about their persistence in the environment after the dicharge and the real interactions they have with living organisms at the concentrations at which they are found in surface waters. With the reference to the persistence, it has been reported that some pollutants may undergo photochemical transformations, via direct light absorption or sensitized reactions [8,9] through which their characteristics can be modified. Only scant indications are found on their removal from wastewaters [10].

Carbamazepine (CBZ) is an antiepileptic drug whose occurrence in municipal STP effluents and in domestic wastewaters has been reported in the literature [7,11]. No indications are available in specialized journals for its persistence in the environment and/or for its toxicity

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towards simple living organisms such as algae nor for its removal from aqueous streams by means of alternative chemical treatments. Recently, some papers have been reported on the assessment of the risk of major congenital abnormalities associated with CBZ assumption [12,13].

In the present paper, all these aspects are faced aiming at assessing the persistence of CBZ in the environment and preliminarily evaluating its toxicity towards algas. The capability of ozonation treatment of removing this species from aqueous streams is also studied.

## 2. Experimental

### 2.1. Solar photodegradation and oxidation experiments

Solar photodegradation experiments were carried out during spring at Naples in south of Italy (40°N–14°E). They were conducted in 0.5 $dm^3$ annular magnetically stirred glass reactors refrigerated at 25°C [14]. Aqueous solutions to be irradiated were prepared by dissolving a proper amount of CBZ in bidistillated water or in natural waters from Liri river (Frosinone, Italy: pH = 7.8, $Cl^- = 5.1 \times 10^{-4}$ mol $dm^{-3}$, $NO_3^- = 1.0 \times 10^{-4}$ mol $dm^{-3}$, $HCO_3^- = 5.1 \times 10^{-3}$ mol $dm^{-3}$).

Solar actinometry was daily carried out by measuring the concentration decay of a solution of *p*-nitroanisole ($2.0 \times 10^{-6}$ mol $dm^{-3}$) in pyridine [15]. The pyridine concentration was adjusted to obtain during the irradiation time an almost complete consumption of *p*-nitroanisole.

According to Dullin and Mill the photolytic degradation of *p*-nitroanisole in a batch reactor is described by means of the following equation:

$$-\frac{d[C]}{dt} = q\alpha[C] = k_p[C] \quad (1)$$

with the dimensionless parameter $q$ given by

$$q = 0.44[\text{pyridine}] + 0.00028 \quad (2)$$

and $\alpha$ being dependent on system characteristics (optical path, volume), sunlight intensity and optical absorption properties of the substrate.

Therefore from experimental values of $k_p$ the sunlight intensity is indirectly evaluated by:

$$\alpha = \frac{k_p}{q}. \quad (3)$$

In fact, for established apparatus and chemical system, the values assumed by the ratio $k_p/q$ depend only upon the sunlight intensity.

For ozonation experiments with continuous ozone feeding, a 0.8 $dm^3$ glass-stirred reactor has been employed [16]. An ozonized oxygen stream with an ozone content of 2.0% per volume has been bubbled in the solution at 36 $dm^3\,h^{-1}$. For batch experiments $3.0 \times 10^{-3}$ $dm^3$ of ozonated aqueous solution have been rapidly mixed with $1.0 \times 10^{-1}$ $dm^3$ of CBZ ($3.3 \times 10^{-6}$ mol $dm^{-3}$) aqueous solution and the resulting solution kept at rest for 10 min.

CBZ and *p*-nitroanisole concentrations were determined by HPLC (1100 Hewlett Packard) using Phenomex C6 reverse phase column with a mixture of water/acetonitrile (60/40) as mobile phase at $5.0 \times 10^{-4}$ $dm^3\,min^{-1}$ and measuring the absorbance at 210 nm for CBZ and 320 nm for *p*-nitroanisole.

The pH of aqueous solutions, adjusted with dilute $HClO_4$ or NaOH, was determined using Orion 960 pH-meter with a glass pH electrode.

The absorbances of humic acid ($5.0 \times 10^{-3}$ g $dm^{-3}$) and CBZ ($8.0 \times 10^{-6}$ mol $dm^{-3}$) were measured with UV-VIS spectrophotometer (HP 8452 A) with quartz cell path length of 1.0 cm.

All the reagents but hydrogen peroxide (30% by weight, Fluka) were purchased from Sigma Aldrich.

