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Waste-Derived Bioorganic Substances for Light-Induced Generation of Reactive Oxygenated Species

Alessandra Bianco Prevot,*[a] Paola Avetta,[a] Debora Fabbri,[a] Enzo Laurenti,[b] Tatiana Marchis,[b] Daniele G. Perrone,[c] Enzo Montoneri,[c] and Vittorio Boffa[c]

Urban waste-derived bioorganic substances (UW-BOS) have shown promise as chemical auxiliaries for a number of technological applications in the chemical industry and in environmental remediation. In this study, the application of these substances in the photodegradation of organic pollutants is addressed. The experimental work is specifically focused on the photolysis mechanism promoted by AC8, a UW-BOS isolated from a 2:1 w/w mixture of food and green residues, composted for 110 days, using 4-chlorophenol (4-CP) as probe molecule. The production of $^{\cdot}OH$ and the $^1O_2$ is monitored by EPR spectroscopy. The correlation between radical species evolution and photodegradation of 4-CP is investigated. The effect of $^1O_2$ and $^{\cdot}OH$ scavengers on the 4-CP degradation process is also checked. The results suggest that the role of these species in the photodegradation of 4-CP depends on AC8 concentration. AC8 is thereby proven to be a photosensitizer for applications in environmental remediation. The results on AC8 further support the use of urban bio-waste as a versatile source of chemical auxiliaries of biological origin for use in diversified applications.

Introduction

Urban bio-wastes (UW) have been reported to contain soluble bioorganic substances (BOS) that show promise as chemical auxiliaries for a number of technological applications in the chemical industry and in environmental remediation.[1–6] As part of a project[7] aimed at promoting sustainable products and processes from UW at industrial level, we report herein the potential of urban refuse-derived bioorganic substances (UW-BOS) as photosensitizers for environmental applications.

In the last thirty years, great attention has been devoted to light-induced oxidation processes for environmental remediation,[8–12] particularly for the treatment of waste waters. These techniques are based on the generation by light energy of highly reactive species, mostly $^{\cdot}OH$ radicals, able to promote the degradation of organic pollutants into nontoxic or less toxic products, and ultimately their mineralization. Natural organic matter present in water and soil contains bioorganic substances (SW-BOS) that have light-absorbing power. They have been demonstrated to be able to promote photochemical reactions and have been widely studied in order to understand water auto-purification mechanisms.[13–18] In principle SW-BOS could be recovered from soil and then added to specific polluted wastewaters to enhance and/or promote the photodegradation of contaminants. However, due to their poor concentration in soil and water, isolation of SW-BOS would be rather costly. Recent work[5,19,20] has demonstrated that UW-BOS also have good photosensitizing properties. Contrary to soil and water, urban wastes are a rather appealing source of photosensitizers for several reasons. As result of increased production due to urbanization, urban wastes are concentrated in confined areas by municipal collection. In addition, depending on the type of treatment and on composition, they may provide high yields of a large variety of bio-based products fitting a wide range of uses (Figure 1). UW-BOS are chemically similar to SW-BOS. They are described[7] as likely mixtures of substances with apparent molecular weights in the range $1–3\times10^5$ Da, formed by long aliphatic carbon chains substituted by aromatic rings and functional groups such as COOH, CON, C=O, PhOH, O–alkyl, O–aryl, OCO, OMe, and NRR′, (R and R′ = alkyl or H). These organic moieties, represented in the virtual molecular fragment shown in Figure 1, are most likely remnants of the main constituents of the source bioorganic refuse matter, which are not completely mineralized by microbial degradation. The proposed mechanism for SW-BOS photoactivity can thus also be proposed for UW-BOS; it is based on the production, under UV/Vis irradiation, of excited triplet states that can in turn react with organic substrates by two main mechanisms: hydrogen transfer and energy transfer.[18,20,21]

