



Mechanism of the photosensitizing action of a mixture humic acid–riboflavin in the degradation of water-contaminants

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ABSTRACT

Kinetic and mechanistic aspects on the photooxidation of 3-hydroxypyridine (3-OHP) and phenol (PhOH), taken as model compounds for hydroxyaromatic water-contaminants, was studied. A mixture of the naturally occurring visible-light absorbers humic acid (HA) and vitamin B₂ (riboflavin, Rf) was employed as a photosensitizing agent. The work was done in pH 7 aqueous solution, in the presence of 0.04 mM Rf and 50 µg/ml HA and employing photoirradiation wavelengths higher than 360 nm, a range where 3-OHP and PhOH are transparent.

The mechanism involved in the photooxidation was mainly evaluated through oxygen consumption measurements. The mixture HA + Rf generates O₂(¹Δ_g) and O₂^{•-}, the latter especially in the presence of electron donors such as the hydroxyaromatic compounds. The rates of oxygen consumption were taken as a measure of the overall oxidation rate of the contaminant model compounds. The photooxidation of 3-OHP at pH 7, sensitized by low Rf and HA concentrations, and standardized by comparison with the photooxidation rate of the known oxidizable target furfuryl alcohol, is an efficient process. PhOH is only degradable in the alkaline range of pH.

A moderate decrease in the rate of oxygen consumption by 3-OHP upon Rf+HA sensitization was observed as compared to the simple addition of the oxygen uptake rates for 3-OHP upon individual Rf and HA sensitization. It is attributed to a catalytic decomposition of O₂^{•-} by HA, which competes with 3-OHP by the oxidative species, inhibiting this oxygen-consumer channel, especially active in the presence of the pyridine derivative.

Noticeable photofading of the sensitizers was detected when photolyzed in the absence of the contaminants, within typical irradiation times employed for the hydroxyaromatics degradation.

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1. Introduction

Humic substances, among which humic acids (HAs) constitute the predominant fraction, are naturally occurring mixtures of organic compounds abundant in terrestrial and aquatic ecosystems [1,2]. In some sense, the same can be said about riboflavin (Rf, vitamin B₂) which is also present in waters of rivers, lakes and seas [3,4]. Both water constituents have been considered to individually play an important role in the photochemical degradation of organic materials in nature [5–7].

Most water contaminants are colorless and cannot be degraded by direct irradiation with daylight. This situation has triggered the study of their photosensitized decomposition [8,9]. In this mechanism, the degradation is carried out through the gener-

ation of reactive species, oxygenated (ROS) or non oxygenated, by a visible light absorbed named photosensitizer. Consequently, the photochemical mechanism of contaminants degradation, in aerated solution and under environmental light irradiation, is a topic of growing interest [6,10,11]. Studies on the photosensitizing ability of individual aqueous HA and Rf have been published by other researchers and by ourselves employing different water-contaminants or model compounds, to mimic the natural decay of all these substances [6,9,12–17]. Nevertheless, the composition of natural waters is not simple, and the simultaneous presence of several light-absorbers may constitute an intricate picture from the point of view of predicting photochemical degradation efficiency based on the individual behavior of isolated sensitizers. In this context the knowledge on the mechanistic steps governing the photodegradation processes and potential synergistic or inhibitory effects of the photosensitizers can help to model and envisage the fate of contaminants spread into a natural medium.

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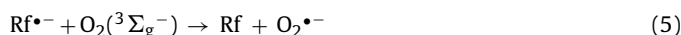
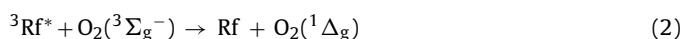
Hydroxyaromatic compounds (Ar-OH) are transparent to visible light and their sensitized phototransformation under different conditions has received considerable attention in the last decades [9,10]. It has been reported that the parent compound phenol and several substituted hydroxyaromatics can be photodegraded with varied efficiency in the individual presence of Rf or HA as photosensitizers, and that the photodegradation occurs mainly through ROS and radical-driven reactions [14,15,18].

In 2003 Zeng et al. [19] published a paper devoted to the photosensitizing effect given by the simultaneous presence of HA and Rf, employing the polycyclic hydrocarbon 6-aminochrysene (6-AC) as a water-contaminant model. Although most effort was directed to the elucidation of photooxidation products and cytotoxicity of the irradiated system, the authors also discuss comparative results on the rate of 6-AC degradation upon photosensitization with different mixtures Rf-HA.

