Available online at www.sciencedirect.com
ScienceDirect
Journal of Photochemistry and Photobiology A: Chemistry 185 (2007) 277–282
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Photocatalytic degradation of paraquat and genotoxicity of its intermediate products

Maria Jlenia Cantavenera [a], Irene Catanzaro [b], Vittorio Loddo [a], Leonardo Palmisano [a,*], Giulia Sciandrello [b]

[a] *Dipartimento di Ingegneria Chimica dei Processi e dei Materiali, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy*
[b] *Dipartimento di Biologia Cellulare e dello Sviluppo, Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy*

Received 5 May 2006; received in revised form 20 June 2006; accepted 21 June 2006
Available online 28 June 2006

Abstract

The photocatalytic degradation of paraquat (1,1-dimethyl-4,4′-bipyridylium dichloride) aqueous solutions in the presence of polycrystalline TiO_2 Degussa P25 irradiated by near-UV light was investigated. The substrate and total organic carbon concentrations were monitored by UV spectroscopy and TOC measurements, respectively: the complete photocatalytic mineralization of paraquat (20 ppm) was achieved after ca. 3 h of irradiation by using 0.4 g l^{-1} of catalyst amount at natural pH (ca 5.8). On the contrary no significant photodegradation of paraquat was observed in the absence of TiO_2 under similar experimental conditions. To evaluate the genotoxicity of paraquat and its intermediates produced during heterogeneous photocatalytic treatment, in vitro tests such as Ames test, with and without rat liver microsomal fractions (S9 mix), and micronucleus test, were used. Results obtained with Salmonella typhimurium (strain TA100) showed that paraquat and photocatalytic products were unable to induce gene mutations when photocatalysis was used in the presence of the optimum amount of TiO_2, i.e. 0.4 g l^{-1}, whereas an increase of revertants his^+ per plate was observed after 300 min irradiation in the presence of very low amount of TiO_2 (0.04 g l^{-1}). The negative results from micronucleus test suggest that mutagenic, but non-clastogenic, late intermediates of paraquat photo-oxidation were formed when the photocatalytic runs of paraquat degradation were carried out by using 0.04 g l^{-1} of photocatalyst.

Keywords: Photocatalytic; TiO_2; Paraquat; Genotoxicity

1. Introduction

Paraquat is one of the most widely used herbicides in the world. It acts by interfering with the photosynthesis desiccating the green parts of all plants with which it comes into contact and its adsorption, through the leaf surface, is increased by high light intensity and humidity. The site of action is the chloroplast, in particular paraquat reacts with electrons produced by the photosystem I and produces free radicals that damage cellular membranes and then plant's tissues. Also it is known that paraquat is toxic for human and animals [1] and its action is due to lipid peroxidation [2] by generation of free radicals such as superoxide ($^{\bullet}O_2^-$) and hydroxyl ($^{\bullet}OH^-$) and hydrogen peroxide (H_2O_2) that are involved in the initiation of membrane damage [3]. As herbicide, paraquat has been available to farmers for over 40 years and it is present as an environmental pollutant both in soil and in surface waters, due to its wide utilization.

Therefore, it appears important to avoid environmental contamination and to look for new systems of remediation. Among several options, the development of processes that can transform a large number of toxic into inactive compounds is one of the most promising alternatives [4–6]. Heterogeneous photocatalysis is an advanced not selective oxidation process, which has been successfully used to oxidize many organic pollutants present in aqueous systems [7–11]. It is characterized by the photoproduction of radicals ($^{\bullet}OH$) through a multistep process. The use of TiO_2 semiconductor as the catalyst for the photo-oxidation of organic compounds has received much interest because not only TiO_2 can be used both as polycrystalline powder and a film onto suitable supports, but also because it is inexpensive, (photo)chemically stable and innocuous. Complete mineralization of different types of organic molecules, including several herbicides as for example monuron (3-(4-chlorophenyl)-1,1-dimethylurea) has been reported [11].

* Corresponding author. Tel.: +39 0916567246; fax: +39 0916567280.
E-mail addresses: palmisano@dicpm.unipa.it (L. Palmisano), gscian@unipa.it (G. Sciandrello).