For the identification of intermediates and products of CBZ ozonation the samples withdrawn from the reactor have been submitted to oxidative, hydrolytic and derivatizing work-up.

### 2.2. Oxidative work-up

A drop of concentrated $H_2O_2$ solution (10 mol $dm^{-3}$) has been added to an aliquot of ozonated sample after having adjusted the pH to 10. Resulting solutions have been heated for 2 h at 50°C. This treatment allows an easy conversion of aldehydic function to carboxylic.

### 2.3. Hydrolytic work-up

An ozonated sample, after addition of one drop of concentrated perchloric acid, has been heated at 150°C in oil-bath for 40 h at reflux. After cooling, the sample is divided into two parts. The first aliquot has been submitted to an oxidative treatment with $H_2O_2$ for determining anthranilic acid, the second has been employed for the ammonia test.

### 2.4. Derivatizing work-up

For the analysis of $\alpha$-dicarbonylic compounds, $1.0 \times 10^{-3}$ $dm^3$ of the ozonated mixture has been mixed in a cap-screw vial with $2.0 \times 10^{-3}$ $dm^3$ of a solution prepared by means of $2.37 \times 10^{-1}$ g of 1,2-phenylenediamine and $2.0 \times 10^{-3}$ $dm^3$ of concentrated sulphuric acid and taking the volume at $2.5 \times 10^{-1}$ $dm^3$. Samples have been heated for 1 h at 100°C and after neutralization analysed by HPLC using a mobile 90/10 buffered solution ($H_2O$:$CH_3OH$:$H_3PO_4$, 500:25:2)/$CH_3CN$.

Evolved carbon dioxide has been trapped by means of $Ba(OH)_2$ solutions and quantified by titration with standardized HCl solution.

Oxalic and oxamic acids were detected by HPLC analyses with an Alltech OA-1000 acid column (mobile phase: $H_2O/H_2SO_4$, 500/0.8).

An iodometric procedure [17] has been adopted for $H_2O_2$ analysis.

Colorimetric procedure has been used for the analysis of ammonia [18]. Nitrate has been reduced to ammonia by means of Devarda's alloy in alkaline medium after heating at 50°C for 3 h [19].

### 2.5. Bioassays

The algae tested against CBZ were *Ankistrodesmus braunii*, strain CCAP 202-7a, from the Culture Collection of Algae and Protozoa, Ambleside, Cumbria (UK) and *Selenastrum capricornutum*, strain UTEX 1648, from the Culture Collection of algae at the University of Texas at Austin (USA). These algae, among the most commonly used in laboratory testing, have been grown in Bold's basal medium (BBM) [20] in 1.0 $dm^3$ Erlenmeyer flasks at $23 \pm 1$°C with a total irradiance of 100 $\mu E\, s^{-1}\, m^2$ provided by a daylight fluorescent Philips lamps (TLD 30w/55). The photoperiod was 16 h light–8 h dark. The flasks have been placed on a plexiglas shaking apparatus as indicated by Shihira and Kraus [21]. The growth of the alga has been followed at 550 nm with a colorimeter Bausch & Lomb spectronic 20. Inocula corresponding to $1 \times 10^3$, $1 \times 10^4$ and $1 \times 10^5$ cells $ml^{-1}$ from axenic cultures in mid-exponential phase have been grown in test tubes containing BBM and, respectively, (a) CBZ at concentrations ranging from $2.1 \times 10^{-6}$ g $dm^{-3}$ (the actual concentration found in same STP effluents of Germany [7]), to $2.0 \times 10^{-2}$ g $dm^{-3}$, (b) aqueous solutions of CBZ ($8.0 \times 10^{-3}$ mol $dm^{-3}$) at different times of ozonation (0, 5, 10, 20, 30 and 40 min). Each test tube containing the algal inocula and a volume ($6.0 \times 10^{-3}$ $dm^3$) of the testing solution has been incubated on a shaking apparatus as previously described. The growth of the cultures has been followed daily either by counting the cell number with a Burker bloodcounting chamber or by measuring the absorbance increase at 550 nm with a colorimeter. During the time course of the experiments (96 h) the algal cultures remained in the exponential phase of growth. The tests were carried out in axenic conditions. Cells have been thoroughly mixed for 10 s by a vortex mixer prior to reading. Control containing only distilled water and BBM was also tested. Growth experiments have been carried out five times and results have been evaluated on the basis of the average of five tests. To test statistical significance of results, one-way ANOVA has been performed at $\alpha = 0.05$. For each solution, a comparison among means was performed using Student-Newman-Keuel test (SNK), at $\alpha = 0.05$. The SPSS statistical package (SPSS Inc. for Windows) was used.