In the present contribution we include a systematic kinetic and mechanistic study on the photosensitizing properties of the mixture HA plus Rf, employing phenol (PhOH) and 3-hydroxypyridine (3-OHP) as models for Ar-OH contaminants. Chemical structures containing hydroxyaromatics and N-heteroaromatics are very frequent in the basic formulation in the most employed biocides [20].

The study reasonably mimics a picture where a mixture of endogenous sensitizers and potentially oxidizable uncolored contaminants are simultaneously present in a given aqueous environment, illuminated by daylight.

The following simplified and self-defined reaction sequence describes the photosensitizing properties of Rf and HA (see references below):

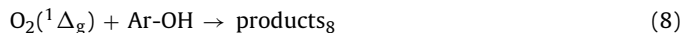
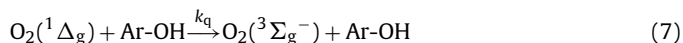


Upon light absorption Rf generates the ROS $\text{O}_2({}^1\Delta_g)$ (reactions (1) and (2) and $\text{O}_2^{\bullet-}$ (reactions (1) and (3)), with quantum yields of 0.49 and 0.009, respectively [4,21]. In the presence of electron donors, such as Ar-OH, the production of $\text{O}_2^{\bullet-}$ may be greatly enhanced due to the electron-transfer reactions shown in processes (4) and (5) [7,22].

According to the reaction scheme and literature reports [5,14,15,23], the natural decay for the species $\text{O}_2({}^1\Delta_g)$ (reaction (6)), its physical quenching (reaction (10)) and the oxidation of Ar-OH by ROS may occur, employing HA and/or Rf as photosensitizers in the presence of 3-OHP and PhOH (reactions (8) and (9)). The vitamin is also autooxidized by $\text{O}_2({}^1\Delta_g)$ with a reported value $k_r = 6 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ for process (8) with Rf instead of Ar-OH [4].

It has been shown that HA contains chromophores capable of sensitizing the production of $\text{O}_2({}^1\Delta_g)$. In fact quantum yields of generation of $\text{O}_2({}^1\Delta_g)$ of ca. 4% as upper limits have been reported [5,15,17]. In 1986 Malcom and MacCarthy demonstrated differences between commercial and natural humic acids (HAs), especially when their respective chemical reactivities are compared [24]. Nevertheless, commercial HAs still being employed to model photochemical properties of the natural analogs [5]. The autooxidation of HA by $\text{O}_2({}^1\Delta_g)$ has been mentioned as one of the possible pathways for its own degradation in natural waters [5] according to reaction (8) with HA instead of Ar-OH. Other HA degradation routes have been reported [25], including the $\text{O}_2^{\bullet-}$ – catalytic decomposition [26], being in this case the observed stoichiometry of $\text{O}_2^{\bullet-}$ the same as for a bimolecular dismutation, as

shown below in reaction (11) in the presence of SOD.



On this basis we designed a systematic kinetic study on the degradation of Ar-OH compounds by the simultaneous photosensitization by HA and Rf in order to elucidate, as best as possible, the mechanism involved in the photodegradation of Ar-OHs and potential mutual effects between the sensitizers in relatively low concentrations.

2. Materials and methods

2.1. Chemicals

Riboflavin (Rf), 3-hydroxy pyridine, phenol, superoxide dismutase from boverythrocytes (SOD), mannitol, sodium azide (NaN_3) and catalase (CAT) from bovine liver (CAT) were purchased from Sigma Chem. Co. Humic acid, Rose Bengal (RB), were from Aldrich. Furfuryl alcohol (FFA) was provided by Riedel de Haën. Phosphate buffer was used to regulate pH. All measurements were carried out with freshly prepared solutions.

Experiments in deaerated solutions were made by bubbling ultrapure Ar.

2.2. Absorption and fluorescence measurements

Ground state absorption spectra were registered employing a Hewlett Packard 8452A diode array spectrophotometer. Stationary fluorescence was measured with a Spex Fluoromax spectrofluorometer at $24 \pm 1^\circ\text{C}$ in air-equilibrated solutions. Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-9000CD instrument. In all cases the sample was placed in a 1 cm \times 1 cm quartz cell. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively. In both cases, stationary and time-resolved fluorescence measurements, a classical Stern–Volmer treatment of the data was applied through Eqs. (10) and (11), where I and I_0 , and ${}^1\tau$ and ${}^1\tau_0$, are the respective fluorescence intensities of Rf in the presence and in the absence of an eventual quencher Q and fluorescence lifetimes in the presence and in the absence of Q.