A group of UW-BOS was previously shown to perform efficiently as sensitizers for the photodegradation of azo-dyes.[5] The presence of dissolved oxygen was also found to favor the degradation process. Hydrogen transfer was hypothesized as the main mechanism responsible for the photosensitization; generated hydrogen atoms can be transferred to the dissolved

[a] *Prof. A. Bianco Prevot, Dr. P. Avetta, Dr. D. Fabbri*
Dipartimento di Chimica Analitica, Università di Torino
Via P.Giuria 5, 10125 Torino (Italy)
Fax: (+39) 116707615
E-mail: Alessandra.biancoprevot@unito.it

[b] *Dr. E. Laurenti, T. Marchis*
Dipartimento di Chimica IFM, Via P.Giuria 7, 10125 Torino (Italy)

[c] *D. G. Perrone, Prof. E. Montoneri, Dr. V. Boffa*
Dipartimento di Chimica Generale e Chimica Organica, Università di Torino
Corso Massimo d'Azeglio 48, 10125 Torino (Italy)

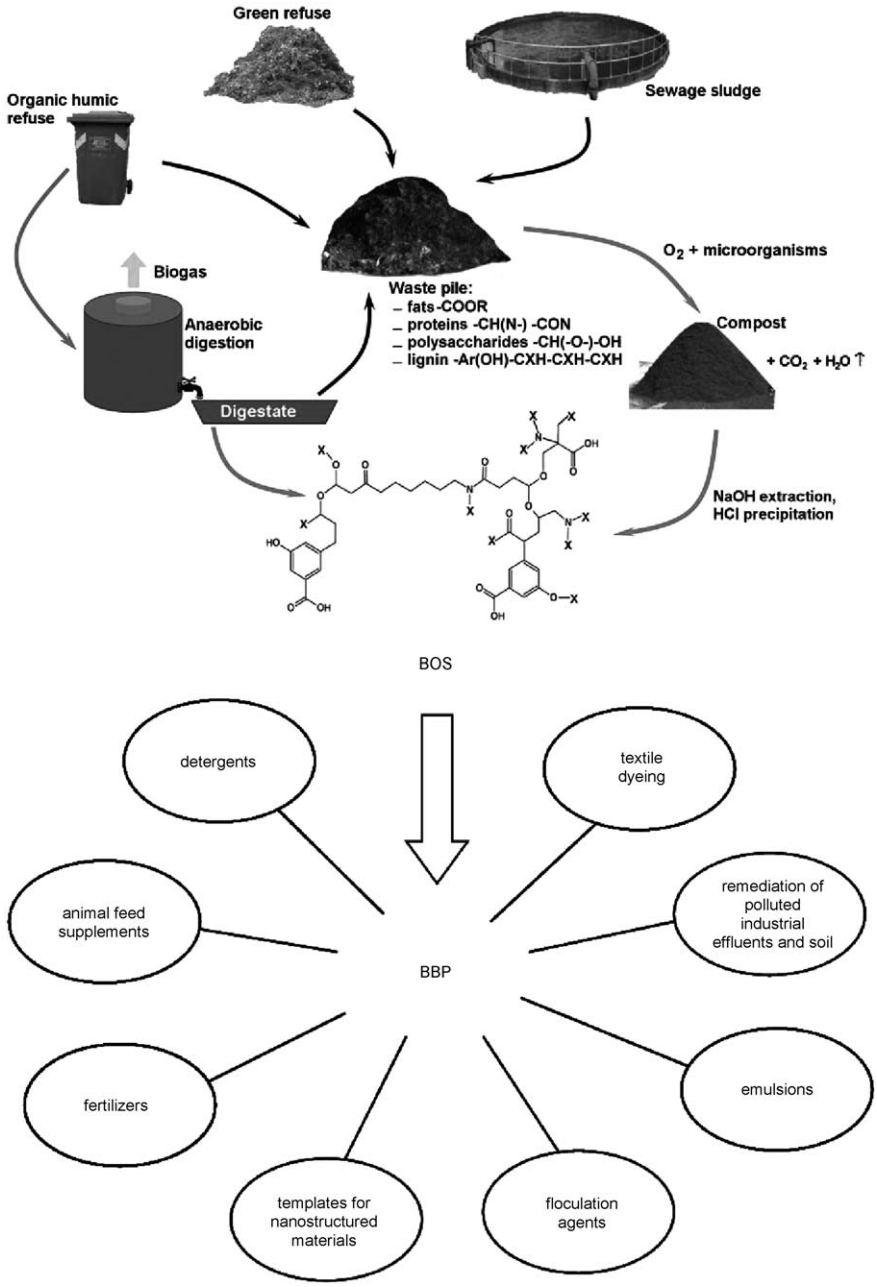


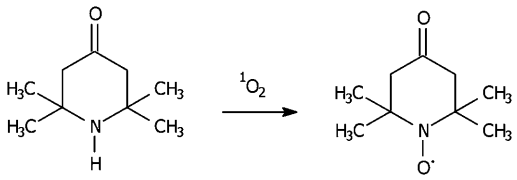
Figure 1. Sourcing bio-refuse, isolated soluble bioorganic substances (BOS; R = alkyl, X = H or C, N, O atoms of other molecular fragments) and related bio-based products (BBP); virtual molecular fragment represents the identified C moieties in BOS (see Experimental Section).

oxygen with the formation of various reactive oxygenated species (ROS) that, in turn, contribute to the degradation of the probe molecule. Particularly, $^{\cdot}OH$ and $^1O_2$, are of prime importance for their contribution to degradation of organic pollutants in irradiated aqueous solutions.[22] However, direct evidence of this reaction mechanism was not obtained, although knowledge of the reaction mechanism was considered important to optimize and address the use of UW-BOS in specific applications.

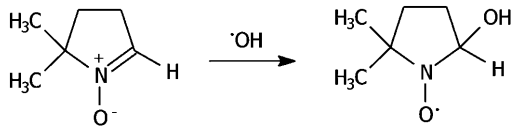