The in vitro long-term effects of CBZ on C 202-7a have been assessed by exposing the algae to the compound for 60 days. The bioassays have been carried out in large conical flasks with 1 l of BBM. CBZ $8.0 \times 10^{-5}$ mol $dm^{-3}$ has been aseptically added and gently shaken for 3 days before the algal inoculum ($1 \times 10^5$ cells $ml^{-1}$). The cultures have been incubated under the above described conditions of light and temperature, and samples ($2.0 \times 10^{-2}$ $dm^3$) from the culture batch have been taken at intervals of 3 days. The samples have been centrifuged (5000 rpm × 15 m) and washed twice with cold BBM (20 ml each). The algal pellet has been lyophilized and extracted with methanol. CBZ has been measured in the sample medium, in washed samples and in the algal pellet.

## 3. Results and discussion

### 3.1. Persistence in surface waters

In Fig. 1 the absorption spectrum of CBZ (black points) is shown which indicates that this molecule absorbs solar UV radiation. In surface waters it could thus photolize and undergo photochemical transformations. This tendency has been checked by means of proper experiments in which an aqueous solution containing CBZ at starting concentration of

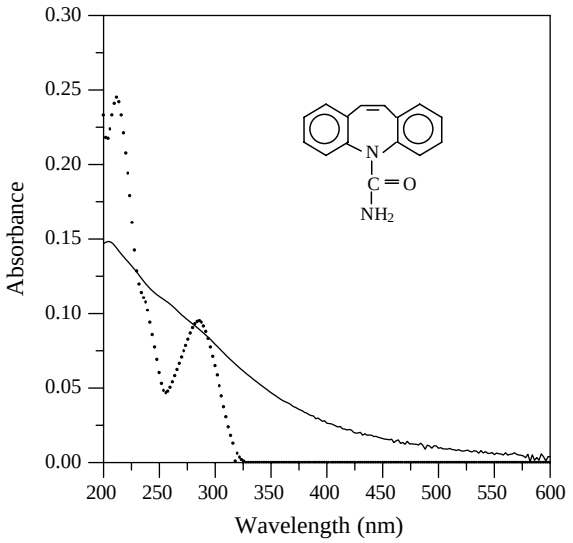



Fig. 1. Absorption spectra of humic acid (solid line) and CBZ (black points) in the region 200–600 nm. [humic acids]$_o = 5.0 \times 10^{-3}$ g $dm^{-3}$; [CBZ]$_o = 8.0 \times 10^{-6}$ mol $dm^{-3}$.

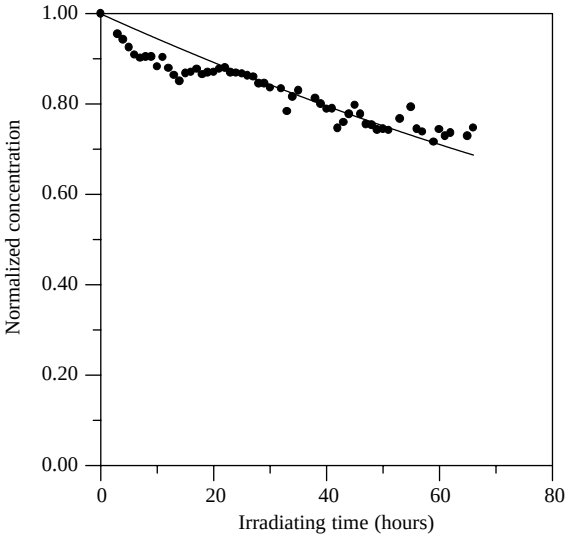



Fig. 2. Photodegradation of CBZ in bi-distilled water during exposure to sunlight at pH = 5.5 and $T = 25°C$. $[CBZ]_o = 8.0 \times 10^{-6}$ mol dm$^{-3}$. Start time after 10 a.m.–end time before 4 p.m. (middle April to early May). Experimental points (full circle), calculated values with first order kinetic model (solid line).

