Photo-Fenton and Slow Sand Filtration coupling for hydroponics water reuse

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Programa de doctorat: Ciència i Tecnologia de Materials

Memòria de tesis doctoral:

PHOTO-FENTON AND SLOW SAND FILTRATION COUPLING FOR HYDROPONICS WATER REUSE

Mª del Mar Micó Reche

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Universitat de Barcelona

APPENDIX I

Comparison between ozonation and photo-Fenton processes for pesticide methomyl removal in advanced greenhouses

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Comparison between Ozonation and Photo-Fenton Processes for Pesticide Methomyl Removal in Advanced Greenhouses

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So-called "Advanced Greenhouses" are a new approach to the concept of protected agriculture. Among other technological and structural improvements, these facilities give the possibility of recycling the irrigation surplus water, rich in lixiviates, salts, pesticides and its metabolites. After many cycles, the current is so concentrated on those substances that it becomes necessary for the presence of a membrane separation stage which brine, highly concentrated on those named pollutants, has to be treated before being sent to the public sewage system. Advanced Oxidation Processes, among other chemical treatments, can be considered an alternative to process this current effluent. In this work, concentrated aqueous solutions of methomyl as model pesticide (200 mg·L^{-1}) have been subjected to two of those processes: ozonation and photo-Fenton reaction. Analysis of the elimination of the pesticide itself and the grade of mineralization achieved have shown how, while the ozonation is the most effective process decomposing the pesticide (eliminating the total concentration in 60 minutes), the photo-Fenton reaction mineralizes successfully the 40% of the total organic load (the ozonation only can cope with 20%) but only decompose a 40% of the pesticide. Evolution of biodegradability and toxicity of the effluent along both processes was also analyzed. Intermediates generated both by ozonation and photo-Fenton did not increase the biodegradability of the treated effluents. Nevertheless, while acute toxicity just after 15 minutes of treatment with ozone is notably higher than for raw solution, and it is maintained till the end of the experiment (120 min), though, toxicity along photo-Fenton reaction has two growing and decreasing regions, always shows lower values than the provoked during ozonation. None of the two assayed processes has been proved to increase biocompatibility of highly concentrated methomyl solutions.

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INTRODUCTION

The persistent demand of fruits and vegetables out of season and the need of better yields have forced to develop a new system of culturing under greenhouses, assuring protected conditions to the crops. This fact and new approaches to the essential sustainability of agriculture have motivated the development of Advanced Greenhouses. These facilities, among other technological improvements, include the infrastructure for recovering surplus water non-assimilated by plants, in order to create a semiclosed recycling system for an efficient use of hydric resources. To avoid phytotoxicity, due to the effluent high concentrations on pesticides and salts by accumulation after several cycles, a treatment before its recirculation is needed. A pertinent option for this process could be a membrane separation stage, although its brine, even more concentrated in harmful products, has also to be refined because the toxic and non-biodegradable characteristics of most pesticides and their metabolites.

Due to the characteristics of insecticides as recalcitrant and bioacumulative, the conventional biologic treatments are insufficient to achieve the parameters required on the processed water. This is why, apart from the physical separation through membranes; a chemical oxidation process to mineralize these substances is needed. The oxidative processes are expected to be able to degrade those typically stable products into carbon dioxide, water and inorganics or, at least, transform them into harmless compounds (Andreozzi et al., 1999). In the last decades a representative kind of these technologies;

based on the production of hydroxyl radicals, has been proved to be effective against recalcitrant compounds (Pera-Titus et al., 2004). They are the so-called Advanced Oxidation Processes (AOP's). Two of these technologies have been tested in this work. Their reliability to eliminate the target pesticide from water was compared, in terms of the very elimination of the product, the diminishment of the total organic carbon and the evolution of biodegradability and toxicity of the hypothetical intermediates generated. For these two last measures, the parameters taken into account were BOD_5/COD ratio as indicator of biodegradability (Yu and Yu, 2000) and EC_{50} as an acute toxicity index (González et al., 2007).

The Photo-Fenton reaction is based on the generation of hydroxyl radicals due to the interaction between hydrogen peroxide and Fe^{2+} as catalyst, with the action of UV radiation (from 180 to 400 nm (Wadley and Waite, 2004)) in the ferrous ion recovering cycle (Evgenidou et al., 2007). The following reactions (Equations [1] and [2] illustrate the basics of the process:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot} \qquad [1]$$

$$Fe^{3+} + H_2O + h\upsilon \rightarrow Fe^{2+} + H^+ + OH^{\cdot} \qquad [2]$$

(Wadley and Waite, 2004)

The global reaction has been already reported in the literature as successful for the treatment of water polluted with pesticides (Tamini et al., 2007; Huston and Pignatello, 1999; Badawy and Ali, 2006; Ballesteros et al., 2008; Hincapié et al., 2006; Lapertot et al., 2007; Segura et al., 2008). Iron is a very abundant and non toxic element, and hydrogen peroxide is easy to handle and environmentally safe and, as main advantage, photo-Fenton process has the possibility to use solar light as source of radiation for the recycling reaction (Equation [2]). However the disadvantages have to be also taken into account. As well as H_2O_2 is an expensive raw material, a low pH is required. According to Pignatello (Pignatello and Dark, 2008) the optimum pH to work with is 2.8, in which the Fe (II) and Fe (III) species coexist in a ratio 1/1. So the addition of an extra reagent to fix the pH is needed, increasing the costs.

On the other hand, the ozonation of water containing pesticides has not been as developed as the photo-Fenton processes, neither at lab scale nor at pilot or industrial scale. Few are the real applications of ozone for the treatment of real wastewaters, because the removal of high concentrations of pollutants always implies the use of large amounts of ozone, making difficult the economical implementation of the technique (Maldonado et al., 2006). Nevertheless the tendency is changing in our days. In ozonation, the attack to the organic molecules takes place through two different paths. In the direct path, the oxidant potential of the molecule of O_3 is able to decompose certain kind of organic molecules (it presents high selectivity) by means of electrophilic, nucleophilic and as dipole actions (Langlais et al., 1991). The other path consists on the formation of hydroxyl radicals in the decomposition of the ozone in water catalyzed by the presence of OH^-, (Sehested et al., 1984; Hoigné et al., 1985). This path is the one that makes the ozonation part of the AOP's classification. The main advantage of ozonation among other techniques is the fact that no alien species are needed to be added to the water to treat. Furthermore the degradation products of the ozone itself have been proved as harmless for the environment. The conflictive point in this treatment is related to the degradation products of the pesticide since cases in which the intermediates generated during the process are more toxic that the raw pollutant has been reported (Miltner et al., 1992).

This present study is a preliminary project focused on the treatment by ozone and photo-Fenton of synthetic waters spiked with one of the most widely used insecticide on greenhouse farming of ornamental herbaceous plants in Almeria (Junta de Andalucía, 2008) (South of Spain) till 2009, methomyl, *S-methyl-N((methylcarbamoyl)oxy) thioactimidate*. Analysis of the elimination of the pesticide itself, mineralization of the intermediates, and the evolution of the biodegradability and toxicity of the effluent along both processes were studied. As can be seen in Figure 1, the target substance is an N-methyl member of the carbamates family, characterized by -NH(CO)O- as their principal functional group. The main attributes that make it an interesting compound to study are its high solubility in water (57.9 g·L^{-1}, 20 °C) and its low affinity for soils (Tamimi et al., 2007). Both properties involve higher levels of this pesticide on the brine waters in comparison to other substances less soluble or more retained by substrates.

The product employed in this study is a commercial formulation named Tomilo 20L, an aqueous solution in which the concentration of the insecticide is 200 g·L^{-1}. This formulation also contains 250 g·L^{-1} of ethanol in order to solubilize the active principle and to make the mixture more volatile to facilitate the absorption by the insects. The idea of using a commercial product is founded on the need of having a scenario as real as possible to test the procedures employed along this research.

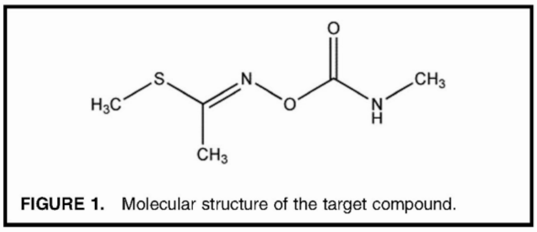

FIGURE 1. Molecular structure of the target compound.

MATERIALS AND METHODS

Chemicals

The experiments of ozonation and photo-Fenton reaction were carried out with solutions of Tomilo (200 g·L^{-1} Methomyl, Aragonesas Agro S.A.) and Millipore water (Milli-Q Millipore system with a 18 MΩ.cm^{-1} resistivity). The Fenton reagents were H_2O_2 (30% w/v) and $FeSO_4 \cdot 7H_2O$ (Panreac). The reaction was quenched with Na_2SO_2 (40%, Panreac). To adjust pH, a dilution of 16% H_2SO_4 was used (prepared from H_2SO_4 98%, Panreac). For HPLC analysis, acetonitrile (99.8%, isocratic grade for HPLC, Merck) and Millipore water were used. For BOD$_5$ analysis, reagents were $FeCl_3 \cdot 6H_2O$ (98%, Probus), $CaCl_2$ (95%, Panreac), NH_4Cl (99.5%, Panreac), $NaH_2PO_4 \cdot H_2O$ (98%, Probus), $MgSO_4$ (97%, Panreac) and NaOH (97%, Merck). For COD analysis, digester and catalysis solutions were respectively $K_2Cr_2O_7$, (0.04 mol·L^{-1} with 80 g·L^{-1} of $HgSO_4$, Panreac) and Ag_2SO_4 (10 g·L^{-1} in sulfuric acid, Panreac).

Analytical Methods

Methomyl concentration was followed by reversed-phase HPLC, through injections of 10 µl of the mixture to analyze. A Waters HPLC apparatus (photodiode array detector 996, autosampler 717, controller 600, Milford, MA, USA) with a Millenium Software was used. The column was a Mediterranea Sea$_{18}$, 5 µm 25x0.46 cm (Teknokroma, Barcelona, Spain). The mobile phase was a mixture of water and acetonitrile (80:20) (Tamimi et al., 2007) isocratically delivered by a pump at a flow rate of 1 mL.min^{-1}.

The wavelength of the UV absorbance detector was 232.5 nm. Total Organic Carbon (TOC) analyses were performed by a Shimadzu TOC-VCSN TOC analyzer (Kyoto, Japan), with a potassium phthalate solution as the calibration standard. Chemical Oxygen Demand (COD) was also followed by means of a photometer (Hach Odyssey) according to the Standard Methods section 5220D. Analysis of samples Biochemical Oxygen Demand (BOD$_5$) were performed according to the procedures stipulated in Standard Methods (American Public Health Association, 1985) section 5210D, by means of an Oxitop system (WTW, Weilheim, Germany). Inoculum was BOD seeds supplied by Cole and Parmer. Acute toxicity of the initial solutions and the photo-Fenton and ozonation final effluents was measured by Microtox toxicity test (Azur Environmental, Carlsburg, CA, USA) using *Vibrio fischeri* strains, with bioluminescence properties. The protocol followed to perform the tests was the one recommended by the device manufacturer and let obtain the index EC$_{50}$. It represents the concentration of sample in percentage that causes a 50% reduction in light emission, in this case, after 15 minutes of contact between the culture and the solution tested.

Ozonation Procedure

The ozonation experiments were carried out in a hermetically closed 1L Pyrex vessel, where the mixture was continuously agitated by a magnetic stirrer. The O_3 was supplied as little bubbles (to ensure a good contact between phases) through gas diffusers at the end of the polymer pipe that connected the reactor with the ozone generator. This device was a lab scale ozonator 301.19 by Sander, Uertze Eltze, Germany. An ozone analyzer BMT 963VENT (Stahnsdorf, Germany) was able to detect the concentration of ozone in the gas phase at the inlet of the vessel, while the concentration in the outlet was monitored by means of an Erwin Sander *Quantozon* device. With the difference between these two measurements (knowing the respective flows), the ozone dose, instantly and along the experiment, was obtained in terms of mg of O_3 used by L of solution treated.

At the beginning of every experiment, the reactor was charged with an aqueous solution with 200 mg·L^{-1} of methomyl (2 mL of Tomilo). The pH of the reaction solution was allowed to evolve freely. Experiments were performed at room temperature (20–25 °C). Samples were periodically withdrawn for pH, HPLC and TOC analysis. This sampling provokes volume decrease, but it was regarded not to exceed the 10% of the initial 2L. This descent, although small, was taken into account in ozone consumption calculations. For the biodegradability characterization of the final effluent depending on the time of ozonation, several experiments were carried out at the same conditions but being finished at different times. The resulting of those experiments was used to execute the BOD$_5$, COD and toxicity analysis, in order to obtain an evolution through time of treatment of those parameters. All the surplus gas streams were driven to devices in where, by means of a reaction with KI, the ozone was destroyed into water and oxygen.

Photo-Fenton Procedure

Aqueous solutions with 200 mg·L^{-1} of methomyl and 70 mg·L^{-1} of Fe^{2+} (as $FeSO_4 \cdot 7H_2O$) were subjected to photo-Fenton reaction in a 2 L jacketed Pyrex vessel, wrapped with aluminum foil to protect the environments from radiation. This reactor was equipped with three blue lamps (Philips TL 8W-08 FAM), axially arranged inside it, with nominal power of 8W each, emitting radiation between 350 and 400 nm, with a maximum at 365 nm. More details of the experimental system is already described by González et al., 2007.

The temperature was kept at 25 °C with a thermostatic bath (Haake C-40) and good mixing was provided using a magnetic stirrer. Once the solution was fed into the reactor and this was closed, 1.8 mL of H_2O_2 30% w/v was added, simultaneously to the switching-on of the lamps, this constituted the beginning of the experiment. The reaction was left to evolve till H_2O_2 was consumed. Quantofix peroxide-reactive sticks were used to control this consumption. Samples were periodically withdrawn and quenched with sodium hydrogen sulfite to carry out HPLC, TOC and toxicity analysis with the same consideration taken for ozonation sampling. To perform BOD_5 and COD analysis, the resulting effluent of the treatment was saved.

RESULTS AND DISCUSSION

To know about the effectiveness of the treatments, solutions with the same initial concentration on methomyl (200 $mg \cdot L^{-1}$) were subjected to ozonation and the photo-Fenton reaction in the best conditions for both processes (based on previous experiments) for the depletion of the pesticide and for the elimination of the total organic carbon (mineralization grade). Photo-Fenton reaction was carried out with 300 $mg \cdot L^{-1}$ of hydrogen peroxide and 70 $mg \cdot L^{-1}$ of Fe^{2+}. On the ozonation, an average inlet ozone concentration of 10.5 $mg \cdot L^{-1}$ was used along the treatment, and the pH, initially around 4.5, evolved freely tending to 4.00. In terms of the concentration of pesticide and of organic carbon remaining, Figures 2 and 3 show the comparison between both technologies.

The depletion of methomyl is not as effective with the photo-Fenton reaction as with the ozonation, as can be seen in Figure 2. The carbamate is completely eliminated in 60 minutes of treatment with ozone. In that time, the photocatalytic reaction is only able to cope with 20% of the pesticide. The total consumption of the H_2O_2 occurs in 300 minutes, with a final methomyl elimination of 40%. In opposition to this, and according to Figure 3, the mineralization achieved by means of photo-Fenton reaction is bigger than at the end of the ozonation experiment. The photo-Fenton mineralizes 40% of the total organic carbon of the effluent, while the ozonation reaches only 20%.

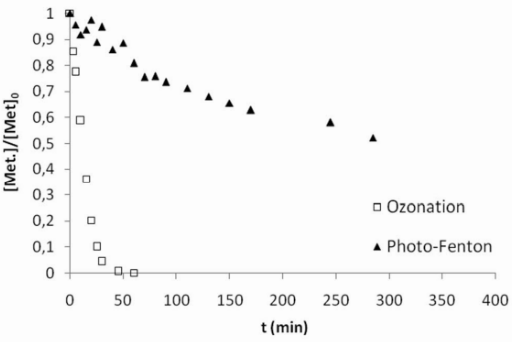

FIGURE 2. Comparison between treatments in terms of the elimination of the pesticide along the processes. Photo-Fenton: pH ≈ 2,7, $[H_2O_2]$ = 300mg·L^{-1}, $[Fe^{2+}]$ = 70mg·L^{-1}, [Met.] = 200mg·L^{-1}. Ozonation: free pH, $[O_3]$ = 10.5 g·m^{-3}, q = 50 L·h^{-1}.

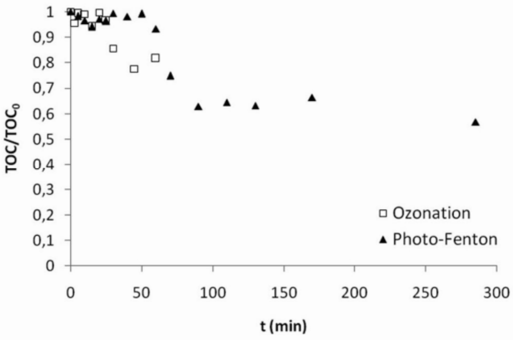

FIGURE 3. Comparison between treatments in terms of the mineralization grade eliminated along the processes. Photo-Fenton: pH ≈ 2,7, $[H_2O_2]$ = 300 mg·L^{-1}, $[Fe^{2+}]$ = 70 mg·L^{-1}, [Met.] = 200 mg·L^{-1}. Ozonation: free pH, $[O_3]$ = 10.5 g·m^{-3}, q = 50 L·h^{-1}.

As stated previously, both processes evolve at acid pH, consequently, in the case of ozone treatment, oxidation by the direct via predominates. In this situation, ozone is the main oxidant species, and although the target molecule has active sites, such as imine or sulfide groups, which O_3 is selective to, their oxidation does not lead to the total mineralization of the product, justifying this low TOC depletion achieved. This fact could be considered as an advantage for the photo-Fenton technology against ozonation: it is not just the pesticide itself which causes an environmental harm, but also its intermediates and inert ingredients, which degradation is also reflected on TOC diminishment.

The marked difference between technologies, in terms of pesticide depletion, could lead one to think that ozonation is the best option for depolluting this kind of waters. But as the elimination of TOC is far from complete, an evaluation of the toxicity and biodegradability characteristics of the ozonation and photo-Fenton resultant effluents is needed. BOD_5, COD and toxicity tests were carried out to establish the effluents compatibility with the environment and with a biological post-treatment to end up with the original pollutant remaining and the metabolites generated. Figure 4 represents the evolution of the ecotoxicity of the effluent depending on the ozone dose. The toxicity is expressed in Toxic Units, $TU = 100/EC_{50}$, the near TU is to 100, the more toxic the sample.

It can be seen that after 15 minutes (around 200 $mg \cdot L^{-1}$ of ozone dose) of treatment the sample toxicity clearly increases. Probably, from the very beginning of the reaction with ozone, toxic intermediates are generated and not destroyed during the process, causing this high toxicity in the effluent. This kind of behavior, where the toxicity increases instead of diminishing during an

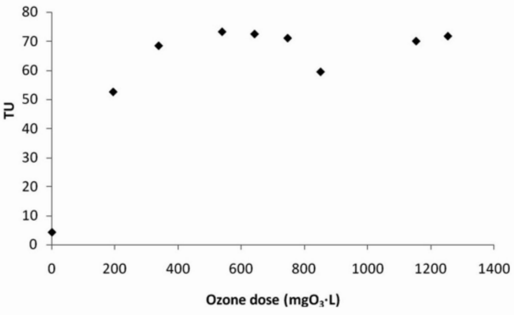

FIGURE 4. Evolution of the toxicity through the ozonation process in terms of TU. free pH, $[O_3] = 10.5\ g{\cdot}m^{-3}$, $q = 50\ L{\cdot}h^{-1}$.

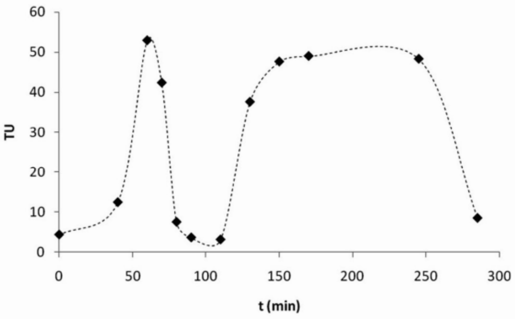

FIGURE 5. Evolution of the toxicity through the photo-Fenton process in terms of TU. pH ≈ 2,7, $[H_2O_2] = 300\ mg{\cdot}L^{-1}$, $[Fe^{2+}] = 70\ mg{\cdot}L^{-1}$. In dots, suggestion of the evolution.

oxidative treatment, has already been reported in literature for chlorinated compounds, (Shang et al, 2006; Dantas et al., 2008). The fact that the first point shows an outstanding lower value does not mean necessary that the raw pesticide is less toxic or more biocompatible. Acute toxicity assays does not take into account long term toxic effects and they neglect the accumulative nature of some substances and possible accumulation of their toxic effects, (Froehner et al., 2000; Laskowski, 2001).

The formulation of the product showed that there is an important biodegradable fraction in the solutions tested, one of the principal components of the commercial product, apart from methomyl, is ethanol. This substance is hardly attacked by the ozone (von Gunten, 2003) but highly biodegradable. The fact that, in spite of the presence of this substance, the biodegradability is so low from the very beginning (BOD_5/COD lower than 0.02), could be due to the existence of one or several products, either the pesticide itself, the intermediates produced or the inert ingredients of the commercial formulation, that apart from being non biodegradable, have an inhibitory effect over the degradative capacity of the microorganisms of the BOD_5 analyses.

On the side of photo-Fenton, Figure 5 shows the changes on toxicity as the photocatalytic reaction progresses.

As can be seen, general values are smaller than the ones obtained for the ozonation process. Moreover, the tendency is completely different. This time, the toxicity has two zones of increase, followed by a plateau in one of them, and a diminishment afterwards. This swinging behavior indicates the evolution of the oxidation through different intermediates in agreement with the mineralization grade achieved along the reaction.

Biodegradability at the end of photo-Fenton reaction is higher than the registered for the raw solution. However all the values along the process are far from the 0.4 index pointed at (Sarria et al., 2002) as characteristic of a biodegradable effluent. According to these low levels of biodegradability, although less toxic metabolites are punctually generated, the effluents can be considered non-biocompatible.

CONCLUSIONS

Ozonation technology is able to eliminate high concentrated aqueous solutions of methomyl in 60 minutes. Meanwhile the photocatalytic process only decomposes 40% of the pesticide loading in 300 minutes. Although the mineralization grade is quite low for both technologies, the photo-Fenton reaction seems to be more effective than the ozonation in this subject, even though it needs a longer operation time to achieve higher levels of mineralization.

The low grade of mineralization achieved by the ozonation suggests that the direct path in which the ozone is the principal oxidant reagent is not effective for the degradation of the organic matter derived from the pesticide and the ethanol contained in the commercial formula. In the case of the methomyl itself, although it has active sites to which the ozone is selective, it does not lead to the total oxidation of the substance.

The reaction between the components of the solution to treat and the ozone causes the generation of intermediates that increase the toxicity of the effluent and act as inhibitors to the biodegradation process. This is why, in spite of the relatively high content in ethanol of the samples, their biodegradability values are quite low.

The photo-Fenton reaction achieves global toxic values lower than in the case of ozonation, although it swings all along the process. Like in the case of the treatment with ozone the biodegradability achieved after the photocatalytic reaction shows the final effluent as non compatible with the environment neither with a biological post-treatment.

The study revealed that when the efficacy of an oxidation technique, like ozonation or photo-Fenton, in pollutant removal is evaluated, the depletion of the target compound needs to be complemented with a characterization of intermediates biocompatibility.

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APPENDIX II

Experimental design applied to photo-Fenton treatment of highly methomyl-concentrated water

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Experimental design applied to photo-Fenton treatment of highly methomyl-concentrated water

María M. Micó, Jordi Bacardit and Carme Sans

ABSTRACT

This work is focused on the study of the suitability of the photo-Fenton process as a pretreatment for water highly contaminated with a methomyl commercial formulation in Advanced Greenhouses devices. Initial concentrations of reagents and pesticide were evaluated according to a central composite experimental design, with methomyl depletion and biocompatibility of the final effluent as response functions. A triad of optimal operation conditions could be determined, $[\text{Met.}]_0 = 50\,\text{mg L}^{-1}$, $[H_2O_2]_0 = 254\,\text{mg L}^{-1}$ and $[Fe^{2+}]_0 = 77\,\text{mg L}^{-1}$ for the best elimination yield and an acceptable BOD_5/COD value, and initial concentration of methomyl can be established as the most important parameter for the performance of the treatment due to the limitations that impose on the hydrogen peroxide doses in the presence of the excipients of the commercial formulation.

Key words | advanced oxidation processes, biodegradability, central composite, pesticide, photocatalytic process, photo-Fenton, water treatment

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INTRODUCTION

The aim of sustainability, more important each day in every aspect of human development, is one of the impelling factors of the new concept of Advanced Greenhouses. These facilities, based on the original idea of protected agriculture, introduce structural, mechanical and functional improvements that make them more environmentally respectful than their predecessors, and even more competitive in terms of cropping yields. Among other improvements, Advanced Greenhouses are specially designed for the efficient use of water resources. The approach on which this work is based lies in the possibility of recovering lixiviates from the culturing substrate and returning the current to the greenhouse to be reused as many times as possible. Nevertheless, after several recirculation cycles, the surplus water concentration in salts and pesticides can be so high as to cause phytotoxicity problems. This is why a membrane separation stage could be used in order to condition the stream to take part again in the irrigation process, generating a permeate suitable for being resent to the cycle, and a brine highly concentrated in those named harmful substances.

In the last few decades, AOP's have been proven to be highly effective for the removal of most pollutants in wastewaters (Pera-Titus *et al.* 2004). This kind of treatment is based on the generation of hydroxyl radicals, whose strong oxidizing potential has been proven as a good alternative to traditional treatments such as photo-oxidation, chemical coagulation or absorption, among others, for the elimination of pesticides (Lafi & Al-Qodah 2006; Badawy *et al.* 2006). In these days, photo-Fenton is considered a suitable treatment for those pollutants with numerous references in literature (Abdessalem *et al.* 2010; Ballesteros-Martín *et al.* 2009), even with practical applications (Kenfack *et al.* 2009).

In photo-Fenton reaction, hydroxyl radicals are produced by the decomposition of hydrogen peroxide when reacting with ferrous ions in the presence of UV light, which contributes with an additional pathway to the generation of

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free radicals in comparison to the dark Fenton reaction (González *et al.* 2007). One of the main advantages of photo-Fenton reaction compared to other AOP's is the fact that solar light could also be a suitable source of radiation for the process, which makes it optimum for an outdoor installation, reducing costs and risks (Mendoza-Marín *et al.* 2010; Zapata *et al.* 2009*b*).

