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Detoxification of wastewater containing five common pesticides by solar AOPs–biological coupled system

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Abstract

A mixture of five pesticides commonly used in intensive agriculture in the southeast of Spain, Methomyl, Dimethoate, Oxamyl, Cymoxanil and Pyrimethanil, has been completely mineralized in a combined solar photocatalytic–biological pilot plant. Two advanced oxidation processes (AOPs: TiO_2 and photo-Fenton) were employed for enhancing the biodegradability of wastewater and an aerobic immobilised biomass reactor (IBR) was used for the following continuous biological treatment. TiO_2 photocatalysis experiments were performed in a 35-L solar pilot plant made up of three compound parabolic collectors (CPCs), whereas photo-Fenton tests were carried out in a 75-L solar pilot plant with four CPCs units. The initial pesticide concentrations in the mixture were 50 mg L^{-1} each. The TiO_2 catalyst concentration employed was 200 mg L^{-1}, and two different Fe^{2+} concentrations, 20 mg L^{-1} and 55 mg L^{-1}, were used in the photo-Fenton tests. Toxicity (*Vibrio fischeri*) and biodegradability assays (Zahn-Wellens test) were also performed to monitor toxicity and biodegradability of samples at different stages of photo-Fenton treatment. Biodegradable compounds generated during the preliminary oxidative process were mineralized in a 60-L activated sludge biological reactor filled with 30 L of propylene Pall Ring supports. Total disappearance of the parent compounds, more than 90% mineralization and complete nitrification were achieved by the combined system.

Keywords: Solar photocatalysis; Photo-Fenton; Immobilised biomass reactor; Organophosphates; Carbamates; Toxicity; Biodegradability

1. Introduction

Surface and underground water sources have a finite (and sometimes low) renewal capacity. The quality and quantity of European water resources in Europe are under threat from pressures, ranging from increasing populations, economic growth, intensive greenhouse agriculture, and rapid urbanization to the lack of proper supply and treatment facilities and water management. Intensive greenhouse agriculture, in particular, has been growing exponentially in the Mediterranean Basin due to its climate (longer daylight hours, almost no frost, etc.). This kind of crop demands special fertilizers and pesticides to fight crop pests and reduce competition from weeds, thus improve yields and protect the quality, reliability and price of produce. However, their use does involve risk because most have inherent properties that make them dangerous to health and the environment if not used properly. Therefore, the EU seeks to ensure their correct use through their regulation to minimise their detrimental environmental impact and public information about use and residue issues [1,2]. In this context, the EU's sixth Environmental Action Programme [3] addresses the need to encourage farmers to change their use of plant protection products.

In the southeast of Spain (mostly in the province of Almeria), greenhouse surface has increased around 15% in the last 5 years, which directly involves a significant increase in the use of pesticides (12% in only 2 years). At the present time, the amount of plant protection products employed in this province is about 6000 t per year at the rate of around 45 kg of pesticide mixtures per hectare. These pesticides, present in agrochemical wastewater, have been designated as persistent organic pollutants (POPs) in EU legislation [4].

Among the different approaches to pesticide elimination, advanced oxidation processes (AOPs) have been recognized as especially efficient compared to conventional technologies, mainly phase-separation (adsorption processes, stripping techniques) and other methods which destroy the contaminants

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(chemical oxidation/reduction) [5–8]. AOPs are characterized by the production of hydroxyl radicals ($^{\bullet}OH$), which are able to oxidise and mineralize almost any organic molecule, yielding CO_2 and inorganic ions. Due to the reactivity of hydroxyl radicals, their attack is unselective, which is useful for the treatment of wastewater containing many different pollutants [9]. The main drawback of AOPs is their relatively high operating costs compared to those of biological treatment [10,12]. Therefore, in recent years, the attention of research has been focused on AOPs that can be driven by solar radiation (photo-Fenton and heterogeneous catalysis with UV/TiO_2) instead of using UV lamps or ozone treatments which are expensive [13–16]. The photo-Fenton process combines Fenton (addition of H_2O_2 to Fe^{2+} salts) and UV–vis light [17], whereas, heterogeneous photocatalysis employs a solid semiconductor (TiO_2) that absorbs radiation (according to its band-gap) to generate hydroxyl radicals.

A step forward in cost reduction would be the combination of AOPs with a conventional biological treatment. This means that wastewater which is toxic, inhibitory or refractory to biological cultures can be chemically pre-treated by AOPs to produce biogenic intermediates [11,18–22].

This paper evaluates the technical feasibility of TiO_2 photocatalysis and photo-Fenton (at two different Fe^{2+} concentrations), for the treatment of wastewater polluted by a mixture of five pesticides commonly used in intensive agriculture (Methomyl, Dimethoate, Oxamyl, Cymoxanil and Pyrimethanil), and classified as POPs due to their persistence and bioaccumulation, and consequently, their long-term toxicity. The efficiency of the two methods is compared with a view to selecting the best option for future combination with a conventional biological treatment. Moreover, toxicity and biodegradability tests were performed at different stages of the photocatalytic process to establish the best time, when the AOP has sufficiently enhanced wastewater biodegradability, for subsequent continuous disposal into an aerobic immobilised biomass reactor.

2. Experimental

2.1. Chemicals

Cymoxanil (98.2% technical grade $C_7H_{10}N_4O_3$, Aragonesas Agro S.A.), Methomyl (99.4% technical grade $C_5H_{10}N_2O_2S$, Aragonesas Agro S.A.), Oxamyl (Vydate 24% commercial grade $C_7H_{13}N_3O_3S$, Du Pont Iberica S.A.), Dimethoate (98.2% technical grade $C_5H_{12}NO_3PS_2$, Aragonesas Agro S.A.) and Pyrimethanil (98.2% technical grade $C_{12}H_{13}N_3$, Agrevo S.A.), were used as received (Diagram 1). Analytical standards for chromatography analyses were purchased from Sigma–Aldrich. Distilled water used in both pilot plants was obtained from the Plataforma Solar de Almería (PSA) distillation plant (conductivity $< 10\ \mu S\ cm^{-1}$, $Cl^- = 0.7–0.8\ mg\ L^{-1}$, $NO_3^- = 0.5\ mg\ L^{-1}$, organic carbon $< 0.5\ mg\ L^{-1}$). The heterogeneous photocatalytic degradation tests were carried out using a slurry suspension ($200\ mg\ L^{-1}$) of Degussa (Frankfurt, Germany) P-25 titanium dioxide (surface area $51–55\ m^2\ g^{-1}$). Photo-Fenton experiments were performed using iron sulphate ($FeSO_4{\cdot}7H_2O$), reagent grade hydrogen peroxide (30%, w/v) and sulphuric acid for pH adjustment (around 2.7–2.9), all provided by Panreac. The photo-treated solutions were neutralized by means of NaOH (reagent grade, Panreac). Neutral pH was maintained during the biological treatment by adjustment with H_2SO_4 and NaOH (reagent grade, Panreac) solutions. Glucose (reagent grade) added to the photo-pre-treated effluent was also purchased from Panreac.