In previous studies concerning photo-assisted degradation processes in water in the presence of natural organic matter, the study of $^1O_2$ and $^{\cdot}OH$ formation and evolution has been studied mainly by using probes/scavengers or by EPR spectroscopy.[16,23,24] In the first case, suitable molecules react selectively with the species under investigation, yielding a specific reaction product (in the case of probes) or inhibiting the kinetics of the photodegradation process of a target organic molecule. In the second case, the detection and identification process involves the formation of a persistent spin-adduct radical from a compound acting as spin-trap and the target species (Schemes 1 and 2).

These adducts have distinctive EPR spectra in which the signal intensities allow estimation of the amount of trapped radicals. However, both approaches suffer from possible drawbacks of indirect methods. Scavengers are not usually specific for a single radical. Spin-adduct formation reactions may be affected by low yield and/or insufficient product stability.

In the present research, the photosensitizing properties of one type of UW-BOS, namely AC8, were studied using 4-chlorophenol (4-CP) as probe molecule. The production of $^{\cdot}OH$ and $^1O_2$ was monitored by EPR spectroscopy. The correlation between the evolution of radical species and the photodegradation of 4-CP was investigated. Finally, the effect of $^1O_2$ and $^{\cdot}OH$ scavengers on the 4-CP degradation process was analyzed.



Scheme 1. Formation of spin-adduct 4-oxo-TMP–$^1O_2$ (4-oxo-TEMPO; 4-oxo-TMP = 2,2,6,6-tetramethyl-4-piperidone hydrochloride).



Scheme 2. Formation. of spin-adduct DMPO–OH (DMPO = 5,5-dimethyl-1-pyrroline-*N*-oxide).