In this preliminary study, Fenton reaction photoactivated by artificial light has been tested applying experimental design procedures as a proposal to decontaminate the brine coming from the separation stage, too concentrated in harmful substances to be sent directly to a public sewage system. The main objective was to establish the suitability of the photo-Fenton reaction as an effective treatment to degrade certain pesticide loads in the influent and increase its intermediates biocompatibility. In addition, thanks to the statistical information derived from the analysis of the experimental design, the most influential parameters of the process were determined for both evaluated aspects.

Biocompatibility is considered an important factor to be taken into account for the design of a real treatment, since it has been already proven that photochemical pre-treatment can be shortened when combined with biological oxidation (Ballesteros-Martín *et al.* 2008). Biocompatibility was tested in order to evaluate the possibility of introducing a biological treatment after photo-Fenton reaction. According to this, the final optimization derived from the experimental design results will be performed taking into account not just contaminant removal capacity, but also the biodegradability of the resulting effluents.

The experiments were carried out with methomyl as target pollutant (*S-methyl-N((methylcabamoxyl)oxy)thioactimidate*). Literature about photo-Fenton applied to different pesticides through experimental design can be found (Segura *et al.* 2008); however, this research introduces the use of a commercial concentrate, instead of the pure substance: Tomilo-20 L, from Aragonesas Agro S.A. (200 g/l of the active product, and ethanol as main solvent). Its *non-active* ingredients could cause interference with the process, which could not be observed, nor taken into account, while working with the pure substance (Zapata, *et al.* 2009*b*). Therefore the use of a commercial product places this study in a more real scenario than working with a pure compound.

METHODS

Experimental conditions and analyses

Photo-Fenton reaction was carried out in a 2 L jacketed stirred vessel with three 8W black light lamps ($\lambda_{máx} = 365$ nm), at controlled temperature between 22 and 25°C. More detailed description of the device and the procedure can be found elsewhere (González *et al.* 2007). pH was kept at 2.7 (optimum for the equilibrium $[Fe^{2+}]/[Fe^{3+}]$ to achieve its highest ratio) at the beginning of the reaction and was monitored during the process, oscillating from 2.7 to 2.5. All the experiments were carried out until the total consumption of hydrogen peroxide from every sample, after withdrawn, was quenched with $NaHSO_3$ (except the one corresponding to the end of the reaction, when no quenching is needed). All the testing was performed according to an experimental design described in the next section. The extreme values for the involved parameters were the following: [50, 200] mg L^{-1} of pesticide, [50, 100] mg L^{-1} of ferric ion and [100, 300] mg L^{-1} of hydrogen peroxide. While the choice of pesticide concentrations is based on membrane separation capability of regulate brine dilution, the range of the concentrations of hydrogen peroxide and ferric ion needed preliminary assessments, not shown in this paper, that justify their tested levels. On the one hand, those experiments revealed that peroxide concentrations higher than 300 mg L^{-1} (near to the stoichiometric concentrations related to 200 mg L^{-1} of methomyl as a pure substance) showed worse reaction yields, probably caused by a radical scavenging effect of the peroxide itself, (Evgenidou *et al.* 2007), or by the interference of the commercial excipients known as organic substances susceptible of consuming radicals. On the other hand, catalytic inactivity of Fe^{2+} at lower concentrations than 50 mg L^{-1} was observed, most likely due to the formation of complexes with any excipient of the commercial product, or pesticide metabolites as it happens with different organic compounds and ferrous iron (Perdue *et al.* 1976).

Pesticide depletion at the end of each experiment was evaluated as a first answer function to be taken into account for the optimization. For this purpose, the concentration of pesticide during the reaction and the possible formation of intermediates (not identified in this work) were monitored by high performance liquid chromatography with a photodiode array detector. The column used was a Mediterranea Sea_{18}, 5 μm 25 × 0.46 cm (Teknokroma). The mobile phase, composed by a mixture of water and acetonitrile (60:40), was delivered at a flow rate of $1\,ml\,min^{-1}$. The wavelength of the UV maximum absorbance was 232.5 nm. Total Organic Carbon (TOC) was also monitored by a Shimatzu TOC-VCSN TOC analyzer.

Biodegradability, as a biocompatibility indicator, was measured in terms of BOD_5/COD (Biochemical and Chemical Oxygen Demand) ratio and was also evaluated as an answer function to be optimized through the experimental design. BOD_5 was analyzed by means of an OxiTop measure system (Weilheim) according to Standard Method 5210D (American Public Health Association *et al.* 1985) for five days of digestion. To analyze for COD, the Standard Method 5220D procedures were followed.

Experimental design

Several works have been published already in the field of experimental design precisely in the frame of pesticide decontamination (Segura *et al.* 2008; Zapata *et al.* 2009*a*). Their positive results are based on the reduction of the number of experiments performed without renouncing the quality information that can be achieved using these statistical methods.

In this study, photo-Fenton reaction was evaluated using these analytical tools and according to those ideas stated by Leardi (Leardi 2009). The effect on the pesticide removal yield and biocompatibility of the mixture after the process, of initial concentrations of Fe(II), H_2O_2 and methomyl, was assessed.

Specifically, the method identifies the first and second response variables, pesticide removal yield, Y (Equation (1)), and biodegradability as BOD_5/COD; with two empiric equations that define hypersurfaces of 4 dimensions, in which independent variables are the named reagents and methomyl initial concentrations, the products between them, and their second potencies.

$$Y(\%) = \frac{[\text{Met.}]_0 - [\text{Met.}]}{[\text{Met.}]_0} \times 100 \tag{1}$$

Only these three parameters were taken into account being aware that there are many other factors that can interfere in photo-Fenton reaction, such as pH or temperature. Nevertheless they were fixed around the values expected in an implementation of the process in the field.

The experiments were performed according to a central composite design consisting on a factorial design and 6 star points, in order to obtain the information needed for defining the response surfaces (3 factors: $[\text{Met.}]_0$, $[H_2O_2]_0$ and $[Fe^{2+}]_0$; and 2 levels: [50, 200], [100, 300] and [50, 100] $mg\,L^{-1}$ respectively). A summary of the experimental design and results is presented in Table 1. Variables were coded on two normalised levels: +1 as the highest and −1 the lowest value. According to this, the central point of the design was coded as (0, 0, 0). Three replicated experiments were carried out at that central point, in order to check the statistical consistency of the data. Star points were distributed at $\sqrt{3}$ times the distance from the central point 0 to +1; except in the case in which that distance implies negative values of one factor. In that case, identified with a star (∗), the negative value was replaced with the corresponding −1 value. Statistical validation was determined by ANOVA test at 95% confidence level.

RESULTS AND DISCUSSION

Pesticide depletion

It can be observed in Table 1 how the removal percentage, Y, oscillates between low values of elimination, 29% at (200, 100, 100), in ($[\text{Met.}]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) ($mg\,L^{-1}$), to the virtually complete depletion at (50, 300, 50). A multivariate regression was performed on this data to obtain a mathematical expression that represents the response hypersurface, describing Y as a function of initial concentrations of pesticide, hydrogen peroxide and ferrous ion. Equation (2) shows a corresponding model with significant coefficients.

$$\begin{aligned} Y(\%) = {} & 45.2 - 0.6[\text{Met.}]_0 + 0.24[H_2O_2]_0 + 1.42[Fe^{2+}]_0 \\ & + 0.0009[\text{Met.}]_0[H_2O_2]_0 - 0.0017[\text{Met.}]_0[Fe^{2+}]_0 \\ & - 0.0010[H_2O_2]_0[Fe^{2+}]_0 + 9.1\times10^{-4}[\text{Met.}]_0^2 \\ & - 4.1\times10^{-4}[H_2O_2]_0^2 - 0.0070[Fe^{2+}]_0^2 \end{aligned} \tag{2}$$

Table 1 | Central composite design of photo-Fenton oxidation of methomyl commercial formula and corresponding results obtained

Experiments	$[\text{Met.}]_0$ (mg L^{-1})	$[H_2O_2]_0$ (mg L^{-1})	$[Fe^{2+}]_0$ (mg L^{-1})	Y (%)	BOD_5/COD
1	50 (−1)	100 (−1)	50 (−1)	86.0	0.151
2	200 (1)	100 (−1)	50 (−1)	34.0	0.010
3	50 (−1)	300 (1)	50 (−1)	99.9	0.327
4	200 (1)	300 (1)	50 (−1)	68.0	0.007
5	50 (−1)	100 (−1)	100 (1)	99.9	0.110
6	200 (1)	100 (−1)	100 (1)	29.0	0.005
7	50 (−1)	300 (1)	100 (1)	97.0	0.242
8	200 (1)	300 (1)	100 (1)	58.8	0.013
9	125 (0)	26.79 ($-\sqrt{3}$)	75 (0)	42.0	0.000
10	125 (0)	373.71 ($\sqrt{3}$)	75 (0)	83.7	0.010
11	125 (0)	200 (0)	31.7 ($-\sqrt{3}$)	66.0	0.006
12	125 (0)	200 (0)	118.5 ($\sqrt{3}$)	58.0	0.006
13	50 (−1)*	200 (0)	75 (0)	99.4	0.205
14	254.9 ($\sqrt{3}$)	200 (0)	75 (0)	51.8	0.000
15	125 (0)	200 (0)	75 (0)	74.8	0.140
16	125 (0)	200 (0)	75 (0)	76.1	0.120
17	125 (0)	200 (0)	75 (0)	77.0	0.110

*Point ($-\sqrt{3}$, 0, 0) had to be replaced by (−1, 0, 0) because the real variable value for ($-\sqrt{3}$) was lower than 0.

The regression coefficient R^2 indicates that the model explains 98.95% of Y variability. Figure 1 presents examples of the response surface derived from Equation (2) for a fixed value of methomyl concentration.

An ANOVA test was carried out in order to compare the model variance with the variance of the residuals (error). If the variances are different enough, the parameters taken into account are significant and have influence on the model. To measure this difference, the F-test is performed, and from it the P-Value is obtained. The parameters would have influence on the model if their P-Values are lower than 0.05, those with the lowest ones would have the highest influence. Table 2 shows the results of the ANOVA test.

P-Value for lack of fit test is higher than 0.05, showing that the model appears to be adequate for the observed data at the 95.0% confidence level.

Since the value of its P-Value is the lowest, initial methomyl concentration can be considered the most influential factor in this design. Furthermore, the negative value of its coefficient in Equation (2) physically implies that a higher concentration of pesticide entails lower depletion yields.

In practical terms, it is not just that the concentration of the pesticide determines the performance of the reaction, but also the lack of enough oxidant reagent to cope with the pollutant load. This fact, together with the previous experiments that bear witness to the worse depletion yields achieved with higher doses of hydrogen peroxide, indicates the presence of an important scavenging effect that consumes radicals without degrading the target compound. This scavenging could be due to the hydrogen peroxide itself however, the highest concentration used in these essays was close to the stoichiometric quantity related exclusively to the highest pesticide concentration (not taking into account the inert ingredients), weakening this first explanation. There is another possible source of radical competition, the excipients of the commercial formulation, in this case ethanol and short esters. These substances could consume radicals and make the reagent not sufficient, even for the higher doses, simulating peroxide scavenging. The presence of this excipient is obviously related to the initial concentration of the pesticide used in the experiments, which in the real proposed application would be related to the performance of the membrane. This is why the initial concentration of

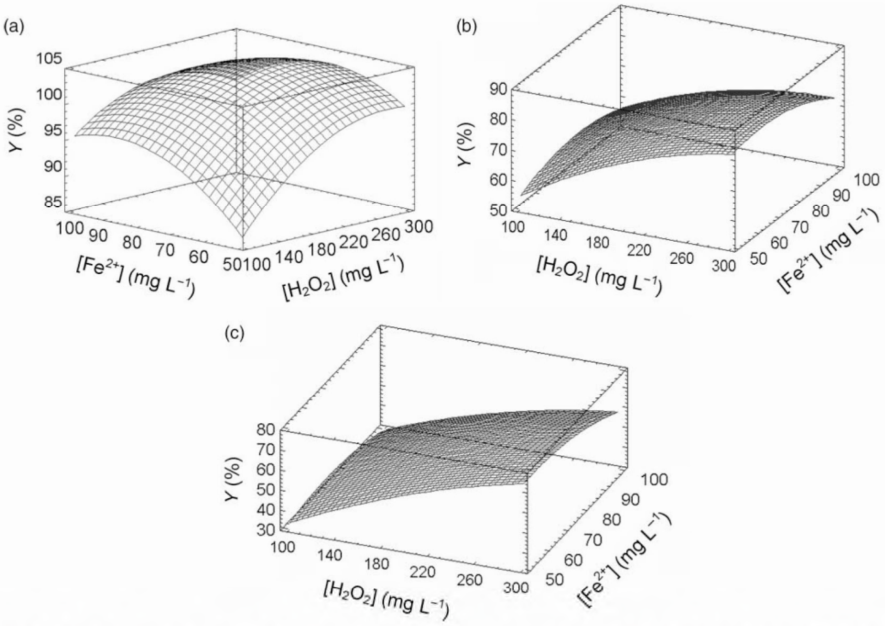

Figure 1 | Biodegradability values of final effluents of photo-Fenton experiments as function of the ratio $[H_2O_2]_0/[Fe^{2+}]_0$ in $mg L^{-1}$. Above the columns BOD_5 and COD numerical values can be seen as $mgO_2 L^{-1}$. Ratio 0: Raw solution (50, 0, 0); Ratio 2: (50, 100, 50); Ratio 3: (50, 300, 100); Ratio 6: (50, 300, 50), in ($[Met.]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) ($mg L^{-1}$).

the pesticide becomes an important parameter to take into account for the design of the separation stage, due to the tight relationship between that extra organic load and the quantity of peroxide that can be added for a successful treatment. Reinforcing this hypothesis mathematically, the initial concentration of H_2O_2 seems to have also a remarkably influence, it has the second lowest *P*-Value.

Synergies are represented by the coefficients preceding the products of factors two by two. $[Met.]_0[Fe^{2+}]_0$ and $[H_2O_2]_0[Fe^{2+}]_0$ cross-effects have the lowest influence,

Table 2 | ANOVA test for coefficients from Equation (2)

Source	Sum of Squares	D.F.	Mean square	*F*-Ratio	*P*-Value
$[Met.]_0$	5,703.31	1	5,703.31	4662.11	0.0002
$[H_2O_2]_0$	1,547.79	1	1,547.79	1265.22	0.0008
$[Fe^{2+}]_0$	20.397	1	20.397	16.67	0.0551
$[Met.]_0^2$	206.141	1	206.141	168.51	0.0059
$[Met.]_0[H_2O_2]_0$	347.952	1	347.952	284.43	0.0035
$[Met.]_0[Fe^{2+}]_0$	79.1282	1	79.1282	64.68	0.0151
$[H_2O_2]_0^2$	221.596	1	221.596	181.14	0.0055
$[H_2O_2]_0[Fe^{2+}]_0$	54.9152	1	54.9152	44.89	0.0216
$[Fe^{2+}]_0^2$	252.363	1	252.363	206.29	0.0048
Lack of fit	84.1079	5	16.8216	13.75	0.0692
Pure error	2.44667	2	1.22333		
Total correlation	8,306.02	16			

while $[\mathrm{Met.}]_0[\mathrm{H_2O_2}]_0$ is the most noticeable cross-term, confirming again the dependence of the quantity of peroxide that promotes the depletion of methomyl with the initial quantity of the pesticide itself and its related inert ingredients.

Although its contribution is also positive, ferrous ion concentration presents no influence (P-Value > 0.05). Nevertheless, since its cross-effects and quadratic expression are significant, the factor $[\mathrm{Fe}^{2+}]_0$ itself cannot be removed from the model in practical terms this is caused mathematically by the quadratic term of Fe(II) concentration, and it could be chemically justified by the existence of a radical scavenging effect by ferrous iron, already referenced in the literature (Ay *et al.* 2009).

The curvature of the response surfaces reveals the weight of the quadratic terms. The most influential one is $[\mathrm{Fe}^{2+}]_0^2$, followed by peroxide and methomyl concentration quadratic terms, with higher P-Values than some synergic effects.

As can be seen in Figure 1(b, c), for upper values of initial concentration of methomyl, the behaviour of the surface reveals an increase of depletion rate for the higher concentration of reagents, achieving the top depletion, around 80%, for the maximum concentrations of H_2O_2 and ferrous ion. Nevertheless, for the lowest concentration of pesticide, 50 mg L^{-1}, an optimum ratio H_2O_2/Fe^{2+} can be seen for the initial concentration intervals of 180 to 260 mg L^{-1} of hydrogen peroxide and from 70 to 80 mg L^{-1} of ferrous ion; conditions in which the depletion reached is complete. Mathematically, the optimum values are identified as $[\mathrm{Met.}]_0 = 50\,\mathrm{mg\,L^{-1}}$, $[\mathrm{H_2O_2}]_0 = 245\,\mathrm{mg\,L^{-1}}$, $[\mathrm{Fe}^{2+}]_0 = 77\,\mathrm{mg\,L^{-1}}$. The presence of this optimum gives an idea of the best operation conditions, from those studied, for the oxidative process with the lowest tested pesticide concentration pesticide.

Biocompatibility

The biodegradability measurements were carried out in order to study biocompatibility of final effluents of the oxidative process with a subsequent biologic reactor which could finish the degradation of the organic matter not mineralized by the chemical process. The low BOD_5 values obtained for the highest concentrations of pesticide and its high carbonic load (due to the inert ingredients) caused low biodegradability values, practically near to 0, in all the pretreatment conditions tested. In opposition to this, 50 mg L^{-1} experiments show remarkably high BOD_5/COD values. Figure 2 depicts the changes in biodegradability depending on the ratio of reagents used for the lowest initial concentration of pesticide, supposing they and their interaction as an important factor on biodegradability values.

Apparently, the experiments show an enhancement of biodegradability with the increase of the reagents ratio which seems to identify these parameters as capitals for obtaining maximum values of BOD_5/COD ratio, close to 0.4, which characterizes easily biodegradable effluents (Sarrià *et al.* 2002). Regarding Figure 2, an increase of BOD_5 is also detected in every case in comparison to the raw dilution of methomyl; while COD suffers a perceptible diminishment for ratios 3 and 6, more than 60%, slightly more intense for the lowest concentration of iron II, possibly due to a scavenging effect by the cationic metal. Both the increase of BOD_5, and the diminishment of COD identify a noticeable oxidation of the organic load. Nevertheless, probably, due to the presence of inert ingredients, that interferes with radical action, TOC results do not coincide with this tendency. The total organic carbon measures show negligible mineralization even in the highest $[\mathrm{H_2O_2}]_0/[\mathrm{Fe}^{2+}]_0$ ratio case, indicating the generation of oxidised by-products from methomyl, and even its excipients (presumably partially oxidized), which cannot be further degraded by the chemical treatment. This fact, together with the positive evolution of biocompatibility

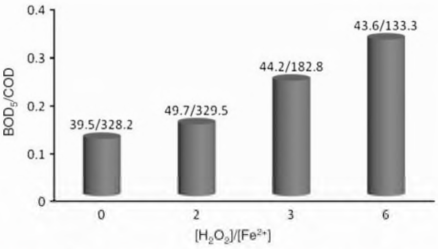

Figure 2 | Biodegradability values of final effluents of photo-Fenton experiments as function of the ratio $[\mathrm{H_2O_2}]_0/[\mathrm{Fe}^{2+}]_0$ in mg L^{-1}. Above the columns BOD_5 and COD numerical values can be seen as mgO_2 L^{-1}. Ratio 0: Raw solution (50, 0, 0); Ratio 2: (50, 100, 50); Ratio 3: (50, 300, 100); Ratio 6: (50, 300, 50), in ($[\mathrm{Met.}]_0$, $[\mathrm{H_2O_2}]_0$, $[\mathrm{Fe}^{2+}]_0$) (mg L^{-1}).

achieved could justify the suitability of an integrated chemical-biological treatment, introducing a subsequent bioreactor as proposed before.

Despite this, the mathematical study of the results of the experimental design should give more information about the real influence of each, and will show that indeed, it is not always what seems to be an important factor at first sight.

To start with, an ANOVA test was carried out over BOD_5/COD results that is reflected in Table 3. As can be seen, *F*-test and the following *P*-Value showed that there is more than one parameter that can be considered non significant for the model.

It can be observed that the highest *P*-Value, far from 0.05, corresponds to the interaction between the two reagents, hydrogen peroxide and iron (II), followed by the individual effect of the metal itself and the interaction between Fe^{2+} and the pesticide. The low influence in the model of the cross effect $[H_2O_2]_0[Fe^{2+}]_0$ invalidates the first consideration explained about biodegradability, showing the non real significance of the ratio $[H_2O_2]_0/[Fe^{2+}]_0$. This means that although apparently positive results have been obtained relating to different reagents ratios, they cannot be considered significant, and in order to obtain optimal conditions for maximizing biodegradability, more parameters have to be taken into account.

Non significance of those terms, namely, $[H_2O_2]_0[Fe^{2+}]_0$ and $[Met.]_0[Fe^{2+}]_0$, are reflected on the final mathematical model where both interactions in which iron takes part have been neglected. The individual effect cannot be ignored so easily again, due to the significance of the quadratic factor related to it. According to this, the equation that defines the BOD_5/COD versus parameters in Table 3 is presented as Equation (3).

$$\begin{aligned} BOD_5/COD = {} & -0.14 - 0.0018[\mathrm{Met.}]_0 \\ & + 0.0020[H_2O_2]_0 + 0.006[Fe^{2+}]_0 \\ & + 5.73 \times 10^{-6}[\mathrm{Met.}]_0^2 - 5.0 \\ & \times 10^{-6}[\mathrm{Met.}]_0[H_2O_2]_0 - 2.8 \\ & \times 10^{-6}[H_2O_2]_0^2 - 4.43 \times 10^{-5}[Fe^{2+}]_0^2 \end{aligned} \tag{3}$$

In this case, R^2 is only 0.898 but, as can be seen on Table 3, *P*-Value of lack of fit test is higher than 0.05, so the model appears to be adequate for the observed data at the 95.0% confidence level.

Regarding the *P*-Values of the coefficients, it can be observed that the parameter that has the most important influence on BOD_5/COD value is methomyl initial concentration, being also significant at its quadratic term. Their negative coefficients in Equation (3) imply that higher quantities of pesticide derive from worse biocompatibility results after the treatment. On one hand there is the possible increase of initial COD inherent to a higher concentration of organic matter at the beginning of the process, which cannot be effectively degraded by photo-Fenton reaction,

Table 3 | ANOVA test for BOD_5/COD regression

Source	Sum of Squares	D.F.	Mean square	*F*-Ratio	*P*-Value
$[\mathrm{Met.}]_0$	0.10868	1	0.10868	358.65	0.0028
$[H_2O_2]_0$	0.00782	1	0.00782	25.81	0.0366
$[Fe^{2+}]_0$	0.00108	1	0.00108	3.56	0.1999
$[\mathrm{Met.}]_0^2$	0.00817	1	0.00817	26.96	0.0351
$[\mathrm{Met.}]_0[H_2O_2]_0$	0.01143	1	0.01143	37.73	0.0255
$[\mathrm{Met.}]_0[Fe^{2+}]_0$	0.00206	1	0.00206	6.81	0.1208
$[H_2O_2]_0^2$	0.01036	1	0.01036	34.19	0.0280
$[H_2O_2]_0[Fe^{2+}]_0$	0.00014	1	0.00014	0.46	0.5688
$[Fe^{2+}]_0^2$	0.01001	1	0.01001	33.25	0.0288
Lack-of-fit	0.01389	5	0.00278	9.17	0.1013
Pure error	0.00061	2	0.00030		
Total correlation	0.16334	16			

giving higher values of final COD. On the other hand, an inhibitory effect to biodegradation by methomyl itself or its metabolites, either by inert compounds of the formulation, could be suggested, even because of their own nature or because of the lack of hydrogen peroxide to perform the adequate oxidation, leading to a limited biodegradability.

After the methomyl individual effect, the most significant influence is the interaction between the pesticide and H_2O_2. Its negative coefficient indicates that higher concentrations of both substances end up with lower biodegradability results, which is a positive fact with respect to reagent consumption. It has been previously stated how the maximum quantity of efficient hydrogen peroxide depends on the quantity of initial pesticide due to the inert ingredients content that provokes radicals consumption, limiting the biodegradability that can be achieved The statement that concentration of hydrogen peroxide could imply higher biodegradability is also supported by the coefficient of H_2O_2 quadratic term although it is contradicted by the individual effect (Figure 3).

Finally, by optimizing the mathematical expression, a trio of best initial conditions can be found corresponding to a maximum biocompatibility of the final effluent. Those initial concentrations are $[\mathrm{Met.}]_0 = 50\,\mathrm{mg\,L^{-1}}$, $[H_2O_2]_0 = 309.1\,\mathrm{mg\,L^{-1}}$ and $[\mathrm{Fe}^{2+}]_0 = 71\,\mathrm{mg\,L^{-1}}$, quite close to ones from the optimum set obtained for the modelization of pesticide depletion, (50, 254, 77) $\mathrm{mg\,L^{-1}}$. This coincidence establishes a relationship between the level of degradation of methomyl achieved and the biocompatibility of the treatment final effluent, and states the importance of limiting the concentration of pesticide treated. This fact should be taken into account in the design of the separation stage. Its performance should provide the system with an enriched effluent, although its concentration should not exceed a certain value.

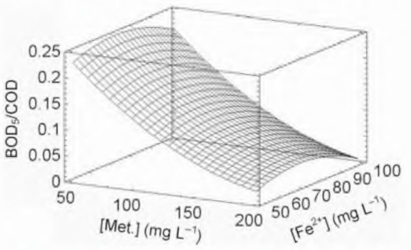

Figure 3 | Estimated response surface showing BOD_5/COD as a function of initial concentrations of methomyl and iron (II) (in $\mathrm{mg\,L^{-1}}$) for $[H_2O_2]_0 = 200\,\mathrm{mg\,L^{-1}}$.

Regarding the possibility of implementing a subsequent biological reactor to oxidize the organic matter non degraded by the process, even with the set of conditions for the maximal degradation, biodegradability achieved is 0.45 (obtained by the model), more than acceptable for considering the effluent as biodegradable (Sarrià *et al.* 2002). As a result, those three conditions $[\mathrm{Met.}]_0 = 50\,\mathrm{mg\,L^{-1}}$, $[H_2O_2]_0 = 254\,\mathrm{mg\,L^{-1}}$ and $[\mathrm{Fe}^{2+}]_0 = 77\,\mathrm{mg\,L^{-1}}$, can be considered the optimal for this study.