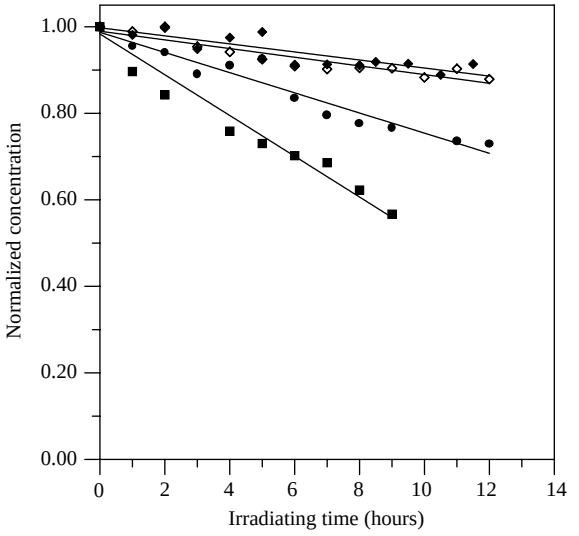



Fig. 3. Nitrate-induced photodegradation of CBZ in bi-distilled water during exposure to sunlight at different concentrations of nitrate at pH = 5.5 and $T = 25°C$. $[CBZ]_o = 8.0 \times 10^{-6}$ mol dm$^{-3}$. Start time after 10 a.m.–end time before 4 p.m. (middle April to early May) ◇, without nitrate; ◆, nitrate ($5.0 \times 10^{-4}$ g dm$^{-3}$); ●, nitrate ($1.0 \times 10^{-2}$ g dm$^{-3}$); ■, nitrate ($1.5 \times 10^{-2}$ g dm$^{-3}$).

$8.0 \times 10^{-6}$ mol dm$^{-3}$ has been submitted to solar irradiation in the apparatus described in the experimental section. The results of these runs are reported in Fig. 2 (full circles) and indicate that the substrate concentration really decreases with increasing the irradiation time. Actinometric measurements with *p*-nitroanisole in pyridine performed each day in a similar reactor with the same exposure with respect to solar irradiation gave an average value of $k_p/q = 72.5$ h$^{-1}$. To fully assess the nature of the observed reaction, an aqueous solution with the same concentration of CBZ has been kept in the dark and analyzed at different reaction times. These results (not shown) indicated that no reaction occurs without irradiation. The data reported in Fig. 2 for the photochemical degradation of CBZ have been analyzed by plotting the natural log of the relative residual concentration against the time and the pseudo first-order kinetic constant derived by means of a least-squares regression (solid line). A kinetic constant value of $5.7 \times 10^{-3}$ h$^{-1}$ has been obtained from which a half-life $t_{1/2} = 121.6$ h has been calculated.

Several authors reported about the capability of humate of generating, after sunlight absorption, reactive oxidant species, such as singlet oxygen [22,23] and hydroxyl radicals [24] or of enabling rapid photosensitized reactions of certain pollutants via energy transfer from molecules in their triplet states [25]. Nitrite and nitrate are also indicated as capable of promoting the formation of OH radicals [26,27]. Some experimental runs have thus been performed by adding to CBZ aqueous solutions nitrates and humic acid (separately). CBZ half-life reduced to 69.0, 24.5 and 11.2 h when the concentrations of nitrates were, respectively, of $5.0 \times 10^{-4}$ g dm$^{-3}$, $1.0 \times 10^{-2}$ g dm$^{-3}$ and $1.5 \times 10^{-2}$ g dm$^{-3}$ (Fig. 3).

The presence of dissolved humic acid ($5.0 \times 10^{-3}$ g dm$^{-3}$) on the other hands resulted into an increase of CBZ half-life (233.7 h) (Fig. 4). The addition of these compounds to CBZ aqueous solutions seems to hinder the spontaneous photochemical degradation of the pollutant. A possible explanation for this behaviour could be found by considering that the adopted humic acid is characterized by strong UV absorptions in the same range as CBZ (Fig. 1). That is, when humic acid is present, only a part of the irradiated power is absorbed by CBZ itself which undergoes a slower photochemical transformation with respect to that observed in bidistilled water. A similar behaviour has been observed by others for the degradation of pesticides [28,29].