1010-6030/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2006.06.021

Therefore, heterogeneous photocatalysis offers a great potentiality to detoxify wastewater producing H_2O, CO_2 and inorganic species as the final products.

It is worth noting that, although many papers report the successful photodegradation of various organic molecules by using heterogeneous photocatalysis, only few investigations have been carried out with the aim to evaluate the toxicity of the effluents before, during and after their treatment [12].

The purpose of the present study has been not only to assess the efficiency of heterogeneous photocatalysis to mineralize paraquat by using polycrystalline TiO_2 (mainly in the anatase phase) as the photocatalyst [13,14] or TiO_2 films [15,16], but also to check if genotoxicity turns up during and/or after the treatment, due to formation of intermediate species more noxious than the starting substrate. Bioassays (fish, invertebrates, cell cultures, bacterial or algae) have been traditionally used to investigate the toxicity of persistent organic pollutants [17,18]. Different bioassays have been used to measure several different genotoxic endpoints because it is not possible to detect all forms of toxicity in a single assay. To this aim the genotoxicity of paraquat and its photocatalytic products was assessed by two screening tests: the Ames test [19] to measure the induction specific locus-gene mutation in Salmonella typhimurium, and micronucleus test to measure the induction of DNA breakage in mammalian cells [20]. In fact, it is known that paraquat has the capability to induce chromosomal aberration and sister chromatid exchange in Chinese hamster cells in a dose-dependent manner [21,22], but non-gene mutations in Salmonella typhimurium [23].

2. Experimental

2.1. Photocatalytic experiments

Aqueous solution of Paraquat with different concentrations, ranging from 5 to 25 mg l^{-1} were prepared to obtain a calibration curve of absorption versus concentration (error: ±0.5 ppm), selecting the wavelength of 257 nm that corresponds to the maximum absorption of the herbicide.

Photocatalytic degradation experiments were carried out in a 0.5 l Pyrex batch cylindrical photoreactor with immersed lamp, continuously bubbling oxygen in the suspension and using amounts of polycrystalline TiO_2 Degussa P25 (ca. 80% anatase; 20% rutile) ranging between 0.04 and 0.4 g l^{-1}. The suspensions were placed inside the photoreactor and before irradiation they were mixed for ca. 30 min by using a magnetic stirrer to achieve the adsorption/desorption equilibrium of the pesticide. The artificial radiation source was a medium pressure mercury 125 W lamp (Helios Italquartz, Milano), with an irradiance of ca. 14 mW cm^{-2}. Samples of the suspensions were withdrawn at selected time intervals and their UV spectra were recorded with a UV–visible Shimadzu spectrophotometer Model UV-2401 PC after separation of the photocatalyst by using 0.1 μm filters (Millex, Millipore). Non-purgeable organic carbon (NPOC) analyses were performed at fixed intervals of time with a Shimadzu 5000A instrument (error: ±1 ppm).

The quantitative determination of anionic species was performed by using an ionic chromatograph system (Dionex DX 120) equipped with an Ion Pac AS14 4 mm column (250 mm long, Dionex). Aqueous solution of $NaHCO_3$ (8 mM) and Na_2CO_3 (1 mM) was used as eluent at a flow rate of 1.67×10^{-2} cm^3 s^{-1}. The quantitative determination of ammonia was performed by an ion sensitive electrode (Orion mod. 9512) in an expandable ion analyser (Orion EA 920).

2.2. Ames test

For all assays, Salmonella typhimurium strain TA100, kindly supplied by Prof. Roberto Barale from University of Pisa, was grown in 20 ml nutrient broth (Oxoid no 2) with ampicillin (0.1 ml of 8 mg/ml ampicillin solution, Sigma–Aldrich) in a shaking incubator at 37 °C until the suspension reached a density of about 10^9 cells/ml (measured as absorbance at 650 nm). From this suspension, 100 μl were added to 2 ml molten overlayer agar (supplemented with 18% glucose and 0.2 ml of 0.5 mM histidine/biotin solution) together with 50 μl of one of the sample from the photocatalytic runs, in absence or in presence of 500 μl S9 mix, and poured onto minimal-agar petri dishes. The inverted plates were held at 37 °C in a dark incubator for 48 h and then the revertant colonies were scored. The strain was tested without any addition of foreign compounds as negative control and with 1 μg/plate methyl methane sulfonate (MMS, CAS 66273 Sigma–Aldrich) as positive control to confirm that the assay worked efficiently. A sample was considered mutagenic when an increase of the number of revertants up to the double one on respect to the negative control was reached. The number of spontaneous revertants ranged between 100 and 200 for strain TA100, under the experimental conditions used. Each determination was carried out in triplicate and two independent experiments were carried out at least.