CONCLUSIONS

Photo-Fenton has been revealed as an effective process to treat waters highly polluted with methomyl in the conditions essayed, since high pesticide depletion yields have been achieved ($Y > 95\%$), together with acceptable values of biocompatibility of the final effluent ($BOD_5/COD > 0.4$).

Experimental design procedures determined the most important variables influencing those two parameters, revealing as a useful tool to obtain quality information minimizing the number of experiments in this kind of research with more than one variable to check. On the one hand, initial pesticide concentration has been shown as the factor with the highest influence in the studied responses, due to the inert ingredients it entails. These substances seem to consume radicals preventing further degradation of methomyl and higher biodegradability values. According to this, the existence of the excipients seems to be capital for hydroxyl radical oxidation processes, which suggests that the organic matter accompanying the active principles have to be taken into account for implementing designs. In this case, this fact implied the need for setting $50\,\mathrm{mg\,L^{-1}}$ as the optimal pesticide concentration to work with. On the other hand, optimal values for the other two factors could be inferred also from the result of the statistical study of pesticide depletion: $[H_2O_2]_0 = 254\,\mathrm{mg\,L^{-1}}$ and $[\mathrm{Fe}^{2+}]_0 = 77\,\mathrm{mg\,L^{-1}}$.

These optimal values were in agreement with the conclusions extracted from the evaluation of BOD_5/COD. This parameter improvement achieved by the photo-Fenton treatment, together with the low TOC depletion obtained, suggested the suitability and necessity of a chemical-biological integrated system, where a subsequent bioreactor could assimilate the organic load not totally oxidized by the Advanced Oxidation Process.

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APPENDIX III

Photo-Fenton reaction applied to imidacloprid highly polluted water removal

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PHOTO-FENTON REACTION APPLIED TO IMIDACLOPRID HIGHLY POLLUTED WATER REMOVAL

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BACKGROUND

Since Imidacloprid release in 1991, neonicotinoid pesticides have continually increased their market share till nowadays, beating consolidated products like carbamates or organophosphates. They comprise an effective solution to the increasing problem of some species cross-resistance to common insecticides. This and the fact that, from the very beginning of the production process, they are conceived as products less harmful than their predecessors, not just for the users, but also for the consumers of the treated crops, the manufacturers and the environment, are advantages that justify a constant improve in sells.

A new concept of protected cropping is being developed in the last few years, known as Advanced Greenhouses. Among other technological improvements, this facilities comprise a semi-closed system of water reuse that recovers lixiviates from irrigation, physically treats that effluent by a reverse osmosis step and resends the permeate to the greenhouse. The main drawback to these devices is the fact that the brine coming from the membrane separation is highly concentrated on pesticides and salts, so it is hardly degraded at conventional wastewater treatment plants.

The aim of this work is to perform a preliminary study of the effects of the photo-Fenton reaction, an Advanced Oxidation Process, as a pretreatment for this new generation pesticide polluted water. The final scope will be to adapt those brines to be re-circulated into the greenhouse or to be emitted towards a wastewater treatment plant. For this research, in order to work in a more real scenario, Imidacloprid was not used as pure substance (Segura et al. 2008) but a commercial formula supplied by Aragonesas Agro S.A., with this neonicotinoid ($200 g \cdot L^{-1}$) as active ingredient. The so called *non-active* ingredients that it contains, small esters mainly, could cause interferences along the process, which couldn't be observed nor taken into account while working with the pure substance.

METHODS

Photo-Fenton reaction was carried out in a 2 L jacketed stirred vessel with three 8W black light lamps ($\lambda_{máx.}$ 365 nm), axially arranged. Pesticide and ferrous ion concentration, 20mg·L^{-1} and 15mg·L^{-1}, respectively haven been selected taking into account a previous stage of reverse osmosis. On the other hand, concentrations of hydrogen peroxide were chosen in order to include the ratio [Imidacloprid]/[H_2O_2] suggested by Segura et al. 2008 inside the range tested, and to establish a comparison btween the results of the cited reference and the obtained at the experiments with the commercial formulation. The concentration of pesticide along the reaction and the possible formation of intermediates were monitored by a high performance liquid chromatographer with photodiode array detector. The column used was a Mediterranea Sea$_{18}$, 5 µm 25x0.46 cm (Teknokroma). The mobile phase, composed by a mixture of water and acetonitrile (60:40), was delivered at a flowrate of 1 mL.min^{-1}. The wavelength of the UV absorbance detector was 269.5 nm. Total Organic Carbon (TOC) was monitored by means of a Shimatzu TOC-VCSN analyzer. Biodegradability was measured in terms of BOD_5/COD ratio.

RESULTS AND DISCUSSION

Figure 1 shows the evolution of pesticide concentration along the photo-Fenton reaction. *Y* represents the percentage of Imidacloprid eliminated in process,

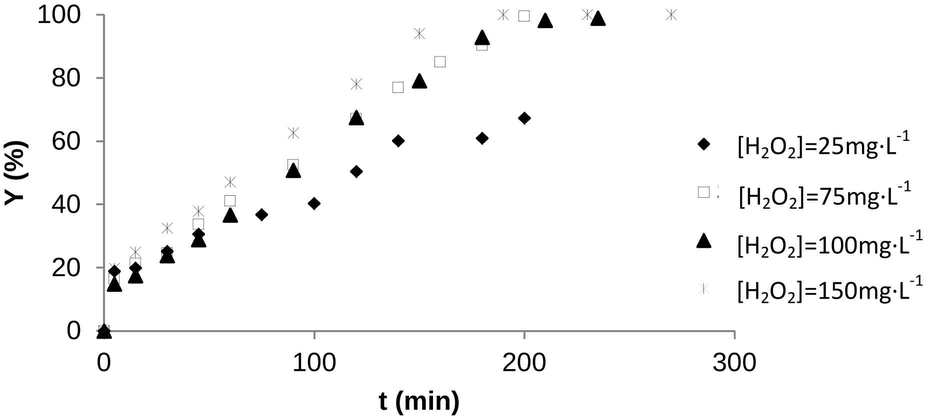
$$Y(\%) = \frac{[Imid.]_0 - [Imid.]}{[Imid.]_0} \cdot 100 \qquad \text{(Equation 1).}$$

Imidacloprid is totally depleted with 75 mg.L^{-1} of H_2O_2. There is no improvement of the reaction rate with higher H_2O_2 doses.

The biodegradability mesurements (BOD_5/COD ratio) coincide with this behaviour. The initial ratio of 20 mg·L^{-1} solutions increases from 0.12 to 0.16 after photo-Fenton reaction with $[H_2O_2]_0$=25mg·L^{-1}, while the values reach a top and remains the same from the experiment with 75 mg·L^{-1} to the last one (0.21, 0.22 and 0.21, for 75, 100 and 150mg·L^{-1}).

These facts could be due to a phenomena of scavenging that is taking part at the reaction, consuming the free radicals in competition with the pesticide. This effect could derivate from the hydrogen peroxyde itself, but also from the *innert* compounds of the commercial formula.

Fig.1 Profile of the elimination of Imidacloprid through performance of the experiments. $[Imid.]_0$=20mg·L^{-1}, [Fe^{2+}]=15mg·L^{-1}.



The Total Organic Carbon (TOC) measurements give more information about pesticide oxidation. On one hand , there is the fact that the concentrations of initial TOC is much higher than the expected for solutions of 20mg·L^{-1} of the pure compound (24,5 mgTOC·L^{-1} versus 8,2 mgTOC·L^{-1}). There is a high contribution on carbonic matter by innerts that can interfer with the reaction, consuming free radicals itself. On the other hand, along the process of elimination, the final depletion of TOC is really low (a 10% in highest quantity of peroxyde case), although pesticide depletion is completed when using 75mg·L^{-1} initial hydrogen peroxide concentration and higher. The scavenging effect by the non-active ingredients could still be present, but it doesn't contributes noticeably to their mineralisation. At Segura et al. 2008, the experiment corresponding to [Imid.]0=100mg·L^{-1}, [H2O2]=150mg·L^{-1} and [Fe^{2+}]=15mg·L^{-1}, achieves a TOC removal yield of 21%, although, as sayed, the experiments were performed with the pure specie.

CONCLUSIONS

Photo-Fenton reaction can be considered as a successful way of treating Imidacloprid in commercial formula in the tested conditions. An optimum initial concentration of hydrogen peroxide for the complete pesticide removal can be established between 25 and 75 mg·L^{-1} ([Fe^{2+}]=15mg·L^{-1}). By the other hand, the possible interferences of non-active ingredients or hydrogen peroxide scavenging were observed and low mineralization was obtained. Pesticide itself shows a moderate biodegradability related with the fact that it was conceived as more environmental respectful than precedents. In any case, BOD_5/COD ratio is not high enough to consider the effluent partially or fully biodegradable. Thus, the AOP is needed.

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APPENDIX IV

Fosetyl-Al photo-Fenton degradation and its endogenous catalyst inhibition

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Fosetyl-Al photo-Fenton degradation and its endogenous catalyst inhibition

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Abstract

Interferences from many sources can affect photo-Fenton reaction performance. Among them, catalyst inhibition can be caused by the complexation and/or precipitation of iron species by the organic matter and salts present in the reaction media. This is the case of the oxidation of effluents containing organophosphorous fosetyl-Al. The degradation of this fungicide generates phosphate anions that scavenge iron and hinder Fe(II) availability. Experimental design was applied to artificially enlighten photo-Fenton reaction, in order to evaluate fosetyl-Al degradation. The performed experiments suggested how iron inhibition takes place. The monitoring of photo-Fenton reaction over a mixture of fosetyl-Al with other two pesticides also showed the interferences caused by the presence of the fungicide on other species degradation. Solar empowered photo-Fenton was also essayed for comparison purposes. Artificial and solar light photo-Fenton reactions were revealed as effective treatments for the elimination of tested fungicide. However, the phosphate ions generated during fosetyl oxidation decrease iron availability, what hampered organic matter degradation. Performed BOD_5/COD tests showed how fosetyl-Al biodegradability increases thanks to the oxidation treatment.

1. Introduction

Light empowered Advanced Oxidation Processes, such as photo-Fenton reaction or photo-catalysis, are based on the light enhancement of the production of highly reactive radical species. They has been proven as efficient processes for the decontamination of waters polluted with different origin emergent pollutants [1, 2] such as endocrine disruptors [3, 4], pharmaceuticals [5, 6], pesticides [7-9], etc. They have been recently shown as promising for the treatment of industrial wastewater [2, 3] and the prevention of groundwater contamination [4, 5]. One of the main advantages of these techniques among other AOPs has to do the versatility of their light/energy source. Although most studies are performed in artificial UV light reactors, very positive results are being obtained in the last two decades from works on solar light empowered devices [1, 10-

13], which makes them optimum for outdoor installation, especially in rural areas, which in turn reduces both costs and risks.

This work is specially focused on the photo-Fenton reaction and certain factors influencing the efficiency of this process. It faces a new perspective of the study of photo-Fenton applied over pesticide polluted water due to the fact that takes into account the direct interferences that the target contaminant to treat can exert over the process.

As said before, photo-Fenton reaction is based on the generation of highly reactive transient species, mainly hydroxyl radical, ·OH, that can rapidly oxidize most organic substances [6]. Reaction (a) shows how hydroxyl radical is obtained from the decomposition of hydrogen peroxide by means of the oxidation of dissolved ferrous ions. The presence of UV light photo-reduces Fe^{3+}, reaction (b), thereby recovering Fe^{2+} and contributing to an additional pathway leading to a greater generation of free radicals in comparison to the dark Fenton reaction [7, 9].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + HO^- \text{ (dark Fenton)} \tag{a}$$

$$Fe(OH)_2^+ + H^+ + h\nu \rightarrow Fe^{3+} + HO\cdot \tag{b}$$

Diverse sources of interferences in these reactions have been reported in literature and could be classified into ·OH scavenging effects and precipitation and/or complexation of iron species:

·OH scavenging:

One of the most reported sources of influence are halide salts. According to Pignatello et al. [14] Cl^- and Br^- consume hydroxyl radical in detriment of the target organic matter, according to the pH dependent reversible reaction (c) [15]. However, there are some cases in which the halide radical generated in the middle of the reaction can contribute enhancing the depletion of certain species from the organic load [9, 16].

$$\cdot OH + X^- \rightleftharpoons HOX\cdot^- \underbrace{\rightleftharpoons}_{H^+(-H_2O)} X\cdot \underbrace{\rightleftharpoons}_{X^-} X_2^-\cdot \tag{c}$$

Halide anions can also exert certain complexing effect over Fe(III) nonetheless they can be considered as relatively weak ligand of ferrous ion [14].

An excess of hydrogen peroxide in comparison to the organic matter content can also act as a hydroxyl radical sink itself [17, 18].

Fe complexing or precipitation:

The existence of strong interactions between Fe(II) and Fe(III) with major and minor ligands SO_4^{2-}, OH^-, HCO_3^-, CO_3^{2-} and HS^- is well known [19]. In the case of sulfate salts, they can hinder organic matter oxidation [20-22], even though they are poor ·OH scavenger, and sulfate iron complexes are soluble. However the SO_4^{2-}coordinated iron species inhibit the ulterior complexation with H_2O_2 needed previous to reaction (a), so they have to be taken into account if sulfate concentration is considerable.

Organic substances and their degradation by-products can also complex Fe(III); some of them compose labile complexes which undergo thermal or photochemical reduction to Fe(II) [23, 24]. Nevertheless certain ligands could stabilize the Fe(III), inactivates its recycling [9, 25] and jeopardizes organic matter oxidation.

Among other inorganic salts Photo-Fenton process seems to be quite sensitive to the presence of phosphate [4]. In the presence of $H_2PO_4^{2-}$ (predominant specie at pH~3) ferric ions undergo a complex reaction with this salt, causing Fe(III) to lose the ability to be recycled to Fe(II) and catalyze hydrogen peroxide decomposition into ·OH radicals [26, 27].

Regarding to the target compound, due to its simple structure, fosetyl-Al is easy to produce, cheap to sell; therefore it is widely used as systemic fungicide against oomycetes (mainly root-attacking *phytophtora* and downy mildews) in a variety of crops, both in agricultural environment and in gardening. The possibility of interfere in photo-Fenton reaction due to its phosphate group components makes it interesting for the study of mentioned endogenous inhibition.

According to all this, the main aim of this study is to evaluate the degradation of the fungicide fosetyl-Al by means of photo-Fenton reaction, and its role as catalyst inhibitor affecting its oxidation and the oxidation of other pesticides coexisting in the same mixture. For the accomplishment of the first purpose, experimental design in an artificially enlightened reactor was used, followed with biodegradability tests. Also solar photo-Fenton experiments were carried out in relation to the results obtained from the experimental design in order to complete the picture.

The second objective pursued in this work was to assess the behavior of fosetyl in the mixture of pesticides. This was accomplished by essaying photo-Fenton reaction over a mixture of fosetyl with other two pesticides structurally very different, methomyl and imidacloprid.

2. Experimental

2.1. Photo-Fenton reaction

The photo-Fenton reaction was carried out in different devices depending on the radiation source. For artificially enlightened reaction, a 2 L jacketed stirred vessel with three 8 W black light lamps (λ_{max}=.365 nm) axially arranged to the reactor was used. This device was connected to a thermostatic bath that permitted the experiments to evolve at a controlled temperature of 21 ºC. A more detailed description of the device and the procedure can be found elsewhere [5]. The pH was set to 2.7 (optimum for the equilibrium [Fe^{2+}]/[Fe^{3+}] to achieve its highest ratio) at the beginning of the reaction with H_2SO_4 (Panreac) diluted solution. No interference is expected from this sulfate due to its low concentration in the final mixture [14]. This value was monitored during the process and kept constant between 3 and 2.5 by the reaction itself. All the trials were carried out until reaching the total consumption of hydrogen peroxide. Every set and every sample was withdrawn and then quenched with $NaHSO_{3,}$ 40% w/v (Panreac) to remove the remaining hydrogen peroxide.

In the case of solar-photo-Fenton, the experiments were performed in two different locations. The smaller device consisted on a bench-scale plant located in University of Barcelona (latitude 41º28'N, longitude 2º06'E, sea level). The photo-reactor comprises a module of 6 parallel CPCs (theoretical concentration factor of 1, C_{CPC}=1) made of polished aluminum, with a total mirror´s area of solar irradiation caption-reflection of 0.228 m^2, tubular quartz receivers, attached to a galvanized surface angled 41º. The irradiated volume was 0.95 L from a total reaction volume of 5 L allocated in a mechanically stirred reservoir tank of 10 L. The solution was continuously recirculated employing a pump (peristaltic pump Ecoline VC-380, ISMATEC) through the photo-reactor piping and the reservoir tank. Temperature was not controlled and it could vary from 20 to 30 ºC.

The pilot photo-reactor was sited in Tabernas, Almería (Spain), at Plataforma Solar de Almería. Based also in CPC's [28], it is made up of two twin systems of three collectors. Each collector (1.03 m^2 each) consists of eight series-connected tubes, mounted on a fixed platform tilted 37º (Local Latitude). The 22 L of a total of 35 L was the irradiated volume. More details of this device can be found elsewhere [2, 29].

2.2. Chemicals

Fosetyl-Al or aluminum ethylphosphonate was chosen as a possible source of interference during its degradation due to its phosphorous derived structure, fig. 1. It is a pesticide with systemic fungicide activity. Fosbel 80 was the source of fosetyl-aluminum. The commercial formulation is a wettable product with 80% of the active principle and around a 19% of kaolin.

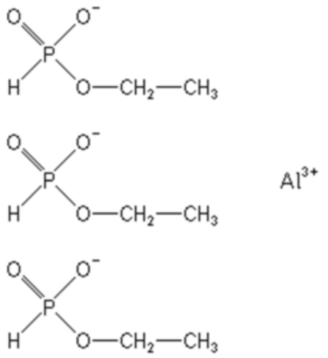

Figure 1: Dissociated molecular structure of fosetyl-Al.

Methomyl and imidacloprid were chosen as model compounds in the mixture due to their extended application in horticulture and the previous experience of the research group. They present different chemical structure and properties. While methomyl is an N-carbamate, imidacloprid is a neonicotinoid, a third-generation pesticide. Commercial formulations of both products were used with no further purification. Tomilo-20L was the carbamate source and Kohinor the neonicotinoid's; 200 $g{\cdot}L^{-1}$ of active principle in each product. Both were obtained from Aragonesas Agro S.L. (Spain).

$FeSO_4{\cdot}7H_2O$ was the source for ferrous ions, and hydrogen peroxide was dispensed as a commercial solution of 30% w/v. All of these substances were purchased from Panreac. Deionized water was used to prepare every solution.

2.3. Chemical analyses

Insecticides depletion in each experiment was monitored using high performance liquid chromatography with a photodiode array detector. The column used was a Mediterranean Sea18, 5µm 25x0.46 cm (Teknokroma). The mobile phase was composed of a mixture of water and acetonitrile (both from Merck). The wavelength of the UV maximum absorbance was 232.5 nm for methomyl, while for imidacloprid, it was 269 nm. Detection limit for both compounds was 0.5 $mg{\cdot}L^{-1}$. Fosetyl-Al is determined by liquid chromatography with electrospray tandem mass spectrometry after the addition of tetrabutylammonium acetate (Sigma-Aldrich) as the ion-pairing reagent, according to Hernández et al., [30]. Detection limit for fosetyl was established around 0.05 $mg{\cdot}L^{-1}$

Dissolved organic carbon (DOC) was also monitored by a Shimatzu TOC-VCSN TOC analyzer. Dissolved ferrous ion (together with total iron) and hydrogen peroxide were followed by spectrophotometric methods [31, 32]. 1,10-phenantroline was purchased from Aldrich, while sodium acetate, acetic acid, and ascorbic acid were purchased from Panreac, also were NH_4VO_3 and H_2SO_4.

Phosphate was monitored by means of ion-exchange chromatography with a Dionex DX-120 ion chromatographer equipped with a Dionex DX-600 ion chromatograph and 4 mm × 250 mm Dionex Ionpac AS11-HC column.

Biodegradability was essayed in the shape of the ratio between Biological Oxygen Demand, after 5 days, BOD_5, and Chemical Oxygen Demand, COD. These last analyses were carried out following the Standard Method 5220 D [33]; while BOD_5 values were obtained according to the procedures described in Standard Methods 5210D for respirometry analysis (APHA 1995), using Oxitop® (WTW Chemical) manometric bottles and BOD-seed capsules supplied by Cole-Palmer.

2.4. Experimental design

In order to determine the level of influence of some of the main experimental parameters, fosetyl-Al, Fe(II) and H_2O_2 initial concentrations were selected as independent variables. Their effects were assessed in the shape of a multivariate surface-response analysis, already used and described by Micó et al. [8]. This experimental design is based on a central composite circumscribed set of experiments, consisting on a factorial design 2^3 (3 factors: $[Fos.]_0$, $[H_2O_2]_0$ and $[Fe^{2+}]_0$; and 2 levels: [10, 50], [15, 100] and [10, 30] $mg\cdot L^{-1}$ respectively), and 6 star points. Pesticide concentration range was chosen around the typical dosage of the product in irrigation for real agricultural applications, while reagents dosage was decided according to previous experiments, not shown here, that determine minimum concentration of reagents for total elimination of 30 $mg\cdot L^{-1}$ fosetyl-Al.

A summary of the experimental design and its results preformed in the lab scale artificial light reactor is presented in Table 1. Variables were coded on two normalized levels: 1 as the highest and -1 the lowest value. According to this, the central point of the design was coded as (0, 0, 0). Three replicates were carried out at that central point, in order to check the statistical consistency of the data. Star points were distributed at $\sqrt{3}$ times the distance from the central point 0 to +1; except for the case which that distance implied negative values of one factor. In that case, identified with a star (*) at table 1, the negative value was replaced with the corresponding -1 value. Data analysis, determination of the empirical model and response surface were carried out using Stat Graphics Plus 5.1 software. Statistical validation was determined by ANOVA test at 95% confidence level.

Table 1: Central composite design of photo-Fenton oxidation of fosetyl commercial formula

Exp.	$[Fos.]_0$ ($mg \cdot L^{-1}$)	$[H_2O_2]_0$ ($mg \cdot L^{-1}$)	$[Fe^{2+}]_0$ ($mg \cdot L^{-1}$)
1	10 (-1)	15 (-1)	10 (-1)
2	50 (1)	15 (-1)	10 (-1)
3	10 (-1)	100 (1)	10 (-1)
4	50 (1)	100 (1)	10 (-1)
5	10 (-1)	15 (-1)	30 (1)
6	50 (1)	15 (-1)	30 (1)
7	10 (-1)	100 (1)	30 (1)
8	50 (1)	100 (1)	30 (1)
9	10 (-1)*	57.5 (0)	20 (0)
10	63.6 (√3)	57.5 (0)	20 (0)
11	30 (0)	15 (-1)*	20 (0)
12	30 (0)	130 (√3)	20(0)
13	30 (0)	57.5 (0)	3.18 (-√3)
14	30 (0)	57.5 (0)	36.82 (√3)
15	30 (0)	57.5 (0)	20 (0)
16	30 (0)	57.5 (0)	20 (0)
17	30 (0)	57.5 (0)	20 (0)

(*) Point (-√3, 0, 0) and (0, -√3, 0) had to be replaced by (-1, 0, 0) and (0, -1, 0) because the real variable value for (-√3) was lower than 0 in both cases.

3. Results and discussion

3.1. Previous tests

First of all, stability tests were performed in order to essay if there could be any decomposition of aqueous fosetyl due to the direct effect of light, temperature or stripping. Solar photolysis was dismissed after 10 h stirred batch experiment under direct sun light in a quartz container, in which no diminishment of the concentration was detected. This resistance was also confirmed comparing solar spectrum with absorption spectrum of a solution of 50 $mg \cdot L^{-1}$ of fosetyl-Al. The maximum absorbed wave length for the solution was located around 210 nm while solar spectrum only gets the 10% of its total radiance. Also thermal decomposition and evaporation were ruled out. 2 batch reactors of 500 mL of 10 $mg \cdot L^{-1}$ of fosetyl were heated to 70 ºC during 2 h, one of them was sealed in order to prevent evaporation. No change in concentration was found, establishing that fosetyl-Al solutions are resistant to degradation by heat and no volatile enough to suffer evaporation at the essayed temperature (far from which is going to be used in the performed experiments and from which it would be submitted in a real scenario). No stripping was either detected after submitting a 10 $mg \cdot L^{-1}$ solution to an air flow of 100 $L \cdot h^{-1}$ during 6 h.

According to this, it could be supposed that fosetyl degradation detected in the following experiments, was strictly due to the radical reactions involved in photo-Fenton reaction.

3.2. Experimental design application

Table 2 summarizes the experimental plan and gathers the results for every evaluated response, $t_{50\%}$, half-life of the pesticide, ΔDOC (%), percentage of depleted Dissolved Organic Carbon, t_F, time for the total consumption of hydrogen peroxide, and Y (%), the remaining percentage of fosetyl-Al when all the H_2O_2 was consumed, just for the essays in which the depletion of the fungicide was not complete.

Table 2: Experimental plan followed according to the experimental design, and results obtained. Shadowed cells indicate which experiments did not arrived to a total depletion of the fungicide.