To fully assess the fate of CBZ in real natural waters, aqueous solutions prepared by spiking river water with this substance have been submitted to solar radiation. In Fig. 5 the relative residual concentration is shown against the time. From these data an half-life of 907 sunlight hours is evaluated with an average $k_p/q$ for the system *p*-nitroanisole equal to 26.8 h$^{-1}$.

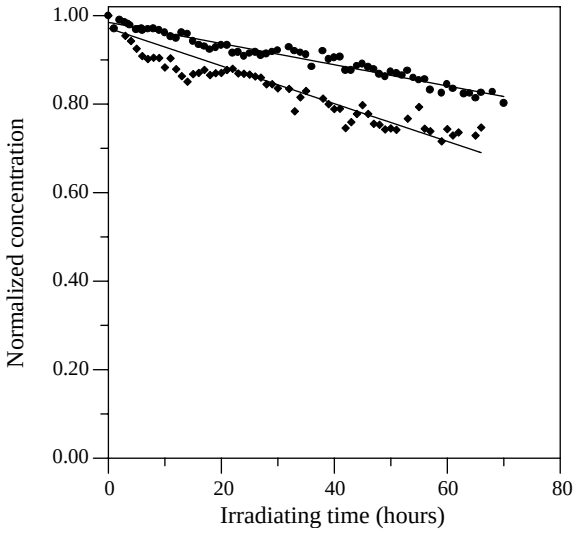



Fig. 4. Humic acids effect on the photodegradation of CBZ in bi-distilled water during exposure to sunlight at pH = 5.5 and $T = 25°C$. $[CBZ]_o = 8.0 \times 10^{-6}$ mol dm$^{-3}$. Start time after 10 a.m.–end time before 4 p.m. (middle April to early May) ◆ without humic acids; ● humic acids ($5.0 \times 10^{-3}$ g dm$^{-3}$).

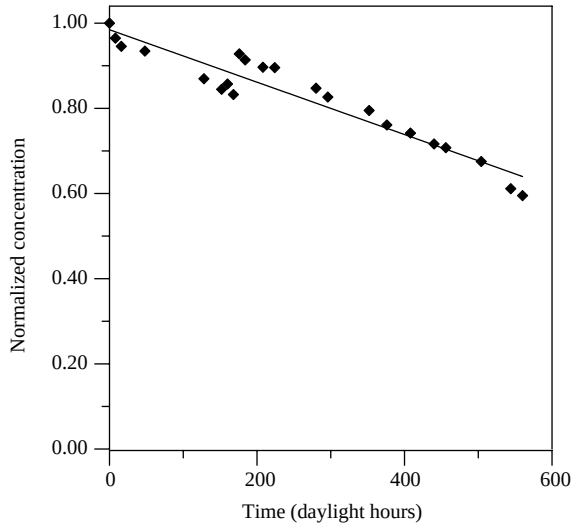



Fig. 5. Phototransformation of CBZ in river water during exposure to spring sunlight (23/03/00–31/05/00). $[CBZ]_o = 8.0 \times 10^{-6}$ mol dm$^{-3}$ – T = 25°C.

### 3.2. Algal bioassays

In the bioassays no inhibition of growth, measured using biomass variation after 96 h from the addition of CBZ, has been found, independently of algal and CBZ concentrations. At the end of the experiment a slight decrease of CBZ in the medium has been observed in *A. braunii* cultures, whereas in the blank and in all the cultures of *S. capricornutum* CBZ concentration remained constant.

These results indicate that CBZ exerts only negligible effects on microalgae within 96 h. However, a toxicity of the substance over longer times could not be excluded. Experiments with 60 days CBZ expo-sure were planned on *A. braunii*, to determine if this alga was capable of accumulating the substrate by adopting high concentrations in comparison with the culture medium. The uptake and concentration into algal cells of CBZ could cause secondary sensitivity of alga to this compound or bioaccumulation through food net to consumer organisms in the upper trophic level.