For metabolic activation, S9 mix was prepared on ice immediately before its usage by adding phosphate buffer at pH 7.4, NADP, G-6-P, KCl solution, $MgCl_2$ solution up to the final concentrations of 0.1 M, 4, 6, 8, 33 mM, respectively. Subsequently 10% rat liver S9 (Araclor-1254 induced, Trinova Biochem.) was added.

2.3. Cell culture

V79 Chinese hamster cells were cultured in D-MEM (BioChrome, Berlin, Germany), supplemented with 5% fetal calf serum (Invitrogen), penicillin (100 U/ml) and streptomycin (100 μg/ml) and maintained at 37 °C in a 5% CO_2 humidified incubator.

2.4. Micronucleus test

Cells (2×10^4) were plated on 18 mm × 18 mm glass cover slips in 60 mm Petri dishes and, after 18 h seeding, they were treated for 6 h with samples deriving from the photocatalytic run performed with 0.04 g l^{-1} of TiO_2. Similar tests were carried out by using the aqueous solution, as positive control, containing paraquat concentrations ranging from 18.22 to 1.40 μg/ml. At the end of the treatment, the cells were washed twice with Hanks' salt solution; sample cells were checked for viability

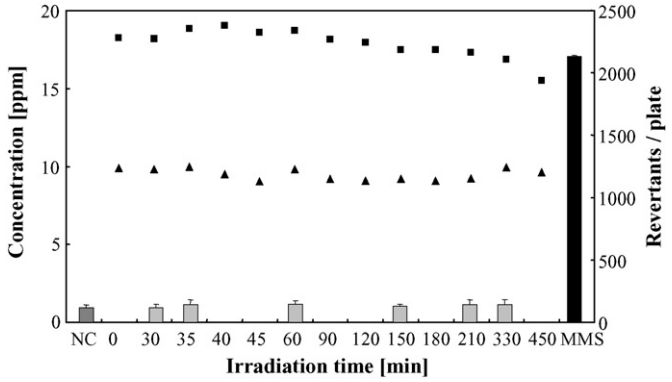


Fig. 1. Paraquat (■) and TOC (▲) concentrations vs. irradiation time. Histograms indicate the number of revertants/plate. NC: negative control, i.e. untreated bacteria. MMS: positive control, i.e. bacteria treated with methyl methane sulfonate.

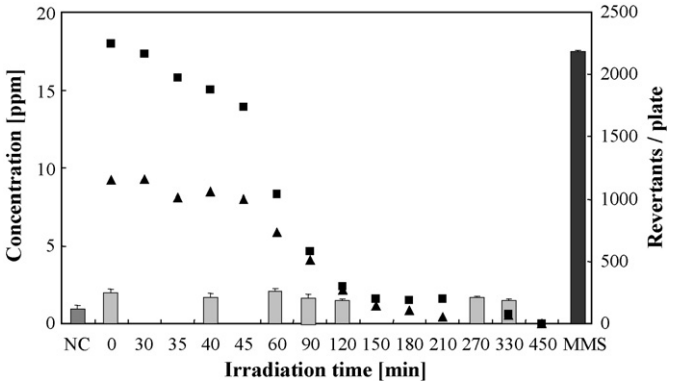


Fig. 2. Paraquat (■) and TOC (▲) concentrations vs. irradiation time (TiO_2 amount 0.4 g l^{-1}). Histograms indicate the number of revertants/plate. NC: negative control, i.e. untreated bacteria. MMS: positive control, i.e. bacteria treated with methyl methane sulfonate.

after treatment by Trypan Blue (Sigma–Aldrich) exclusion. Viability of untreated and treated cells was always greater than 90%. After 6 h recovering in fresh medium, cells were prefixed with methanol, added to the culture medium in the ratio 1:1 for 5 min at room temperature, fixed with methanol for 7 min and then stained with 2.5% Giemsa (Gurr) or with 5 μg/ml Hoechst 33258 (Sigma–Aldrich) in distilled water. At least 1000 cells of each culture were examined to determine nuclear abnormalities. Each determination was made in triplicate and two independent experiments were carried out at least.