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (10)$$

$$\frac{{}^1\tau_0}{{}^1\tau} = 1 + {}^1k_q {}^1\tau_0[Q] \quad (11)$$

2.3. Stationary photolysis

Stationary aerobic photolysis of aqueous solutions was carried out employing a home-made photolyzer, provided with a 150 W quartz-halogen lamp, employing a 360 nm cut off filter.

The participation of photogenerated ROS was tested by comparing spectral changes of photoirradiated solutions of the sensitizers plus the contaminants in aerated and deaerated solutions contained in 1 cm \times 1 cm quartz cells. The latter were Argon-bubbled by 20 min.

2.4. Laser flash photolysis experiments

Argon-saturated 0.04 mM Rf aqueous solutions were photolysed using a laser flash photolysis apparatus. A ns Nd:YAG laser sys-

tem (Spectron) at 355 nm was the excitation source, employing a 150 W Xenon lamp as analyzing light. The apparatus has been already described [27]

$^3\text{Rf}^*$ was generated by a 355 nm laser pulse, and the $^3\text{Rf}^*$ disappearance was monitored from the first-order decay of the absorbance at 670 nm, a zone where the interference from other possible species is negligible. The triplet decay was measured at low Rf concentration (typical 0.05 mM) and at low enough laser energy, to avoid self-quenching and triplet-triplet annihilation.

For the determination of the rate constant 3k_q , for interaction of $^3\text{Rf}^*$ -FFA, the Stern–Volmer expression (Eq. (12)) was employed:

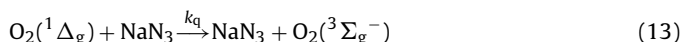
$$\frac{1}{3\tau} = \frac{1}{3\tau_0} + ^3k_q[\text{FFA}] \quad (12)$$

where 3τ and $3\tau_0$ are the experimentally determined lifetimes of $^3\text{Rf}^*$ in the presence and in the absence of FFA, respectively.

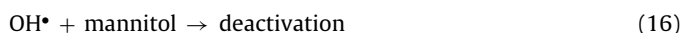
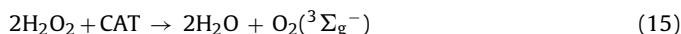
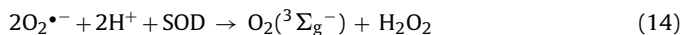
2.5. Oxygen uptake measurements

Oxygen uptake in water was monitored with a 97-08 Orion electrode. The photoirradiation set-up has been already described [28]. Rate values of oxygen uptake for each system represent the mean value of a set of three runs under identical conditions. All rate values of the set did not differ more than 5% of the mean value. Standard deviation values for the individual runs gave values lower than 1%. Nevertheless, we consider that $\pm 3\%$ as an error bar is a more realistic estimation that assists in the interpretation of the actual magnitude of the observed effects. The Rf sensitized photooxygenation rates of 3-OHP and PhOH were determined by evaluation of the initial slopes of substrate consumption through the decrease in the respective absorption bands at pH 7 and pH 11 for 3-OHP and at pH 7 for PhOH.

Auxiliary oxygen uptake experiments by the contaminants, photosensitized by Rf and/or HA, in the individual presence of 5 mM sodium azide, 1 $\mu\text{g}/\text{ml}$ superoxide dismutase, 1 $\mu\text{g}/\text{ml}$ catalase and 10 mM mannitol were performed. Similar experiments with ROS-interceptors have been formerly employed to confirm/discard the participation of $\text{O}_2^{\bullet-}$, hydrogen peroxide (H_2O_2) and hydroxy radical (OH^\bullet), respectively, in a given oxidative event [29–31]. Sodium azide is a well known physical quencher of the oxidative species $\text{O}_2(^1\Delta_g)$, with a pH-independent reported k_q value of $4.5 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ in pH 7 water [32] (reaction (13)).



The enzyme SOD dismutates the species $\text{O}_2^{\bullet-}$ (reaction (14)), whereas CAT decomposes H_2O_2 (reaction (15)) and mannitol deactivates the species OH^\bullet (reaction (16)).