Results and Discussion

AC8 chemical structure

In the present study, a UW-BOS (AC8) was isolated from food and green wastes mixed in a 1:2 weight ratio. The refuse mixture was piled and periodically turned to promote aerobic biodegradation of the bioorganic matter. After 110 days, the biomass was reduced to one third of its initial volume. Indeed, during this biodegradation treatment, part of the refuse organics was completely mineralized, whereas lignin and other recalcitrant biopolymers underwent structural modifications becoming soluble in alkali. AC8 was extracted from the residual refuse by a facile procedure (see Experimental Section).

Once isolated, AC8 was characterized according to a previously reported procedure.[2] The AC8 functional group distribution, the relative ratios of which are represented in the virtual molecular fragment in Figure 2, are given in Table 1.

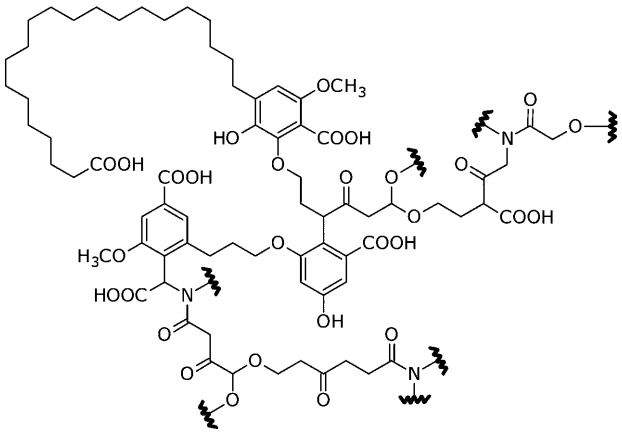


Figure 2. Virtual molecular fragment fitting analytical data from Table 1.

Table 1. AC8 functional group distribution.

Functional group	Concentration/meq g^{-1}
aliphatic C	17.1
OMe	0.0
ammine C (NC)	2.9
O–alkyl C (OR)	4.0
di-O–alkyl C (ROCOR)	1.3
aromatic and/or olefinic C (C=C), excluding PhO	6.8
phenol (PhOH)	1.3
phenyl ether (PhOX, X = R, Ar)	2.5
carboxylic acid C (COOH)	3.8
amide C (CON)	0.0
keto C (C=O)	1.6

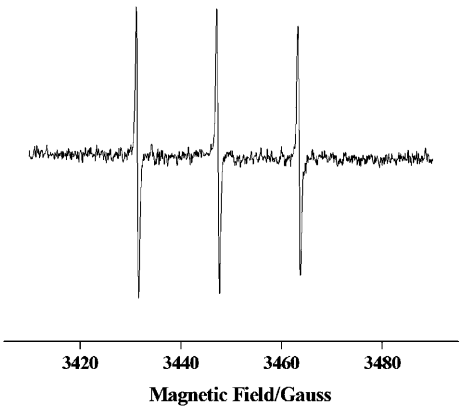


Figure 3. EPR spectrum of 4-oxo-TMP–$^1O_2$ adduct. AC8 concentration = 2000 mg L^{-1}; TMP concentration = 45 mM; irradiation time = 15 min.

EPR analysis of AC8

Figure 3 shows a typical EPR spectrum of the 4-oxo-TMP-$^1O_2$ spin adduct (Scheme 1) obtained by irradiating a 2000 mg L^{-1} aqueous solution of AC8 in the presence of 45 mM TMP. The spectrum shows the typical triplet, with a 16.1 G hyperfine splitting constant. To optimize the irradiation time before the EPR measurement, two sets of experiments were performed at 20 and 150 mg L^{-1} AC8 concentration and irradiation times ranging from 0 to 120 min.

