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Degradation of chlorophenoxy herbicides by coupled Fenton and biological oxidation

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2,4-D; MCPA; Fenton process; sequencing batch reactor, ecotoxicity; biodegradability

1. Introduction

In the last decades, the use of agrochemicals (herbicides) has been a common practice in intense agriculture, which has considerably increased pollution problems of surface and ground water. Contamination includes point sources like water from cleaning pesticide containers and agricultural industries (10-100 mg L⁻¹) and water from pesticide manufacturing plants (1-1000 mg L⁻¹) (Malato et al., 2001). Chlorophenoxy herbicides, such as 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), are mainly used as plant growth regulators. These herbicides are chemicals of main concern for the environment due to their toxicity (EC₅₀ values of 21.1 for 2,4-D and 11.6 for MCPA from Microtox® test and 213 and 144 mg L⁻¹ from activated sludge respiration inhibition test, respectively) and low biodegradability (less than 25% of TOC reduction after 28 d) (Tobajas et al., 2010; Polo et al., 2011). These characteristics make them hardly biodegradable compounds, being detected in the effluents of wastewater treatment plants (Hu et al., 1999; Peschka et al., 2005).

To prevent the environmental hazard and human risks of herbicides it is necessary to develop methods allowing their effective breakdown. In this context, the application of Advanced Oxidation Processes (AOPs) has been considered a promising solution dealing with recalcitrant compounds (Chiron et al., 2000; Pera-Titus et al., 2004), industrial effluents (Azbar et al., 2004; Bautista et al., 2008) or landfill leachates (Wu et al., 2004; Primo et al., 2008a, 2008b). However, to achieve high percentages of mineralization, namely complete oxidation, requires in most cases costly amounts of H_2O_2 . The use of coupled treatments for the efficient breakdown of organic pollutants has received increasing attention. One of the most attractive solutions appears the combination of AOPs and biological oxidation (Oller et al., 2011). Regarding herbicides removal, different AOPs like photo-Fenton (Farre et al., 2008), TiO_2 photocatalysis (Oller et al., 2007), electrochemical oxidation (Brillas et al., 2004) and ionizing radiation (Drzewicz et al., 2004) have been used coupled with biological systems.

In particular, Fenton oxidation, based on the catalytic decomposition of H_2O_2 into hydroxyl radicals by means of Fe^{2+} at acid pH, has been used in combination with biological treatment for the removal of pentachlorophenol (Zimbron and Reardon, 2011) and 2,4-D (Sun et al., 2005). Application of Fenton oxidation as pre-treatment can convert initially persistent organic pollutants into biodegradable intermediates, which could be treated in a biological system with considerable cost reduction. In this sense, the efficient use of H_2O_2 in the oxidation step is a critical issue for the economy of the treatment. Insufficient oxidation can lead to intermediates structurally similar to the starting pollutants, of even higher toxicity and recalcitrant character (Osano et al., 2002; Sinclair and Boxall, 2003; Hernando et al., 2005). Thus, establishing the optimal H_2O_2 dose to be used in the combined treatment requires evaluating the toxicity and biodegradability of the effluents going to the biological step.

Among standardized bioassays for determining the toxicity of a certain pollutant, the Microtox® test is one of the most widely used, due to its high sensitivity and precision (Dalzell et al., 2002). Although some authors have reported that EC₅₀ values obtained by this method could give an overestimation of the toxic effect on the biomass of an activated sludge unit, it can be useful for a rapid toxicity detection in wastewater treatment plants since it has been found a good agreement between the response of *Vibrio fischeri* and activated sludge to different chemicals (Ricco et al., 2004; Lapertot et al., 2008; Polo et al., 2011).

Methods for measuring biodegradability include OECD tests (OECD, 1992, 1993), which are commonly used to predict the potential hazard of a pollutant in a natural environment and thus low biomass concentrations and fairly large experimental times are employed. Recently, Polo et al. (2011) have proposed a new biodegradability test to predict in an easy and rapid way the behavior of a target compound or a wastewater in a biological reactor by means of respirometry. This test is based on the direct relationship between the specific oxygen uptake rate (SOUR) and the biomass activity. The bioassay simulates the typical operation conditions of an activated sludge unit, using a high biomass to COD ratio, which allows a significant reduction of the testing time to no more than 24 h, comparable to the common hydraulic retention times in a conventional biological aerobic reactor.