Exp.	($[Fos.]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) ($mg \cdot L^{-1}$)	DOC_0 ($mg \cdot L^{-1}$)	$t_{50\%}$ (min)	ΔDOC (%)	t_F (min)	Y(%)
1	(10, 15, 10)	2.6	3.3	26.2	165	1.01
2	(50, 15, 10)	12.7	74.4	24.7	1100	2.01
3	(10, 100, 10)	2.6	2.9	79.7	270	
4	(50, 100, 10)	12.7	61.1	37.8	1380	1.30
5	(10, 15, 30)	2.6	1.6	30.2	75	
6	(50, 15, 30)	12.8	4.76	42.0	90	8.01
7	(10, 100, 30)	2.6	1.36	84.4	130	1.19
8	(50, 100, 30)	12.7	2.36	77.6	800	
9	(10, 57.5, 20)	2.7	1.5	55.2	130	
10	(63.6, 57.5, 20)	16.2	14.1	34.6	600	
11	(30, 15, 20)	7.6	2.1	36.4	130	
12	(30, 130, 20)	7.7	2.1	78.9	1380	
13	(30, 57.5, 3.18)	7.6	60.7	34.6	1350	
14	(30, 57.5, 36.82)	7.6	1.6	69.7	150	
15	(30, 57.5, 20)	7.6	1.9	49.2	510	
16	(30, 57.5, 20)	7.5	2.0	48.3	450	
17	(30, 57.5, 20)	7.4	2.4	50.8	510	

As can be seen, half -life of pesticide, $t_{50\%}$, ranges from 1 to 74 min, with the majority of the experiments laying in the lower values. It is difficult to directly find a pattern that determine which is the combination of factors related to a low rate of fosetyl degradation. However, a quadratic mathematical model, eq. 1, could be obtained, relating this response with the experimental conditions, in order to predict the values of this variable.

$$t_{50\%} = 22.37 + 2.35^{*}[Fos.] - 0.22[H_2O_2] - 3.97^{*}[Fe(II)] - 1.5 \cdot 10^{-3^{*}}[Fos.]^2 - 2.21 \cdot 10^{-3}[Fos.][H_2O_2] - 78.28 \cdot 10^{-3^{*}}[Fos.][Fe(II)] + 1.45 \cdot 10^{-3}[H_2O_2]^2 + 3.24 \cdot 10^{-3}[H_2O_2][Fe(II)] + 11.10 \cdot 10^{-3^{*}}[Fe(II)]^2 \qquad (1)$$

Submitting the mathematical formula parameters to an ANOVA test, the significance evaluation indicates that only the variables marked with * have a p-value lower than 0.05, so only those can be considered of significance for the model. According to this, the model can be simplified into eq. 2 (parameters are accompanied by their respective confidence semi-interval).

$$t_{50\%} = 15.97(\pm 24.37) + 2{,}2(\pm 0.6)[\mathrm{Fos.}] - 3{,}70(\pm 1.97)[\mathrm{Fe(II)}] - 0{,}078(\pm 0.028)[\mathrm{Fos.}][\mathrm{Fe(II)}] + 0{,}109(\pm 0.043)[\mathrm{Fe(II)}]^2 \qquad (2)$$

The R^2 statistics for this multivariate regression is 0.96.

Depending on the value of each parameter, the magnitude of the influence can be determined, in the same way their signs identify the sense of that influence. The higher parameter, with lower p-value, is related to ferrous ion concentration, which seems to have the strongest weigh over $t_{50\%}$, due to the fact that also its quadratic value and its interaction with the fungicide concentration are both significant to the mathematical model. The presence of the quadratic term indicates that the negative relationship between this factor and half- life is not linear. Taking into account that the sign of its parameter influence of Fe(II) principal effect is negative, the higher this variable the lower the final value of $t_{50\%}$ is. In opposition to this, the second more important term, with positive sign, is the principal effect of fosetyl-Al initial concentration. Unexpectedly the amount of hydrogen peroxide or its related values do not have mathematically significant effects over the response, which does not mean that the process does not depend also in this reagent, photo-Fenton reaction cannot take place without it, but the influences of the other terms are so high that mask peroxide's.

The contour plot represented by this model is depicted in Figure 3. It can be seen how the model predicts an area where the half time values are <0, what have no physical meaning. Due to the quite high standard deviations of the residues, 7.18, and the dispersion of the results themselves, it could be expected that a realistic prediction could not be made in this region. Nevertheless, these negative values point out the region where the minimum half time is expected, although its value cannot be truly predicted. Optimizing the mathematical model, the optimum point is characterized by the following combination of parameters ($[\mathrm{Fos.}]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) as (10, 44, 21) $mg\cdot L^{-1}$, although, as have been said and can be seen in Fig. 2, the correspondent half time, mathematically is lower than 0; what lacks of practical value but indicates a region where good conditions can be found.

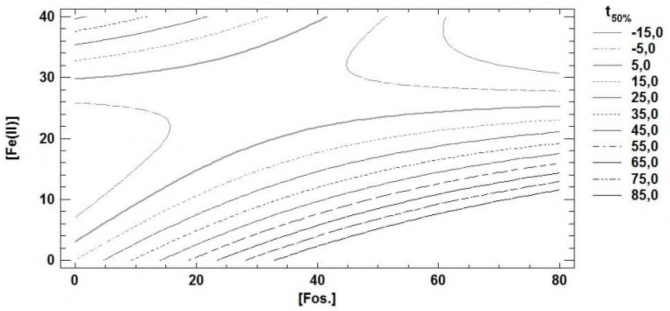

Figure 2: Contour plot representing the behavior of fosetyl half- life according to the concentration of ferrous ion and the fungicide (both expressed in mg·L^{-1}) for experiments with 100 mg·L^{-1} of hydrogen peroxide.

Fig. 3 depicts the comparison of fosetyl-Al degradation profiles for different experiments. One with the closest conditions to the calculated optimum, (10, 57.5, 20) mg·L^{-1}, another also with a low $t_{50\%}$ value, but lower [H_2O_2], (10, 15, 30), and the experiment with the highest $t_{50\%}$ measured (50, 15, 10).

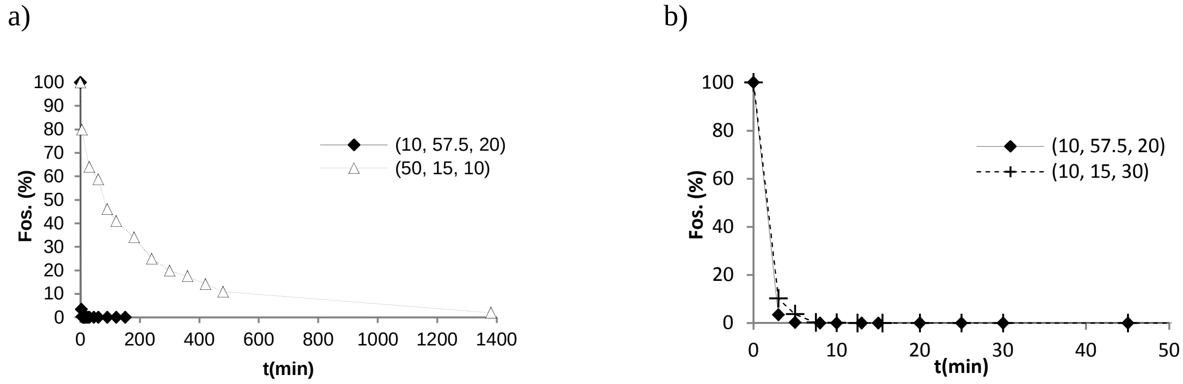

Figure 3: 3a and 3b. 3a. Comparison of fungicide degradation in different experiments. ([Fos.], [H_2O_2], [Fe^{2+}]) mg·L^{-1}. 3b. X axis is reduced for better understanding.

In these figures, it can be seen how the depletion is almost immediate in the case of the lower quantity of fosetyl together with a higher ferrous ion concentration, as was expected according to the model, and taking into account the signs of their associated parameters. In the case of two experimental points similar in [Fos.] and [Fe^{2+}] to the optimum, the depletion is also very quick and the half- life of the pesticide is lower than 1.6 min. However, just looking at the values of fosetyl and ferrous iron, a lower $t_{50\%}$ and quicker degradation were expected for (10, 15, 30), not for (10, 57.5, 20), which is slightly faster as can be seen in 3b. H_2O_2 concentration seems to exert a certain influence, despite the fact that its effect has not significance in the mathematical model. It seems that low hydroxyl radicals availability in the first case (due to lower initial peroxide concentration) could compensate the benefic effect of lower [Fos.]/[Fe^{2+}] ratio.

While for most of the experiments the fungicide was totally depleted, only in some of them the mineralization exceeded 70%. In general, low DOC depletion was achieved, being (50.6$\pm$10.4) % the average remaining percentage of DOC. However, a different mathematical model can also be obtained for this response data. Ec. 3 represents this model once the non-significant terms (ANOVA test, 95.0%, p-value<0.05) had been excluded.

$$\Delta\text{DOC}\ (\%) = 18.77(\pm 15.63) - 0.40(\pm 0.46)[Fos.] + 0.69(\pm 0.14)[H_2O_2] + 0.01(\pm 0.61)[Fe^{2+}] - -0.0087(\pm 0.0042)[Fos.][H_2O_2] + 0.030(\pm 0.018)[Fos.][Fe^{2+}] \qquad (3)$$

Once again, the sign and value of the parameters let determine the character of their influence. In this case, the strongest effect is exerted by hydrogen peroxide initial concentration, stating that it is the most important factor in mineralization as the main source of hydroxyl radicals. The second most important factor is the concentration of ferrous ion, which also plays an important role in the generation of radicals. As it was expected, the influence of the initial concentration of fosetyl-Al, although it is lower in absolute value compared to the interaction between the fungicide and H_2O_2 is negative. The last factor taking part in the mathematical model is the interaction between [Fos.] and [Fe^{2+}], which is positive.

Fig. 4 represents response surfaces of the mathematical model for DOC. It can be seen how the tendencies according to parameters values are reflected, consistently with the interpretation of the parameters of the equation 3. As in the case of $t_{50\%}$, DOC representation for [Fe^{2+}]=10 mg·L^{-1} also includes a region with no physical meaning, with percentages over 100%. Although the values cannot be accurately calculated, the shape of the surface indicates the tendency of obtaining the best values around 10 and 30 mg·L^{-1} of fosetyl-Al and hydrogen peroxide over 120 mg·L^{-1}. However, this fact does not warranty better results with higher concentration of H_2O_2 than the essayed in this study, due to the risk of scavenging exerted by the hydrogen peroxide itself, over the hydroxyl radical [34].

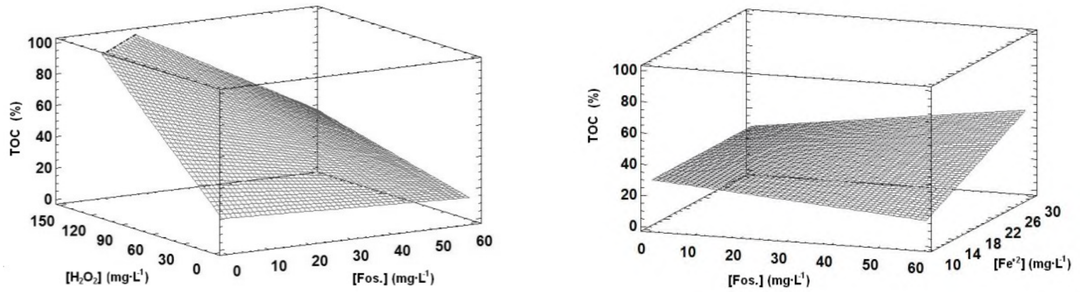

Figure 4: Surface plots representing the remaining DOC (recorded here as TOC) according different combinations of concentrations of fosetyl and hydrogen peroxide, for experiments with 10 mg·L^{-1} of ferrous ion and [H_2O_2]=15 mg·L^{-1}, respectively.

From the point of view of a maximum DOC mineralization, the optimum value for total DOC depletion was obtained mathematically for ($[Fos.]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) as (15, 130, 37). The concentration of the reagents is quite high, despite the low concentration of fosetyl, what implies an economical drawback. However, taking into account the good results for pesticide degradation in almost every case, and so it is expected also for this set of conditions, the coupling with a posterior biological treatment could be suggested (depending on biodegradability resulsts), where total DOC depletion is not required but contraidicated. With this integrated system, the doses of reagents could be reduced This will be detrimental to DOC depletion, nevertheless the bioreactor is expected to be able to metabolize that remaining organic load not mineralized by the chemical process.

The named mathematical inconsistencies of the models could not be attributed to the range of reagents concentration tested. The evaluated intervals for hydrogen peroxide an iron concentration were chosen around previous experiences that showed that central values could cope with 30 $mg \cdot L^{-1}$ of the fungicide. Moreover, they included such high values (compared to fosetyl-Al concentrations) in order to prove that not always the highest reagent conditions guarantee the best results, what it is especially evident in the case of fosetyl degradation.

Regarding to t_F, acquired data was so disperse that a representative mathematical model could not be presented. However, submitting the data to ANOVA test, the strongest influences could be determined according to the p-value of the different factors, their quadratics terms and their interactions. Table 3 summarizes those parameters and their p-values with other parameters of the statistical test. As can be seen, the most important parameter in this case is the concentration of fosetyl (lowest p-value), followed by ferrous ion concentration, H_2O_2 concentration and the interaction H_2O_2 and Fe^{2+}.

Table 3: ANOVA tests for responses. R^2, coefficient R squared, R^{2*} adjusted coefficient R squared, related to the number of degrees of freedom (17-1=16 d.f.).

	t_F	
	parameters	p-values
[Fos.]	50.32	0.0008
[H2O2]	-9.43	0.0087
[Fe2+]	-42.94	0.0010
$[Fos.]^2$	-0.40	0.0547
$[Fos.][H_2O_2]$	0.12	0.1700
$[Fos.][Fe^{2+}]$	-0.85	0.0406
$[H_2O_2]^2$	0.07	0.0937
$[H_2O_2][Fe^{2+}]$	0.11	0.5063
$[Fe^{2+}]^2$	0.85	0.1622
R^2		93.13
R^{2*}		78.68

3.3. Iron endogenous inhibition

Taking into account the evaluation of the three responses it can be observed that Fe^{2+} and fosetyl concentrations appears as critical factors in the observed parameters, and always with opposite sign. This is due to a verified chemical interaction between both of them due to the molecular formula of the fungicide. Fosetyl-Al oxidation ends up in the release of phosphate salts, these anions precipitate ferric ions, inhibiting their recycling into Fe^{2+}. This effect reduces the rate of catalytic decomposition of H_2O_2, causing a low availability of radicals that can affect the rate of elimination of the very fungicide and hinder the mineralization of the organic matter.

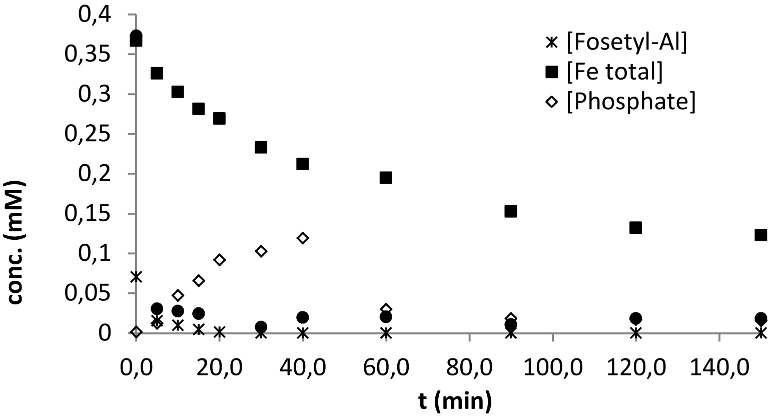

Figure 5: Evolution of the iron and phosphates along the reaction. Belongs to the experiment (25, 50, 20) $mg \cdot L^{-1}$.

Fig. 5 represents an example of the evolution of dissolved iron, in the shape of Fe(II) and total iron, together with the concentration of fosetyl and free phosphates. Despite fosetyl is quickly degraded, corresponding phosphates (3 times initial molarity of foestyl-Al) are not released to the media at the same rate but gradually, during the first minutes of the reaction. Although in general Fe^{3+} and PO_4^{3-} tend to precipitate as ferric phosphate, the small fraction of total iron concentration reduced during those first minutes cannot be blamed for the precipitation of all the missing phosphates. Saturated fosetyl carbon bonds of suggest that its main reaction mechanism with hydroxil radical will run through hydrogen abstraction followed by a subsequent possible formation of phosphate containingpolimeric by-products, among others (Samuni and Neta 1973). Only through the advance of the oxidation these species end up decomposing and releasing PO_4^{3-} to the media.

In fig. 5 also can be seen how Fe(II) is rapidly converted into Fe(III) at the beginning of the reaction. This phenomenon takes place in every experiment with the presence of fosetyl in the moment the hydrogen peroxide is added. In a solution free of other interferences, at the acid pH, ferric ion remains soluble and photoactive in the shape of hydroxyl complexes. These complexes are susceptible to undergo photoreduction by ligand-to-metal charge transfer (MLT) (Pignatello et al. 2006) to release Fe^{2+} and hydroxyl radical to the media. This enables the progress of the degradation of the pesticide and its intermediates. In this particular case, when noticeable amounts of phosphates and iron are present in the media, a rapid precipitation of $FePO_4$ is expected, causing the decomposition of hydroxyl-ferric ion complexes and preventing it from MLT process. Nevertheless, as can be seen in fig. 5, this precipitation takes place more progressively than anticipated. According to inorganic chemistry literature (Lente et al. 2000), a plausible explanation suggests the presence of another kind of complexes generated by the interaction between Fe (III), water and phosphates that prevent phosphate salt from precipitate. Fig. 6 intends to illustrate the sequence of the main chemical species which can influence the degradation of Fosetyl-Al in the studied conditions.

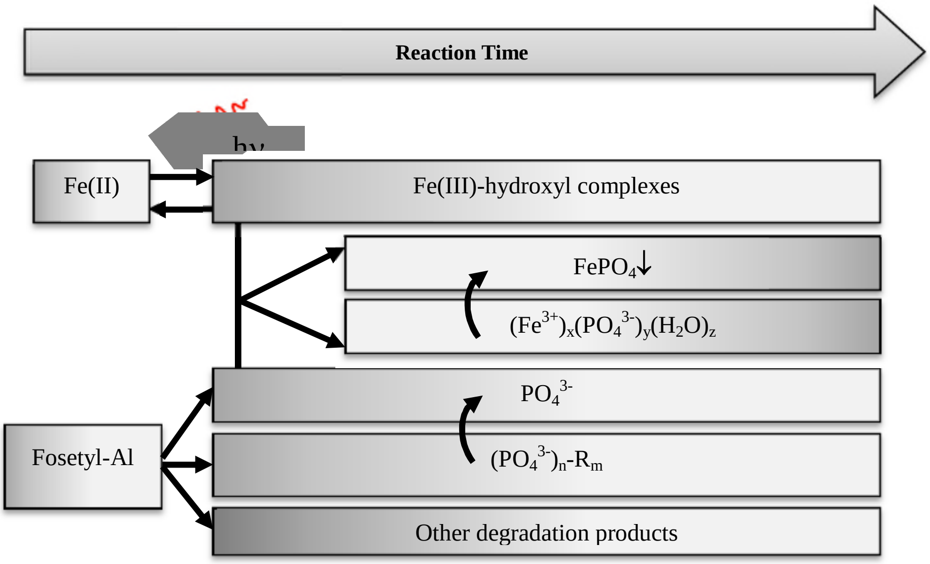

Figure 6: Schematic figure of the chemical evolution of iron and phosphate species during photo-Fenton reaction. $(Fe^{3+})_x(PO_4^{3-})_y(H_2O)_z$ refers to complexes proposed, while $(PO_4^{3-})_n$-R_m stands degradation phosphate containing degradation by-products.

Table 4 shows how the molar mass balance between total dissolved iron and dissolved phosphate is fulfilled after surpassing the point to maximum concentration of phosphate, the moment from when iron removed from the solution closely corresponds to the precipitated phosphate. This confirms that although the possible existence of PO_4^{3-} stabilizing complexes, the depletion of phosphates and iron are related to their precipitation. Table 5 summarizes the final mass balances of other two similar experiments which are also equilibrated.

Table 4: Mass balance of phosphates and iron depletion with time for (25, 50, 20) experiment.

$[Fos.]_0$ (mM)	$[PO_4^{3-}]_{máx.}$ (mM)	$[Fe]_0$ (mM)
0.07	0.21	0.36

t (min)	$[PO_4^{3-}]_t$ (mM)	$[Fe]_t$ (mM)	$\Delta[Fe]$ (mM)	$\Delta[PO_4^{3-}]$ (mM)
40	0.12	0.25	**0.11**	**0.09**
60	0.03	0.19	**0.18**	**0.17**
90	0.02	0.15	**0.19**	**0.21**
120	0.01	0.13	**0.20**	**0.23**
150	0.01	0.12	**0.20**	**0.24**

Table 5: Mass balance of phosphates and iron final depletion for (15, 30, 15) and (50, 100, 30) experiments**.**

	$[Fos.]_0$ (mM)	$[PO_4^{3-}]_{máx.}$ (mM)	$[Fe]_0$ (mM)	$[PO_4^{3-}]_f$ (mM)	$[Fe]_f$ (mM)	$\Delta[Fe]$ (mM)	$\Delta[PO_4^{3-}]$ (mM)
(15, 30, 15)	0.04	0.12	0.26	0.01	0.11	**0.15**	**0.11**
(50, 100, 30)	0.14	0.42	0.54	0.12	0.15	**0.29**	**0.30**

3.4. Biodegradability results

Table 6 recaps values of biodegradability, represented as BOD_5/COD, corresponding to effluents before being treated, and after their treatment under the conditions that gave best results for $t_{50\%}$. It is worth to mention that in all the essayed cases, the depletion of the fungicide was complete.

Table 6: Chemical and biochemical oxygen demand for effluents before and after being treated. Conditions: ($[Fos.]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) ($mg\cdot L^{-1}$).

Conditions	Previous to treatment				After treatment			
	BOD_5 ($mgO_2\cdot L^{-1}$)	COD ($mgO_2\cdot L^{-1}$)	BOD_5/COD	TU	BOD_5 ($mgO_2\cdot L^{-1}$)	COD ($mgO_2\cdot L^{-1}$)	BOD_5/COD	TU
(10, 57.5, 20)	3.26	25.35	0.13	0.52	1.45	5.87	0.25	0.52
(30, 57.5, 36.8)	3.60	61.43	0.06	0.48	1.6	8.32	0.19	0.60
(50, 100, 30)	3.93	95.23	0.04	0.62	5.45	30.45	0.18	0.20

As can be seen, initial higher concentrations of the pesticide imply lower values of biodegradability due to the fact that, in opposition to the proportional increase of COD, the value of BOD_5 is very similar for every case, probably due to a certain effect of inhibition exerted by the pesticide or its inert ingredients over the test biomass.

After the treatment, in the case of 10 $mg\cdot L^{-1}$ of pesticide biodegradability increases, connected to a noticeable COD diminishment after chemical treatment, that compensates a slight reduction in BOD_5 (due probably to the mineralization of the organic content by photo-Fenton). In the cases of 30 $mg\cdot L^{-1}$ of fosetyl-Al, biodegradability also follows the same pattern, a diminishment of BOD accompanied by decrease of COD (not as pronounced as in the case of 10mg·L^{-1}). According to this, the final biodegradability is higher than at the beginning but lower than for 10mg·L^{-1} experiments. The same happens with the highest pesticide concentration, the biodegradability increases noticeably compared to the initial value. In this case, while the final BOD increases with respect to initial BOD, a diminishment (nearly 50%) of COD thanks to the chemical treatment is registered.

According to the results obtained, the effluent final biodegradability is determined (at least partially) by the initial concentration of the pesticide. Only in the case of 10 mg·L^{-1} of pesticide, the final values achieved make the effluent eligible of being considered at least partially biodegradable (Ballesteros Martín et al. 2009b). However, the results point out that although the biodegradability achieved after treatment is not optimum, the chemical process is essential to adapt the effluent to be sent to the effluent to a biological post-treatment, either at the public sewage system or to an adapted bioreactor.

3.5. Solar photo-Fenton reaction over fosetyl-Al and mixture of pesticides

The applicability of solar light to enhance the decontamination of fosetyl-Al was also essayed, compared to the performance of electrically enlighten photo-Fenton. The first implies an inexpensive source of light that would reduce operational costs and constitute a more environmental friendly alternative, avoiding the need of an artificial energy supply to empower the UV lamps.

The UV experiments were performed in the same reactor as previous runs, while solar light essays were carried out in SOLEX device, a 35 L composed parabolic converter. pH was fixed around 2.8, and temperature evolved freely (also did for artificial light experiments in this case). This set comprises the experimental conditions which are summarized together with the results in Table 7. Pesticide concentration, mineralization, dissolved and total Fe, and PO_4^{3-} were monitored along the process.

Table 7: Comparison between the results obtained for artificially and solar powered photo-Fenton. . Conditions: ([Fos.]$_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) (mg·L^{-1}).

		UV lamps				Solar light			
Exp.	Conditions	$Q_{50\%}$ (KJ·L^{-1})	DOC_0 (mg·L^{-1})	DOC_f (mg·L^{-1})	ΔDOC (%)	$Q_{50\%}$ (KJ·L^{-1})	DOC_0 (mg·L^{-1})	DOC_f (mg·L^{-1})	ΔDOC (%)
A	(15, 30, 15)	0.46	3.5	2.0	43.50	0.46	3.4	1.5	55.88
B	(25, 50, 20)	1.18	5.7	3.0	48.10	0.26	5.5	0.7	87.27
C	(30, 100, 25)	2.07	12.5	6.0	52.35	0.31	11.7	5.0	57.26

Instead of $t_{50\%}$, the parameter compared in this case is $Q_{50\%}$, representing the energy required to achieve the decomposition of 50% of the pesticide load. This response was chosen to make it comparable two different sources of light (Tokumura et al. 2008), obviously different, regarding to their power. Figure 7 shows the evolution of the concentration of fosetyl-Al versus the accumulated energy for the experiments with solar and UV light. At the same time, it is evident how the fastest experiments are those made under solar radiation. The depletion profiles are similar for every experiment of this set; the fungicide concentrations have a quick decrease at the beginning. However the profiles are clearly steeper, what indicates higher reaction rates, and implies lower $Q_{50\%}$, as can be seen in Table 7. Besides, also DOC depletion percentage is superior, much more for experiment B, which solar light improves to almost ΔDOC =90%. The higher light incidence intensity over the solar device (between 17 to 25 $W \cdot m^{-2}$, in front of around 7 in $W \cdot m^{-2}$ for the UV lamps device), together with possible photo-chemical reactions that would undergo beneath the visible range of solar light spectrum, could justify the increase in fosetyl-Al degradation velocities in the case of sun powered photo-Fenton, in opposition to UV lamp performed process. Regarding to DOC depletion, the same factors could contribute to its increase, but the temperature could also play a part, given that the solution suffers a noticeable temperature increase while being irradiated by sun. In fact, the experiments were performed in October, with an initial average temperature of 23 ºC, just after 30 minutes of treatment reached around 26 ºC, and arrived to 35ºC at the end of the experiments. No influence is supposed to be exerted directly by temperature over the degradation of the fungicide due to the fact this event happens at the beginning of the process, when the temperature change is not noticeable yet.

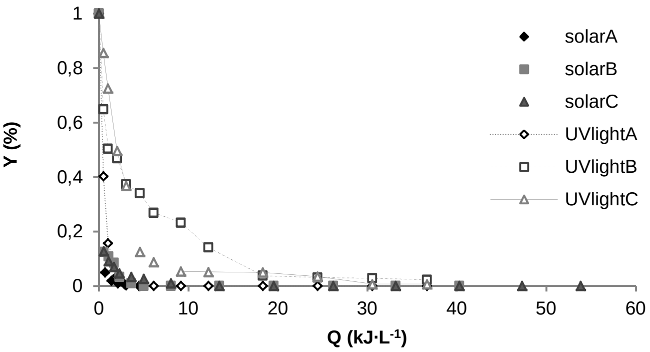

Figure 7: Pesticide degradation profiles comparing photo-Fenton processes powered by solar light or by UV lamps.