Figure 4 shows that the signal intensity for both AC8 concentration values increases upon increasing the irradiation time to reach a maximum value at 15 min irradiation and then decreases. This behavior is likely to arise from a progressive degradation of 4-oxo-TMP and/or of 4-oxo-TEMPO, due to their reactions with other reactive species produced during the AC8 irradiation. For this reason, the successive EPR experiments were performed after 15 min of irradiation. Figure 4 also shows that the 4-oxo-TEMPO signal intensity at 150 mg L^{-1} AC8 concentration is higher than that at 20 mg L^{-1} over the whole irradiation-time experimental range. Thus, to assess the

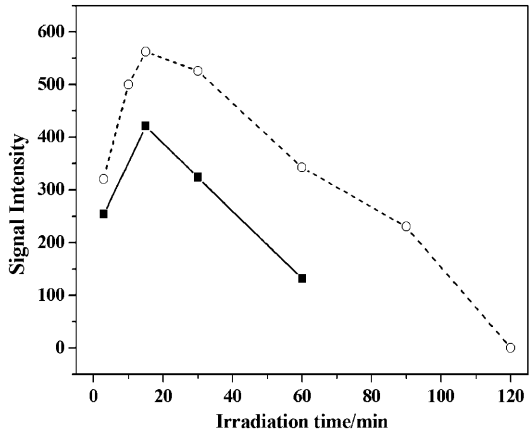


Figure 4. Intensity of 4-oxo-TEMPO EPR spectrum versus irradiation time. AC8 concentration = 20 mg L^{-1} (■) and 150 mg L^{-1} (○); signal intensity calculated as average of the triplet spectral lines.

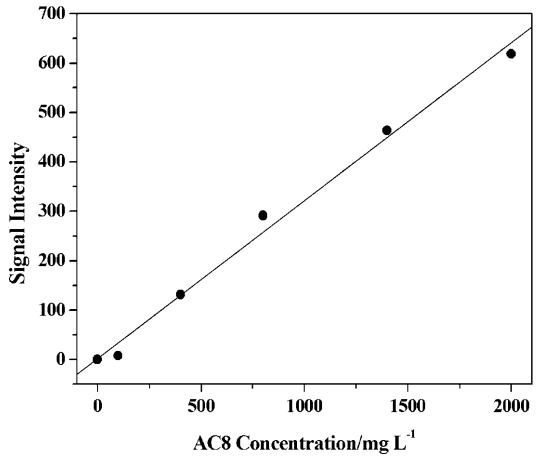


Figure 5. Intensity of 4-oxo-TEMPO EPR signal, after background subtraction, versus AC8 concentration. 4-oxo-TMP concentration = 45 mM; irradiation time = 15 min. Linear fit of data; slope = 0.3198, intercept = 1.7913, $r^2 = 0.9920$.

AC8 concentration effect on $^1O_2$ production, further irradiation experiments were performed in the 0–2000 mg L^{-1} AC8 concentration range.

The observed trend of the signal intensity (Figure 5) suggests that $^1O_2$ production is directly proportional to AC8 concentration. Other experiments were performed to evidence

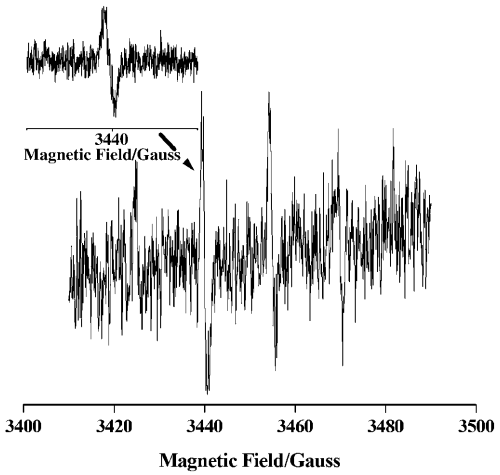


Figure 6. EPR spectrum of DMPO–OH adduct EPR. AC8 concentration = 30 mg L^{-1}; DMPO concentration = 17,4 mM; irradiation time = 3 min.

the formation of OH radicals. The typical EPR spectrum of the DMPO–OH spin-adduct (Figure 6) formed according to Scheme 2 is a 1:2:2:1 quartet, with a 14.8 G hyperfine splitting constant. Due to the overall poor signal/noise ratio, for quantitative analysis the line showing the highest signal/noise ratio was chosen as an analytical tool and the number of accumulated scans was increased without changing the scan rate or recording time. This procedure led to a significant improvement of the signal/noise ratio (Figure 6, inset).