3. Results and discussion

Zeng et al. [19] studied the effect of different concentration mixtures of Rf (up to 0.1 mM) and river humic acid (up to 80 $\mu\text{g}/\text{ml}$) on the photodegradation rate of 6-AC employing a UVA lamp as luminic source. In that case all components of the mixture, HA, Rf and 6-AC, absorbed light within the photoirradiation range. The presence of $>20 \mu\text{g}/\text{ml}$ HA in irradiated solutions containing 6-AC and Rf, inhibited the phototransformation, due to the inner filter effect exerted by HA. On this knowledge, our work was made under concentration conditions of the sensitizers in which the addition of absorbances of HA plus Rf within the irradiation wavelengths range ($>360 \text{ nm}$) was ca. 1.0 as a maximum value (Fig. 1, inset A), whereas

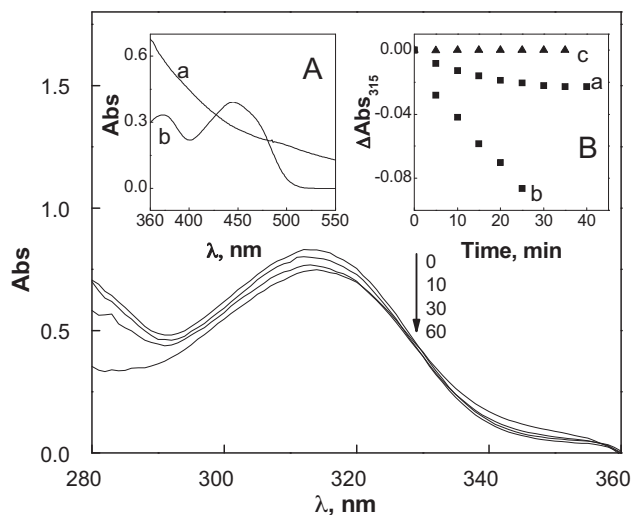


Fig. 1. Spectral changes in a pH 7 aqueous solution of 0.2 mM 3-hydroxypyridine upon photoirradiation in the presence of 0.04 mM riboflavin plus 50 $\mu\text{g}/\text{ml}$ humic acid. Numbers on the spectra represent irradiation time, in min. Inset A: absorption spectra of pH 7 aqueous solutions containing 50 $\mu\text{g}/\text{ml}$ humic acid (a) and 0.04 mM riboflavin (b). Inset B: absorbance changes at 315 nm as a function of photoirradiation time of a pH 7 aqueous solution containing 0.03 mM 3-hydroxypyridine plus 0.5 mM humic acid (a). The same as (a) for a pH 11 aqueous solution (b). The same as (b) in the presence of 5 mM NaN_3 (c).

as said, the Ar-OH and auxiliary substrates employed in this work do not absorb at all in this spectral region. Under these conditions, the simultaneous presence of HA and Rf as daylight photosensitizers could represent the case of superficial layers in lakes and water courses, where the concentration of the sensitizers is not sufficient to reach optically dense conditions.

The visible-light irradiation of air-equilibrated pH 7 aqueous solutions of 0.2 mM 3-OHP in the presence of 0.04 mM Rf plus 50 $\mu\text{g}/\text{ml}$ HA as sensitizers, modified the absorption spectrum of the substrate (Fig. 1). It can be seen as a decrease in the absorption band centered at 315 nm, qualitatively similar to that already described for the photoconsumption of the pyridine derivative due to Rf-sensitized photooxidation of 3-OHP [23].

The same experiment was performed for 3-OHP at pHs 7 and 11 in the absence of Rf, i.e. using only HA as a sensitizer. The respective rate profiles of photoconsumption show a much higher degradation rate for the OH-ionized species of 3-OHP (Fig. 2), being pK_a 3-OHP = 8.72 [33]. The photolysis of HA alone, at both pH values, only produced a comparatively slight absorbance decrease at the monitoring wavelength. No spectral changes at all were observed upon photoirradiation in the presence of 5 mM NaN_3 , as shown in Fig. 2 for 3-OHP + HA at pH 11 as a representative example.

Similarly, no photoconsumption of 3-OHP at all was observed upon irradiation of deaerated solutions in the presence of Rf + HA or HA as photosensitizers, within typical times employed under aerobic conditions.

Rf could not be employed in the alkaline pH range due to its known fast decomposition upon photoirradiation [34].

