Journal of Photochemistry and Photobiology B: Biology 135 (2014) 48–54

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

On the natural fate of maleic hydrazide. Kinetic aspects of the photochemical and microbiological degradation of the herbicide

Adriana Pajares [a,b,*], Mabel Bregliani [b], Walter Massad [c], José Natera [c], Cecilia Challier [c], Laura Boiero [d], Mariana Montenegro [d,e], Norman A. García [c,*]

[a] Facultad de Ingeniería, Universidad Nacional de la Patagonia SJB, 9000 Comodoro Rivadavia, Argentina
[b] Unidad Académica Río Gallegos, Universidad Nacional de la Patagonia Austral, 9400 Río Gallegos, Argentina
[c] Departamento de Química, Universidad Nacional de Río Cuarto, 5800 Río Cuarto, Argentina
[d] Facultad Regional Villa María, Universidad Tecnológica Nacional, 5900 Villa María, Argentina
[e] Instituto de Ciencias Básicas y Aplicadas, Universidad Nacional de Villa María, 5900 Villa María, Argentina

ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form 5 March 2014
Accepted 7 April 2014
Available online 18 April 2014

Keywords:
Biodegradation
Humic acid
Maleic hydrazide
Photodegradation
Riboflavin

ABSTRACT

Kinetic and mechanistic aspects of the photochemical and microbiological degradation of the herbicide Maleic Hydrazide (MH) have been studied.

Riboflavin (Rf, vitamin B_2) was employed as a main photosensitizer whereas Humic Acid (HA) was included as a second sensitizer in order to more closely simulate natural environmental conditions. MH quenches excited singlet and triplet states of Rf, with rate constants close to the diffusion limit. The herbicide and dissolved molecular oxygen competitively quench triplet excited Rf. As a consequence the reactive oxygen species (ROS), superoxide radical anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and singlet molecular oxygen ($O_2(^1\Delta_g)$) are produced by electron- and energy-transfer processes, respectively, as demonstrated by auxiliary experiments employing selective auxiliary quenchers and the exclusive $O_2(^1\Delta_g)$ generator Rose Bengal (RB). As a global result, the photodegradation of Rf is retarded, whereas MH is degraded by the generated ROS. The bacteria *Pseudomonas aeruginosa* (Ps) and *Bacillus subtilis* (Bs), recognized as contaminants surface-water and soil and microbial antagonists of phytopathogenic, were used in the microbiological experiments. Results of the individual incubation of both bacteria in in the presence of MH indicate a stimulation on the Ps growth, implying the biodegradation of the herbicide, whereas MH only exerted a bacteriostatic effect on Bs.

1. Introduction

Many agricultural biocides of profuse use have molecular structures with a six-membered nitrogen-containing aromatic heterocycles. Significant amounts of these compounds could contaminate surface waters and soils [1,2,3]. MH (Scheme 1), a plant growth regulator and herbicide that acts by inhibiting cell division in plants, belongs to this class of chemicals [4]. High doses of its potassium salt appear to be genotoxic. Besides, some relatively recent laboratory studies demonstrated that the pesticide may cause liver and kidney toxicity in mice [5]. In this context, all possible information about the fate of the pesticide under environmental conditions is very important, due to its profuse employment in crop protection and other practical applications.

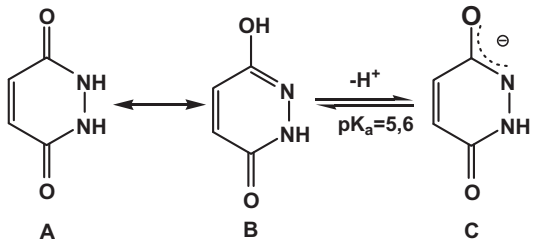
After release into the environment, pesticides in general may have different destines, being unavoidable its immediate exposition to natural light and native microorganisms. As a consequence, systematic studies on the kinetics of photo- and bio-degradation pathways of such a contaminants are topics of growing interest that could help to foresee the environmental decay of these compounds [1, 6, 7].

According to our knowledge, only two works were published on direct MH photoirradiation. Stoessl, in 1964, described the photodegradation of MH in aqueous solution, being a number of acidic compounds, including succinic, maleic and fumaric acids, identified as photoproducts [8]. Recently, Reva et al., studied the UV-induced photoisomerization of maleic hydrazide. UV irradiation of matrix-isolated maleic hydrazide induced two isomerization processes yielding the dihydroxy tautomer and N-aminomaleimide [9].

The optical absorption spectrum of MH is extended up to 370 nm. This fact predicts a limited extent for the direct degradation of the herbicide by the action of direct sunlight irradiation. For this class of compounds an interesting possibility of photodeg-

* Corresponding authors. Address: Facultad de Ingeniería, Universidad Nacional de la Patagonia SJB, 9000 Comodoro Rivadavia, Argentina (A. Pajares). Tel.: +54 586 4676439; fax: +54 586 4676233 (N.A. García).
E-mail address: ngarcia@exa.unrc.edu.ar (N.A. García).

http://dx.doi.org/10.1016/j.jphotobiol.2014.04.002
1011-1344/



Scheme 1. Accepted tautomeric forms and acid–base equilibria for MH.

radation to be explored is through a photosensitized process. This means the irradiation of the polluted medium in the presence of a photosensitizer, using light of wavelengths longer than those absorbed by the pollutants. This procedure takes advantage of the presence in surface natural waters of some colored compounds that can act as the primary environmental light-absorbers. Traces of sensitizers are usually present in water courses, lakes and seas. Among them, HA and, in special, the pigment Rf are relevant in the sensitized photooxidation of contaminants [10,11,12]. Both HA and Rf generate $O_2(^1\Delta_g)$, with quantum yields of 0.04 (upper limit) and 0.47, respectively [13,14]. The vitamin also generates $O_2^{\cdot-}$ with a quantum yield of 0.009 [15].