Focusing just in the performance of the solar powered process, figure 8a presents scale reduced degradation profiles for solar experiments. As can be seen, the essay with lower fungicide concentration suffered a quicker decomposition, followed by experiments B and C, although in the first minutes of the experiments both profiles were very similar. According to DOC depletion results, presented in figure 8b, mineralization seems higher for intermediate values of fosetyl and Fe(II), while is very similar between experiments A and C.

A mixture of three studied pesticides was also essayed in the bench-scale solar device in Barcelona, to see the influence that the presence of dissimilar organic substances can exert on the solar photo-Fenton reaction. The experiment performed had the following initial conditions: [Fosetyl], [Methomyl] and [Imidacloprid]=20 mg·L^{-1}; [H_2O_2]=200 mg·L^{-1}; [Fe^{2+}]=10 mg·L^{-1}. Figure 9 is a representative graphic of these experiments.

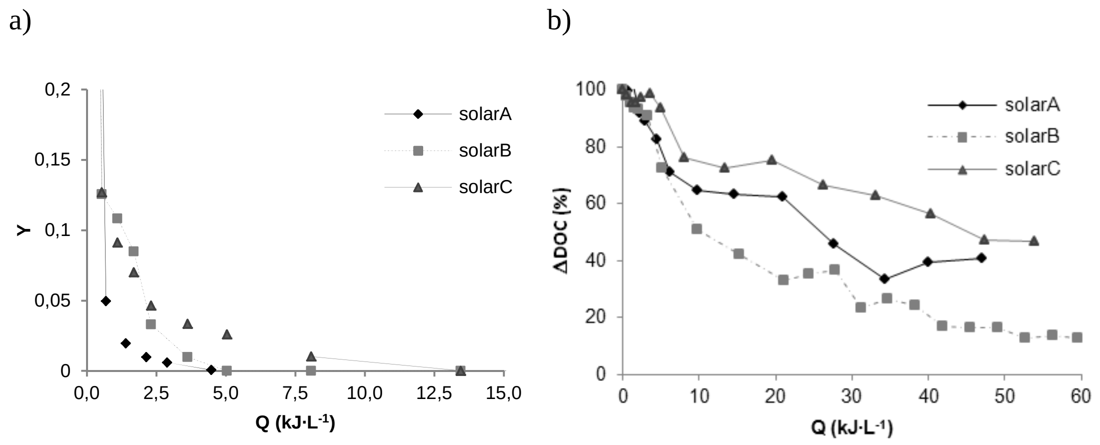

Figure 8: 8a and 8b; 8a. Fosetyl-Al degradation profiles, the axes have been scaled with respect to fig. 7 in order to distinguish the different curves for solar experiments, due to the quick depletion of the fungicide in the first minutes of the process. 8b. DOC depletion profiles for solar experiments.

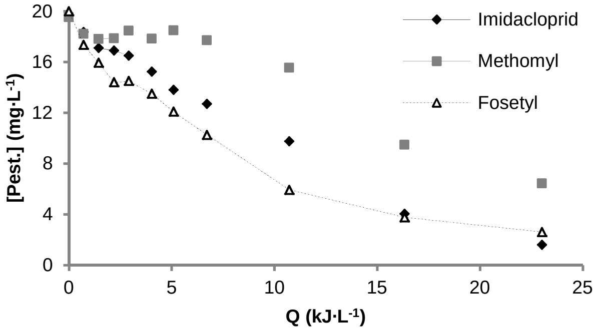

Figure 9: Degradation profiles for the mixture.

The segregation on the elimination rates of the different compounds highlighted that, even though hydroxyl radical, in which is based this technology, does not react selectively with organic matter, it does it indeed with different initial degradation rate, related to the structure of the molecule degraded (Walling 1975). Nevertheless the most interesting results obtained in this essay are the uncompleted elimination of every species, and the shape of the shown profiles. In previous works with the pesticides treated separately and together, but in absence of Fosetyl-Al, by means of photo-Fenton reaction (Micó et al. 2010a; Micó et al. 2013), their profiles always presented a gradual concentration decrease from the very beginning of the process. Even in the case of fosetyl-Al, as seen before, the pesticide suffers an initial notably sharp descent. In opposition to this, Figure does not show that quick decrease for fosetyl-Al, and for imidacloprid and methomyl it seems there is a delay in their degradations while fosetyl-Al is being oxidised. These facts reveal the existence of a competition for the radicals between pesticide species (and/or their inert ingredients and by-products); furthermore, a noticeably influence by the phosphates generated by the degradation of fosetyl could be expected. It will gradually precipitate iron (III) as discussed before, inhibiting the characteristic photo-recycling of the photo-Fenton reaction, and preventing the total depletion of the target compounds.

4. Conclusions

For most of the experiments performed under the fosetyl experimental design, pesticide degradation is complete and very fast, averaged $t_{50\%}$= 14.13 min (3.14 min excluding the three experiments with $t_{50\%}$ higher than 60 min). DOC degradation is quite low in general. Only some experiments exceed 70% of mineralization. However, both responses enables to obtain mathematical models with R^2= 96.97 and 97.37 respectively. These models allowed determining the most important parameters that influence $t_{50\%}$ and ΔDOC.

In the case of half-life time model, the most influential factor turned to be Fe^{2+} concentration, through a non-lineal relationship, for which the higher ferrous $[Fe^{2+}]$ is, the lower $t_{50\%}$ is achieved. The second term in influence is the interaction between fosetyl and ferrous ion, justified by the importance for the process of the phosphates released by the degradation of the fungicide, which precipitate Fe^{3+} preventing it from being recycled and generate more hydroxyl radicals. The optimum conditions for the lowest $t_{50\%}$ are ($[Fos.]_0$, $[H_2O_2]_0$, $[Fe^{2+}]_0$) as (10, 44, 21).

Mathematical model for DOC removal stated that the most influential parameters are hydrogen peroxide and ferrous ion concentrations, which are both critical factors for the generation of hydroxyl radicals. Also an optimum trio of conditions was obtained for maximum mineralization, (15, 130, 37).

Experimental results have shown how phosphates released by fosetyl-Al degradation interferes with the oxidation process precipitating Fe(III) and disabling it from recycling. However, the existence of other species related to PO_4^{3-} that avoid an immediate precipitation of the ferric salt are postulated. Further study should be performed to obtain more detailed information about the entangled complexation chemistry.

BOD_5/COD results showed an increase of biodegradability of the effluent after the chemical treatment, encouraging the use of a subsequent biological reactor that could cope with no mineralized organic load, enabling the reduction of reagents. According to this, the convenience of a maximum DOC removal, for which an optimal set of condition was determined, may be subjected to the interest of integrating the subsequent bioreactor.

Solar light powered photo-Fenton showed that for every tested case, the pesticide depletion was complete. The comparison between this technique and UV light photo-Fenton indicated that solar experiments had better performance regarding to the speed of the fungicide degradation and DOC depletion. This system is especially advisable for outdoor environments with high solar irradiation.

The essay of solar photo-Fenton over a mixture of pesticides shows clear influence by the different coexisting organic species, invalidating one-specie-only experiments if there is the intention of making an exercise of studying a real scenario, where numerous substances are expected to coexist.

To sum up, photo-Fenton seems to be an effective way of treating pesticides solutions containing fosetyl-Al. Its solar version is even more effective than UV light powered process, which is an advantage regarding to the use of an inexpensive source of radiation. It has been stated that fosetyl containing effluents compose cases in which endogenous inhibition should be taken into account, and the optimization of the working conditions is extremely troubled by it. In this case, regarding to multivariate analysis results and in opposition to what was expected, the concentration of the catalyzer (affected by fosetyl degradation) seemed to be more influential than the oxidant agent itself.

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APPENDIX V

Enhancement of pesticide photo-Fenton oxidation at high salinities

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Enhancement of pesticide photo-Fenton oxidation at high salinities

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ABSTRACT

The performance of the photo-Fenton reaction under high salinity conditions (up to 50 mS cm^{-1}) has been tested regarding the oxidation of two pesticides, imidacloprid and methomyl, in the shape of their commercial formulations. In opposition to what is commonly outlined in the literature, an enhancement in pesticides depletion has been found due to the positive participation of halogen radicals, Cl$^\bullet$. However, this beneficial effect does not improve organic carbon elimination but rather displays worse total organic carbon (TOC) depletion. This effect is most likely due to the hydroxyl radicals scavenging and the complexation of Fe^{3+} by chloride salts, which reduce the availability of reagents. Although salts/Fe^{3+} interaction was observed, Fe^{3+} interaction with the organic content was revealed as more relevant, especially for imidacloprid.

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1. Introduction

1.1. Photo-Fenton process

Advanced oxidation processes (AOPs), which are based on the generation of very reactive and oxidizing free radicals, especially hydroxyl radicals, have been reported to be powerful oxidative techniques [1]. Among these processes, the homogeneous photo-Fenton reaction and the titanium dioxide-mediated heterogeneous photocatalytic treatment have recently shown great promise for the treatment of industrial wastewater [2,3] and the prevention of groundwater contamination of [4,5]. HO$^\bullet$ is a highly reactive transient specie that can rapidly oxidize most organic substances [6]; furthermore, catalytic behavior and light enhancement can improve its potentialities.

In the photo-Fenton reaction, the generation of hydroxyl radicals is achieved by the decomposition of hydrogen peroxide by means of the oxidation of dissolved ferrous ions, reaction (a), in the presence of UV light, which photo-reduces Fe^{3+}, reaction (b), thereby recovering Fe^{2+} and contributing to an additional pathway leading to a greater generation of free radicals in comparison to the dark Fenton reaction [7–9].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^\bullet + HO^- \quad \text{(dark Fenton)} \tag{a}$$

$$Fe(OH)_2{}^{+} + H^+ + h\nu \rightarrow Fe^{3+} + HO^\bullet \tag{b}$$

Photo-catalytic processes present several drawbacks in practical applications, such as the amount of chemicals needed and the pH 3 requirement (to prevent $Fe(OH)_3$ precipitation). However, photo-Fenton also has some advantages over heterogeneous catalysis. For instance, the employed reagents are harmless once the process is complete, and if Fe^{2+} working conditions meet reuse standards, there is no need for catalyst separation and recovery. In addition, solar light could also be a suitable source of radiation for the process, which makes it optimum for outdoor installation, especially in rural areas, which in turn reduces both costs and risks [10–12].

Extensive work has addressed the photo-Fenton reaction as applied to pesticides decontamination [8,13–17]. Studies have established this process as a suitable treatment for bio-recalcitrant pollutants. Most of this research has been performed in countries with rural regions under high hydric stress where the reuse of even agricultural water is required. The most important characteristics of this water are their content of salts, mainly chlorides, nitrates and sulfates, a certain amount of BOD and COD, traces of pesticides, and inert ingredients that comprise commercial products and their degradation by-products.

The photo-Fenton reaction has been widely studied from all possible perspectives; although notable contributions can be found [18–20], a weakness of the extant research is the scarce study of two important aspects that the present work addresses: this reactions' performance with commercial formulations versus moderate/high salinities. In the recycling hydroponic system envisaged in this study the effluents might function under either condition.

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1.2. Hydroponics scenario

Hydroponics is a culture method conducted in a soil-less environment that is usually installed inside greenhouses of varying sophistication. The nutrient source is provided directly to the roots by means of a flowing solution. These systems allow for the control of nutrients given to the plants, thereby assuring the nutrient sources' nutritive and sanitary qualities [21]. Their advantages include their high production per unit area compared to soil cultures and the independence of external climate conditions. Both of these factors are particularly interesting for extreme climate regions, which are often present in developing countries in need of higher productivity.

Hydroponics typically work as closed systems, preventing the leaching of chemicals into ground or surface water and enabling the recycling of the nutrient effluent as many times as needed; while the increasing concentration on salts and pesticides does not harm the crops. It is considered an efficient technique of saving water from which regions such as Spain, where water scarcity provokes the overexploitation of wells and their consequent salinization by seawater intrusions [22] can benefit. In particular, an area that may take profit of is Spanish struggling South Basin, where the use of protected agriculture [23] is already extensive and where technification by hydroponics has not yet been widely utilized as a way of economizing water.

Despite the positive aspects of recycling after a number of rounds, the effluent cannot be directly discharged to the environment due to its enriched salt content and pesticide-derived products. Treatment is necessary to recondition the effluent prior to sending it back to the system or for it to meet the sewage system's standards.

1.3. Objectives

The aim of this work is to study the suitability and performance of the photo-Fenton reaction for degrading a mixture of two commercial pesticide formulations based on methomyl and imidacloprid in the presence of high salinity. The working scenario is a simulated hydroponic greenhouse where the effluents can be reused several times as a semi-closed system until they reach a conductivity threshold, at which point the flows are diverged to treatment once they have high concentrations of salts, pesticides and related organics.

2. Experimental

2.1. Photo-Fenton reaction

The photo-Fenton reaction was carried out in a 2 L jacketed stirred vessel with three 8 W black light lamps (λ_{max} = 365 nm) axially arranged to the reactor. This device was connected to a thermostatic bath that permitted the experiments to evolve at a controlled temperature of 21 °C. A more detailed description of the device and the procedure can be found elsewhere [7]. The pH was set to 2.7 (optimum for the equilibrium $[Fe^{2+}]/[Fe^{3+}]$ to achieve its highest ratio) at the beginning of the reaction with H_2SO_4 (Panreac) diluted solution. This value was monitored during the process and kept constant between 3 and 2.5. Except for the 60 min experiments, all of the trials were carried out until reaching the total consumption of hydrogen peroxide. Every set and every sample was withdrawn and then quenched with $NaHSO_3$, 40% (w/v) (Panreac) to remove the remaining hydrogen peroxide.

2.2. Chemicals

Methomyl and imidacloprid were chosen as target compounds due to their extended application in horticulture. They present different chemical structure and properties. While methomyl is an N-carbamate (Fig. 1a), imidacloprid is a neonicotinoid (Fig. 1b), a third-generation pesticide. Commercial formulations of both products were used with no further purification. Tomilo-20 L was the carbamate source and Kohinor the neonicotinoid's; 200 g L^{-1} of active principle in each product. Both were obtained from Aragonesas Agro S.L. (Spain).

KNO_3 with no antipackaging, $CaCl_2 \cdot 2H_2O$, NH_4Cl, $MgSO_4 \cdot 7H_2O$, $NaHCO_3$, NaCl, and $CaSO_4 \cdot 2H_2O$ were used for simulating the salinity content. $FeSO_4 \cdot 7H_2O$ was the source for ferrous ions, and hydrogen peroxide was dispensed as a commercial solution of 30% (w/v). All of these substances were purchased from Panreac. Deionized water was used to prepare every solution.

2.3. Chemical analyses

Pesticides depletion in each experiment was monitored using high performance liquid chromatography with a photodiode array detector. The column used was a Mediterranean Sea18, 5 μm 25 cm × 0.46 cm (Teknokroma). The mobile phase, composed of a mixture of water and acetonitrile (both from Merck) (60:40), was delivered at a flow rate of 1 mL min^{-1}. In the cases with higher salinity content, a peak related to a NaCl-masked methomyl peak; therefore, another method was used, having a mobile phase composed of 2.5 mL of 80% H_3PO_4, 25 mL of methanol diluted to 500 mL with milli-Q water. In this case, the flow was 0.7 mL min^{-1}. The wavelength of the UV maximum absorbance was 232.5 nm for methomyl, while for imidacloprid, it was 269 nm. Dissolved organic carbon was also monitored in the shape of TOC, total organic carbon, by a Shimatzu TOC-VCSN TOC analyzer. Dissolved ferrous ion (together with total iron) and hydrogen peroxide were followed by spectrophotometric methods [24,25]. 1,10-Phenantroline was purchased from Aldrich, while sodium acetate, acetic acid, and ascorbic acid were purchased from Panreac, as were NH_4VO_3 and H_2SO_4.

2.4. Experimental plan

The photo-Fenton reaction was tested with different salinity conditions, from no salts to a concentration of 42.13 g L^{-1}. Table 1 shows the averaged parameters of each type of effluent, grouped in Set A, before chemical treatment. The added salts were determined according to the previous literature regarding the typical minerals needed by crops and soilless cultures [26–28]. In the case of A1, only the essential salts for crop nutrition were added, while in A2, A3, and A4, the principal species that caused salinity problems (NaCl and $CaSO_4$) were also added. While the conductivities of A1 and A2 represent intermediate points of conductivity that could be still used in the hydroponic system, A3 accounts for the threshold salinity limit, the extent to which plants can be forced, according to Fornes and Montesano [29,30]. A4 represents a highly saline effluent closed to the conductivity of seawater. The aim of this experiment was to analyze the extension of the salinity effect under extreme conditions.

The photo-Fenton reaction was carried out with initial reagents concentrations of 10 mg L^{-1} of ferrous iron ($FeSO_4$) and 100 mg L^{-1} of hydrogen peroxide. These values were chosen according to previous experiments in which these concentrations enabled the total depletion of 20 mg L^{-1} of imidacloprid without any salt [8]. All experiments, except otherwise is noted, were carried out until hydrogen peroxide was totally consumed.

Samples of the photo-Fenton reactor were withdrawn along the reaction for this set, and successive experiments and the following

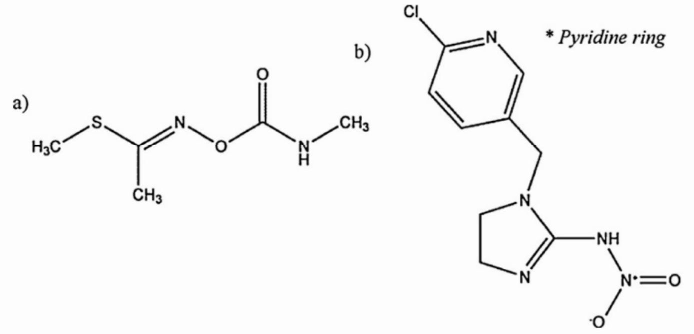

Fig. 1. (a) and (b) molecular structures of methomyl and imidacloprid, respectively.

species concentrations were analyzed: pesticides, total organic carbon, dissolved ferrous iron and dissolved total iron, and hydrogen peroxide.

3. Results and discussion

3.1. *Effect of salinity on pesticide and organic matter depletion*

Methomyl degradation within the mixture of two pesticides is represented in Fig. 2, where changes in elimination curves behavior can be observed according to the conductivity of the effluents.

As seen, the elimination of methomyl is complete in any case at the end of the experiment. For higher salinities, faster degradations of the carbamate were achieved. The lowest conductivity effluent shows a slight decrease in the elimination rate, compared to the control type. When salinity achieves 5 μS cm^{-1}, the oxidation seems to go faster and slightly increases, doubling the salt content. The degradation pattern is also different. For lower salinities, the oxidation begins more slowly and increases its rate after 1 h of treatment, while for the highest conductivities; the degradation is quite rapid from the beginning.

In the case of imidacloprid performance, Fig. 3 illustrates how, for every conductivity, the depletion of imidacloprid is complete.

It is notable that despite the concentration of salts, imidacloprid is degraded in a similar pattern and rate, while a more rapid oxidation step in the first minutes of the reaction and subsequent slowing until total elimination have been reported elsewhere, [31,32], even using different oxidation technologies [33].

Comparing pesticide elimination to H_2O_2 consumption for the extreme experiments, as depicted in Fig. 4, it is shown how the tendencies are different depending on the salt content. The process seems to be slower in the absence of salts, where the decomposition of the hydrogen peroxide follows the same pattern as methomyl elimination; the latter is noticeably slower than imidacloprid depletion. Nevertheless, Fig. 4b represents how, for experiment A4,

Table 1
Summary of salts concentration and conductivity of each experiment performed.

Set A, mixture (10 mg L^{-1} imidacloprid, 10 mg L^{-1} methomyl, 44 mg L^{-1} TOC$_0$)					
	Specie	Salts conc. (g L^{-1})	Total salts conc. (g L^{-1})	Conductivity (mS cm^{-1})	[Cl–] (M)
A0	Non added salts			~1.5×10^{-3}	0
A1	KNO_3	0.60	0.9	1.00	2×10^{-3}
	$CaCl_2$	0.10			
	NH_4Cl	0.05			
	$MgSO_4$	0.05			
	$NaHCO_3$	0.10			
A2	KNO_3	0.60	4.6	5.06	0.05
	$CaCl_2$	0.10			
	NH_4Cl	0.05			
	$MgSO_4$	0.15			
	$NaHCO_3$	0.10			
	NaCl	3.00			
	$CaSO_4$	0.60			
A3	KNO_3	1.25	9.05	11.06	0.1
	$CaCl_2$	0.10			
	NH_4Cl	0.05			
	$MgSO_4$	0.30			
	$NaHCO_3$	0.10			
	NaCl	6.00			
	$CaSO_4$	1.25			
A4	KNO_3	1.25	42.13	50.00	0.6
	$CaCl_2$	0.10			
	NH_4Cl	0.05			
	$MgSO_4$	0.90			
	$NaHCO_3$	0.08			
	NaCl	36.00			
	$CaSO_4$	3.75			

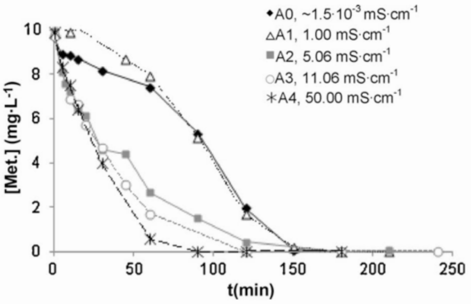

Fig. 2. Concentration of methomyl along the process. The reaction reached completion when all of the hydrogen peroxide was consumed.

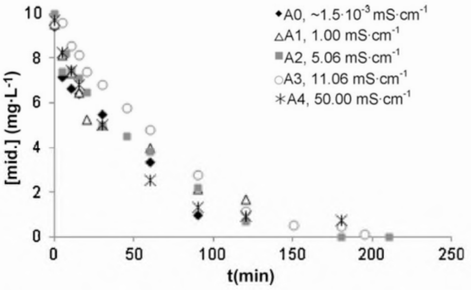

Fig. 3. Concentration of imidacloprid along the reaction, Set A. The reaction was finished when all of the hydrogen peroxide was consumed.

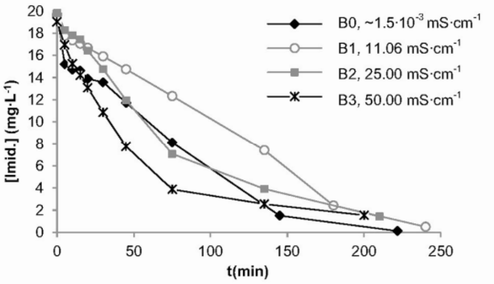

Fig. 5. Concentration of imidacloprid along the reaction, Set B. The reaction was complete when all the hydrogen peroxide was consumed.

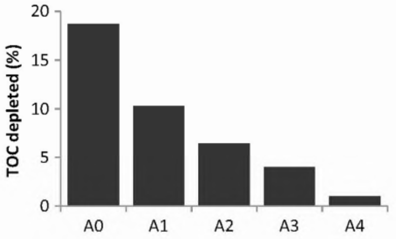

Fig. 6. Total percentage of TOC depletion for Set A experiments.

the carbamate consumption is somewhat faster than the degradation of the other target compound and how peroxide was also consumed at a higher rate.

Higher salinity experiments were performed with 20 mg L^{-1} of the neonicotinoid as the single target compound, with the same concentration in reagents and the same salt proportions. Experimental conditions for Set B are summarized in Table 2.

As observed in Fig. 5, effectively, the influence of salt increases for imidacloprid. Apparently, the lowest salinity has a negative influence on the degradation of imidacloprid, while higher salinities seem to cause faster degradation, as occurred in the presence of methomyl. However, the depletion achieved is reduced by almost 10% for the two highest values of conductivity.

The weak but positive impact that salinity seems to have in the case of imidacloprid for Set B, together with the noticeable improvement of the methomyl degradation rate in the presence of salts, undermines the notion of inorganic ions, such as NO^{3-}, $SO_4{}^{2-}$ and Cl^-, [19,32–35] provoking interferences in the photo-Fenton reaction, implying lower oxidation results, as has been reported in previous literature. On one hand, these anions could be blamed for a scavenging effect of hydroxyl radicals [18,19,36]; for chloride, these effects were established to be noticeable above 0.01 M Cl^- [37]. On the other hand, these anions have also been found to cause complexation with dissolved Fe(III), forming less photoactive species [18].

The influence of the anions over hydroxyl radicals could explain the faster hydrogen peroxide degradation observed in Fig. 4b, while the combination of both effects could justify the low TOC diminishment at the end of the process. Although depletion is low even in the experiments without any salt, conductivity seems to clearly affect the mineralization of the organic load, reducing the TOC [38] elimination while conductivity increases.

Fig. 6 presents those values. Even in the most beneficial case, the total TOC removal is quite low compared to the total degradation that pesticide content achieves. As proposed by Kavitha and Oturan [39,40], the formation of hardly oxidizable by-products, such as short chain carboxylic acids, which are refractory to radical oxidation, deters TOC removal. Although higher salinity seems not to affect the depletion of the pesticides themselves, it hinders the oxidation of the organic content, confirming what the previous literature found, most likely due to the lack of availability of hydroxyl

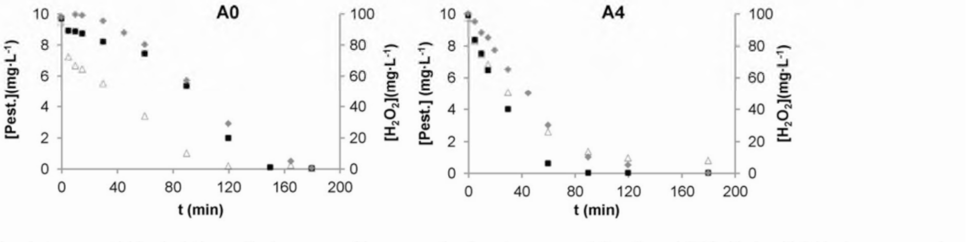

Fig. 4. (a and b) Comparison between pesticides depletion and hydrogen peroxide consumption for extreme cases. (■) methomyl, (△) imidacloprid, (◆) hydrogen peroxyde. A0, 1.5×10^{-3} mS cm^{-1}, A4, 50.0 mS cm^{-1}.

Table 2
Summary of salts concentration and conductivity of each experiment performed as set B.