2.5. Optical microscopy

An epi-fluorescence microscope (Nikon Microphot-FXA/SA) equipped with a HBO 100 mercury lamp and a suitable filter, was used to visualize the blue (Hoechst-stained) fluorescence cells. Photomicrographs were processed using Adobe Photoshop 6.0 software.

2.6. Statistical analysis

The results from bioassays were analyzed with the χ^2-test [24].

3. Results and discussion

Fig. 1 shows paraquat and total organic carbon (TOC) concentrations versus irradiation time for a run performed in homogeneous system. The experiment indicated that no degradation occurred when the solution was irradiated in the absence of catalyst. A blank test in the dark and in the presence of TiO_2 showed similar results.

Paraquat was degraded only in the contemporaneous presence of oxygen, photocatalyst and light. Fig. 2 reports the results from a run carried out in the presence of 0.4 g l^{-1} TiO_2 (Degussa P25).

It can be observed an increase of both paraquat degradation and mineralization rates after an induction time of 45–60 min. An almost complete disappearance of paraquat and TOC was achieved after ca. 3 h.

Analyses carried out by ionic chromatography and by ion sensitive electrode revealed the presence of nitrate ions and ammonia at the end of the experimental runs.

Fig. 3 shows some absorbance spectra recorded versus irradiation time during the same run. It can be noticed that the absorption band at 257 nm decreases during the irradiation time, while some absorption at ca. 220 nm slightly grows and then decreases, due probably to formation of intermediate species subsequently mineralized.

Similar results were obtained by using 0.08 g l^{-1} of TiO_2, although a longer time was needed to achieve the mineralization (data not shown for the sake of brevity).

Photodegradation occurred instead more slowly by using a lower amount of TiO_2 (0.04 g l^{-1}) and 8–10 h of irradiation (Fig. 4) were needed to obtain a decrease of TOC down to ca. 1 ppm. Fig. 5 shows the absorbance spectra recorded during the run carried out with a catalyst amount of 0.04 g l^{-1}.

It can be noticed, still after 270 min, the presence of the band at 254 nm due to paraquat and some absorption at 220 nm due to some unknown degradation intermediates. The different behaviours found by carrying out experimental runs with different catalyst amounts could be due to different numbers of active sites on the photocatalytic surface. Indeed, when the number of

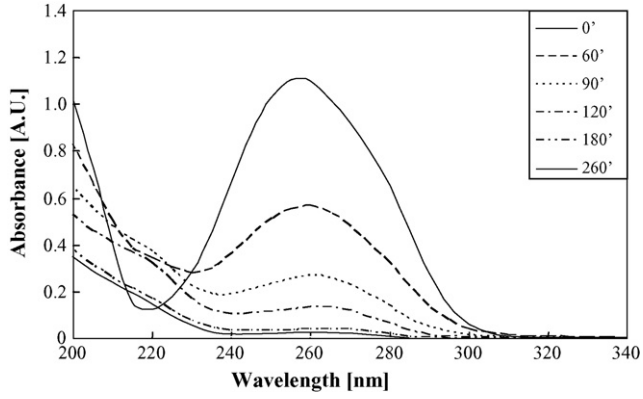


Fig. 3. Absorbance spectra of samples withdrawn at increasing irradiation times for a representative run. Initial paraquat concentration: 20 ppm; TiO_2 amount: 0.4 g l^{-1}.