2.2. Analytical determinations

Mineralization was monitored by measuring the dissolved organic carbon (DOC) by direct injection of filtered samples into a Shimadzu-5050A TOC analyser calibrated with standard solutions of potassium phthalate and provided with a NDIR detector.

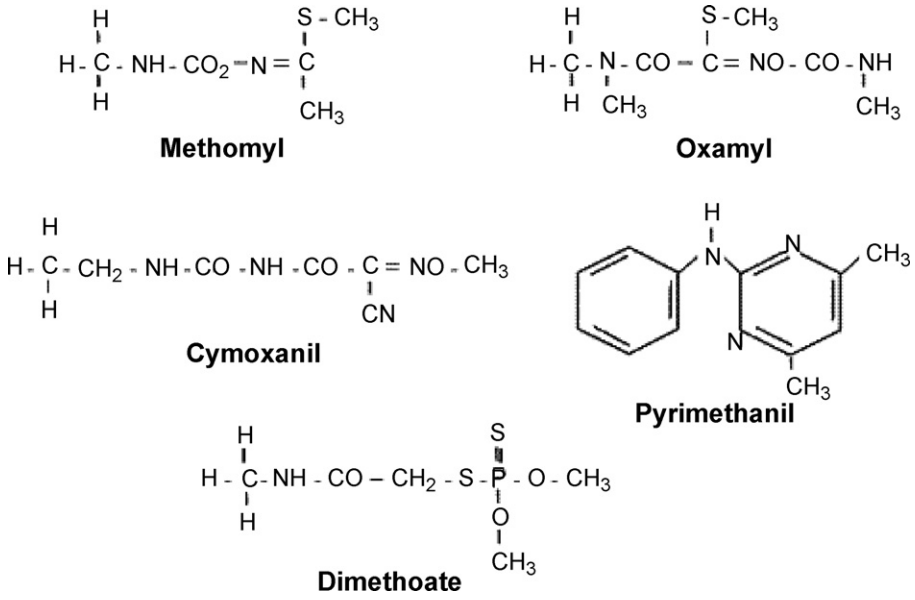

Diagram 1. Pesticides chemical structure.

The concentration of the five pesticides present in the mixture was analysed using reverse-phase liquid chromatography (flow rate: 0.5 mL min^{-1}) with UV detector in an HPLC-UV (Agilent Technologies, series 1100) with C-18 column (LUNA 5 μm, 3 mm × 150 mm from Phenomenex). Ultra pure distilled-deionised water obtained from a Milli-Q (Millipore Co.) system and HPLC-graded organic solvents were used to prepare all the solutions. The mobile phase composition was 50% acetonitrile/50% Milli-Q water at four different wavelengths depending on the pesticide detected in the mixture: 210 nm (Dimethoate), 234 nm (Methomyl and Oxamyl), 240 nm (Cymoxanil) and 270 nm (Pyrimethanil).

Ammonium concentration was determined with a Dionex DX-120 ion chromatograph equipped with a Dionex Ionpac CS12A 4 mm × 250 mm column. Isocratic elution was done with H_2SO_4 (10 mM) at a flow rate of 1.2 mL min^{-1}. Anion concentrations (NO_3^-, NO_2^-, PO_4^{3-} and carboxylates) were determined with a Dionex DX-600 ion chromatograph using a Dionex Ionpac AS11-HC 4 mm × 250 mm column. The gradient programme for anion determination was pre-run for 5 min with 20 mM NaOH, an 8-min injection of 20 mM of NaOH, and 7-min with 35 mM of NaOH, at a flow rate of 1.5 mL min^{-1}. Analysis of carboxylate ions (acetate, formiate, pyruvate and oxalate) was done with a different gradient programme, pre-run 10 min with 1 mM of NaOH, an 8-min injection of 1 mM of NaOH, 10-min with 15 mM of NaOH, 10-min with 30 mM of NaOH and 10-min with 60 mM of NaOH, at 1.5 L min^{-1}.

Colorimetric determination of total iron concentration with 1,10-phenantroline was used according to ISO 6332. Hydrogen peroxide analysis was carried out by iodometric titration, although, since this method is very time consuming (around 45 min), it was frequently also determined in fresh sample solutions using Merckoquant Paper (Merck Cat. no. 1.10011.0001).

2.3. Toxicity and biodegradability assays

A commercial assay marketed as Biofix®Lumi-10 was used for toxicity evaluation of samples partially oxidized by photo-Fenton. The reagent is a freeze-dried preparation of a specially selected strain of the marine bacterium *Vibrio fischeri* (NRRL no. B-11177). Sample inhibition effect on bacteria was analysed by measuring the drop in light emission after contact periods of 15 min and comparing it with a toxicant-free control (2% sodium chloride solution). Hydrogen peroxide present in the samples from photo-Fenton experiments was removed using catalase (2500 U/mg bovine liver, 100 mg/L) acquired from Fluka Chemie AG (Buchs, Switzerland) after adjusting the sample pH to 7.

The biocompatibility of the mixture of pesticides at different stages of the photo-Fenton process was followed by the Zahn-Wellens test (an adaptation of the EC protocol, Directive 88/302/EEC). Activated sludge from the Aqualia Wastewater Treatment Plant in Almería, mineral nutrients and test material as the sole carbon source were placed together in a 0.25-L glass vessel equipped with an agitator and aerator. The test was continued for 28 days at 20–25 °C and under diffuse illumination (or in a dark room). Degradation was monitored by DOC determination of the filtered solution, daily or at other appropriate regular time intervals. The ratio of DOC eliminated after each interval to initial DOC is expressed as the percentage of biodegradation. Samples analysed are considered biodegradable when the biodegradation percentage is over 70% [23].