In a 60-day experiments the initial algal concentration was $1 \times 10^5$ cells ml$^{-1}$ and CBZ has been axenically added to give a concentration of $1.9 \times 10^{-2}$ g dm$^{-3}$. During these tests growth rate, morphology of cells, number and shape of sporangia and autospores have been monitored. In the blank cultures as well as in CBZ-treated ones the stationary phase of growth was reached after 15 days. In this phase the algae presented numerous droplets and grains in the protoplasm, and cells of irregular shape were frequently observed. Moreover, algal cultures showed an increasing number of sporangia. A comparison with the sporangia observed in the exponential cultures evidenced that in the stationary phase the sporangia were larger and formed more autospores. These changes can be attributed to the senescence of algal population [30] and no significant differences between control and CBZ-treated cells were observed

On the other hand, the concentration of CBZ progressively decreased in the algal culture (Fig. 6), and, after 60 days, over 50% of CBZ was disappeared in

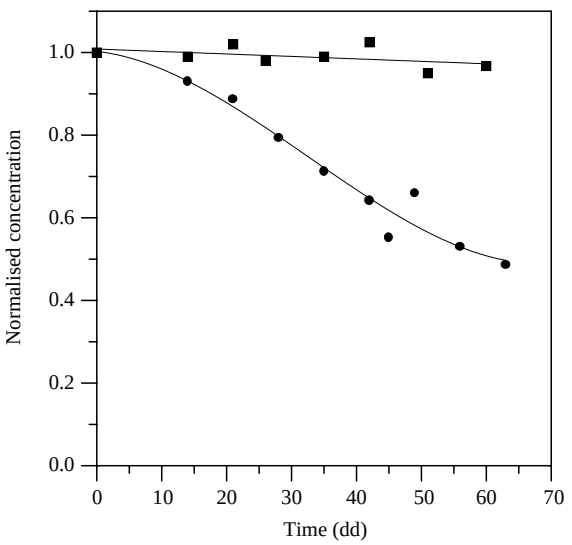



Fig. 6. Transformation of CBZ in Bold's basal medium (BBM) without algae (■) and in algal culture (●). $[CBZ]_o = 8.0 \times 10^{-5}$ mol dm$^{-3}$ – T = 25°C.

the medium. No significant amounts of CBZ could be detected in *A. braunii* cells during the course of the experiment. As previously reported, the strain 202.7a of *A. braunii* is able to subtract from the medium α-asarone, a toxic phenolic substance, and no significant amount of this compound could be detected in the cell [31]. In the present case, it could be put forward that CBZ is taken up by algal cells and enter into biochemical processes. The utilization of unusual compounds as metabolites has been described for other unicellular algae. It has been reported [32] that the red alga *Galdieria sulphuraria* could be grown on a wide range of rare sugar-alcohols, whilst Sample and Cain [33] reported that the chrysophyte *Ochromonas danica* metabolized phenol by the metacleavage pathway.

### 3.3. Oxidative treatments

The results reported in the previous paragraphs show that CBZ is characterized by a certain persistence in the environment and by the absence of toxic effects towards and accumulating capability in tested aquatic organisms (algae).

Since these results cannot rule out the existence of some toxic effects exerted by this species towards other living organisms [34], a conservative approach to the problem of the presence of CBZ in the environment would thus suggest its removal at least from water streams for human use. To this purpose some investigations have been firstly performed to assess if CBZ could be destroyed during the oxidative treatments to which drinking waters are currently submitted [10]. When ozonation is employed, an ozone dosage of $1.0 \times 10^{-3}\,g\,dm^{-3}$ and a residence time of 10 min are adopted.

Some ozonation runs have thus been carried out, according to the procedure described in Section 2, by using CBZ aqueous solutions at starting concentration of $3.3 \times 10^{-6}\,mol\,dm^{-3}$. In this way, with an ozone dosage of $2.08 \times 10^{-5}\,mol\,dm^{-3}$, a ratio of approximately 10 (ozone moles/CBZ moles) is realized. A complete abatement of CBZ has been recorded in all the performed runs.

This result indicates that a complete removal of this pollutant may be easily achieved in drinking waters, due to the fact that expected CBZ concentrations in real water streams are below the values adopted in these tests [7].

A kinetic analysis has been also attempted by using the results collected during semi-continous ozonation runs. Since negligible ozone concentrations have been found in the liquid bulk during the early ozonation stages, the process has been considered to develop under a "quasi-diffusive" regime of absorption with reaction.