Compared to the $^1O_2$ detection (Figure 4), for hydroxyl radical determination, the effect of irradiation time on DMPO–OH signal intensity showed a similar behavior in a time range of 3–60 min. Moreover, there was a significant increase in the background spectral intensity arising from the solvent, leading to a worsening of the signal/noise ratio. The irradiation time occurring between the spin-trap addition and the EPR measurement was thus fixed at 3 min.[16]

Figure 7 shows the intensity of the EPR spectrum of the DMPO–OH adduct with respect to AC8 concentration. In contrast to 4-oxo-TEMPO (Figure 5), the DMPO–OH adduct spectrum intensity reaches a maximum value at about 20 mg L^{-1} and then decreases at higher AC8 concentrations. This profile can be explained hypothesizing that two different processes, both production and scavenging of $^{\cdot}OH$ by irradiated AC8, occur in this case.

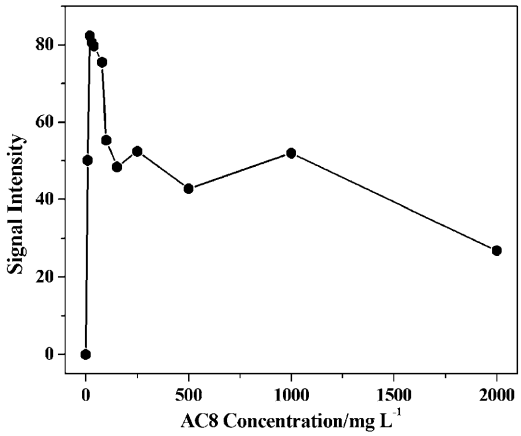


Figure 7. Intensity of DMPO–OH EPR signal intensity versus AC8 concentration. DMPO concentration = 17.4 mM; irradiation time = 3 min.

Photodegradation of AC8 is proven by the significant decrease of the UV/Vis absorbance with irradiation time (Figure 8). This process involving radical species may well contribute to the decrease of hydroxyl radicals available for the

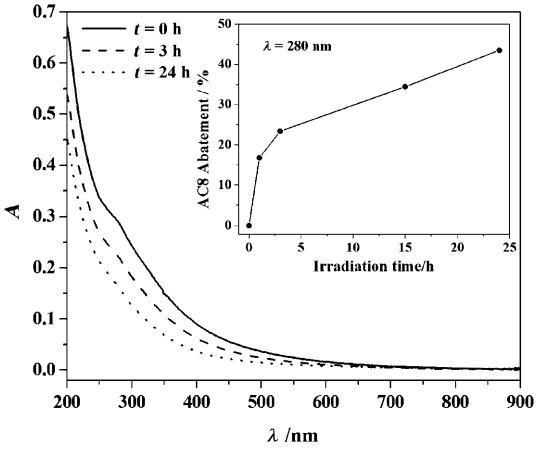


Figure 8. UV/Vis spectrum evolution of AC8 (150 mg L^{-1}) at different irradiation times recorded after diluting the samples by a factor of 10. Inset: AC8 abatement versus irradiation time.

DMPO–OH adduct formation and thus cause the behavior observed in Figure 7.

4-Chlorophenol photodegradation

4-CP was chosen as a model compound to investigate the existence of a correlation between the kinetics of its photodegradation, the $^1O_2$ and OH radical production, and the addition of $^1O_2$ and OH radical scavengers.