2.4. Experimental set-up

2.4.1. Solar reactors

Solar TiO_2 photocatalytic experiments were carried out in a 35-L pilot plant (22 L illuminated volume) installed at the Plataforma Solar de Almería (PSA). It consists of three compound parabolic collectors (CPCs), one tank and one recirculation pump (20 L min^{-1}). The temperature inside the reactor was continuously recorded by a PT-100 inserted in the tank. A plant diagram has been published elsewhere [24].

Photo-Fenton tests were also performed at the PSA, under sunlight in a different 75-L pilot plant (44.6 L illuminated volume), specially developed for photo-Fenton applications. This solar reactor is composed of four CPC units, a tank and a recirculation pump (25 L min^{-1}). A diagram of this system has also recently been published elsewhere [25]. The temperature inside the reactor was kept at 30 °C using a temperature control system consisting of a heating by thermal resistances in the tubing, and cooling by a heat exchanger with a secondary cooling water cycle.

Solar ultraviolet radiation (UV, λ < 400 nm) was measured by a global UV radiometer (KIPP&ZONEN, model CUV 3), mounted on a platform tilted 37° (the same as the CPCs). With Eq. (1), combination of the data from several days' experiments and their comparison with other photocatalytic experiments is possible.

$$t_{30\,W,n} = t_{30\,W,n-1} + \Delta t_n \frac{UV}{30}\frac{V_i}{V_T}; \quad \Delta t_n = t_n - t_{n-1}; \quad t_0 = 0 \ \ (n = 1) \tag{1}$$

where t_n is the experimental time for each sample, UV is the average solar ultraviolet radiation (λ < 400 nm) measured between t_{n-1} and t_n, and $t_{30\,W}$ is a "normalized illumination time". In this case, time refers to a constant solar UV power of 30 W m^{-2} (typical solar UV power on a perfectly sunny day around noon).

Photo-Fenton experiments were carried out at a pH adjusted to 2.7–2.9 (H_2SO_4, 2N), two different Fe^{2+} concentrations (20 mg L^{-1} and 55 mg L^{-1}) and hydrogen peroxide concentration kept between 200 mg L^{-1} and 500 mg L^{-1} throughout the process. The mixture of pesticides was added directly into the pilot plant and properly homogenized by turbulent recirculation during half an hour at 30 °C. At the beginning of the process, the collectors were covered, the pH was adjusted and the ferrous iron salt was added. After each addition of reagents, the plant was well homogenised by recirculation. Finally, an initial amount of hydrogen peroxide was added (100 mL), and after 15 min a sample was taken to evaluate the

Fenton process. Then the collectors were uncovered and photo-Fenton began. The hydrogen peroxide was measured frequently and consumed reagent was continuously replaced.

The mixture of pesticides was also added directly into the pilot plant for TiO_2 photocatalysis. Once it was homogenised (initial pH 6), the catalyst was added (200 mg L^{-1} of TiO_2), and after 15 min, a sample was taken and the collectors uncovered.

2.4.2. Aerobic biological reactor

The aerobic biological reactor installed at the PSA for combined AOP–biological experiments consists of four modules, a 60-L neutralization tank, a 25-L conditioner tank, a 35-L immobilised biomass reactor (IBR) and a 60-L decanter tank. A flow diagram of the biological reactor system is shown in Fig. 1.

The IBR is a flat-bottomed container filled with 30 L of propylene Pall® Ring supports (nominal diameter: 15 mm, density: 80 kg m^{-3}, specific area: 350 m^2 m^{-3}, void fraction: 0.9 m^3 m^{-3}), colonized by activated sludge from the municipal wastewater treatment plant in Almería. This bioreactor is also equipped with an air blower to supply oxygen to the microorganisms and maintain saturated oxygen conditions in the system (8 mg L^{-1}).

This biological system was operated in batch and continuous mode. The mixture of pesticides partially oxidized by photo-Fenton was discharged into the neutralization tank where the pH was roughly adjusted to 7 with NaOH 2.5 M. Then, the photo-pre-treated effluent was transferred into the conditioner tank where the pH was manually adjusted between 6.5 and 7.5 throughout the biotreatment. Afterwards, the effluent was pumped through the IBR which operated as an up-flow reactor, at a recirculation flow rate of 1.6 L min^{-1} between the conditioner tank and the IBR, until the decrease in DOC reached characteristic end biological system values (20–30 mg L^{-1}). At that moment, combined system treatment of the effluent in batch mode was considered completed.

During continuous operation, the neutralized pre-treated effluent was pumped from the neutralization tank to the conditioner tank by a peristaltic pump (0.5–2.5 L h^{-1}). Once stationary state was reached between the conditioner tank and the IBR, the completely treated effluent (DOC between 20 mg L^{-1} and 30 mg L^{-1}) was continuously discharged from the IBR to the decanter at the same flow rate as the inlet.

3. Results and discussion

3.1. Solar photochemical treatment

The degradation of five well-known pesticides, Methomyl, Dimethoate, Oxamyl, Cymoxanil and Pyrimethanil, each mixed at 50 mg L^{-1} in a distilled water matrix, has been evaluated employing heterogeneous photocatalysis with TiO_2 (200 mg L^{-1}), and homogeneous photocatalysis with photo-Fenton at two different Fe^{2+} concentrations: 20 mg L^{-1} and 55 mg L^{-1} (see Fig. 2). These catalyst concentrations were selected based on previous results [26] pointing to 200 mg L^{-1} of TiO_2 and 0.2–1 mM of Fe^{2+} as optimum for experiments in the 35-L pilot plant and 75-L pilot plant, respectively. The comparison of these three methods made it possible to select the most efficient AOP for enhancing biodegradability for subsequent combination with an aerobic biological treatment (IBR).