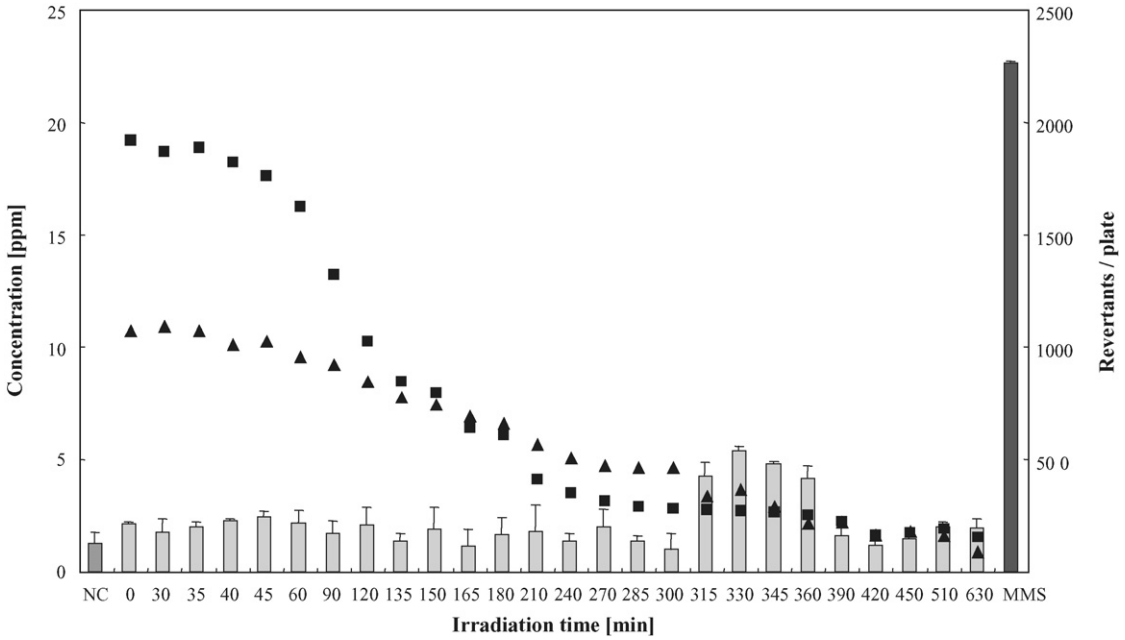


Fig. 4. Paraquat (■) and TOC (▲) concentrations vs. irradiation time (TiO_2 amount 0.04 g l^{-1}). Histograms indicate the number of revertants/plate. NC: negative control, i.e. untreated bacteria. MMS: positive control, i.e. bacteria treated with methyl methane sulfonate.

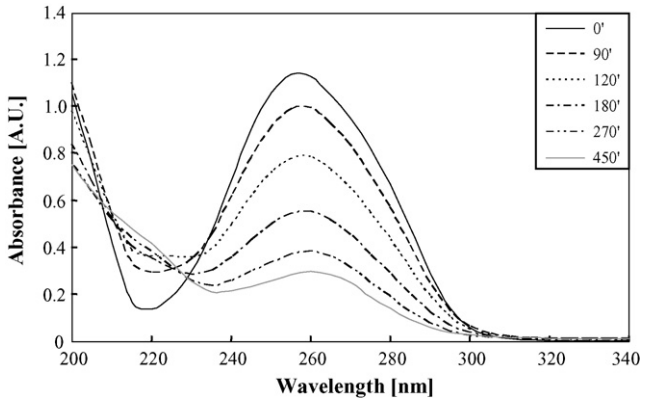
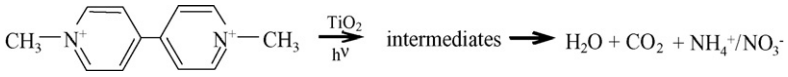


Fig. 5. Absorbance spectra of samples withdrawn at increasing irradiation times for a representative run. Initial paraquat concentration: 20 ppm; TiO_2 amount: 0.04 g l^{-1}.

active sites is lower than the optimum one, the substrate could compete with its degradation intermediates for the adsorption on the catalytic surface, favouring the accumulation of late intermediates and the observed decrease of degradation rate. Moreover, the low number of active sites could induce a modification of the reaction pathway(s).