Set B, mixture (20 mg L^{-1} imidacloprid, 0 mg L^{-1} methomyl, 41 mg L^{-1} TOC_0)				
	Specie	Salts conc. (g L^{-1})	Total salts conc. (g L^{-1})	Conductivity (mS cm^{-1})
B0	Non added salts			~1.5×10^{-3}
B1	KNO_3	1.25	9.05	11.06
	$CaCl_2$	0.10		
	NH_4Cl	0.05		
	$MgSO_4$	0.30		
	$NaHCO_3$	0.10		
	NaCl	6.00		
	$CaSO_4$	1.25		
B2	KNO_3	1.25	24.15	25.00
	$CaCl_2$	0.10		
	NH_4Cl	0.05		
	$MgSO_4$	0.90		
	$NaHCO_3$	0.10		
	NaCl	18.00		
	$CaSO_4$	3.75		
B3	KNO_3	1.25	42.13	50.00
	$CaCl_2$	0.10		
	NH_4Cl	0.05		
	$MgSO_4$	0.90		
	$NaHCO_3$	0.08		
	NaCl	36.00		
	$CaSO_4$	3.75		

radicals and Fe^{3+} ready to be returned to Fe^{2+}; these conditions could promote the formation of these recalcitrant substances.

Additional sets of experiments with less-complex matrices were performed. In all of them, chloride was most suspected of influencing the photo-Fenton reaction, as it was the most significant contributor to the total conductivity of the effluents. As methomyl seems to suffer more acute effect on degradation, the following study was primarily focused on this pesticide. The remaining pesticide was measured along a 60 min photo-Fenton reaction; to which three solutions were submitted (these experiments were stopped before all the H_2O_2 was consumed). All of them were prepared with 20 mg L^{-1} of methomyl and 10 mg L^{-1} of Fe^{2+}. No salt was added to the first solution, while 15.8 g L^{-1} of KCl was added to the second solution and 18.5 g L^{-1} of KNO_3 to the third solution to obtain two solutions with the same electric conductivity, 25 mS cm^{-1}, and compare the effect of both anions. No conclusions about TOC depletion could be made in these experiments due to the low values obtained even for the solution without salts. Nevertheless, regarding pesticide elimination, Fig. 7 shows how the presence of high chloride concentration in fact improves the degradation of the carbamate in comparison to the nitrate solution and the solution without any salt, which both had similar behaviors, indicating that chloride plays a special role in promoting the degradation of the pesticide. It was concluded that Cl^- was the main contributor to enhanced pesticide depletion.

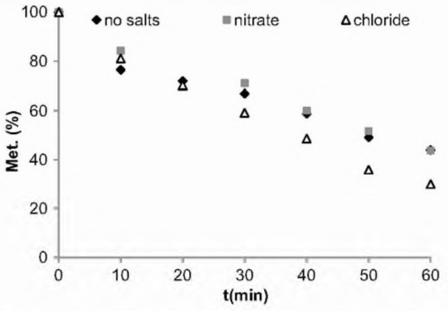

Fig. 7. Evolution of methomyl in different salinity conditions; no salts, negligible conductivity; with chloride or nitrate, *C* = 25 mS cm^{-1}, $[Met.]_0$ = 20 mg L^{-1}, TOC_0 = 46 mg L^{-1}.

Photo-Fenton reactions were also performed over a mixture of pesticides in a solution with no salts and a solution with 15.8 g L^{-1} of KCl, both spiked with 20 mg L^{-1} of methomyl and 20 mg L^{-1} of imidacloprid (with 10 mg L^{-1} of Fe^{2+}, each). Comparing Figs. 7 and 8a (different scale), it can be seen how the depletion is higher for methomyl with or without chlorides added but lower in the presence of imidacloprid. This finding is most likely due to the higher quantity of initial organic content in these experiments. Nevertheless, Fig. 8a itself depicts how depletion of the methomyl is clearly improved by the presence of halide salts in comparison to the experiments without them, while Fig. 8b shows an insignificant influence of the halide in imidacloprid elimination. Both facts confirm what was seen in Figs. 2 and 5, respectively. No conclusion could be drawn from TOC depletion results due to their low value.

As stated above, OH· scavenging and Fe^{3+} complexation could be attributed to halide ions, chloride in this case, which may have a negative effect even on pesticides. Nevertheless, it is also noted in the literature that under certain conditions, the reactive halogen species that is derived from hydroxyl scavenging can be more than a HO· sink and may participate in contaminant destruction [41]. Reactions (c)–(f) [18,38] represent the processes taking place between the hydroxyl radicals and the halide anions.

$$HO^{\bullet} + X^- \rightleftharpoons XOH^{\bullet -} \tag{c}$$

$$XOH^{\bullet -} + H^+ \rightleftharpoons X^{\bullet} + H_2O \tag{d}$$

$$XOH^{\bullet} - + X^- \rightleftharpoons X_2^{\bullet -} + OH^- \tag{e}$$

$$X^{\bullet} + X^- \rightleftharpoons X_2^{\bullet -} \tag{f}$$

While halogen radical anions, $X_2^{\bullet -}$, are generally less reactive than hydroxyl radicals, the magnitudes of halogen atoms $X^{\bullet}$ rate constants with organic compounds are comparable to those for $OH^{\bullet}$. This monoatomic species, favored by acidic pH, can react by one-electron oxidation by H-abstraction and by addition to unsaturated C-C bonds, while hydroxyl radical oxidation is mostly based on the latter [41].

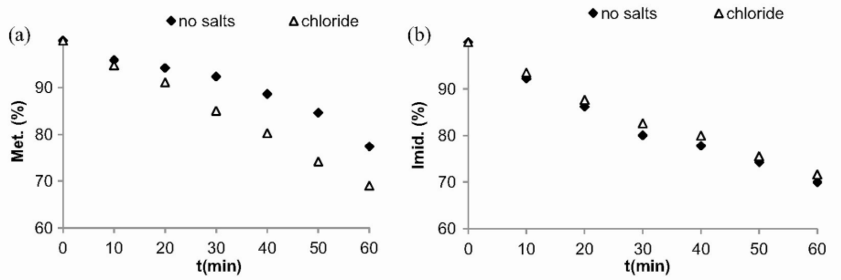

Fig. 8. (a and b) Evolution of the pesticides in different salinity conditions; no salts, negligible conductivity; chloride, 25 mS cm^{-1}; [Met.] = [Imid.] = 20 mg L^{-1}, TOC$_0$ = 83 mg L^{-1}.

Accordingly, the participation of this type of radicals could justify the improvement of methomyl and imidacloprid depletion and even the changes in their profiles. Furthermore, due to the molecular formula of methomyl, chloride radicals could promote the formation of extremely nucleophilic compounds based on its chlorosulfenyl group. These substances could collaborate in the oxidative process. In the case of imidacloprid, the improvement is not so obvious due to its molecular structure, mainly its pyridine ring, which tends to stabilize any type of radical instead of promoting the expected chain reactions. From the point of view of TOC depletion, on one hand, the reactive halides themselves are not powerful enough to lead to mineralization; on the other hand, halide anions seem to have a negative effect, most likely due to the scavenging effect over hydroxyl radicals or the complexation of Fe(III) that hinders its recycling.

3.2. Effect of salinity on iron catalyst

The influence of inorganic ions was also explored by analyzing the presence of dissolved ferrous iron along the photo-Fenton reaction, as was reflected in Fig. 9, where results for Set A are represented.

A general behavior can be distinguished among the different experiments. Ferrous ion suffers an extreme decrease when the hydrogen peroxide is added to the media, no matter the conductivity of the effluent, and its concentration is kept low during the process until 70–80% of the peroxide is consumed, coinciding with the lowest remaining concentrations of the pesticides. At this point, a certain level of recovery is detected with respect to the salt content: the strongest recovery is registered for experiment A0 and the weakest for A4.

Inorganic ions are known to exert some coordinating effect over ferric ions [18], with the formation of thermodynamically favored complexes such as $FeCl^{2+}$, $FeCl_2^{+}$, and $Fe[(SO)_4]_2^{-}$ reducing the capability of the photo-Fenton reaction to recycle ferrous iron [42]. In fact, the complexation of iron by chloride and sulfate ions could be primarily responsible for the diminishment of the process' efficiency [35], depending on its concentration. Complexation is reflected on the different recovery rates in the final phases of the reaction.

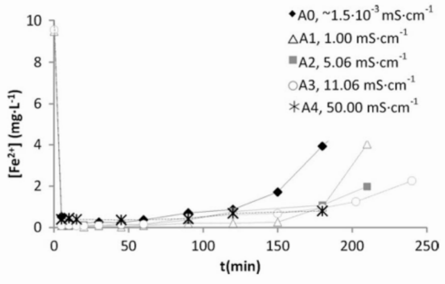

Fig. 9. Progress of dissolved Fe^{2+} versus reaction time for Set A.

Focusing on the decrease of Fe^{2+} at the beginning of the reaction, when hydrogen peroxide is added, there is a rapid interaction between this reagent and Fe^{2+} dissolved ions, causing their oxidation into Fe^{3+}, according to the well-known Fenton reaction, Eq. (1) in Fig. 10. Once ferrous iron is converted into Fe(III), apart from the formation of photoactive aquo-complexes, reaction (2) is favored in acidic pH [38]; in the presence of H_2O_2, the equilibriums detailed in reactions (3) and (4) are established almost instantly [18].

In the absence of any other complexing agents, this complexation is reverted by the reduction of ferric ion, reactions (5) and (6) [18], in which the complex breaks into a ferrous ion and radicals. Nevertheless, these processes have very low reaction rates in comparison to the photo-enhanced reaction (7), which is supposed to be the main path for the recovery of Fe^{2+} in the photo-Fenton process [43,44]. This reaction does not require the participation of hydrogen peroxide and constitutes an additional means to generate hydroxyl radicals [18]. According to the catalytic and cyclic character of the photo-Fenton reaction, a near-total recovery of ferrous iron was anticipated in the process, far from what was actually observed.

All of these reactions are expected to happen constantly throughout the photo-Fenton process; however, there may be, as in this case, other complexing reactions that disable Fe^{3+} from participating in this cycle as efficiently as expected.

No interactions between the phytosanitary products themselves and Fe^{2+} were detected, as no initial depletion of this specie was observed previous to the addition of hydrogen peroxide. However, it is accepted that apart from inorganic ions, Fe(III) may also complex with certain organic compounds [44], especially those acting as polydentate ligands, which would explain the low levels of dissolved Fe(II) until nearly the end of the trials. Therefore, particular experiments were carried out to determine whether the commercial formulations of each pesticide could prevent Fe^{3+} from being

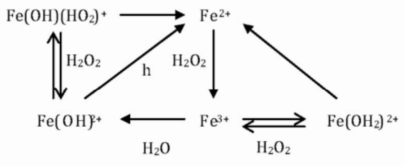

Fig. 10. Simplified diagram of iron cations chemistry. Reactions are not balanced. Reaction (2) is representative of the several reactions that take place between Fe(III) and water.

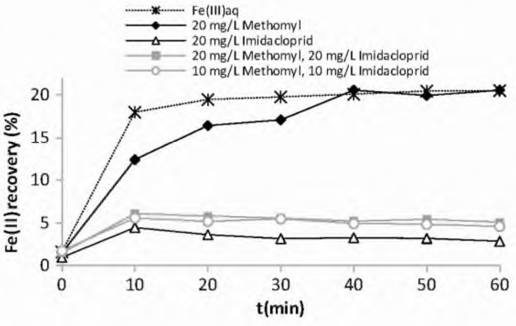

Fig. 11. Recovery of dissolved Fe(II) along the exposition to UV light of Set E experiments, pH = 2.7.

photo-reduced. The experiments, depicted in Fig. 11, consisted of Fenton-like reactions in which different solutions of $FeCl_3$ ($[Fe^{3+}]_0 = 10\ mg\ L^{-1}$) and commercial pesticides ([Met.] = 20 mg L^{-1} and/or [Imid.] = 20 mg L^{-1}) were submitted to UV-light for 60 min, with no addition of hydrogen peroxide, in the same reactor where previous photo-Fenton experiments were performed. A comparison of the photo-reduction of Fe(III) into Fe(II) in a solution without the presence of any pesticides was established.

It can be observed that the effect of UV light is the ability to convert more than 20% of the Fe(III) into Fe(II) in a solution with only $FeCl_3$; this recovery is also achieved in the presence of methomyl. In contrast, the imidacloprid experiments showed a notably high negative effect by this pesticide or its inert ingredients in the photo-reduction of ferric iron, even in the case of the mixture with methomyl, in which the influence of imidacloprid overcame the neutral effect of the carbamate, corroborating what was observed for set A experiments.

Furthermore, Fig. 12 shows the evolution of ferrous iron in contrast with the pesticide depletion. It depicts how the imidacloprid concentration did not diminish, while methomyl slightly decreased as the percentage of dissolved Fe(II) increased. Because previous experiments proved that no photolysis of methomyl is caused at working wavelengths, the depletion of the pesticide is likely due to the hydroxyl radicals generated by the photo-reduction of Fe(III) complexes. As imidacloprid or its inert ingredients seem to block iron reduction, a low amount of hydroxyl radicals are generated and, consequently, extremely low depletion of imidacloprid was observed.

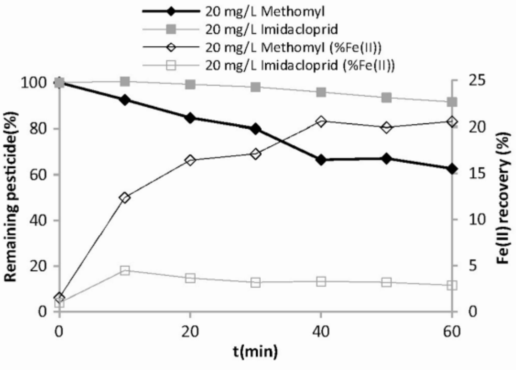

Fig. 12. Comparison between the depletion of pesticides and Fe(II) recovery along the exposition to UV light.

The results indicate that methomyl or its inert ingredients (mainly dibasic esters) can complex ferric iron of a certain grade [45]; the recovery is lower than for the experiment without organics, but they are either easily photo-decarboxyled or oxidized by the radicals generated in the iron photo-reduction, freeing Fe^{3+} for its recycling. On the other hand, even in the case of salts' absence, imidacloprid commercial formulation prevents Fe(III) photo-reduction. No complexing effect can be attributed to the organic solvents that the commercial formulation contains, dimethyl sulfoxide and propylene carbonate. Instead, there is an imidacloprid pyridine ring, Fig. 1b, that provokes the chelating effect over ferric ions [46]. This ring is unharmed during the first stages of the degradation of the compound [32]. However, with the evolution of the reaction, this cycle is also decarboxyled and decomposed by the produced radicals, promoting the regeneration of a slightly higher concentration of Fe(II) near the end of the process.

Apparently, in Set A experiments, ferrous iron evolution along the reaction followed three stages. The first consisted of an extreme drop due to the addition of H_2O_2, while the second step was related to the nature of the present pesticides. Both substances, together with Tomilo-20 L inert ingredients but mainly imidacloprid and its by-products, complexed Fe(III) until radical reactions degraded them sufficiently to cause the recovery of Fe(II) that took place in the third stage depending on the salt content and inorganic complexes generated. Not even in the A0 case was the Fe(II) upturn complete; therefore, it may be assumed that although the pesticides were consumed, some by-products still prevented ferric ions from being recycled.

4. Conclusions

The photo-Fenton reaction was revealed to be an efficient way to oxidize mixtures of imidacloprid and methomyl, even for salt contents and conductivities close to those of seawater.

Contrary to what is commonly found in the literature, chloride anions seem to favor the degradation of both target compounds, especially methomyl (even in a mixture with imidacloprid). This enhancement can be attributed to the generation of halide radical species, $X^{\bullet}$, that contribute to the degradation of the pesticides by new reaction paths. However, TOC depletion is not enhanced but worsened, potentially due to the global effect of chlorides through hydroxyl radicals scavenging and the complexation of Fe(III) by chloride and sulfate salts. It can be concluded that salinity has a particularly positive effect on the decomposition of the pesticides, which cannot be perceived by taking into account global organic matter depletion that is worsened by the presence of inorganic ions.

Available Fe(II) should be taken into account for future optimizing works due to the possible complexation of Fe^{3+} by the oxidizing substances, depending on their molecular structure. In this case, the imidacloprid pyridine ring is suspected to complex a notable amount of Fe(III), while the effect of methomyl and its inert ingredients is minimal.

Additionally, chlorides have been proved to influence ferrous ion recovery, although, in this case, their effect was overshadowed by the organic ferric complexes until the final minutes of the reaction. In those moments, the direct influence of salinity could be seen in the different rates of Fe^{2+} recovery, which were higher for the lower conductivity values.

Further work should be performed to test whether the participation of chloride radicals in the oxidation of organic matter could promote the generation of organochlorinated substances. Further studies should also assess the viability of a subsequent biological treatment loaded with photo-Fenton processed salty effluents to

address the remaining organic content not degraded in the photo-Fenton process.

Acknowledgments

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APPENDIX VI

Slow sand filtration as part of an integrated system for pesticides removal in high salinity effluents: Performance and biomolecular assessments

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Abstract

Greenhouses with hydroponics culture usually work in closed and semi-closed irrigation systems. The addition of water treatments to process the drainage and the eventual leaching are strongly recommended given its content in crops protection products and high amounts of salts. This work pretends to study the suitability of the integration of photo-Fenton reaction and a slow sand filtration column to treat those pesticide contaminated effluents at different salinity conditions. As a first step the performance of a series of sequencing batch reactors was monitored in order to check biocompatibility of photo-Fenton pretreated effluents depending on their salinity content.

On the second step, those same pretreated effluents were loaded to the slow sand filtration column. Its performance was also monitored to verify if the integration between this bioreactor and photo-Fenton reaction is plausible. Finally, bacterial 16S rRNA gene sequencing will be applied to analyze microbial diversity of the biomass developed in the column depending on the salinity of the load. Results stated that the coupled system combining the chemical treatment and a subsequent bioreactor is effective for the treatment of water effluents containing pesticides. Its robustness makes the integrated system able to deplete more than 80% of the organic load, even for high salinity contents. Molecular biology techniques allowed determining the diminishment of the Slow Sand Filtration biomass diversity with the increase of conductivity.

1. Introduction

In hydroponics, traditionally terrestrial plants are grown with their roots submerged in a mineral nutrient solution (aquaponics or true hydroponics) or in an inert (organic or inorganic) medium, such as perlite, gravel, mineral wool, expanded clay or coconut husk, through which the nutrient solution flows continuously. This soilless culture requires frequent irrigation and high fertilization rates (Rouphael, et al., 2006), therefore in order to safe water resources, plantation usually work as closed or semi-closed irrigation systems. In those schemes the nutrient solution, after its pass through the root mass, is recovered and recycled back to the system. In the case of semi-closed irrigation circuit, some fresh nutrient solution is supplied along the circle, while another fraction is discarded and the rest is recycled. The logical accumulation of salts in the recycled stream can cause phytotoxicity problems on the crops. This fact is of especial concern in the case of already saline native irrigation waters, such in coastal area of Mediterranean climate (Flowers, 1998). On the other hand, although hydroponics avoids possible soilborne pests, these crops do not escape the need to control other pests and diseases so rational use of pesticides is inevitable metabolites will also accumulate on the recycled streams of closed and semi-closed systems, endangering the crops, the producers and the final consumer. Both salinity and toxic substances accumulations justified the need of discarding part of the nutrient solution in the semi-closed system. However, these named characteristics impede to discard directly to the public sewage system, not least dump it directly to the environment.

From the point of view of the content in phytosanitary products, pesticides, etc., can be blamed for a major impact on the environment when they are discharged to the environment without control. In the case of pesticides, they are considered a major concern due to the fact that most of them are biorecalcitrant substances with a relatively high average life (Hayasaka, et al., 2012) that hardly degrade in natural ecosystems. Their presence has been detected in air, water and soil, and at all trophic levels, from plankton to large mammals, given that these compounds tend to bioaccumulate in many living beings. This increases their toxic and polluting potential as they move through food chains (Baranowska, et al., 2005; Hayasaka, et al., 2012; Pérez-Ruzafa, et al., 2000; Wendt-Rasch, et al., 2004). In intensive farming effluents the concentration of pesticides may reach considerably high levels (up to several hundred $mg\cdot L^{-1}$) and the methods conventionally used in sewage treatment plants, as filtration or secondary reactors, etc., are insufficient (Malato, et al., 2001). As an answer to this, in the last decades several research works have been focused on testing the performance of photo-Fenton reaction over this kind of emerging pollutant (Ballesteros Martín, et al., 2009; Huston and Pignatello, 1999). Those studies revealed the adequacy of this procedure to depollute aqueous effluents containing pesticides, even in presence of high salinity concentrations (Luna, et al., 2012; Micó, et al., 2013).

Though in most of the cases the named chemical treatment is able to degrade xenobiotics to concentrations lower than $1mg\cdot L^{-1}$, organic carbon depletion was revealed as not very efficient at times, or implied an excessive use of reagents (Muñoz, et al., 2005; Oller, et al., 2011). In this regard, several works suggested the need of implementing a subsequent biological process after the chemical treatment that could cope with remaining organic matter (Liberatore, et al., 2012; Oller, et al., 2011; Sarria, et al., 2002). Different biosystems distributions have been tested for the integration: packed bed bioreactors (Lapertot, et al., 2007), immobilized biomass reactors (Moreira, et al., 2012; Oller, et al., 2007), membrane biofilm reactors (Sánchez Pérez, et al., 2013), etc. However, among these technologies, moderate to high salinities are known to produce inhibitory or toxic effects on bacteria not specifically adapted to high salinity (Reid, et al., 2006).

(Rittmann 2006)Slow sand filtration column (SSF) is one of the earliest forms of water treatment. In these devices raw water flows by gravity (0.1-0.3 $m \cdot h^{-1}$ (Campos, et al., 2002)) through a column of sand with high surface area, which is susceptible to be colonized by microorganisms. The combination of the physical filtration and absorption, determined by the effective size of the filling; and the activity of the biomass layer that grows attached to the sand bed, *schmutzdecke* (Huisman and Wood, 1974), contributes to the efficiency of this uncomplicated device. This technology is considered a suitable method for the removal of suspended solids (Ellis, 1987; Logsdon, et al., 2002), pathogens (Bauer, et al., 2011; Martínez, et al., 2010) and dissolved organic matter (Linlin, et al., 2011; Zheng, et al., 2009; Zheng, et al., 2010). In fact, in recent years, a resurgence of interest in this technique has occurred based on its proven efficiency removing dissolved organic matter remaining after an oxidation pretreatment (Graham, 1999; Moncayo-Lasso, et al., 2012; Moncayo-Lasso, et al., 2008).

According to this, in this particular study, SSF is suggested as part of a photo-Fenton/bioreactor coupled arrangement. Its simplicity, its low capital requirements and operating costs (Campos, et al., 2002), together with its robustness against fluctuations in feed quality (Moncayo-Lasso, et al., 2008) makes it a good candidate for the integration, even more considering its inexpensive installation in an agricultural media, where the source of polluted effluents is located. Furthermore, attached grow systems are specially interesting for the particular matter of this research, due to the fact that biofilms self-assemble to provide the community with optimal access to substrates in addition to protecting them from a variety of environmental insults, such as toxicity, salinity, predation, desiccation and washout. Biofilms also establish gradients of substrates, creating specialized niches where microorganism with different and seemingly incompatible metabolic functions can co-exist in the same media (Rittmann, 2006).

2. Objectives

The main aim of the present study is to essay the suitability of the integration between photo-Fenton reaction and slow sand filtration column to treat pesticide contaminated effluents at different salinity conditions. This essay comprises a first step in which the effect of salinity on the biocompatibility of the different photo-Fenton pre-treated effluents is tested by means of monitoring the performance of sequencing batch reactors (SBR) for different conductivity loads. This step is of special importance given that crucial limitation for using slow sand filtration is the lack of a way to predict a priori the treatability of source water (Logsdon, et al., 2002). On the second step, photo-Fenton final effluents are loaded to the slow sand filtration column. Its performance is monitored to verify that indeed the integration is plausible and robust against salinity changes in the load. Bacterial 16S rRNA gene sequencing, a culture independent technique, will be applied to analyze microbial diversity of the schmutzdecke at different conductivity environments. In opposition to other techniques proposed in previous works, most of them based on culture dependent techniques (Duncan, 1988; Nakamoto, 1993; Yordanov, et al., 1996), gene sequencing does not limit the study to those species susceptible of being culturable in lab conditions, improving the bacterial diversity monitoring and understanding.

3. Material and methods

Feed composition

The performance of photo-Fenton pesticide removal at high salinities has already been studied and presented in a previous work (Micó, et al., 2013). Simulated hydroponic effluents for the current study were prepared with the conditions summarized in Table 1 and submitted to photo-Fenton reaction, as seen in (Micó, et al., 2013), before its load into the SBR's. Salt species were chosen according to those that Fornes and colleagues stated as critical for high salinity issues in hydroponics (Fornes, et al., 2007). B0 solution was tested as a control being submitted to photo-Fenton reaction in absence of added salts. In opposition to it, BC was nor pretreated before its load but directly loaded to the bioreactor. Experiments B1, B2 and B3 represents three different levels of salinity among which B3 averages the maximum conductivity that different plants can cope, between 10.5 to 14.4 $mS \cdot cm^{-1}$, according to literature (Fornes, et al., 2007; Montesano, et al., 2010; Villarino and Mattson, 2011). B4 represents extreme salinity value. It was tested in order to prove the suitability of the integrated technology to treat the concentrate of a possible reverse osmosis process applied to hydroponics effluent (to reduce the volume of the polluted stream and to resend the permeate to the cycle). Photo-Fenton initial concentration of reagents for the pretreatment were $[H_2O_2]_0$= 100 $mg \cdot L^{-1}$ and $[Fe^{2+}]$= 10 $mg \cdot L^{-1}$ (in the shape of $FeSO_4$), and commercial formulations of methomyl and imidacloprid were used as target compounds. As mentioned in (Micó, et al., 2013), reagent values were chosen according to previous experiments (Micó, et al., 2010).

Table 1. Summary of averaged conditions previous and after photo-Fenton for the effluents loaded to the sequencing batch reactors.