The mineralization of the pesticide mixture (i.e. disappearance of DOC) is shown in Fig. 2. The DOC removal rate observed at the end of each AOP tested was 90% of the initial DOC, although the illumination time required was twice as long with TiO_2 photocatalysis (1197 min) as with photo-Fenton treatment (512 min). In a direct comparison between the two different Fe^{2+} concentrations employed in photo-Fenton experiments, with the same illumination time (500 min) mineralization was the same (90%, final DOC = 14 mg L^{-1}), so no significant differences were detected, except at shorter illumination times where 50% and 72% was mineralized with Fe^{2+} = 20 mg L^{-1} and Fe^{2+} = 55 mg L^{-1}, respectively, at 200 min of illumination time. It was concluded that light

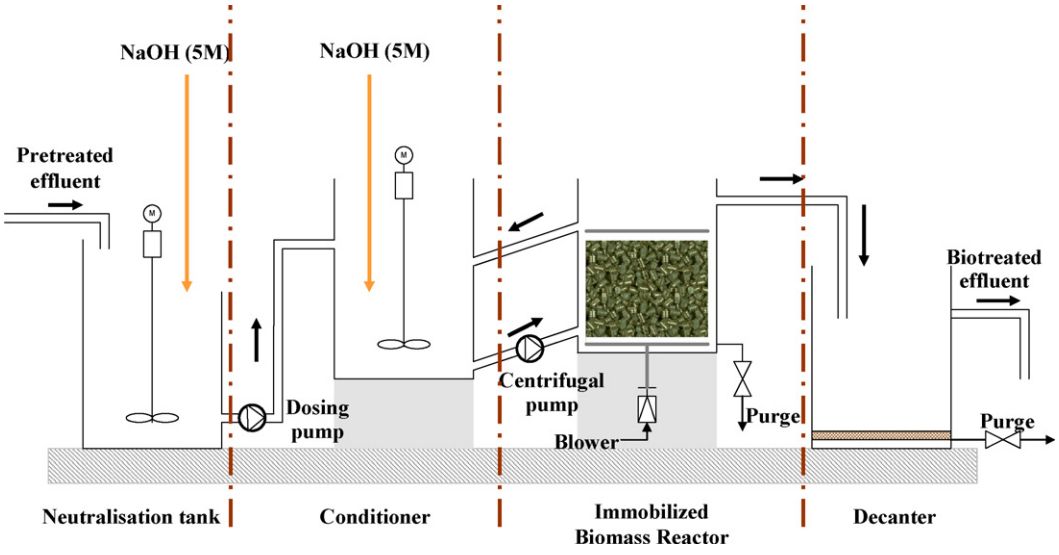

Fig. 1. Flow diagram of the aerobic biological system.

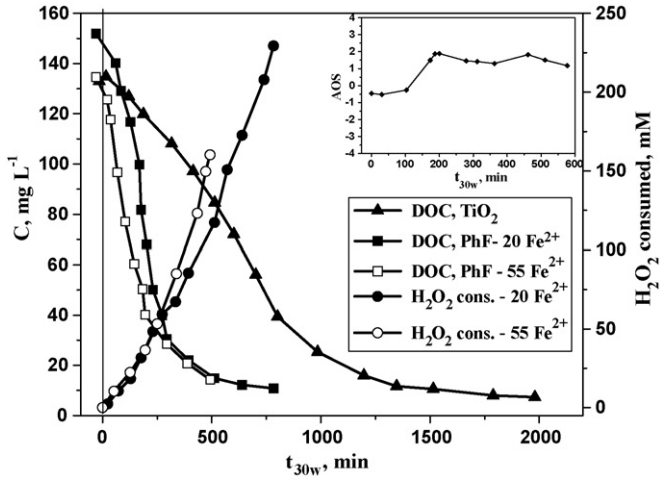

Fig. 2. Mineralization of the pesticide mixture by both AOPs and H_2O_2 consumed during the photo-Fenton experiments. AOS evolution shown in figure inserted.

absorption was quite similar at 20 and 55 mg Fe^{2+} L^{-1} in a solar photoreactor with a 5-cm light pathlength. The experiment at Fe^{2+} = 55 mg L^{-1} was quicker at the beginning due to the Fenton reaction (more pronounced at higher iron concentration) but at the moment that Fe^{2+} regeneration from Fe^{3+} (light absorption species) became the rate limiting step, the two situations were similar. Furthermore, H_2O_2 consumption during both photo-Fenton tests after 500 min of illumination time was 160 mM with 55 mg L^{-1} of Fe^{2+} and 117 mM with 20 mg L^{-1}, so less hydrogen peroxide, and a lower catalyst concentration were needed for attaining similar mineralization.

It is worth mentioning that due to the presence of an organophosphate pesticide in the mixture treated (Dimethoate), the phosphorous released at the beginning precipitated with the Fe^{3+} produced by Fe^{2+} oxidation (Fenton reaction) as ferric phosphate (around 60% of initial iron in both cases). This made it necessary to add more Fe^{2+} in the middle of both photo-Fenton treatments to compensate for iron precipitation and complete degradation of the pesticide mixture [27].

From Table 1, it may be observed that each pesticide present in the mixture was completely eliminated at least more than twice as fast by photo-Fenton as TiO_2 photocatalysis. Furthermore, the illumination time required for total degradation of each pesticide was not significantly reduced when 55 mg L^{-1} of Fe^{2+} were employed for photo-Fenton. The individual pesticide degradation rate may be described as a first-order reaction, and the pseudo-first order constant corresponding to each has previously been published elsewhere [28]. The initial degradation rates for each pesticide also shown in Table 1 are similar in both photo-Fenton experiments (except for Oxamyl, Cymoxanil and Pyrimethanil), and they were also clearly higher than with TiO_2 photocatalysis.

The mixture of five pesticides commonly used in intensive greenhouse agriculture was successfully treated by both photo-Fenton and TiO_2 photocatalysis within a reasonable length of time. Nevertheless, photo-Fenton was more efficient than TiO_2, not only for pesticide degradation, but also for DOC mineralization. Moreover, in view of the similar mineralization results in the photo-Fenton experiments with 20 and 55 mg L^{-1} of Fe^{2+}, the lower Fe^{2+} concentration was decided to be used for photo-Fenton treatment of the pesticide mixture, avoiding the need for iron removal before harmlessly discharging into the aerobic biological treatment (IBR).