The removal of the biodegradable oxidation byproducts generated upon Fenton oxidation can be carried out by means of different biological treatments, using sequencing batch reactors (SBR) (Farre et al., 2008; Ballesteros Martin et al., 2009), immobilized biomass reactors (Oller et al., 2007) or aerated biofilters (Wang et al., 2012). SBR have been widely used in the last decades for industrial wastewater treatment (Wilderer et al., 2001; Singh and Srivastava, 2011) due to their low area and

energy requirements, easy control and the possibility of nutrients removal by combining anaerobic, anoxic and aerobic stages in the same reactor (Zanetti et al., 2012). In addition, SBR allow changes in the operational and control strategies, so they are suitable for the treatment of variable organic loads (Monsalvo et al., 2009, 2012).

In this work, the combination of Fenton and biological oxidation in SBR is proposed for the removal of the chlorophenoxy herbicides 2,4-D and MCPA. Optimization of the hydrogen peroxide dose is analyzed through toxicity and biodegradability assays of the resulting Fenton effluents.

2. Materials and methods

2.1. Fenton oxidation

The experiments were carried out in batch mode in a 3 L glass reactor at controlled temperature (30 °C) and stirred at 200 rpm. The initial pH was adjusted around 3, which is within the optimal range for Fenton oxidation (Zazo et al., 2007; Bautista et al., 2008). The starting concentration of 2,4-D and MCPA was fixed at 180 mg L⁻¹, since these herbicides can appear at concentrations up to their solubility limits in point-pollution sources, such as effluents from agricultural industries and pesticide containers cleaning. The dose of hydrogen peroxide was varied between the theoretical stoichiometric amount for complete oxidation up to CO₂, H₂O and HCl to a 20% of that value, maintaining a H₂O₂/Fe²⁺ ratio of 10/1 (M/M). The experiments were made by duplicate.

The evolution of the concentration of herbicide, TOC and H₂O₂ upon reaction time was followed at every H₂O₂ dose tested. Final samples were also analyzed for COD, BOD₅ and reaction byproducts, as well as ecotoxicity (Microtox®). Respirometric tests for

biodegradability assessment were also performed. The samples were previously neutralized with NaOH 6 N and filtered (Albet FV-C).

2.2. SBR experiments

The biological treatment of the effluents from Fenton oxidation was performed in a 3 L SBR. It was equipped with pH and dissolved oxygen probes to evaluate the biomass activity by respirometry. Air was introduced through a ceramic diffuser at a flow rate of 9 L min⁻¹ to avoid oxygen limitations. Peristaltic pumps were used to feed and discharge the bioreactor, as well as for the addition of sodium hydroxide solution (12 N) for pH control.

The experiments were conducted at 30 °C, 200 rpm and pH 7 in sequences of 8 h as follows: anoxic filling (1 h), aerated reaction (5.5 h), settling (1 h) and draw (0.5 h), using a hydraulic retention time of 12 h. Samples were withdrawn along the biological process for measuring TOC, COD, organic byproducts and inorganic nitrogen species. SOUR profiles were also recorded during the aerobic stages.

Biomass concentration was maintained around 1 g VSS L⁻¹ and a cell retention time of 30 d was used. The organic load was different for each herbicide, since the only carbon source was that provided by the corresponding effluents from Fenton oxidation. Ammonium sulfate and phosphoric acid were used as nitrogen and phosphorous sources, respectively, and mineral salts (CaCl₂, KCl y MgSO₄) were also added as micronutrients supply at COD:N:P:micronutrients ratio of 100:5:1:0.05 (w/w).

2.3. Inoculum source

The inoculum used in the bioreactor was collected from an activated sludge sewage treatment plant. The biological sludge was maintained with sodium acetate (150 mg

COD L⁻¹) and glucose (150 mg COD L⁻¹) as carbon sources in a SBR operated at 25 °C for its use as inoculum in the BOD₅ and biodegradability tests.

2.4. Biodegradability and ecotoxicity tests

The biodegradability of the effluents from Fenton oxidation was assessed by respirometry, in a LSS respirometer (Chica et al., 2007), following the fast biodegradability procedure established by Polo et al. (2011). The sample (1 L) was mixed with biomass (350 mg VSS L⁻¹) and aeration was maintained for 24 h, continuously measuring SOUR and TOC. For the sake of reproducibility, the respirometric tests were carried out by duplicate. The reaction vessels, placed in a thermostatic bath and continuously stirred by magnetic bars, were stoppered so that oxygen transfer from the gas to the liquid can be neglected.

BOD₅ measurements were carried out in a Velp Scientifica equipment, following the standard procedure 5210 (APHA, 1992). A sample volume of 400 mL was used, with a biomass concentration of 75 mg VSS L⁻¹. N-allylthiourea was added as nitrification inhibitor at 1.25 mg L⁻¹. The pH of the medium was maintained around a value of 7.2 by using phosphate buffer, and CaCl₂, KCl and MgSO₄ were added to the solution as micronutrients. Tests were carried out by triplicate.