Preliminary experiments performed on 4-CP aqueous solutions, irradiated up to six hours in the absence of AC8, showed that direct photolysis of 4-CP does not occur to an appreciable level (Figures 9 and 10). Afterwards, the substrate (5 mg L^{-1}) was irradiated in the presence of AC8 at two different concentrations; 100 mg L^{-1}, whereby low $^1O_2$ production but the highest $^{\cdot}OH$ production take place (Figures 5 and 7, respectively), and 1200 mg L^{-1}, whereby the ratio between $^1O_2$ and $^{\cdot}OH$ production is greatly increased. At 100 mg L^{-1} AC8 concentration, in the absence of scavengers, after 24 h irradiation about 65% of 4-CP is degraded (Figure 9). Based on the relatively high $^{\cdot}OH$ production (Figure 7) and low $^1O_2$ production (Figure 5), it could be hypothesized that $^{\cdot}OH$ is mainly responsible for the 4-CP degradation.

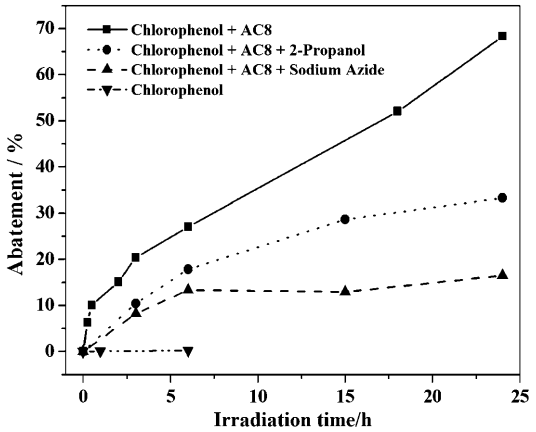


Figure 9. Degradation of 4-CP (5 mg L^{-1}) in the presence of AC8 (100 mg L^{-1}), 2-propanol (0.01 M) and sodium azide (0.03 M).

The addition of 2-propanol, a $^{\cdot}OH$ scavenger, led to significant reduction of the 4-CP abatement from 65% to 33%, in agreement with the previous hypothesis. However, in the presence of sodium azide, a $^1O_2$ scavenger, the 4-CP abatement decreased from 65% to 15%. Since sodium azide is mainly reported as s highly selective $^1O_2$ scavenger but is also known to react with OH radicals, this result could be explained if both $^{\cdot}OH$ and $^1O_2$ play an active role in the 4-CP degradation, and the higher effect of sodium azide could thus be explained on the basis of its additional $^{\cdot}OH$-scavenging property.

The 24 h irradiation of 4-CP in the presence of 1200 mg L^{-1} AC8 concentration and in the absence of scavengers, yielding a 75% abatement, is slightly more efficient than that at the lower AC8 concentration (Figure 10). In agreement with the results obtained with 100 mg $L^{-1}$of AC8, the 4-CP degradation

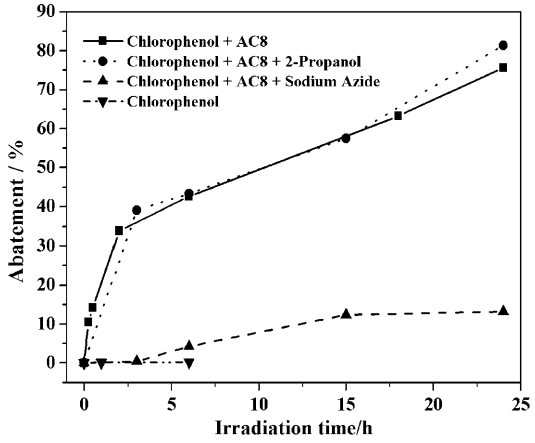


Figure 10. Degradation of 4-CP (5 mg L^{-1}) in the presence of AC8 (1200 mg $^{-1}$), 2-propanol (0.01 M) and sodium azide (0.03 M).