The chemical oxygen demand (COD) was also measured during photo-Fenton treatment of the pesticide mixture with 20 mg L^{-1} of Fe^{2+} in order to evaluate the average oxidation state (AOS) of the solution (see inset, Fig. 2), calculated using Eq. (2) [29,30].

$$\mathrm{AOS} = \frac{4(\mathrm{DOC} - \mathrm{COD})}{\mathrm{DOC}} \tag{2}$$

where DOC and COD are expressed in moles of C L^{-1} and moles of O_2 L^{-1}, respectively. AOS takes values between +4 for CO_2, the most oxidized state of C, and −4 for CH_4, the most reduced state of C. As observed in Fig. 2 inset, the maximum AOS of 1.9 was reached after approximately 180 min of illumination time and remained almost constant until the end of the treatment. AOS usually increased with treatment time until almost reaching a plateau. These results suggest that more oxidised organic intermediates are formed at the beginning of the treatment, and after a certain time, the chemical nature of most of them no longer varied substantially, even if the photo-Fenton treatment continued. Formation of more oxidized intermediates indirectly demonstrates that the treatment can improve biodegradability. At the moment that AOS stabilizes, the chemical treatment is only mineralizing organic contaminants, but with no oxidation.

Table 1
Initial degradation rate of each pesticide in the mixture when treated by TiO_2 photocatalysis and photo-Fenton (20 mg L^{-1} and 55 mg L^{-1} of Fe^{2+})

Target compounds (50 mg L^{-1} initial concentration)	TiO_2		Fe 20 mg L^{-1}		Fe 55 mg L^{-1}	
	$t_{30\ w}$ [a] (min)	r_0 [b] (mg L^{-1} min^{-1})	$t_{30\ w}$ [a] (min)	r_0 [b] (mg L^{-1} min^{-1})	$t_{30\ w}$ [a] (min)	r_0 [b] (mg L^{-1} min^{-1})
Methomyl	360	0.21	168	1.04	103	0.88
Dimethoate	248	1.00	35	1.99	36	1.78
Oxamyl	360[c]	0.33	72[c]	1.75	52[c]	0.20
Cymoxanil	316	0.34	150	1.08	75	0.45
Pyrimethanil	510	0.42	150	0.95	86	2.59

[a] Treatment time necessary to completely eliminate each pesticide.
[b] Initial degradation rate.
[c] Commercial Oxamyl containing 76% unidentified compounds.

The changes in AOS were taken into account in determining when to perform the biodegradability tests.

3.2. Toxicity and biodegradability analyses of the partially oxidized solutions

Toxicity and biodegradability assays of samples taken at different stages of the photo-Fenton process were performed for the purpose of determining the best moment to stop the photo-Fenton pre-treatment and discharge the effluent into an aerobic biological reactor. The mixture of the five pesticides was treated by photo-Fenton at 20 mg L^{-1} of Fe^{2+} and small amounts of H_2O_2 (around 100 mg L^{-1} each time) were added to obtain partially oxidized samples free of hydrogen peroxide, which would be harmful for biological analysis (see Fig. 3(a)). The addition of H_2O_2 in small amounts also ensured sample stability (no further degradation) for possible subsequent biological tests.

Fig. 3(a) shows the percentage of *V. fischeri* inhibition during the photo-Fenton experiment. At first, *V. fischeri* showed 95% inhibition after 15 min of contact with the mixture of pesticides, but as the photo-Fenton treatment continued, the percentage of inhibition decreased to 60% when DOC was reduced to 86 mg L^{-1}, then increased again to 95% due to the generation of more toxic intermediate products during oxidation. From this point to the end of photo-Fenton treatment, toxicity varied between 72% and 47%, remaining under the 50% limit until the end of the treatment. As observed, *V. fischeri* toxicity tests are useful analytical tools for quick decisions concerning the advisability of further oxidative treatment. In this case, the toxicity results show that photo-Fenton must be continued until DOC drops to less than 50 mg L^{-1}. Nevertheless, these toxicity tests are usually more sensitive than activated sludge, in particular, 47% of inhibition detected at the end of the photo-Fenton treatment is already rather higher than desirable for disposal in the environment, but it could be perfectly alright for combination with a biological system. Toxicity tests are also useful for quickly deciding on the samples selected for Zahn-Wellens tests. It should be remarked that Zahn-Wellens tests last several weeks and toxicity tests only take a few minutes. Therefore, Zahn-Wellens tests were performed on those samples (at different stages of photo-Fenton treatment) in which toxicity revealed substantial changes in the treated waste water, but without substantial mineralization, as Zahn-Wellens should be applied to those samples containing at least 50 mg L^{-1} of DOC: (i) when toxicity was constant but DOC was mineralized; (ii) when toxicity decreased, and (iii) when toxicity increased. The Zahn-Wellens test was also done when AOS remained constant. Therefore, Zahn-Wellens biocompatibility analyses were carried out to select a reliable point of discharge the pre-treated effluent into an aerobic biological reactor (IBR).

Fig. 3(b) shows the percentage of biodegradability of each sample taken at different stages of the photo-Fenton process, accordingly to toxicity results. When DOC was up to 126 mg L^{-1}, samples were hardly biodegradable, while when DOC was lower than 107 mg L^{-1}, 70% biodegradability was achieved in less than 12 days and the combination with an aerobic biological treatment could then be performed.

The Zahn-Wellens tests validated toxicity results, as for DOC over 126 mg L^{-1} (105 min of photo-Fenton treatment), the effluent was non-biodegradable and also toxic, with an inhibition percentage of around 80%. On the other hand, samples with lower DOC were more biodegradable, although toxicity analyses showed a high percentage of inhibition (94% for DOC = 82 mg L^{-1}, and 70% for DOC = 62–55 mg L^{-1}). These results underline the usefulness of toxicity testing for selecting the treatment sampling time for biodegradability tests, but they also show that toxicity tests are not a good approach to biodegradability prediction.

An additional vessel containing diethylene glycol, a well-known biodegradable substance recommended by EC Directive 88/302/EEC, was tested in parallel to the rest in order to check proper activity of the activated sludge. Complete disappearance of DOC was detected in less than 5 days (100% of biodegradability).