The toxicity of the effluents from Fenton experiments was evaluated by the Microtox® Acute Toxicity Test (ISO 11348-3, 1998), using a Microtox® M-500 analyzer from SDI. The toxic effect is calculated from the decay of the luminescence of the bacteria *Vibrio fischeri*, after 15 minutes of incubation at 15 °C. Toxicity is evaluated as IC₅₀, defined as the percent dilution of the initial solution causing 50% reduction of the luminescence. Results were expressed in toxicity units (TU), calculated from IC₅₀ values (TU = 100/IC₅₀).

2.5. Analytical methods

Total organic carbon was measured using a TOC-Vcsh apparatus from Shimadzu. COD and biomass concentration were analyzed following the APHA procedures 5220A and 2540E (APHA, 1992), respectively. Residual hydrogen peroxide concentration was determined by colorimetric titration through the titanium sulfate method (Eisenberg, 1943), using a Shimadzu UV-1603 spectrophotometer.

Herbicides and their aromatic byproducts were quantified by HPLC (Varian Prostar 325) with a UV detector and a Teknokroma Mediterranea Sea-18 column (25 cm length, 4.6 mm i.d.) as the stationary phase. Analyses of 2,4-D and MCPA were carried out at 280 nm using a mixture of acetonitrile/H₂O (80/20% – 65/35% (0 – 15 min) and 65/35% – 25/75% (15 – 30 min)) as the mobile phase, with a constant flow of 0.60 ml min⁻¹. Chlorophenols, chlorocatechol and their non-chlorinated byproducts formed upon Fenton oxidation were analyzed at 280 nm using a 50/50% (v/v) mixture of acetonitrile/H₂O as the mobile phase.

Short-chain organic acids and chlorine were analyzed by means of an ion chromatograph with chemical suppression (Metrohm 790 IC) using a conductivity detector. A Metrosep A supp 5-250 column (25 cm length, 4 mm i.d.) was used as the stationary phase and 0.7 mL min⁻¹ of an aqueous solution of 3.2 mM Na₂CO₃ and 1 mM NaHCO₃ as the mobile phase.

The dark brown solid formed upon Fenton oxidation of 2,4-D was characterized by elemental analysis (Perkin-Elmer analyzer, model 210 CHN) and inductively coupled plasma mass spectroscopy (ICP/MS) by means of a Perkin-Elmer analyzer, model Elan 6000 Sciex.

2.6. Data analysis

The results reported were the average values from at least duplicate runs. In all the cases, the standard errors were lower than 10%.

3. Results and discussion

3.1. Fenton experiments

Figure 1 shows the time-evolution of H_2O_2 , TOC and conversion of 2,4-D and MCPA upon Fenton oxidation at 30 °C using the stoichiometric dose of hydrogen peroxide and a $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ ratio of 10/1 (M/M). Both target compounds were quite rapidly converted being the disappearance rate faster in the case of MCPA in spite of the fact that the H_2O_2 decomposition rate constant in the 2,4-D experiment was almost double than in the MCPA one (0.22 min^{-1} vs. 0.12 min^{-1}). Mineralization, namely TOC reduction, proceeded faster in the case of 2,4-D, indicating that the hydroxyl and hydroperoxy radicals generated upon H_2O_2 decomposition are being consumed in great part in the oxidation of the byproducts from 2,4-D breakdown since the earlier stages of reaction. On the opposite, the evolution of TOC upon MCPA oxidation shows a lag time indicating that the radicals attack affects mostly to the starting MCPA molecules rather than to the resulting byproducts which must be more resistant to oxidation. Once MCPA is almost completely converted, further oxidation of the reaction byproducts proceeds rapidly. In both cases complete conversion of the starting target compound was achieved in less than 45 min, but mineralization did not reach more than about 63% upon the 3 h reaction in spite of the fact that H_2O_2 was completely decomposed.

Complete conversion of the starting herbicide and also total decomposition of H_2O_2 was achieved even at the lowest H_2O_2 dose tested. Beyond 40% of the stoichiometric H_2O_2 dose further reduction of COD and TOC was slower and H_2O_2 decomposition becomes

increasingly inefficient due to the formation of refractory byproducts (see Figure S1 in the Supplementary data for COD and TOC conversion at different H_2O_2 doses).

3.2. Oxidation byproducts

The effluents from Fenton oxidation of 2,4-D and MCPA at different H_2O_2 doses were analyzed in order to identify the main oxidation byproducts. To learn more, an additional experiment at 10% of the stoichiometric H_2O_2 was performed.