In separate experiments, oxygen uptake was monitored for the photoirradiated mixtures 0.5 mM 3-OHP plus 50 $\mu\text{g}/\text{ml}$ HA and 0.5 mM PhOH plus 50 $\mu\text{g}/\text{ml}$ HA both at pHs 7 and 11. A much higher rate of oxygen uptake was observed for both substrates in pH 11 solutions. The rate for oxygen uptake for a pH 7 solution containing 50 $\mu\text{g}/\text{ml}$ HA alone consumed oxygen at the same rate than in the presence of 0.5 mM PhOH, suggesting again the absence of reactivity of this substrate at neutral pH. The profiles of oxygen photoconsumption for phenol are shown in Fig. 2.

The rate of oxygen consumption by a solution composed by 50 $\mu\text{g}/\text{ml}$ HA plus 0.5 mM 3-OHP was neatly decreased in the pres-

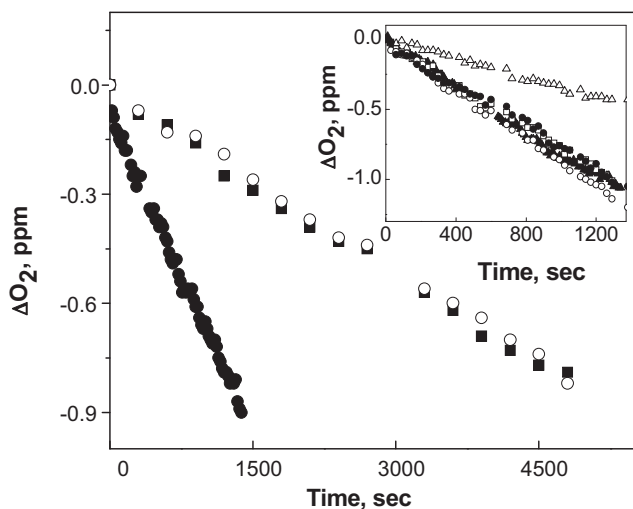


Fig. 2. Oxygen consumption as a function of photoirradiation time for: a pH 7 aqueous solution of 50 $\mu\text{g}/\text{ml}$ humic acid (\circ); the same in the presence of 0.5 mM phenol at pH 7 (\blacksquare) and a pH 11 aqueous solution of 50 $\mu\text{g}/\text{ml}$ humic acid plus 0.5 mM phenol (\bullet). Inset: oxygen consumption as a function of photoirradiation time for a pH 7 aqueous solution of 50 $\mu\text{g}/\text{ml}$ humic acid plus 0.5 mM 3-hydroxypyridine (\bullet). The same in the individual presence of: 0.5 mM NaN_3 (Δ); 1 $\mu\text{g}/\text{ml}$ SOD (\square); 1 $\mu\text{g}/\text{ml}$ CAT (\circ); 10 mM mannitol (\circ). In all cases cut off at 360 nm.

ence of 0.5 mM NaN_3 at pH 7 (Fig. 2, inset). The rate was practically not affected by the individual presence SOD, CAT and Mannitol (Fig. 2, inset).

The observed 4-fold decrease in the oxygen uptake rate in the presence of NaN_3 (Fig. 2, inset), approximately matches the expected quantitative effect due to the quenching of $\text{O}_2(^1\Delta_g)$ by the salt as evaluated by a simple calculation employing the Stern–Volmer equation ($\tau^0/\tau = 1 + k_q \tau^0 [\text{NaN}_3]$), with a lifetime $\tau^0 = 4 \mu\text{s}$ for the oxygen excited species in water [32]. τ^0 and τ are the respective lifetimes of $\text{O}_2(^1\Delta_g)$ in the absence and in the presence of 0.5 mM NaN_3 . Employing these data, a value $\tau^0/\tau = 4.5$ is obtained, close to the quotient of the corresponding oxygen uptake rates.

Oxygen consumption in neutral medium was reported by ourselves upon visible-light irradiation on system Rf – 3-OHP, attributed to a combination of $\text{O}_2(^1\Delta_g)$ and $\text{O}_2^{\bullet-}$ mediated photooxidation of the pyridine derivative [23]. The $\text{O}_2(^1\Delta_g)$ -driven rate constants values, obtained employing RB as a dye-sensitizer, indicate a highly pH-dependent process, being the respective k_r values 0.26 and $2.60 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ in unbuffered water and water pH 11 (process (8) [9]).

Following, we determined oxygen uptake rates upon photoirradiation of the system 0.5 mM 3-OHP with the mixture 0.04 mM Rf + 50 $\mu\text{g}/\text{ml}$ HA at pH 7 as a photosensitizing system. The rate of oxygen uptake suffers a delay in the presence of 1 $\mu\text{g}/\text{ml}$ SOD (Fig. 3, inset), whereas oxygen consumption is greatly inhibited by a concentration 5 mM of NaN_3 . As expected, experimental evidence at this point strongly supports the participation of the oxidative species $\text{O}_2(^1\Delta_g)$ and $\text{O}_2^{\bullet-}$ in the photooxidation of 3-OHP employing the mixture Rf + HA as photosensitizers. Both ROS are quenched through reactions (13) and (14), respectively.