Finally, Zahn-Wellens tests not only validated toxicity results but also the AOS, which predicted that biodegradability of the oxidised effluent would not significantly change after the maximum AOS was reached at 180 min of photo-Fenton treatment (see Fig. 2(a)). According to the above, Zahn-Wellens

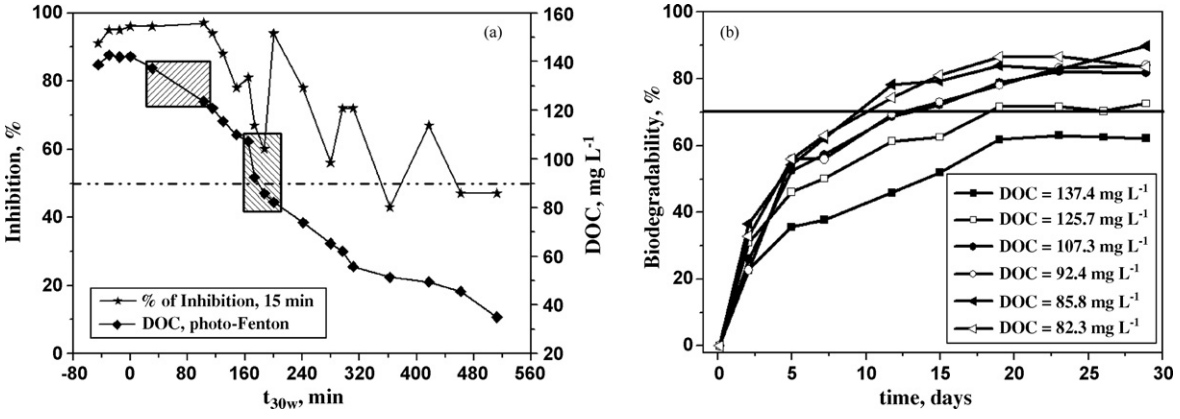

Fig. 3. (a) Percentage of *Vibrio fischeri* inhibition after 15 min exposure to samples partially treated by photo-Fenton at 20 mg/L of Fe^{2+}. (b) Zahn-Wellens biodegradability analysis of photo-Fenton samples (labelled in (a)).

biodegradability tests demonstrated that all the pre-treated samples taken after 165 min of illumination were more than 70% biodegradable in less than 12 days. This similarity between chemical and biological assays (AOS and Zahn-Wellens) does not mean that biocompatibility of solutions could be predicted by calculating only the AOS, however, this does allow biodegradability tests to be performed only on a small number of samples closest to the maximum AOS.

3.3. Combined solar photo-Fenton–biological system

Once successful enhancement of photo-treated effluent biodegradability by photo-Fenton with 20 mg L^{-1} of Fe^{2+} had been demonstrated, the combined photo-Fenton/biological treatment was employed for the complete mineralization of water containing a mixture of five pesticides: Methomyl, Dimethoate, Oxamyl, Cymoxanil and Pyrimethanil.

The solar photochemical part of the combined process was always performed in batch mode, while the biological reactor was operated both in batch and continuous.

Before performing the experiments in the combined system and taking previous studies into account [31,32], the IBR was inoculated with 45 L of concentrated activated sludge from the Municipal Wastewater Treatment Plant of Almería (Biomass concentration = 9 g L^{-1}). Then, recirculation was maintained between the conditioner tank and the IBR for nearly 10 days in order to ensure optimum fixation of the sludge on the propylene Pall® Ring supports. The total suspended solids, DOC and inorganic ions concentration (mainly ammonia and nitrate) were measured daily.

The total suspended solids analysis (TSS) assessed bacteria fixation on the supports during IBR inoculation, and 0.20 g L^{-1} to 0 variation in approximately 7 days was detected. At that moment, the conditioner tank and the IBR were refilled with the normal influent from the Wastewater Treatment Plant of Almería, in order to increase the concentration of biomass fixed on the supports and the diversity of bacteria population, which is when the IBR was ready to treat the wastewater from the photo-Fenton pre-oxidation process. Nevertheless, to avoid any possible shock to IBR bacteria which could reduce their activity, several small amounts of the photo-Fenton pre-treated effluent (DOC around 50–70 mg L^{-1}) were added to the system before completely replacing the whole volume. Initial DOC was reduced to a concentration of 20–30 mg L^{-1}, corresponding to background noise from the physiological bacteria activity normally found in conventional biological media.

According to the results from the Zahn-Wellens tests on the samples partially oxidized by photo-Fenton, the optimal moment for finishing pre-treatment and discharging the effluent into the aerobic biological reactor was when DOC dropped to below 110 mg L^{-1} ($t_{30\ w}$ = 150 min) and a small concentration of some of the pesticides still remained in the solution: 2.4 mg L^{-1} of Cymoxanil, 3.4 mg L^{-1} of Methomyl and 6.2 mg L^{-1} of Pyrimethanil. This resulted in removal of around 30% of the initial DOC by photo-Fenton. To achieve these results, as shown in Fig. 2, the exact amount of H_2O_2 (22 mM) necessary for DOC to become biocompatible was added. Afterwards, the pre-treated effluent was pumped into the neutralization tank so the pH could be adjusted roughly to 7 before entering the conditioner tank and then into the immobilised biomass reactor. In batch mode operation, recirculation (flow rate = 1.6 L min^{-1}) was maintained between the conditioner tank and the IBR until the effluent was bio-mineralized (final DOC between 20 mg L^{-1} and 30 mg L^{-1}). The chemical characterisation of the photo-Fenton pre-treated effluent (DOC = 110 mg L^{-1}; Fe^{2+} = 20 mg L^{-1}; Na^+ = 0.14 g L^{-1}; NH_4^+ = 6 mg L^{-1}; K^+ = 3 mg L^{-1}; Mg^{2+} = 1 mg L^{-1}, Ca^{2+} = 10 mg L^{-1}; Cl^- = 1 mg L^{-1}, NO_3^- = 7 mg L^{-1}; NO_2^- = 3 mg L^{-1}; PO_4^{3-} = 6 mg L^{-1}) showed that the ratios between C and N, P, Fe (from photo-Fenton) and Ca were very close to the required in conventional biological systems as published elsewhere: C:N:P of 100:20:5 and C:Fe:Ca of 100:2:2 [33]. Therefore, no mineral medium was added to the pre-treated effluent so it would be as realistic as possible. Apart from ammonium, nitrate and nitrite, a large amount of methylamine was also detected in the ionic chromatograph although no quantification was possible. Therefore, no extra NH_4^+ was added to force nitrifying bacteria to consume methylamine nitrogen for metabolizing the organic carbon.