As an attempt to standardize the photodegradability of MH upon Rf-photosensitization, its stability was compared with those of two recognized molecules: phenol (ph), a paradigmatic water-contaminant model compound and tryptophan (trp), one of the most studied biological targets in photodynamic action.

Regarding the possible bio-degradation route of MH, many soil and water microorganisms have the ability to degrade certain pesticides [16]. Since the disappearance of contaminants due to microbial degradation obeys to different general conditions, the feasibility and effectiveness of such a process must be studied as a particular case for the different contaminant-microorganism combinations.

On this basis, the purpose of the present research work was to evaluate the rates, and mechanism involved in the photo and bio-degradation of the pesticide MH under simulated environmental conditions.

In doing this, we employed Rf and HA as potential natural sensitizers for the degradation of MH. For the biological assays the microorganisms Ps and Bs were used. Ps is a natural contaminant bacteria of surface waters whereas Bs is frequently found in soils and marine waters [17,18,19]. Both bacteria can exert antagonistic effects on certain phytopathogens, through the production of bacteriocins or antibiotics that adversely affect the development thereof, being able to be employed in the biological control of plagues [20–22].

2. Materials and methods

2.1. Materials

Maleic hydrazide, Riboflavin, superoxide dismutase (SOD) from bovine erythrocytes and catalase (CAT) from bovine liver, were purchased from Sigma Chem. Co., Rose Bengal, furfuryl alcohol (FFA), deuterium oxide (D_2O; 99.9 atom% D), phenol, L-tryptophan and humic acid were from Aldrich (Milwaukee, WI, USA). Sodium azide (NaN_3) was from Merck. The chemicals K_2HPO_4, Na_2HPO_4, KH_2PO_4, NH_4Cl, NaCl, NaOH, $MgSO_4$, $CaCl_2{\cdot}6H_2O$, $NaHCO_3$, and $FeCl_3{\cdot}6H_2O$ were provided by Cicarelli (Santa Fe, Argentina). Glucose peptone and cetrimide agar were from Britania (Buenos Aires, Argentina). Argon (99.9 purity) was provided by Oxígeno Unión (Río Cuarto, Argentina).

All these compounds were used as received. Water was triply distilled and Buffered KH_2PO_4, $NaHCO_3$, and solutions (each 0.01 M) were employed for pHs/pDs 7 and 9 [23]. In all the cases, pHs/pDs were controlled with a MP220 Mettler-Toledo pH-meter.

2.2. Absorption and fluorescence measurements

Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer. Steady-state fluorescence was measured with a Spex Fluoromax spectrofluorimeter at 25 ± 1 °C in air-equilibrated solutions. Excitation and emission wavelengths for Rf were 445 and 515 nm, respectively.

2.3. Continuous photolysis

Continuous aerobic photolysis of aqueous solutions containing Rf or RB as photosensitizers plus different substrates were carried out in a PTI (Photon Technology International) unit provided with a high pass monochromator and a 150-W Xe lamp, irradiating at 440 ± 10 nm, or, for non-monochromatic irradiation, in a home-made photolyser with a 150-W quartz-halogen lamp with a cut-off filter (>400 nm).

The reactive rate constant, k_r, for the chemical reaction of $O_2(^1\Delta_g)$ was determined as described in the literature [24], using the expression $\text{slope}/\text{slope}_R = k_r\,[MH]/k_{rR}\,[R]$, for which the knowledge of the reactive rate constant for the photooxidation of a reference compound, R, at similar concentration, is required. The reference R was FFA, with a reported pH-independent k_{rR} value in water of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ [25].

Slope and slope_R are the respective slopes of the first-order plots of oxygen consumption by the substrate and the reference compound, under sensitized irradiation. Oxygen uptake in water was monitored with a 97-08 Orion electrode. Employing RB as a sensitizer, it was assumed that the reaction of $O_2(^1\Delta_g)$ with MH is the only way of oxygen consumption.

2.4. Laser flash photolysis experiments

Argon-saturated aqueous solutions of Rf 0.04 mM were irradiated with a flash photolysis apparatus. A ns Nd:YAG laser system (Spectron) at 355 nm was used for excitation, employing a 150-W Xenon lamp as a source for the analyzing light. The detection system comprised a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital oscilloscope (Hewlett–Packard 54504A), was transferred via a HPIB parallel interface to a PC where it was analyzed and stored. The disappearance of $^3Rf^*$, a species generated by the 355 nm pulse, 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 decay was measured at low Rf concentration (typically 0.05 mM) and at low enough laser energy to avoid self-quenching and triplet–triplet annihilation.

2.5. Time resolved phosphorescence detection (TRPD) of $O_2(^1\Delta_g)$

The overall quenching rate constant (k_t) for the deactivation of $O_2(^1\Delta_g)$ by MH was determined using a previously reported system [26]. This rate constant is the sum of k_q, the rate constant for physical quenching of $O_2(^1\Delta_g)$ plus the already described k_r. A Nd:YAG laser (Spectron) was employed for the excitation (532 nm) of the sensitizer RB ($Abs_{532} = 0.4$), and the emitted radiation ($O_2(^1\Delta_g)$ phosphorescence at 1270 nm) was detected at right angles using an amplified Judson J16/8Sp germanium detector, after passing through two Wratten filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer for the signal processing. Usually, 16 shots were needed for averaging,