In order to gain insight in the elucidation of the possible mechanism governing these systems an auxiliary experiment was performed, employing RB as a sensitizer. The xanthene dye is a recognized exclusive $\text{O}_2(^1\Delta_g)$ -generator, with a quantum yield of 0.81 for the production of the ROS [35]. We made comparative runs of oxygen uptake on pH 7 and pH 11 individual solutions of 0.5 mM 3-OHP and 0.5 mM PhOH in the presence of RB ($A_{530} = 0.5$). As a result, oxygen consumption was observed on 3-OHP at both pH values and only at pH 11 for PhOH (Fig. 3). This is not a surprising result, on

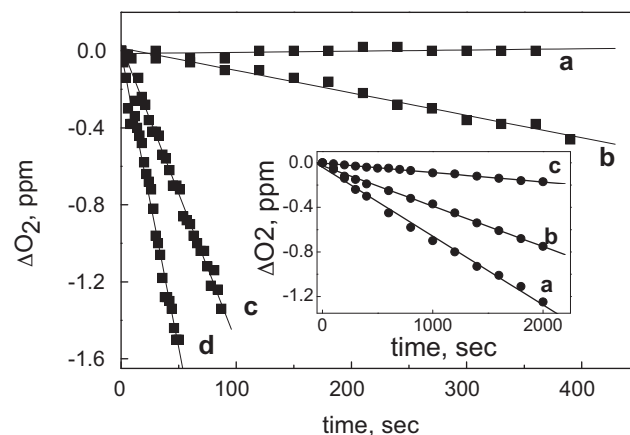


Fig. 3. Oxygen consumption as a function of photoirradiation time in the following aqueous solutions of RB ($A_{530} = 0.5$): in the presence of 0.5 mM phenol, pH 7 (a); in the presence of 0.5 mM 3-hydroxypyridine, pH 7; in the presence of 0.5 mM phenol, pH 11 (c); in the presence of 0.5 mM 3-hydroxypyridine, pH 11. Inset: oxygen consumption as a function of photoirradiation time in pH 7 aqueous solutions of 0.04 mM Rf in the presence of: 0.5 mM 3-hydroxypyridine (a); 0.5 mM 3-hydroxypyridine plus 1 $\mu\text{g}/\text{ml}$ superoxide dismutase (b); 0.5 mM 3-hydroxypyridine plus 5 mM sodium azide (c). In all cases cut off at 360 nm.

the basis that the molecular form of PhOH ($\text{p}K_a = 10$, [36]) is only a physical quencher of $\text{O}_2(^1\Delta_g)$ (process (7)) whereas the reported rate constant k_r (process (8)) for the partially ionized PhOH at pH 10.3, is $2.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ [32].

Aguer and Richard [12] and Kepczynski et al. [15], studied the photodegradation of PhOH in the presence of nanodispersed HA. The first authors did not observe PhOH phototransformation in neutral medium, whereas Kepczynski et al. reported that PhOH degradation is considerably effective in alkaline medium. Our experimental results employing RB as a dye sensitizer are in agreement with these findings, pointing to an exclusive $\text{O}_2(^1\Delta_g)$ -mediated degradation of PhOH when HA is employed as a sensitizer.

In the next set of experiments, we performed a series of photoirradiation runs employing 0.04 mM Rf, 50 $\mu\text{g}/\text{ml}$ HA and its mixture as sensitizing systems and 0.5 mM FFA as an oxidizable substrate. The furfuryl derivative quenches $\text{O}_2(^1\Delta_g)$ exclusively by the reactive fashion (reaction (11)) with a reported pH-independent k_r value of $1.2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ [37]. FFA, that does not absorb any light within the photoirradiation window, is one of the most frequently employed references in kinetic measurements of $\text{O}_2(^1\Delta_g)$ chemistry [32].

Fig. 4 clearly shows the photooxidation of the individual sensitizers, the sensitizing mixture HA + Rf and the effect of the individual sensitizers and the mixture on the photodegradation of FFA.