A simple scheme is reported below to represent the complete mineralization of paraquat:



Samples irradiated in the absence of TiO_2 or in the presence of 0.4 and 0.08 g l^{-1} of photocatalyst did not show any genotoxicity in the Ames test; indeed the number of revertants per plate was very close to the 100–200/10^8 spontaneous revertants of strain TA100. The above findings confirm that paraquat and its photocatalitic products are unable to induce gene mutation with or without metabolic activation [23]. On the contrary, samples collected from 315 to 390 min of irradiation during heterogeneous photodegradation in the presence of low concentration of TiO_2 (0.04 g l^{-1}), showed a significant increase of revertants per plate. Table 1 shows, for the sake of comparison, the results obtained with samples collected from 300 to 390 min irradiation using different photocatalyst amounts.

The observed positive response strongly suggests that the lowest photocatalyst amount induced the formation of some unknown late genotoxic intermediates during the photodegradation of paraquat, although up to date we do not know their chemical nature. It is worth noticing that they were completely degraded at the end of the photocatalytic treatment because the number of revertants per plate returned down to the spontaneous background.

Micronucleus test was utilized in order to further test photocatalytic samples deriving from the run carried out in the presence of 0.04 g l^{-1} of TiO_2. In particular some samples that resulted negative (0, 180, 450 min) or positive (330 min) in the Ames test, were selected to assay their clastogenic activity.

Micronucleus is DNA structure that can be formed by either chromosomal fragment or whole chromosome. This material derives from DNA breaches that during the cell division is distributed only to one daughter, but it is excluded from the main nucleus. Therefore, in order to evaluate the formation of micronuclei, the cell must be allowed to progress through the cellular division and micronuclei can be seen in the resulting interphase cells.

Table 1
Revertants induced by photocatalytic samples obtained under different experimental conditions

TiO_2 (mg/l)	Irradiation time (min)					
	300	315	330	345	360	390
0	125 ± 33	148 ± 47	156 ± 31	137 ± 19	149 ± 43	135 ± 33
0.04	101 ± 9	424 ± 26*	539 ± 105*	481 ± 18*	415 ± 21*	160 ± 31
0.08	174 ± 6	167 ± 14	149 ± 6	184 ± 12	141 ± 3	169 ± 31
0.4	152 ± 27	132 ± 39	167 ± 20	140 ± 12	168 ± 11	158 ± 30

* $p < 0.001$ statistically significant differences vs. negative control (126 ± 23) according to χ^2-test.