$10 mg\cdot L^{-1}$ Imidacloprid
$10 mg\cdot L^{-1}$ Methomyl
$\sim 44 mg\cdot L^{-1}$ DOC_0 (previous to photo-Fenton treatment)

Exp.	Specie	Salts conc. ($g\cdot L^{-1}$)	Total salts conc. ($g\cdot L^{-1}$)	Conductivity ($mS\cdot cm^{-1}$)	DOC_f* ($mg\cdot L^{-1}$)	[Imid.]* ($mg\cdot L^{-1}$)	[Met.]* ($mg\cdot L^{-1}$)
BC**	Non added salts			$\sim 1.5\cdot 10^{-3}$	45.19	10	10
B0	Non added salts			$\sim 1.5\cdot 10^{-3}$	35.76	0.01	Not detectable
B1	KNO_3	0.60	0.9	1.00	39.57	0.03	0.01
	$CaCl_2$	0.10					
	NH_4Cl	0.05					
	$MgSO_4$	0.05					
	$NaHCO_3$	0.10					
B2	KNO_3	0.60	4.6	5.06	41.15	0.03	0.05
	$CaCl_2$	0.10					
	NH_4Cl	0.05					
	$MgSO_4$	0.15					
	$NaHCO_3$	0.10					
	NaCl	3.00					
	$CaSO_4$	0.60					
B3	KNO_3	1.25	9.05	11.06	42.24	0.13	Not detectable
	$CaCl_2$	0.10					
	NH_4Cl	0.05					
	$MgSO_4$	0.30					
	$NaHCO_3$	0.10					
	NaCl	6.00					
	$CaSO_4$	1.25					
B4	KNO_3	1.25	42.13	50.00	43.51	0.77	Not detectable
	$CaCl_2$	0.10					
	NH_4Cl	0.05					
	$MgSO_4$	0.90					
	$NaHCO_3$	0.08					
	NaCl	36.00					
	$CaSO_4$	3.75					

**DOC values after photo-Fenton treatment*
*** This experiment was not chemically pre- treated at all*

As can be seen in Table 1, photo-Fenton pretreatment was able degrade total pesticide load in all salinity conditions studied (exp. B0, B1, B2, B3 and B4). However, mineralization of the organic content composed by the pesticides themselves and the inert ingredients from their respective commercial formulation was not so efficient.

Previous to their addition to any of the bioreactors, all the effluents were neutralized to pH 6.5-7.5 with KOH solution, and were spiked with nutrients which were not already part of the processed solutions, including trace elements according to Standard Method 5210D (APHA, 1995).These nutrients are crucial in stimulating the microorganisms to perform their metabolic functions. If these proper quantities are not present, balanced biomass growths are unable to occur and treatment performance will be impaired (Chan, et al., 2010).

Sequencing batch reactors

Six SBRs in 1 L working volume Pyrex Erlenmeyer flasks, loaded with one type of solution each, were operated by cycles of four stages: fill, react, settle, and decant. In the first cycle, 900 mL of fresh effluent was mixed with 100 mL of aerobic sludge (Volatile Suspended Solids (VSS) = 1720 mg/L) taken from a wastewater treatment plant placed in Barcelona. For subsequent cycles, again 900 mL of fresh effluent was mixed with 100 mL of settled biomass from the content of the previous cycle, decanted beforehand (centrifuged). Therefore the system worked with 90% volumetric exchange ratio, expecting a fast start-up (De Clippeleir, et al., 2009), which was conducted directly contacting the effluent with the secondary liquor.

The reactors were operated through different periods of time, the cycles succeeded as Dissolved Organic Carbon (DOC) values were stabilized, and kept at room temperature (22 ± 2 ºC). Fine stone air diffusers, connected to aeration pumps, provided oxygen ensuring aerobic conditions to the six reactors, laying in their bases and supplying 3 $L\cdot min^{-1}$. Even distribution of the wastewater was achieved through continuous stirring. The reactors were covered with aluminum foil to prevent light interactions.

Samples from the reactors were taken, filtered, and analyzed regularly, in order to monitor the concentration of DOC, methomyl and imidacloprid.

Slow sand filtration column

A scheme of the slow sand filtration column is depicted in fig. 1. Filtration device consisted of a glass cylindrical reactor of 110 cm height, 5 cm diameter, covered with aluminum foil to avoid light penetration and the development of algae. The column was filled (up to 101.5 cm) with expanded clay particles (2.5-5.0 mm), Filtralite ®, and loaded, with an average down flow of 0.3 $mL\cdot min^{-1}$, supplied by a peristaltic pump, from a stirred tank constantly aerated ensuring oxygen saturation. The empty volume not occupied by clay was calculated approximately as 850 mL, the elapsed time for the effluent front to traverse the column length was about 46h. The SSF was continuously operated at a filtration rate of 0.01 $m\cdot h^{-1}$. The filters were not back-washable to make the application as simple as possible (Zheng, et al., 2009).

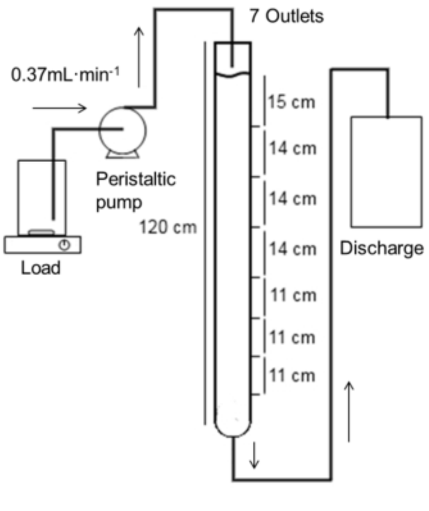

Figure 1. Slow sand filtration scheme.

The column counted with seven sampling points distributed all along its length. First outlet was 15 cm under the surface of the solid media. Over this surface the supernatant water column measured just 2.5 cm. Samples were taken around every 20-24 h from the lowest sample point, to check the global performance of the column in terms of O_2 consumption, DOC depletion, and NH_4^+ and NO_3^- elimination. Samples from all along the column were taken eventually in order to assess the performance at different filtering depths.

Analytical procedures

DOC and pesticides concentrations were monitored with the purpose of assessing the performance of the system. DOC was measured by means of a Shimadzu DOC-VCSN DOC analyzer. The concentration of the pesticides was quantified through a HPLC with photodiode array detector, Waters Corporation. The column used was a Mediterranean Sea18, 5 µm 25x0.46 cm (Teknokroma). The mobile phase, composed by a mixture of water and acetonitrile (both from Merck) (60:40), was delivered at a flow rate of 1 $mL\cdot min^{-1}$. In the cases with higher salinity content, a peak related to NaCl masked methomyl peak, so another method was used with a mobile phase composed by 2.5 mL of 80% H_3PO_4, 25 mL of methanol, diluted to 500 mL with milli-Q water. In this case the flow was 0.7 $mL\cdot min^{-1}$. The wavelength of the UV maximum absorbance was 232.5 nm for methomyl, while for imidacloprid it was 269 nm. A dissolved oxygen (DO) probe (Symphony, VWR) was used to measure this parameter in the feeding tank. NO_3^-, NH^{4+} and total N concentrations were analyzed to find out the fate of the N contained in the load. Total Nitrogen was also measured by Shimadzu DOC-VCSN DOC, while nitrate and ammonia was registered by ionic chromatographer. Finally, volatile suspended solids, VSS, were determined according to the Standard Method 2540E, (APHA, American Public Health Association, 1989).

Molecular biology techniques

(1) DNA extraction and purification. Indirect method was used to extract DNA from active sludge samples. 0.5 g of Filtralite ® sample was added to an Eppendorf tube and PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.) protocol was applied. Then the crude DNA was further purified using PowerClean® DNA Clean-Up Kit (MO BIO Laboratories, Inc.). The ratio between light absorbance at 260 and 280 nm and agarose gel electrophoresis were used to ensure good quality DNA extraction.

(2) Polymerase chain reaction amplification (PCR) of 16S rRNA gene was performed using Taq DNA Polymerase (Quiagen), with 8F and 1492R bacterial universal primers,

according to the quantities from table 2. ABI 9700 (Applied Biosystems) was the thermocycler device that worked on the conditions described in table 3.

Table 2. PCR ingredients.

	v (µL)
Water	10.75
8F	0.25
1492R	0.25
Taq Polymerase	12.50
DNA template	1.25
Total volume	25.00

Table 3. PCR cycling description.

Step	**T (ºC)**	**t (min)**	
Preparation	from 20 to 95	2	
Denaturation	94	0.5	
Annealing	50	0.5	**32 cycles**
Extension	72	0.5	
Hold	68	10	
Conservation	4	∞	

As salinity causes lower PCR yields, dilutions of the extracted DNA were needed to obtain positive results, again checked by gel electrophoresis.

(3) The construction of gene clone library started with the cloning of the PCR products by TOPO® TA Cloning® Kit for Sequencing with One Shot® TOP10 Chemically Competent *E. coli*. The DNA from the resulting colonies was extracted and sequenced by means of Robosec 4204SE automat robot (Applied Biosystems) with Plasmid Miniprep Kit (Millipore). That resultant DNA was sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit and V3F and V3R primers. Thermal reaction was also performed in the ABI thermocycler, while the reading took place by means of ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

(4) The sequences were compared to previously identified organisms using Basic Local Alignment Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi, National Centre for Biotechnology, USA). This free access online app finds regions of local similarity between query sequences and sequences from GenBank database (National Centre for Biotechnology, USA) and calculates the statistical significance of matches. Together with the taxonomic database of National Centre of Biotechnology, http://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/, it allows inferring functional and evolutionary relationships between sequences as well as helping identify members of gene families, enabling the construction of a graph with the distribution of the whole bacterial population that was supposed to be in the initial sample.

4. Results and discussion

a. Sequencing Batch Reactors Results

On the first load, the reactors were feed with 900 mL of the corresponding effluent and 100 mL of secondary liquor from WWTP in Gavà, Barcelona, achieving an initial VSS average of 172 mg·L^{-1}. This biomass load evolved while metabolizing the organic content of the effluents along four consecutive cycles of fill-react-settle-decant, after which bacterial population was considered acclimated to the effluents and stable. Fig. 2 represents the DOC evolution for the successive cycles.

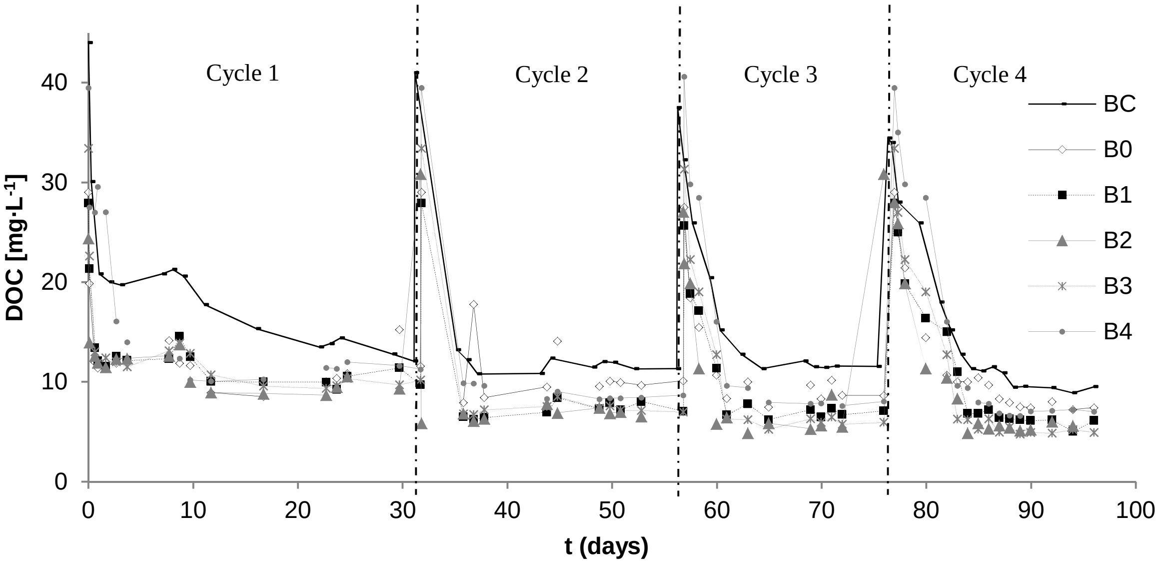

Figure 2: DOC evolution along the four cycles in sequencing batch reactors.

The very first cycle of SBR performance gives an idea of the readily biodegradability content of the effluents (González, et al., 2008; Servais, et al., 1987). As can be seen DOC depletion is evident in every case, which is an indication the presence of a fraction susceptible of being promptly metabolized, even for the non-treated experiment, and the resistance of the inoculum that did not suffer toxic or osmotic shock that collapse the system.

For B0, B1, B2, and B3 the capability of biomass to degrade the readily biodegradable organic fraction seems to be similar from the very first moment of feeding for all the reactors at different salinity conditions, and final DOC depletion at the end of the cycle noticeably high, from 60.1% to 69.5% as can be seen in table 4. It is worthy to note how B4 achieved almost the highest DOC depletion (71.5%) despite its high conductivity, in opposition to what was seen in previous works (Lefebvre, et al., 2007), where chlorine content hindered the performance of the studied bioreactor. However a certain influence could be seen in the slight elapsed time that shows B4 profile at the beginning of the cycle. In the case of BC, extreme pesticide conditions causes lengthier degradation profiles and slower DOC depletion, however, more than acceptable percentage of organic matter depletion was achieved, 72.6%.

Table 4. Averaged DOC depletion percentages for each load and reactor.

	Cycle 1	**Cycle 2**	**Cycle 3**	**Cycle 4**
BC	72.6	72.4	69.2	72.4
B0	60.1	65.2	68.7	74.5
B1	65.2	74.7	72.3	78.2
B2	69.8	78.9	79.7	81.8
B3	69.5	79.2	81.1	85.2
B4	71.5	78.1	80.2	82.3

After this first cycle more uniformity and more DOC removal was achieved for every experiment as biomass acclimated to each feeding conditions. It can be seen how maximum carbon depletion is achieve approximately during the first ten days of every load, and ended up with relative final DOC depletion between 5 and 10% higher that precedent cycle, achieving 85.2% for almost the highest salinity conditions, B3. Nevertheless BC keeps a constant final DOC degradation percentage around 72.5%. The fact that this value is lower than the rest of experiments suggests the capability of photo-Fenton to increase biodegradability of the effluent.

Good results were obtained also for B4 (83.2%), however, it could be seen how its degradation rates remained lower than the rest during the first days after reactor feeding, although final DOC removal reached those high values, more than 80%. This behavior suggests that hydraulic retention time and organic loading rate of biological reactors should be adjusted accurately (to avoid an overloading when working at maximum salinity conditions.

Fig. 3 shows the contribution of each process to final DOC elimination. As said before, even in the case of no previous pre-treatment, the mineralization achieved by the biological reactor is quite high. However, pesticide concentrations showed no changes comparing the effluent and the influent of the reactors. In the cases which their corresponding concentration was still detectable in the effluent (see table 1), the values for the influent remained the same, also in the case of BC, where no treatment was applied. On one hand this reflects the biorecalcitrance of methomyl and imidacloprid; on the other, it states the no evidence of an inhibitory effect given the performance of the biomass given the good metabolizing results. According to this it is important to keep in mind that although the bioreactor itself can considerably diminish organic content, the pesticide content remains unharmed with the environmental risk that entails, so chemical oxidation pretreatment is indispensable.

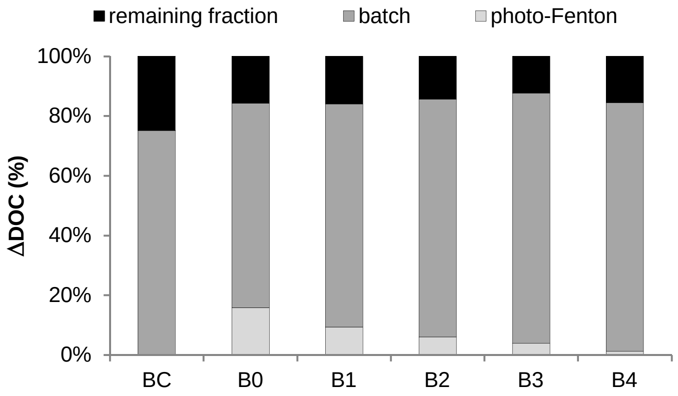

Figure 3. DOC depletion percentages for photo-Fenton and biological processes.

Fig. 3 shows the contribution of each process to final DOC elimination. As said before, even in the case of no previous pre-treatment, the mineralization achieved by the biological reactor is quite high. However, pesticide concentrations showed no changes comparing the effluent and the influent of the reactors. In the cases which their corresponding concentration was still detectable in the effluent (see table 1), the values for the influent remained the same, also in the case of BC, where no treatment was applied. On one hand this reflects the biorecalcitrance of methomyl and imidacloprid; on the other, it states the no evidence of an inhibitory effect given the performance of the biomass given the good metabolizing results. According to this it is important to keep in mind that although the bioreactor itself can considerably diminish organic content, the pesticide content remains unharmed with the environmental risk that entails, so chemical oxidation pretreatment is indispensable.

In line with this, cases B3 and B4 are specially promising due to the fact that pesticide depletion was almost total through photo-Fenton reaction (results shown (Micó, et al., 2013)), and the metabolizing of the organic content in both reactors is even higher than in the case of B0, where no high salinity interferences were taking place. This confirms the suitability of coupling a chemical/biological systems in high salinity applications related to pesticide pollution suggested before.

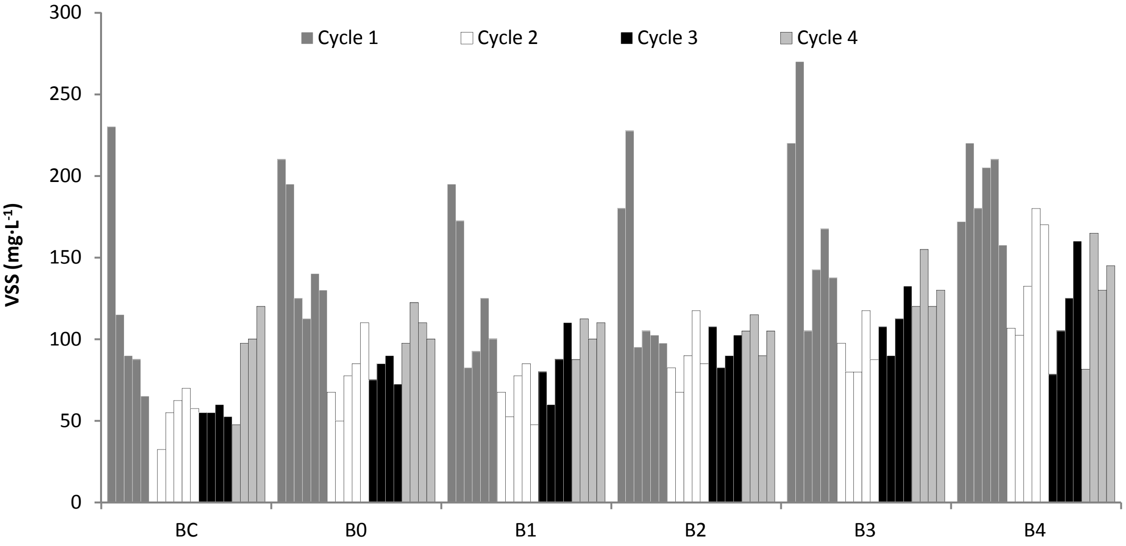

Figure 4. VSS evolution along the four cycles in sequencing batch reactors.

One of the parameters that reflect the stability of the bioreactor is the biomass content in the expressed as volatile suspended solids (VSS). The evolution of VSS of the 6 SBR for the four cycles is depicted in fig. 4. In the first cycle, the biomass, showed initial decrease on every reactor except for B4. This decrease could be considered an adaptation of the initial biomass to the organic matter availability of the media. The descent in VSS is followed also by a certain increase afterwards, which could indicate the effective acclimation of the microbes' consortium to the characteristics of every load. In the case of BC, the significant population recovery in the last cycle indicates that pesticide load is not exerting a noticeable inhibition to the biomass, which seems to be quite stable with no indications of collapsing. In the case of B3 batch reactor, loaded with maximum salinity accepted by the crops, this final increase also indicates that salinity adaptation of a non-halophile inoculum is possible. It also justifies the application of this feeding condition to the slow sand filtration column expecting a good performance at those salinity levels.

Regarding B4, although DOC degradation profile was quite similar to the rest's, the evolution of the solids is significantly different given that no decrease was observed in the first cycle of the SBR but a small descent in the second cycle. However, VSS values in the last cycles were on the average of the other batch reactors indicating again that biomass can be considered stable, and the collapse in the working conditions applied is not expected.

According to all this, it could be concluded that photo-Fenton process increases biodegradability of pre-pretreated effluent to a certain point. Though it leaves a quite low biorecalcitrant fraction that could not be metabolized by biomass as represented in fig. 3, salinity seems not to hinder biodegradation. Once the biomass overcome the osmotic shock during the first two cycles, the effluents with higher conductivities seems to degrade organic matter until the same or even lower final DOC values achieved in the batches with the lower salinity effluents. As a result, taking also into account the pesticide removal achieved by chemical pretreatment, it suggests the suitability of the photo-Fenton integration and a subsequent chemical treatment in cases of high chlorine content pesticide polluted waters.

b. Slow Sand Filtration Colum Results

i. SSF Performance

Previous experiments were performed in order to check the capability of column filling to retain pesticide content or DOC, not noticeable adsorption was observed.

To begin with the operation of the slow sand filtration column, 2 L of chemically pre-treated effluents from type B1, neutralized with KOH, was mixed with 1L of sewage from secondary treatment from Gavà WWTP. This mixture was oxygenated to a DO concentration of 8.5 $mgO_2 \cdot L^{-1}$, then it was continuously recirculated, supplying oxygen when needed, during 48 h.

After that period, the recycling system was open and the feed was renewed with 2 L of fresh KOH neutralized chemically pre-treated effluents load from type B1 (table 1), that were then persistently feed to the surface of the supernatant layer of the column by a peristaltic pump that dosed it by constant dripping. The feed, recharged every two-three days, was aerated periodically in order to keep oxygen concentration between 7.8 to 8.5 $mgO_2 \cdot L^{-1}$. Samples were taken from the lowest outlet in order to monitor dissolve oxygen and measure DOC, pesticide content and concentrations of ammonia, nitrates and total nitrogen. Experimental time started 48 h after the first drop of the new feed, according to the calculation that 46 h needed for the column front to reach the last outlet. The change between one load type to another was drastic in every case. The new effluent just substituted the old one once its last recharge was about to finish.

In opposition to what was expected (Rooklidge, et al., 2005(Rooklidge et al. 2005), no clogging was detected along the whole operational period due to the artificial origin of the effluent. Given that there were no solids in the load, the anticipated clogging was just due to the biomass growth and it was not enough to block the system. Therefore, there was no need to clean the filter media draining the filter neither removing the top layer (Rooklidge, et al., 2005) and no backwash system was necessary after more than a year of operation of the column.

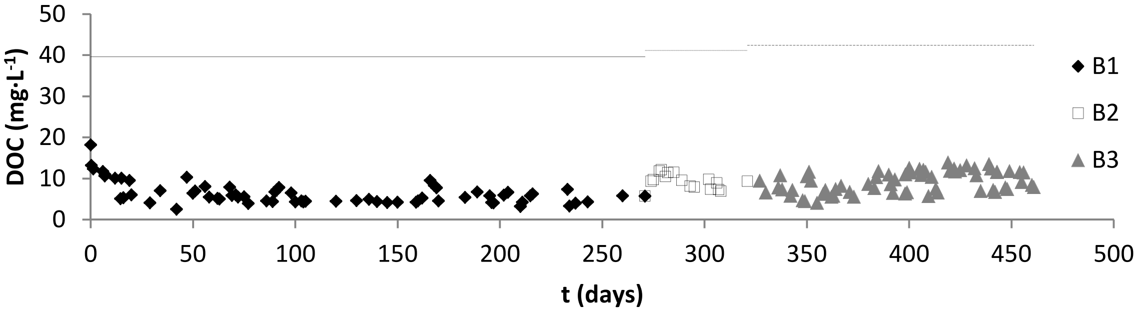

Figure 5. DOC evolution along slow sand filtration column performance. On the legend, B1, B2, B3 correspond to the type of effluent loaded in each different stage, see table 1. Upper lines indicate inlet DOC value.

Fig. 5 represents DOC measures for the different load stages of the column. It can be seen that from the very first moment, just a few hours of start of the process, DOC reduction is noticeable and it keeps diminishing till achieves values between 4 and 5 mgC·L^{-1}. According to the assumption of no significant adsorption process is taking place, the existence of an established biomass could be assumed. This is confirmed by the fact that DO values at the column outlet for those first hours were around 2.3 and 1.2 mgO_2·L^{-1}, what points out the aerobic biodegradation of this organic matter, and the fact that this depletion does not take place just to a physical phenomenon of filtration.

Decreasing tendency is followed by the other two higher conductivities loads. Although DOC values are slightly higher than in the first period, achieved DOC depletion is around 75%, what indicates the capability of the schmutzdecke to metabolize the organic matter that remains after oxidation, even for the highest salinity condition tested. All this shows that the activity and development of microorganism population is able to metabolize a noticeable amount of organic matter present in Photo-Fenton effluents, an average of 30 mg·L^{-1} related to every load DOC content, consuming 6.5 mg·L^{-1} O_2 in the process. No osmotic shock was observed either, given that there were no stiff changes in the measured parameters for increased salinity. This quick adaptation and the resistance to relatively harsh conditions could be related to the fact that supported biomass is more resistant to adverse media conditions compared to suspended biomass reactors (Bishop, 1997; Shieh and Keenan, 1986), and confirms the performance response previously observed in the SBR’s.

Although the nutrients were added fulfilling stoichiometric needs, in relation to the chemical oxygen demand (COD) income, no nitrification or denitrification seemed to take place significantly (TN, [NH_4^+], [NO_3^-] differences between inlet and outlet were negligible). That fact could be explained by the low levels of DO caused by organic matter biodegradation, together with the continuous low values of DOC disposal, precisely due to an intense biological oxidation. Furthermore in literature it is already stated that under normal design and operation, SSF are generally not capable of removing nitrogen (Amy, et al., 2006).

Pesticide degradation was not noticed either for the loading effluents B1 and B2 (in type B3 degradation of pesticide by photo-Fenton reaction was complete previous to the biological reactor). This indicates that if their concentration after photo-Fenton is not low enough for the public standards, it should be optimized for achieving higher depletions. However, the active presence of biomass indicates that remaining pesticides and metabolites do not inhibit its growing and the second can be mostly depleted.

Results just discussed were obtained analyzing samples acquired from the final sampling point of the column. In order to check the distribution of this biological activity along the column, samples from every outlet were also drawn and DOC and DO were measured. Results showed that most part of DOC consumption and DO decay takes place just before the first sampling point -only 15 cm under the surface of the solid- as can be seen in fig. 6. DO measurements also shown a noticeably drop from 7.5-8 mgO_2·L^{-1} in the feeding tank to 2.5-1.8 mgO_2·L^{-1} just in the first outlet, keeping similar values for the rest of them. All this makes evident that the biomass layer activity is mostly located on the first centimeters of the column, a fact that previous researchers have already stated (Campos, et al., 2002). This fact could suggest the need of the optimization of the filtering media depending on the characteristics of the load. High solid content in real effluents will require higher (or deeper) columns, while in the cases where the requirements are more related to biomass activity, the column could be reduced.