Next, we made a similar experiment of oxygen uptake replacing 0.5 mM FFA by 0.5 mM 3-OHP. Results, expressed as relative photodegradation rates, are shown in Fig. 4, inset A. The figure compares results for FFA and 3-OHP obtained under identical conditions. An arbitrary relative rate value = 1 was given to the rate of oxygen consumption by Rf + HA, the same for both set of runs. Of course, the rates for Rf and HA alone were also identical for both set of runs. The respective rates of oxygen uptake can be considered as a relative measure of the overall photooxidability of the substrates and sensitizers under work conditions.

From the observation of the data in Fig. 4, inset A, it arises that the relative rates of oxygen uptake by 3-OHP and FFA employing the sensitizing mixture Rf + HA do not behave in a parallel fashion. The rate for FFA is practically the same as the corresponding one for the addition of the rates employing the individual sensitizers Rf and HA. A similar analysis for the 3-OHP runs indicate a decrease of

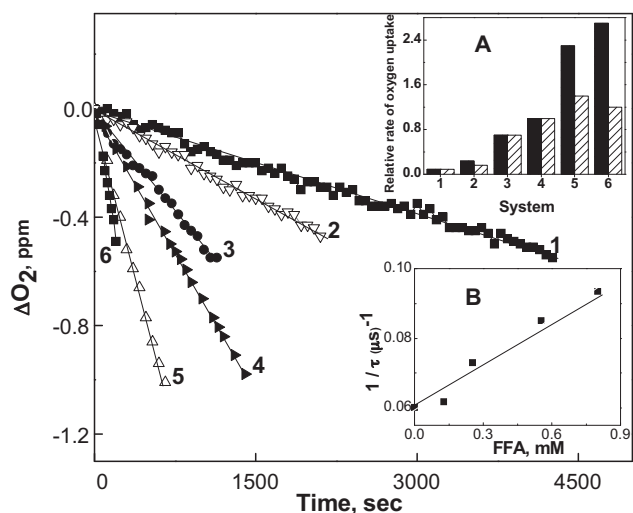


Fig. 4. Oxygen consumption as a function of photoirradiation time for the following pH 7 aqueous solutions: 50 $\mu\text{g}/\text{ml}$ humic acid (1); 50 $\mu\text{g}/\text{ml}$ humic acid plus 0.5 mM furfuryl alcohol (2); 0.04 mM riboflavin (3); 50 $\mu\text{g}/\text{ml}$ humic acid plus 0.04 mM riboflavin (4); riboflavin 0.04 mM plus 0.5 mM furfuryl alcohol (5); 50 $\mu\text{g}/\text{ml}$ humic acid plus 0.04 mM riboflavin plus 0.5 mM furfuryl alcohol (6). Inset A: bar diagram for relative rates of oxygen consumption upon sensitized photoirradiation of the solutions represented in the main figure (black bars), and the same solutions replacing 0.5 mM furfuryl alcohol by 0.5 mM 3-hydroxypyridine (lined bars). Inset B: Stern–Volmer plot for the quenching of triplet electronically excited riboflavin by furfuryl alcohol in pH 7 water. In all cases cut off at 360 nm.

the rate of oxygen consumption upon Rf + HA sensitization as compared to the simple addition of the rates for individual Rf and HA sensitization. The explanation to account for these differences lies on mechanistic grounds, and two possibilities can be envisaged: (I) the quenching of electronically excited Rf by the substrates, FFA, 3-OHP or even by HA, generating additional and/or different reactive species that could participate in the oxygen uptake circuit or (II) the presence of quenchers/deactivators of ROS that could selectively favour or inhibit a given oxygen consumer channel.

The quenching of $^1\text{Rf}^*$ may be disregarded: due to the short lifetime of the excited species ca. 5 ns [22], it cannot be intercepted by the substrates in the sub-mM concentration range. Regarding $^3\text{Rf}^*$, we reported earlier a rate constant of $8.9 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ for the quenching of the excited species by 3-OHP [23]. Through laser flash photolysis experiments, we determined in this work that $^3\text{Rf}^*$ is not quenched at all by HA up to 50 $\mu\text{g}/\text{ml}$ and that FFA quenches $^3\text{Rf}^*$ with a rate constant $k_{q3} = 4.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, the latter result obtained from the Stern–Volmer plot shown in Fig. 4, inset B.