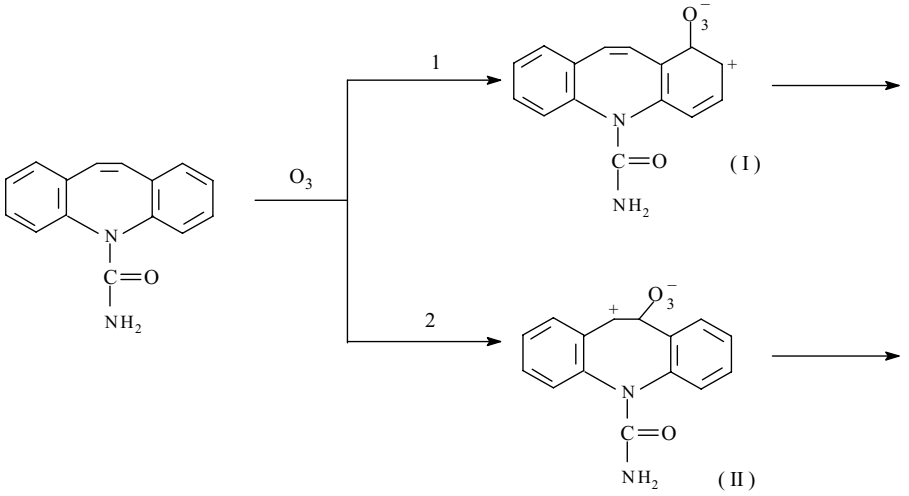
A proper fluid-dynamic submodel reported elsewhere [35] has been coupled with a simplified kinetic one in which a single overall reaction was used to described the ozone attack to the substrate in the initial ozonation stages:

$$CBZ + zO_3 \xrightarrow{k_{O_3}} \text{products}.$$

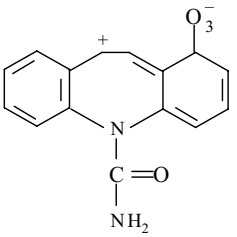
The adopted model has been used to estimate the best values of $k_{O_3}$ and $z$ (Table 1) by means of a suitable optimizing procedure [36].

Some attempts to identify, during the above-reported ozonation runs, the intermediates and products of CBZ oxidation failed probably because of the low concentrations at which they were present in the reacting solution. To more deeply investigate this point a second set of ozonation experiments have been performed by using CBZ solution at starting concentration of $5.0 \times 10^{-4}\,mol\,dm^{-3}$.

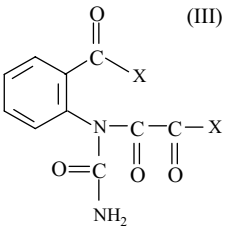
Due to the electrophilic nature of the ozone attack to the substrate, two pathways can be foreseen for the ozonation process depending on the centre to which the oxidant initially binds:



Since for species (I) a benzylic-like cation structure can be written among the others too,



it is more stabilized by resonance than species (II), suggesting that the oxidation process proceeds mainly through path 1 with minor occurrence of path 2. According to both path 1 and path 2 the formation of an anthranilic acid precursor,



(III)



C1 ($CO_2$), C2 (glyoxal, glyoxilic, oxalic and oxamic acids) and C3 compounds (ketomalonic acid) can be predicted.

HPLC analyses performed on directly injected samples indicated a complete disappearance of the substrate after 4 min of ozone feeding with a specific ozone consumption of 1.0 mol $mol^{-1}$ of CBZ and the presence—at trace level—of oxamic acid. Since no standard was available for the species (III) it has been considered that it gives rise to the formation of anthranilic acid after an hydrolitic and oxidative treatment:

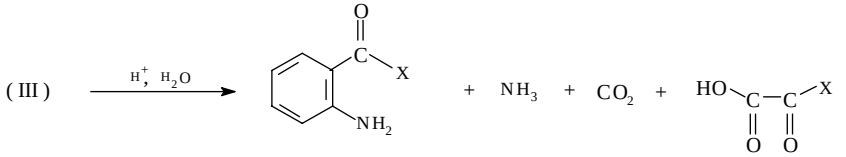
The analyses performed on samples submitted to hydrolytic and oxidative work-up confirmed the presence of anthranilic acid (Fig. 7). Successive analysis on samples hydrolyzed and derivatized with 1,2 phenylenediamine allowed the identification of glyoxal, glyoxilic and oxalic acid (Fig. 7).