Fig. 4, shows the evolution of DOC and nitrogen from NH_4^+ in two combined photo-Fenton/biological experiments performed in batch mode. In both, the DOC at the outlet of the photo-Fenton pre-treatment was between 110 mg L^{-1} and 106 mg L^{-1}, but as observed in the first (solid symbols), although the amount of DOC decreased, the N-NH_4^+ concentration increased up to 25 mg L^{-1} (higher than the legal wastewater discharge limit). This means that the nitrification process was not working properly, probably due to an inhibition effect caused by the high nitrogen content in the system (methylamine) [34]. According to this, an extra biodegradable DOC added to the pre-treated effluent could allow nitrifying bacteria to consume the excess of nitrogen. So, glucose was added twice, once with a DOC of 85 mg L^{-1} and then 47 mg L^{-1} in order to check whether the N-NH_4^+ was reduced at

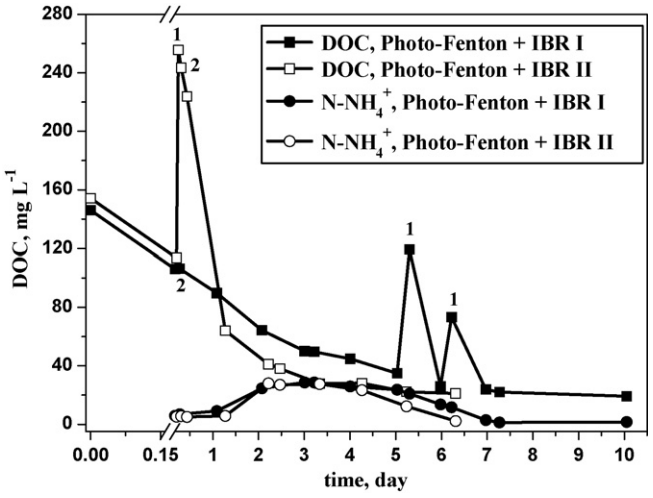

Fig. 4. Immobilised biomass reactor in batch mode operation. DOC and nitrogen during biological treatment. Point 1 is when glucose is added and point 2 is when the pre-treated effluent is discharged into the IBR.

the same time DOC decreased. As observed in Fig. 4, the concentration of N-NH_4^+ started to decrease from the moment glucose was added until a constant value around 2 mg L^{-1}.

In the second combined experiment operated in batch mode, the necessary amount of glucose was added at the beginning of the biological treatment, by mixing it with the pre-treated effluent in the neutralization tank (see Fig. 4). This time, the initial DOC was 255 mg L^{-1}, of which 142 mg L^{-1} was glucose, and after 5 days of bio-treatment, DOC was reduced to 22 mg L^{-1} (92% of the initial overall DOC). By the fifth day of bio-treatment, the concentration of nitrogen from NH_4^+ had decreased to 12 mg L^{-1} which was already within legal wastewater discharge limits (20 mg L^{-1}), however, it continued to fall to only 2 mg L^{-1} on day 6. As mentioned in the introduction, the goal is to combine AOP and an aerobic biological treatment, or discharge AOP pre-treated water into a conventional biological treatment plant. Under the above conditions, mixing water pre-treated by photo-Fenton with other wastewater containing easily biodegradable compounds could lead to a similar behaviour to that observed when added glucose.

As observed in Fig. 4, overall efficiency of the combined photo-Fenton and biological treatment in batch mode for the degradation of wastewater containing a mixture of pesticides was over 85%, of which 23% correspond to the solar photochemical process and 62% to the biological treatment.

The aerobic biological system was also operated in continuous mode, taking into account the results of batch mode operation. The pesticide mixture continued to be treated in batch cycles by photo-Fenton, and supplying biodegradable water to the biological reactor. The mixture of the pre-treated effluent and glucose (around 375 mg L^{-1} based on batch results), was added to the neutralization tank and pumped continuously into the conditioner tank by a peristaltic pump. Recirculation was kept up between the conditioner tank and the IBR throughout the operation and pH was manually controlled by adding NaOH or H_2SO_4 to the conditioner tank. The biologically treated effluent went from the IBR to the decanter continuously, where the suspended solids removed from the IBR were recovered in the decanter conic bottom. The residence time (t_R) of the effluent in the biological system depended on the continuous inlet flow to the conditioner tank, which was varied from 0.72 L h^{-1} (t_R = 83 h) to 2.25 L h^{-1} (t_R = 27 h) in order to find the maximum biological treatment capacity. Fig. 5 shows the changes in the continuous flow and the evolution of DOC, N-NH_4^+, formiate and the concentrations of the remaining pesticides in the IBR outlet. The biological reactor was operated continuously for 11 days, during which time each flow rate tested was maintained at least until twice the whole volume of the system had been replaced so that results would be reliable.

At the beginning of continuous operation, the pre-treated effluent (DOC between 265 mg L^{-1} and 255 mg L^{-1}) was continuously introduced into the biological system at 0.72 L h^{-1} and DOC in the IBR outlet remained the lowest found in the batch mode experiments (23 mg L^{-1}, COD = 55 mg L^{-1}). After 3 days, the flow rate was increased to 1.32 L h^{-1} and DOC in the outlet only increased to 32 mg L^{-1} (COD = 63 mg L^{-1}) which is