The generation of $\text{O}_2^{\bullet-}$ by direct electron transfer from $^3\text{Rf}^*$ to dissolved oxygen (reaction (6)) is a very low quantum yield process and can be neglected. However, $\text{O}_2^{\bullet-}$ may be also produced from $\text{Rf}^{\bullet-}$ by the sequence of processes (7)+(8). This sequence operates in the case of 3-OHP [23] and could be possible also in the case of FFA. Process (8) can compete with the generation of $\text{O}_2(^1\Delta_g)$ (process (2)), and the operation of each reactive channel would depend, in principle, on the kinetic balance between processes (2) and (4). The energy-transfer process (3) occurs with a rate constant k_{ET} in water of $7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, equivalent to 1/9 of the diffusion-controlled value [38]. Taking into account the conditions used in the experiments with continuous irradiation ($[\text{FFA}] = [\text{3-OHP}] = 0.5 \text{ mM}$), with a similar value for the oxygen concentration in air-equilibrated water solution [39], and using the reported k_{q3} values 3-OHP [23] and the value herein obtained for FFA (8.9×10^8 and $4.4 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$, respectively) it can be estimated that: (a) the rate of process (5) is practically the same as the rate of $\text{Rf}^{\bullet-}$ generation – the main $\text{O}_2^{\bullet-}$ precursory species – for 3-OHP (process (4)) and (b) the rate of process (2) would be ca. 16

times faster than the corresponding one for process (4) in the case of FFA.

In other words, kinetic analysis indicates that upon Rf + HA photosensitization, 3-OHP is photodegraded by a combined mechanism of $\text{O}_2(^1\Delta_g)$ and $\text{O}_2^{\bullet-}$ whereas practically an exclusive $\text{O}_2(^1\Delta_g)$ -driven mechanism operates in the case of FFA.

On this basis, we can understand why the rate of oxygen consumption in the system FFA + Rf + HA is close to the simple addition of the respective rates employing the individual sensitizers. On the other hand, the observed delay in a similar comparison employing 3-OHP instead of FFA in the Rf + HA sensitization (Fig. 4 inset A) could be due to the already mentioned catalytic decomposition of the ROS $\text{O}_2^{\bullet-}$ by HA [26], only generated in the presence of 3-OHP. This reaction competes with 3-OHP by the oxidative species. As demonstrated in the preceding kinetic analysis, the generation and consumption of the species $\text{O}_2^{\bullet-}$ may be significant in the presence of the pyridine derivative.

4. Final remarks

As an overall result, and since the experiments of oxygen uptake for 3-OHP and FFA shown in Fig. 4 were made under identical experimental conditions, including the concentration of the oxidizable substrates, the respective oxygen uptake rates can be straightforwardly compared. In this context we can say that the visible-light-mediated photooxidation of 3-OHP at pH 7, sensitized by low Rf and HA concentrations, and standardized by comparison to the photooxidation rate of the known oxidizable target FFA, is an efficient process, reaching ca. one half of the oxidative rate for FFA (Fig. 4, inset A).

The degradation of PhOH only operates in the alkaline pH range, a quite different scenery from current environmental conditions.

Experimental evidence indicates that HA mainly photogenerates $\text{O}_2(^1\Delta_g)$ whereas Rf, in the presence of electron-donor substrates such as 3-OHP, produces also $\text{O}_2^{\bullet-}$. Hence, the degradation efficiency depends on the mechanism involved in the photoprocesses, and specially on eventual interactions of the substrates with electronically excited Rf.

A second important factor is represented by the absorbances exhibited by the photosensitizers in the visible-light spectral range. Concentrations of HA up to 50 $\mu\text{g}/\text{ml}$ in the presence of ca. 0.05 mM Rf constitute an adequate combination for the sensitizing mixture and relatively effective photodegradation of contaminants can be expected. Under these conditions Rf is the most effective photosensitizer. This combination Rf–HA represents an hypothetical situation in natural waters with relative transparency in the superficial layers, in which hydrophilic or hydrophobic organic contaminants could be primarily dissolved or suspended.

Noticeable photofading of the sensitizers was detected, when photolyzed in the absence of the contaminants within typical irradiation times employed for contaminants degradation. Nevertheless, the replenishing of HA and Rf is permanent due to the constant restoring cycle of both natural-water components under environmental conditions. Furthermore, their photodegradation is minimized by the competitive reactive scavenging of contaminants on photogenerated ROS. A typical picture could be represented by a relatively high local concentration of the contaminants and low concentration of the sensitizers. Under these conditions the photosensitizing process not only produces the contaminants degradation, but also exerts a protective effect against the bleaching of the very sensitizers.

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