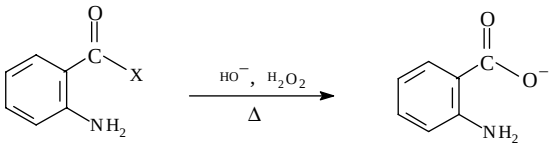
Table 1
Kinetic rate constant ($k_{O_3}$), stoichiometric coefficient ($z$) and percentage standard deviations (% $\sigma$) obtained from experimental runs in bi-distillated water. $T = 25.0°C$, $[CBZ]_o = 5.0 \times 10^{-4}$ mol $dm^{-3}$

| | |
|---|---|
| $k_{O_3}$ ($mol^{-1}\,dm^3\,s^{-1}$) | $7.81 \times 10^4 \pm 1.31 \times 10^4$ |
| $z$ | $9.85 \times 10^{-1} \pm 2.115 \times 10^{-2}$ |
| $\sigma_{O_3}$ (%) | 4.7 |
| $\sigma_{CBZ}$ (%) | 3.7 |

From the diagrams of Fig. 7 it is clear that a low degree of mineralization is achieved for even long ozonation times, evolved carbon dioxide accounting for up to 30% of the initial carbon content. Moreover, total carbon balance results lacking even for prolonged ozonation thus indicating the presence in the reacting solution of some non-identified compounds. Since the formation amidic intermediates can be predicted in the ozonation process [37], hydrolytic treatments have been carried out on ozonated samples to favour the release of ammonia. Ammonia determination on aqueous samples starting with initial CBZ concentration equal to $5.0 \times 10^{-4}$ mol $dm^{-3}$ withdrawn after 60 and 90 min of ozonation account for up to, respectively, 68.9% and 100% of the initial nitrogen content. No nitrates have been found during the ozonation of CBZ since ammonia measurements gave the same results after reductive treatment with Devarda's alloy.

Test for toxicity assessment of CBZ solutions after treatment with $O_3$ at different reaction times (0, 5, 10, 20, 30 and 40 min) have been carried out on *S. capricornutum* and *A. braunii*. The duration of the test ranged from 96 h to 30 days. No inhibition has been observed.





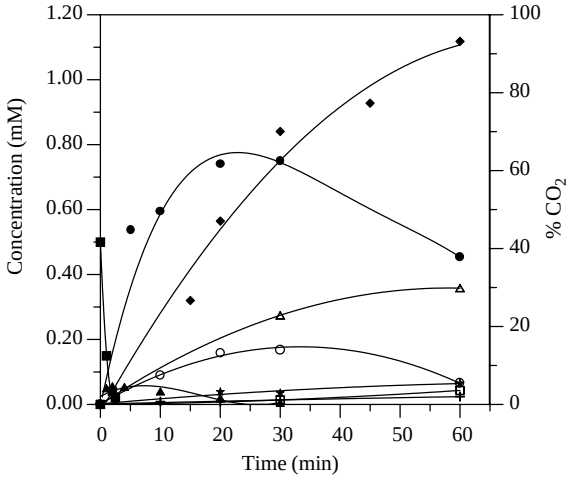



Fig. 7. Ozonation of CBZ and reaction intermediates at pH = 5.5. $[CBZ]_o = 5.0 \times 10^{-4}$ mol dm$^{-3}$–$T = 25$°C ■ CBZ, ●, hydrogen peroxide, ◆, oxalic acid, ○, glyoxylic acid, △, carbon dioxide, +, glyoxal, ★, oxamic acid, ▲, anthranilic acid and □, ketomalonic acid.

## 4. Conclusions

The persistence in the environment of CBZ, its preliminary toxicity towards algas and its oxidative degradation by means of ozone have been studied in the present work. Collected results demonstrate that the studied substrate is capable of photolyzing in distilled and river waters. Nitrate and humic acid have opposite effects on its degradation, the first promoting the second inhibiting. These preliminary bioassays seems to indicate that CBZ is not toxic towards *Selenastrum capricornutum* and *Ankistrodesmus braunii*. Moreover, no accumulation of the compound into the algal cells have been observed. However, further experiments on other test organisms belonging to algae and other aquatic species are required to fully assess the toxicity of CBZ in the environment. The ozonation has been demonstrated to be a suitable tool for CBZ abatement even at the process conditions usually adopted in drinking water facilities. The product distribution during ozonation has been also investigated. A low degree of mineralization is observed after 60 min of ozonation treatment. The carbon balance remains lacking for even long ozonation times.

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