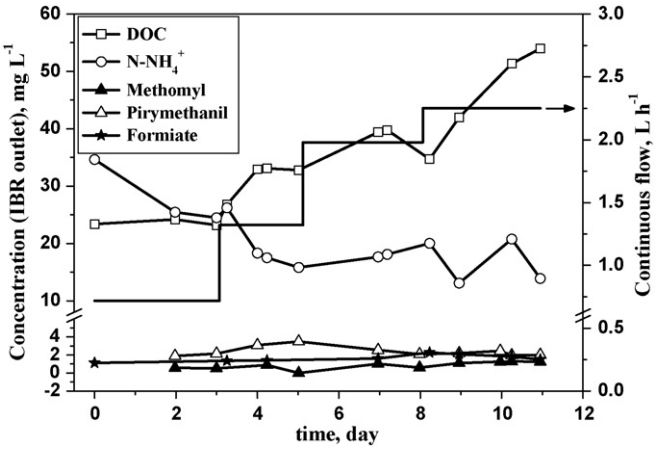

Fig. 5. Continuous operation of the IBR. Changes in the continuous flow, evolution of the DOC, nitrogen, formiate and the pesticide concentration in the effluent after treatment.

clearly due to background noise from normal physiological bacteria activity as mentioned above. Therefore, this flow was maintained until twice the biological reactor system volume (60 L) had been treated, then changed again to 1.98 L h^{-1}. Three days later, outlet DOC was 35 mg L^{-1} (COD = 80 mg L^{-1}). Finally, the flow rate was increased to 2.25 L h^{-1}, and after the last 3 days of biological treatment, the DOC in the IBR outlet had increased to 54 mg L^{-1} (COD = 127 mg L^{-1}), significantly higher than the DOC range corresponding to background noise of physiological bacteria activity (DOC = 20–30 mg L^{-1}), so the maximum treatment capacity of the biological system had therefore been found. If we consider a maximum continuous flow rate of 2.2 mg L^{-1}, the adapted biomass fixed on the IBR supports could continuously treat a photo-Fenton pre-oxidized effluent with an initial DOC of around 265 mg L^{-1} (mixed with glucose), in a t_R of 27 h. The maximum treatment capacity of the biological system per volume of IBR occupied by Pall® Ring supports is 16 mg of DOC h^{-1} L^{-1}.

Fig. 5 also shows how the concentration of nitrogen from NH_4^+ in the IBR outlet decreased during the first 3 days of biological treatment from 34 mg L^{-1} to 20 mg L^{-1} and remained almost constant at this value until the end of the experiment, demonstrating that the nitrification process was working properly throughout continuous operation. It is worth mentioning that concerning the concentration of pesticides remaining in the solution after photo-Fenton pre-treatment of the initial mixture of pesticides, Cymoxanil had completely disappeared and Methomyl had been significantly reduced from 3.4 mg L^{-1} to 0.5 mg L^{-1} and Pyrimethanil was reduced from 6.2 mg L^{-1} to 2 mg L^{-1} during biological treatment, probably due to its adsorption on the supported biomass. However, it can be observed also that, as the continuous flow was increasing the concentration of Methomyl increased also to 1.3 mg L^{-1} demonstrating, in this case, the maximum adsorption capacity of this biological system.

After the photochemical step the following carboxylic acids were detected in the pre-treated effluent: 9 mg L^{-1} of acetate, 3 mg L^{-1} of formiate, 80 mg L^{-1} of propionate and 2 mg L^{-1} of pyruvate and oxalate respectively (these represented

approximately 60% of the total DOC). So, they were also measured in the IBR outlet in order to follow their evolution after the biological treatment. Only formiate was detected since the beginning of the continuous operation mode (see Fig. 5), but when the continuous flow was increased to the maximum treatment capacity, also 0.3 mg L^{-1} of acetate and 2 mg L^{-1} of propionate were detected.

Finally, *V. fischeri* toxicity tests were also applied to monitor the reduction of percentage of inhibition from the inlet to the outlet of the biological reactor in continuous mode operation. The percentage of inhibition after 15 min of *V. fischeri* contact to the solar pre-oxidised effluent (mixed with glucose) was 90%. This percentage of inhibition was reduced to 2–8% at the outlet of the IBR. It is important that the percentage of inhibition was reduced only to 35% when the continuous flow was increased to the maximum biological treatment capacity (2.25 L h^{-1}). These results demonstrate the high sensibility of the toxicity tests compared to the biodegradability ones. In the *V. fischeri* test the pre-oxidised effluent showed high toxicity, which was greatly reduced, when the effluent was biologically treated by activated sludge in the IBR. Therefore, not only the pre-treated effluent by photo-Fenton was non-toxic for the biomass fixed on the supports but also toxicity was completely reduced at the end of the biological treatment, the same as DOC.

4. Conclusions

A combined solar photocatalytic–biological process has been evaluated for the treatment of wastewater contaminated by a mixture of five pesticides commonly used in intensive agriculture (Methomyl, Dimethoate, Oxamyl, Cymoxanil and Pyrimethanil). It has been demonstrated that this pesticide mixture can be successfully treated by photo-Fenton and TiO_2 photocatalysis within a reasonable length of time, although photo-Fenton treatment resulted much more efficient for pesticide degradation and DOC mineralization. Two different Fe^{2+} concentrations (55 and 20 mg L^{-1}) were tested for photo-Fenton experiments, but no significant difference was detected between them. Therefore, photo-Fenton at 20 mg L^{-1} of Fe^{2+} has been selected as the best AOP option for combining with an aerobic immobilised biomass reactor. The use of so low amount of catalyst avoids the necessity of iron removal before discharge of the pre-treated effluent into the subsequent biological treatment.

It can also be concluded that the combination of toxicity (*V. fischeri*) and biodegradability (Zahn-Wellens) assays is a useful technique to select the moment when photo-Fenton pre-treatment has sufficiently enhanced the biocompatibility of the pesticides mixture for a subsequent combination with an aerobic biological treatment.

The global efficiency attained in the coupled solar photo-Fenton/biological system operated in batch mode was 85% of DOC elimination (initial DOC of 256 mg L^{-1}), from which 23% corresponded to the solar photo-Fenton step and 62% to the aerobic biological treatment.

This two-steps field treatment operated in semi-continuous mode (batch mode for the photo-Fenton and continuous mode for the biological process) established the maximum treatment capacity of the aerobic IBR in 16 mg of DOC h^{-1} L^{-1} occupied by polypropylene supports colonized by activated sludge.

Combined solar photo-Fenton and biological system has been demonstrated to be an effective approach for the treatment of wastewaters polluted with a mixture of pesticides.

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