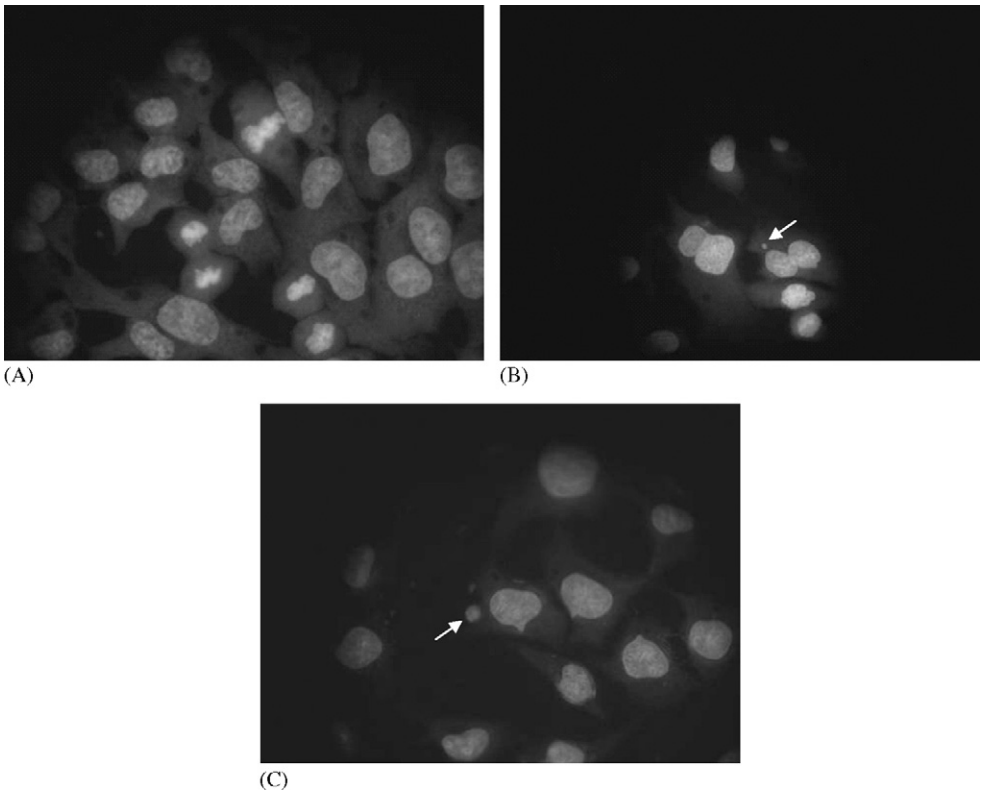


Fig. 6. Optical micrographs of V79 Chinese hamster cells stained with Hoechst 33258 (bisBenzimide, H, Sigma): (A) untreated cells; (B and C) cells treated with paraquat. Magnification: (A) 40×, (B) 40×, and (C) 60×.

The micronucleus test confirmed the clastogenic potential of paraquat [25,26]. In fact, when cells were treated with paraquat for 6 h followed by 6 h of recovery time, the frequency of micronucleated cells decreased depending on the herbicide concentrations (Fig. 6); in particular 18.22 μg/ml of paraquat induced a ca. three times increase of micronucleated cell in comparison to untreated cells (3.02 ± 0.2 versus 1.13 ± 0.3). On the contrary, when cells were treated with samples collected at the beginning and after 180 min of the photocatalytic experiment, a statistically significant induction of micronuclei was observed (Fig. 7).

The above results confirm that bubbling oxygen may primarily contribute to the induction of micronuclei by paraquat, according to Sawada et al. [27]. It is worthy to be pointed out that the positive sample in the Ames test (330 min) was unable to induce micronuclei and this means that the detected genotoxicity can be attributable to mutagenic, not clastogenic intermediates of paraquat photodegradation underlining the importance to utilize bioassays to measure different genotoxic endpoints.

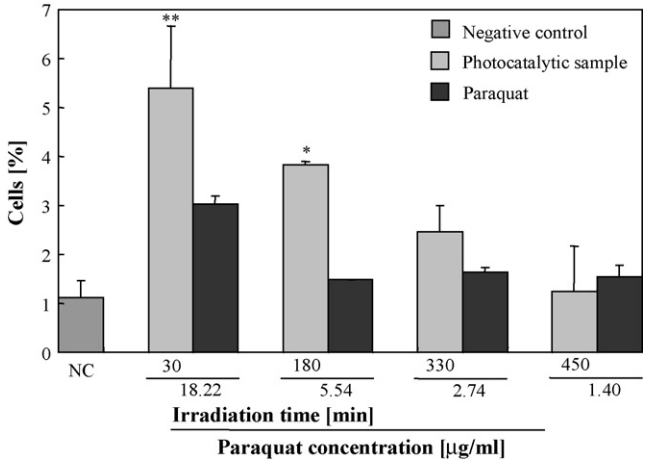


Fig. 7. Frequencies of micronucleated cells (%) induced by photocatalytic samples at different irradiation times (in the presence of O_2) and by different paraquat concentrations. Statistically significant differences vs. untreated cells are indicates as $^{**}p < 0.001$, $^{*}p < 0.01$ according to χ^2-test. The standard deviations per each determination are also indicated.

4. Conclusions

Paraquat was successfully oxidized by heterogeneous photocatalysis. Micronuclei tests showed clastogenic activity for paraquat. The results of photocatalytic runs carried out with an optimum photocatalyst amount did not show any genotoxicity. Nevertheless by using a photocatalyst amount one order of magnitude lower than the optimum one, genotoxic intermediates were formed and subsequently degraded, thus indicating that the reaction pathway was different under these experimental conditions. The results reported demonstrate that the possible production of genotoxic species should be carefully taken into consideration when a photocatalytic process is going to be planned especially for application purposes.