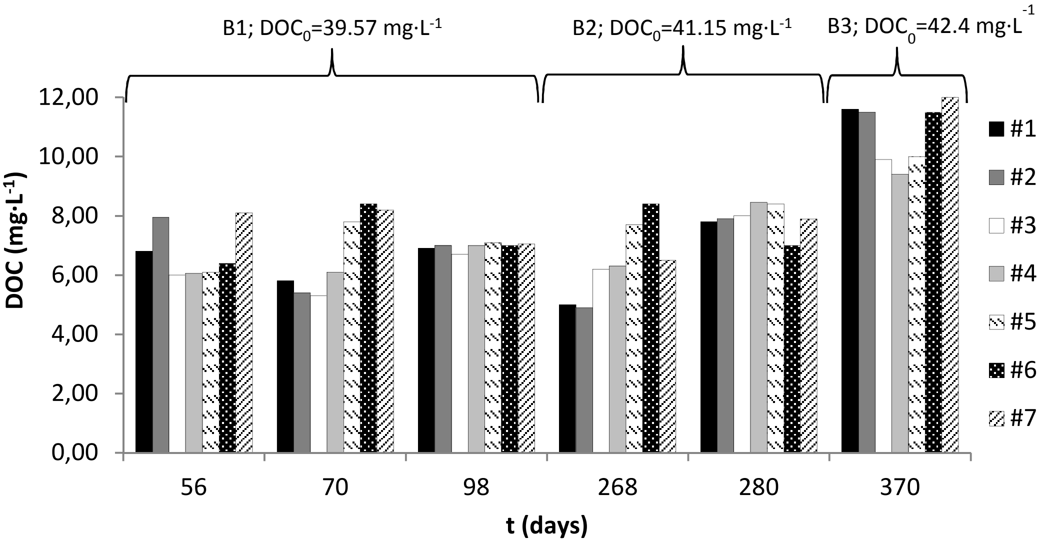

Figure 6. Representative DOC measures for samples taken from every outlet in a row. The first three correspond to the first period with 1.6 mS·cm^{-1} effluent, next two were taken during the period of 5.6 mS·cm^{-1} loading, and the last one belongs to the highest salinity concentration charge. Listed on the left, the number of the outlets, being #1 the closest to the surface and #7 the last one, from where samples were taken regularly.

Regarding to final DOC elimination, mineralization achieved by the biological reactor was between a 72% and a 78%. B3 case is especially interesting given that although the mineralization grade by photo-Fenton reaction is very low, biomass was able to cope with 75% of the total organic content. In the case of SSF, the biorecalcitrant remaining fraction is higher for higher salinity cases (10.3%, 6.48% and 3.99% for B1, B2 and B3, respectively).

ii. *Genomic characterization*

Assuming that the attached biomass is mainly established between the surface of the column and its first inlet, expanded clay samples from the middle point of this region were withdrawn at the end of every cycle. Those solids are manually grinded in a ceramic mortar and between 0.5 and 0.9 mg of the resultant milled matrix are transferred to the first tube of the extraction kit in order to characterize bacterial diversity in tested samples.

According to Wagner and Loy (Wagner and Loy, 2002), *Beta-*, *Alpha-* and *Gammaproteobacteria* are frequently retrieved in wastewater treatment plants, especially *Betaproteobacteria* that play important roles degrading a variety of pollutants. Among this order, *Rhodocyclales* have been stated very important for bioremediation and agriculture, as many member of this class are considered responsible for the removal of anthropogenic compounds in the environment or in biotechnological systemst (Loy, et al., 2005). Particularly, *Methyloversatilis* are microorganism able to grow on single carbon compounds (Kalyuzhnaya, et al., 2006), which presence could be related to the degradation of the photo-Fenton oxidation by-products loaded to the column. For its part, *Lepthothrix,* classified as facultative autotrophic-heterotrophic, seems to be mainly related to iron oxidation and can be found in biofilters intended for iron and manganese elimination from raw and waste water (Tekerlekopoulou, et al., 2013). In the case of *Burkhoderiales* is especially interesting given that a certain strain of *Comamonas* was revealed as capable of degrading certain organochlorine herbicides (Müller, et al., 1999). In this case no pesticide depletion was (Feng et al. 2012)evident; however this kind of bacteria could be making its living by degrading oxidation by-products. Other studies indicate that *Comamonadaceae* could also be involved in denitrification pathways, but no evidences of nitrogen metabolism could be observed.

Regarding to *Gammaproteobacteria,* they are known to exist normally in aerobic biosystems as the main bacterial groups (Lee, et al., 2003; Wong, et al., 2005; Xia, et al., 2010) and in DOC degrading in nutrient-rich environment (Poretsky, et al., 2010), even in sea-salinity media (Manes, et al., 2011). Among them to, bacterial groups such as *Pseudomonas* are linked with aquatic environments containing high concentration of biorecalcitrant species, even in the presence of biocide substances such as oxytetracycline and penicillin G (Deng, et al., 2012). Its presence has been stated also in atrazine mineralizing media (Masaphy and Mandelbaum, 1997) and related to parathion, together with *Xanthomonadales*. It is not strange then to find *Pseudomonas* in every stage of the column. Specially promising is the fact that this order was also identified as nicotine degrader in tobacco wastes by (Zhong, et al., 2010). This suggests this kind of bacteria could also perform neonicotinoid pesticide degradation as imidacloprid, with structures derived from the alkaloid's, if the optimal conditions of feed and aeration could be found.

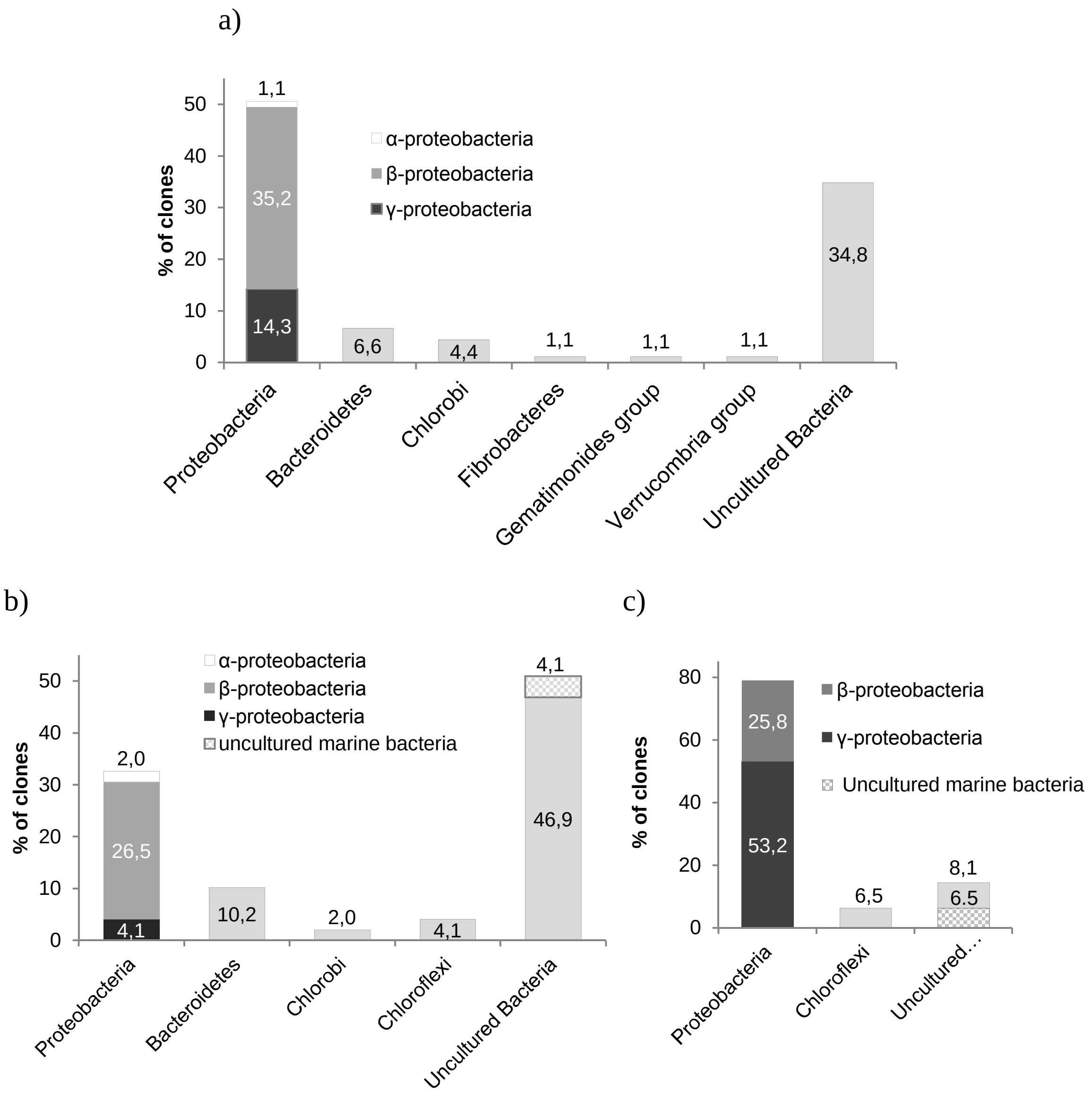

Figure 7. 7a, 7b, 7c. Percentages of the most abundant phylums and groups for the three stages of the slow sand filtration column. The breakdown of the classes among the *Proteobacteria* can be also seen, together with the percentage corresponding to the uncultured marine bacteria in relation with the total uncultured bacteria found for stages 2 and 3. 7a. B1, 1 mS·cm^{-1}. 7b. B2, 5.06 mS·cm^{-1}. 7c. B3, 11.06 mS·cm^{-1} .

Chlorobi and/or *Cholorfelxi* bacteria are also present in every sample. These phyla correspond to photosynthetic prokaryotes, featured with reaction centers that contain bacterio-chlorophyll, that testify the penetration of light to the column, despite the aluminum covering. *Chlorobi* phylum comprises green-brown anaerobic photoautotrophs, while *chloroflexi* are green filamentous anoxygenic phototrophs (Bryant and Frigaard, 2006). The metabolic limitations of *chlorobi* phylum restrict their functions to the oxidation of sulfur compounds (in this case, mainly coming from the byproducts of chemical degradation of methomyl), and the remaining ferrous iron not reduced by pH adjustment; and fix carbon by the reverse tricarboxylic acid cycle (Bryant and Frigaard, 2006; Shah, et al., 2009). In the case of *chloroflexi*, its metabolic diversity allows this phylum to grow as an aerobic chemoheterotroph or as an aerobic photoheterotroph, in both cases fixing CO_2 through different routes (Bryant and Frigaard, 2006; Kunisawa, 2006). According to this, the hererotrophicity of these phyla exclude them from the function of mineralizing the organic matter contained in the load, although they undoubtedly contribute to the equilibrium of the media. From the point of view of the study of the organic content biodegradation, the presence of these phototrophic phyla may reflect an inevitable negative bias in the PCR performance, which manifests more abundant but not critical communities in detriment of most interesting not so numerous organisms.

In addition to referred photosyntetizers, typical heterotrophs such as *Bacteroidetes* were also found in B1 and B2. Together with *Proteobacteria*, these phylums are known to be the most prominent heterotrophic organisms in marine surface waters (Stevens, et al., 2005) and exist normally in aerobic biosystems (Deng, et al., 2012), what fits the envrionment of the column. *Bacteroidetes* are also found as dominant phylum in 16s rRNA libraries from agricultural soil samples (Jangid, et al., 2008; Janssen, 2006), especially *Sphingobacteria* class, which are common in named stages, *Chitinophagaceae* and *Saprospiraceae* in stage 2, and *Terrimonas* in stage 1. The relationship of this phylum with agricultural earth could indicate its resistance to pesticide products. Furthermore, Deng and colleagues stated that this phylum is involved in the degradation of a variety of pollutants, and play important roles in wastewater treatment (Deng, et al., 2012).

Among the uncultured bacteria not associated to any particular specie, especially numerous in B2, a marine subgroup appeared in this stage and in B3, with higher salinities. Corresponding sequences showed similarities with bacteria located in sea samples in relation to marine microbes playing important roles in nitrogen transformation of denitrification and nitrification (Yang, 2011), although none of these processes were visible in the present study.

The fact that microbiological testimonies of nitrification and denitrification are present in all stages of the column, although chemical proves could not be found, could indicate that the process takes place but in such a small proportion that it was invisible analytically. This could be due to the high quantities of nitrates, in the shape of KNO_3, present in the media that could be masking subtle changes in nitrogen content.

5. Conclusions

Regarding to the above commented results and those obtained in (Micó, et al., 2013), it can be concluded that the integration between photo-Fenton reaction and Slow Sand Filtration could be an efficient process for the treatment of hydroponics effluents, even for reasonably high salinities.

SSB performance during the first cycle allowed to determine the readily biodegradable fraction from every load, and stated that photo-Fenton reaction is able to increase this fraction for every tested condition. However a refractory portion was also observed in every case.

The acclimation of the biomass was observed after several loading cycles, with an increase of DOC depletion. This proved the stability of the bioreactors even for maximum salinity values, achieving organic matter degradations higher than 80%

Neither SSB nor SSF biomass was able to eliminate the remaining pesticide content remaining in certain experiments. Although integration is highly recommended, photo-Fenton reaction needs to be previously optimized to ensure total pesticide depletion in the effluent previous to its load to the bioreactor.

In the case of SSF, although regarding to DOC depletion, the three cycles run very similar, with DOC depletion between 72 and 78%, microbial population and its distribution was quite different and it could be seen how diversity diminishes with salinity. This fact shows how different microbial consortiums can develop similar functions in a certain ecosystem.

Proteobacteria, especially β and γ phyla, were abundant in every case what is explained by its relationship to aerobic environments and organic matter degrading functions. Halophile bacteria were found associated to higher salinity effluents, in particular several clones related to marine uncultured bacteria.

Photosynthetic microorganisms were found also in every case. This fact indicates the penetration of light into the solid media, at least through the first layers of expanded clay.

Finally, microbial testimonies of nitrogen metabolizing were found. It could suggest that the related processes could be indeed taking place (although no chemical proofs could be found) in such a small proportion that high NO_3^- concentration could be masking it.

6. Acknowledgements

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Supplementary information. Different clones sequenced in each stage and their closest relatives.

B1

N. of Clones		Most similar Blast® hit	Max. Identity	Phylum	Class	Order
1	EF648106.1	Uncultured alpha proteobacterium clone HB125	100%	Proteobacteria	Alphaproteobacteria	
1	AY509464.1	Uncultured beta proteobacterium clone LiUU-9-138	100%	Proteobacteria	Betaproteobacteria	
1	EU642205.1	Uncultured beta proteobacterium clone Gap-2-95	97%	Proteobacteria	Betaproteobacteria	
2	HM153651.1	Uncultured beta proteobacterium	100%	Proteobacteria	Betaproteobacteria	
2	JQ772239.1	Uncultured beta proteobacterium	99-100%	Proteobacteria	Betaproteobacteria	
1	JF815033.1	Uncultured Oxalobacteraceae	99%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JN083455.1	Uncultured Burkholderiales bacterium clone AgN-20	96%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JN806314.1	Uncultured Burkholderiales bacterium clone GSN-19	99%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	AF235013.1	Acidovorax sp. UFZ-B530	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	GQ273898.1	Uncultured Comamonas sp.	98%	Proteobacteria	Betaproteobacteria	Burkholderiales
3	HM150298.1	Uncultured Comamonas sp. clone kua132	99-100%	Proteobacteria	Betaproteobacteria	Burkholderiales
4	JF681262.1	Uncultured Comamonas sp.	95-100%	Proteobacteria	Betaproteobacteria	Burkholderiales
2	JN226395.1	Comamonas sp. CB22	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JQ818823.1	Comamonas testosteroni genomic sequence	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	FJ982929.1	Thiobacillus sp. ST22	99%	Proteobacteria	Betaproteobacteria	Hydrogenophilales
2	JN125483.1	Uncultured Rhodocyclaceae bacterium clone MBfR_Mix-29	98-99%	Proteobacteria	Betaproteobacteria	Rhodocyclales
4	JF522329.1	Uncultured Leptothrix sp. clone I10	99-100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JN177630.1	Uncultured Methyloversatilis sp.	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JN177681.1	Uncultured Methyloversatilis sp. clone K4S130	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JN648270.1	Uncultured Thauera sp. clone 244	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	HE800809.1	Acinetobacter lwoffii	100%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
2	CP003588.1	Pseudomonas putida	100%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
4	JQ811768.1	Pseudomonas sp. RB001	99-100%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
1	HM798936.1	Uncultured Xanthomonadales bacterium clone PRTBB8498	100%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1	JN217047.1	Uncultured Dokdonella sp. clone S70	96%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
2	JN679193.1	Uncultured Dokdonella sp. clone 5.17m12	98-99%	Proteobacteria	Gammaproteobacteria	Xanthomonadales

B1						
N. of Clones	**Most similar Blast® hit**		**Max. Identity**	**Phylum**	**Class**	**Order**
2	JN679196.1	Uncultured Xanthomonas sp. clone 5.17m24	99%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1	JQ435729.1	Pseudoxanthomonas sp. EE4	100%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1	GQ255608.1	Uncultured Nitrosospira sp.	100%	Nitrospirae	Nitrospira	Nitrospirales
1	JQ684443.1	Uncultured Nitrosospira sp. clone Ba204	98%	Nitrospirae	Nitrospira	Nitrospirales
1	DQ501290.1	Uncultured Fibrobacteres bacterium clone BL11-22	100%	Fibrobacteres/Acidobacteria group		
1	FM207902.1	Uncultured Chlorobi bacterium	100%	Chlorobi	Chlorobia	Chlorobiales
1	FN668201.2	Uncultured Chlorobi bacterium	100%	Chlorobi	Chlorobia	Chlorobiales
1	JN412343.1	Uncultured Chloroflexi bacterium clone PM17	96%	Chlorobi	Chlorobia	Chlorobiales
1	JN695869.1	Uncultured Bacteroidetes bacterium clone 4.21h5	100%	Bacterioidetes		
1	JN936813.1	Uncultured Bacteroidetes	100%	Bacteroidetes		
2	HM193113.1	Uncultured Flexibacteraceae	99-100%	Bacteroidetes	Cytophagia	Cytophagales
2	JN217083.1	Uncultured Terrimonas sp. clone S226	99%	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
1	JN656861.1	Uncultured Verrucomicrobia bacterium clone KWK12S.29	99%	Chlamydiae/Verrucomicrobia group		
1	JN825454.1	Uncultured Gemmatimonadetes bacterium	99%		Gemmatimonadetes	
2	AJ518727.1	Unidentified bacterium	99%			
1	FN827199.1	Uncultured bacterium	100%			
1	FQ659381.1	Uncultured bacterium	100%			
1	HM150333.1	Uncultured bacterium clone kua167	99%			
1	HQ476947.1	Uncultured bacterium clone Pohang_WWTP	100%			
1	JF341478.1	Uncultured bacterium clone 36MIC074	97%			
1	JF728084.1	Uncultured bacterium clone BacNitrate-11-50	98%			
1	JF925033.1	Uncultured marine bacterium clone Tc-95	100%			
1	JN191848.1	Uncultured bacterium clone RBMU-B27	100%			
1	JQ180419.1	Uncultured bacterium clone WBA-10	98%			
8	JQ327982.1	Uncultured bacterium clone sh-xj104	98-99%			
1	JQ413524.1	Uncultured bacterium clone WW1_LAB_F5	100%			
1	JQ624282.1	Uncultured bacterium clone OTU-7-AFB	99%			
11	JX040399.1	Uncultured bacterium clone a-122	97-100%			
1	KC605578.1	Uncultured bacterium clone bacBiof_0868	99%			

B 2

N. of clones	Most similar Blast® hit		Max. Identity	Phylum	Class	Order
1	JF900619.1	Sinorhizobium sp.	1	Proteobacteria	Alphaproteobacteria	Rhizobiales
2	JF808735.1	Uncultured Methylibium sp.	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JQ670734.1	Acidovorax sp.	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	HM150298.1	Uncultured Comamonas sp. clone kua132	99%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JN125483.1	Uncultured Rhodocyclaceae bacterium clone MBfR_Mix-29	97%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JN177681.1	Uncultured Methyloversatilis sp. clone K4S130	98%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JF522329.1	Uncultured Leptothrix sp. clone I10	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JQ811768.1	Pseudomonas sp. RB001	99%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
1	JQ435729.1	Pseudoxanthomonas sp. EE4	100%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
3	JQ791929.1	Uncultured Saprospiraceae	95-98%	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
1	FN821840.1	Sediminibacterium sp. PYM5-6	100%	Bacteroidetes	Sphingobacteriia	Sphingobacteriales
1	HM193113.1	Uncultured Flexibacteraceae	99%	Bacterioidetes	Cytophagia	Chitophagiales
2	EU298116.1	Uncultured Anaerolineae bacterium clone	99%	Chloroflexi	Anaerolineae	Anaerolineales
1	JQ177516.1	Uncultured Anaerolineaceae	95%	Chloroflexi	Anaerolineae	Anaerolineales
3	GQ255608.1	Uncultured Nitrosospira sp. clone P96	98-100%	Nitrospirae	Nitrospira	Nitrospirales
1	GU504446.1	Uncultured bacterium clone F1Q32TO05F3VN1	94%			
2	JF925036.1	Uncultured marine bacterium clone Tc-122	99-100%			
1	JN391617.1	Uncultured bacterium clone	97%			
11	JQ327982.1	Uncultured bacterium clone sh-xj104	97-99%			
2	JQ413524.1	Uncultured bacterium clone WW1_LAB_F5	96-99%			
6	JX040399.1	Uncultured bacterium clone a-122	99-100%			

B3						
N. of clones	**Most similar Blast® hit**		**Max. Identity**	**Phylum**	**Class**	**Order**
1	HQ222278.1	Acidovorax sp.	99%	Proteobacteria	Betaproteobacteria	Burkholderiales
3	JN217068.1	Uncultured Acidovorax sp. clone S169	99-100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	JQ689177.1	Acidovorax delafieldii strain A3RO7	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
4	JF681262.1	Uncultured Comamonas sp. clone R1_56	100%	Proteobacteria	Betaproteobacteria	Burkholderiales
1	FJ982929.1	Thiobacillus sp. ST22	97%	Proteobacteria	Betaproteobacteria	Hydrogenophilales
1	JN177630.1	Uncultured Methyloversatilis sp.	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	JN648270.1	Uncultured Thauera sp. clone 244	100%	Proteobacteria	Betaproteobacteria	Rhodocyclales
1	DQ989450.1	Uncultured Pseudomonas sp. clone HKT912	98%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
1	EU434442.1	Pseudomonas sp. a220	92%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
1	FJ013297.1	Pseudomonas sp. ITRH25	96%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
28	HQ418485.1	Pseudomonas sp. F2(2010)	97-100%	Proteobacteria	Gammaproteobacteria	Pseudomonadales
1	JQ435729.1	Pseudoxanthomonas sp. EE4	99%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1	EF027001.1	Xanthomonas sp. XLL-1	96%	Proteobacteria	Gammaproteobacteria	Xanthomonadales
1	JQ177516.1	Uncultured Anaerolineaceae	96%	Chloroflexi		
2	JQ726863.1	Uncultured Chloroflexi bacterium clone LakeCentre25	96-98%	Chloroflexi		
1	AB638622.1	Uncultured Chloroflexi	96%	Chloroflexi	Anaerolineaceae	
1	HM146554.1	Uncultured bacterium clone SD102-3_36	93%			
1	JF925016.1	Uncultured marine bacterium clone Tc-2	100%			
3	JF925036.1	Uncultured marine bacterium clone Tc-122	98-100%			
2	JN366643.1	Bacterium enrichment culture clone 3_92	99-100%			
1	JN995429.1	Uncultured bacterium clone BUT3_15F06	92%			
1	JQ413524.1	Uncultured bacterium clone WW1_LAB_F5	90%			

OTHER CONTRIBUTIONS AND PUBLICATIONS BY THE AUTHOR

During the development of this thesis, the author took part in several conferences and meetings presenting different part of her work. Hereby there is a list with the contributions to those events.

- **Brussels, Belgium, May 2008:** ORAL presentation: "COMPARISON BETWEEN OZONATION AND PHOTO-FENTON PROCESSES FOR PESTICIDE METHOMYL REMOVAL IN ADVANCED GREENHOUSES."

International Conference of the IOA: Ozone & Related Oxidants in: Advanced treatment of water for human health and environment protection. Disinfection, elimination of persistent pollutants and control of by-products.

- **Berlin, Germany, March-April 2009:** POSTER: "EXPERIMENTAL DESIGN APPLIED TO PHOTO-FENTON TREATMENT OF HIGHLY METHOMYL-CONCENTRATED POLLUTED WATER"

AOP5-5th IWA-Conference on Oxidation Technologies for Water and Wastewater Treatment.

- **Prague, Czech Republic, June 2010:** POSTER: "SOLAR PHOTO-FENTON APPLIED TO PESTICIDE REMOVAL ON ADVANCED GREENHOUSES WATERS."

SPEA6 Solar Chemistry and Photocatalysis: Environmental Applications.

- **Amsterdam, Netherland, June 2011:** POSTER: "INTEGRATION OF ADVANCED OXIDATION TREATMENT SLOW SAND FILTRATION FOR THE REUSE OF ADVANCED GREENHOUSE WATER."

8th IWA Leading-Edge Conference on Water and Wastewater Technologies.

- **Barcelona, Spain, September 2011:** ORAL communication: "CHANCES OF HYDROPONICS WATER REUSE IN ADVANCED GREENHOUSES."

8th IWA Conference on Water Reclamation and Reuse.

- **Barcelona, Spain, November 2011:** POSTER: "MICROBIAL COMMUNITY STRUCTURAL AND FUNCTIONAL INSIGHTS GAINED FROM MOLECULAR BIOLOGY TOOLS IN ENVIRONMENTAL ENGINEERING."

12th Mediterranean Congress of Chemical Engineering.

- **Las Vegas, Nevada, September 2013:** ORAL communication: "ENHANCEMENT AND INHIBITION OF PESTICIDE PHOTO-FENTON OXIDATION IN GREENHOUSES WATER REUSE".

IOA-IUVA 2013 World Congress & Exhibition.

Apart from that, the author collaborated also in the work and interpretation of the genomic content of the following article.

- Simón,F.X.; Penru,Y.; Micó, M.M.; Llorens,J.;Explugas,S.;Baig,S. *Biological activity in expanded clay (EC) and granulated activated carbon (GAC) seawater filters*. Approved with minor revisions, 2013, *Desalination.*

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