could be due to both $^{\cdot}OH$ and $^1O_2$ (Figures 5 and 7). However, only sodium azide seemed to significantly inhibit the photodegradation process, whereas the addition of 2-propanol had no appreciable effect at this concentration. The sodium azide effect seems consistent with the high $^1O_2$ production, about 40 times higher than that at 100 mg L^{-1} AC8 concentration. The negligible effect of 2-propanol at 1200 mg L^{-1} AC8 concentration may suggest that, in this case, $^{\cdot}OH$ does not play a significant role in 4-CP degradation and indirectly confirms that singlet oxygen is mainly responsible for 4-CP degradation. These results suggest that the role played by OH radicals and singlet oxygen in 4-CP degradation is strictly related to their relative production and depends on the AC8 concentration.

Conclusion

Information about radical species involved in the AC8 photosensitizing mechanism and evidence of its good performance as a photosensitizer in 4-CP photodegradation have been obtained. The results offer scope for extending the investigation to other BOS materials obtained from different UW sources (Figure 1) to assess possible source–property relationships of BOS photosensitizers, and thus to help focus product application to specific cases. Together with previous results,[1–5] this study further supports the use of urban bio-wastes as a versatile source of chemical auxiliaries of biological origin for use in diversified applications.

Experimental Section

Materials

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone hydrochloride (4-oxo-TMP), 4-CP, 2-propanol, sodium azide, acetonitrile, and potassium hydroxide were purchased from Aldrich. Ultrapure water was obtained from a Millipore Milli-Q™ system. The AC8 bioorganic substance was extracted as previously reported[8] by aqueous alkali followed by precipitation at pH < 1.5 with HCl from refuse supplied by ACEA Pinerolese. This company runs a waste treatment plant performing anaerobic digestion and/

or aerobic digestion of the organic humid fraction of urban refuse obtained by separate source collection practice and of sewage sludge (Figure 1). The AC8 sourcing material was the product of the aerobic digestion line fed with a 2:1 food/green residues mixture aged for 110 days. This material was characterized by the supplier by 35.2% humic and 46.0% w/w (referred to dry matter) volatile solids content.

Irradiation and analytical procedures

All samples (5 mL) were placed in Pyrex closed cells and irradiated, under continuous stirring, with a 1500 W xenon lamp (Solarbox, Co. Fo. Megra, Milan, Italy) equipped with a 340 nm cut-off filter. EPR spectra were registered at room temperature with a Bruker ESR 300E spectrometer operating at X-band and equipped with a flat quartz EPR cell. The acquisition parameters were as follows: Frequency = 9.69 GHz, microwave power = 5.024 mW, center field = 3450 G ($^1O_2$) or 3440 G ($^{\cdot}OH$), sweep width = 80 G ($^1O_2$) or 20 G ($^{\cdot}OH$), number of scans = 62 ($^1O_2$) or 120 ($^{\cdot}OH$), receiver gain = 1×10^5, modulation amplitude = 0.52 G, conversion time = 40.96 ms ($^1O_2$) or 10.24 ms ($^{\cdot}OH$). 4-oxo-TMP (45 mM) and DMPO (17.4 mM) were employed as trapping agent for $^1O_2$ and $^{\cdot}OH$ study, respectively (Schemes 1 and 2). All experiments were carried out by adding the spin trap to the cell before irradiating AC8. EPR spectra were acquired immediately after the irradiation. The degradation of 4-CP was monitored by HPLC, employing a Merck-Hitachi instrument, equipped with Lichrospher RP-C18 (125 mm × 4 mm i.d., particle diameter = 5 μm, from Merck), L-6200 pumps and UV/Vis L-4200 detector. Elution was carried out with 60:40 v/v water/acetonitrile solvent fed at 1 mL min^{-1} flow rate. The analytical detector wavelength was 225 nm. A CARY 100 SCAN-VARIAN spectrophotometer was used to follow the AC8 photobleaching.

Keywords: biomass · phenols · photodegradation · reactive intermediates · sensitizers

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