Acknowledgement

AA wish to thank MIUR for financial support.

References

[1] T.J. Haley, Clin. Toxicol. 14 (1979) 1–46.
[2] J.S. Bus, S.D. August, J.E. Gibson, Biochem. Biophys. Res. Commun. 58 (1974) 749–755.
[3] Paraquat IPCS Diquat, Environmental Health Criteria 39, WHO, Geneva, 1984, pp. 13–128.
[4] W.J. Weber Jr., E.J. LeBouef, Water Sci. Technol. 40 (2002) 11–19.
[5] A. Kunz, H. Mansilla, N. Duran, Environ. Technol. 23 (2002) 911–918.
[6] A. Kunz, P. Peralta-Zamora, N. Duran, Adv. Environ. Res. 7 (2002) 197–202.
[7] M. Schiavello (Ed.), Heterogeneous Photocatalysis, John Wiley & Sons, New York, 1995.
[8] N. Serpone, E. Pelizzetti (Eds.), Photocatalysis. Fundamentals and Applications, John Wiley & Sons, New York, 1989.
[9] A. Fujishima, K. Hashimoto, T. Watanabe, TiO_2 Photocatalysis: Fundamentals and Applications, Bkc, Tokyo, 1999.
[10] V. Augugliaro, V. Loddo, M.J. López-Muñoz, G. Marcì, L. Palmisano, J. Catal. 166 (1997) 272–283.
[11] V. Augugliaro, L. Cavallero, G. Marcì, L. Palmisano, E. Pramauro, Stud. Surf. Sci. Catal. 82 (1994) 713–720.
[12] A. Kunz, J. Reyes, N. Duran, Proceedings of the International Conference on Differential Pollution, Australian Waste Water Association, Perth, Australia, 1999, pp. 525–531.
[13] M.H. Florêncio, E. Pires, A.L. Castro, M.R. Nunes, C. Borges, F.M. Costa, Chemosphere 55 (2004) 345–355.
[14] M. Kang, Appl. Catal. B: Environ. 37 (2002) 187–196.
[15] K. Tennakone, I.R.M. Kottegoda, J. Photochem. Photobiol. A: Chem. 93 (1996) 79–81.
[16] E. Moctezuma, E. Leyva, E. Monreal, N. Villegas, D. Infante, Chemosphere 39 (1999) 511–517.
[17] S.S. Rao, B.A. Quinn, B.K. Burnison, M.A. Hayes, C.D. Metcalfe, Chemosphere 31 (1995) 3553–3566.
[18] P.S. Melo, N. Duran, M. Haun, Toxicol. Lett. 16 (2001) 237–242.
[19] D.M. Maron, B.N. Ames, Mutat. Res. 113 (1983) 173–215.
[20] W. Frieauff, F. Pötter-Locher, A. Cordier, W. Suter, Mutat. Res. 413 (1998) 57–68.
[21] T.M. Nicotera, A.W. Block, Z. Gibas, A.A. Sandberg, Mutat. Res. 151 (1985) 263–268.
[22] W.E. Ross, E.R. Block, R.Y. Chang, Biochem. Biophys. Res. 91 (1979) 1302–1308.
[23] M. Bignami, A. Carere, G. Conti, L. Conti, R. Crebelli, E. Dogliotti, G. Gualandi, A. Novelletto, V.A. Ortali, Mutat. Res. 68 (1979) 183–193.
[24] J. Stuart Wolf Jr., D.S. Smith, Urology 47 (1996) 2–13.
[25] G.G. Ortiz, R.J. Reiter, G. Zuñiga, D. Melchiorri, E. Sewerynek, M.I. Pablos, C.S. Oh, J.J. García, O.K. Bitzer-Quintero, Mutat. Res. 464 (2000) 239–245.
[26] A.C.C. Rios, D.M.F. Salvadori, S.V. Oliveira, L.R. Ribeiro, Mutat. Res. 328 (1995) 113–118.
[27] M. Sawada, T. Sofuni, M.J. Ishidate, Mutat. Res. 197 (1988) 133–140.