For 2,4-D, chlorophenols such as 2,4-dichlorophenol, 2- and 4-chlorophenol and 4-chlorocatechol, as well as phenol and catechol were detected at the lowest dose (10% of the stoichiometric). 2,4-dichlorophenol was the main aromatic byproduct and its concentration never exceeded 6 mg L^{-1} when working at 20% of the stoichiometric H_2O_2 . This byproduct was also identified by other authors (Drzewicz et al., 2004; Li et al., 2004) from 2,4-D oxidation. Relating to MCPA, 4-chlorophenol, 4-chlorocatechol, phenol and catechol were also observed for the lowest H_2O_2 tested. At higher H_2O_2 doses only short-chain organic acids were identified as oxidation byproducts. The results are shown in Figure 2. Acetic and oxalic acids were the main oxidation byproducts of both herbicides at low and high H_2O_2 doses, respectively. The increase of oxalic acid concentration under more oxidizing conditions lowered the efficiency of H_2O_2 consumption since it is known to be refractory to Fenton oxidation at near-ambient temperature (Zazo et al., 2005).

From the analytical results, the reaction pathways of Figure 3 are proposed for 2,4-D and MCPA Fenton oxidation. According to Brillas et al. (2004), the removal of 2,4-D by means of electro-Fenton and photoelectro-Fenton oxidation generated 2,4-dichlorophenol and glycolic acid. The dehydrogenation of this acid, followed by hydroxilation of the resulting glyoxylic acid originated oxalic acid. The removal of 2,4-

dichlorophenol by means of both oxidation processes originated 2-chlorohydroquinone and 2-chlorobenzoquinone (Badellino et al., 2007). 4-chloro-o-cresol was found to be the first intermediate in the oxidation pathway of MCPA by means of photoelectro-Fenton (Brillas et al., 2003). Further oxidation of this compound would lead to the generation of methylhydroquinone and methyl-p-benzoquinone, which would be oxidized to fumaric acid and, finally, to oxalic acid.

A dark brown solid was observed in a concentration of 21.1 and 13.5 mg L⁻¹ in the effluents from 2,4-D oxidation at 20 and 40% of the stoichiometric H₂O₂, respectively. The elemental analysis of this solid gave 43% C, 22% Cl and 32% O with low percentage of H and traces of Fe. This type of solid has been also observed by Munoz et al. (2011) upon Fenton oxidation of 2,4-dichlorophenol under substoichiometric conditions and was assessed to condensation byproducts typically consisting of a complex mixture containing carboxylic and phenolate groups which are able to form complexes with different metallic ions, such as Fe²⁺ and Fe³⁺. The simultaneous presence of H₂O₂ and Fe²⁺/Fe³⁺ together with the existence of chlorinated organic compounds may lead to the formation of dioxins upon Fenton oxidation (Vallejo et al., 2013).

Measured and calculated TOC (from the identified compounds, not including the residual solid, since it was separated from the solution by filtration previous to the analysis) and chlorine balance (including the residual solid and 2,4-dichlorophenol in the 2,4-D effluents) from Fenton oxidation of both herbicides are shown in Figure 4. The TOC in identified species was around 60% of the measured TOC at the less and reached 90 and 80% with the stoichiometric dose of H₂O₂ for 2,4-D and MCPA, respectively. In both cases, differences between the measured and the calculated TOC increased under substoichiometric conditions, confirming the increasing presence of

unidentified byproducts as the H_2O_2 decreased. The chlorine balance was completely matched at H_2O_2 doses above 60% of the stoichiometric where all the chlorine in the starting herbicide was analyzed as chloride ion in the effluent. Thus, the non-identified byproducts formed beyond 60% of the stoichiometric H_2O_2 are chlorine-free.

3.3. Ecotoxicity and biodegradability of Fenton effluents

Figure 5 shows the ecotoxicity (expressed as TU), the BOD_5/COD ratio (biodegradability index) and the composition in TOC fractions of short-chain organic acids, chlorinated compounds and non-identified byproducts of the effluents from Fenton oxidation of the two target herbicides at different H_2O_2 doses. The biodegradability of 2,4-D effluents was, in general, lower than the observed for the MCPA ones. This can be due to the higher relative amount of non-identified byproducts in the former.

As can be seen, the oxidation byproducts yielded higher Microtox® ecotoxicity values than the starting herbicide solutions. This fact had been previously observed by Lu and Chen (1997), in photocatalytic oxidation (TiO_2/UV) of 2,4-D. Fairly significant increase of ecotoxicity was observed at the lowest H_2O_2 dose tested and then a monotonical decrease occurs at increasing doses although the ecotoxicity values remained above the starting ones even after using the stoichiometric H_2O_2 . However, biodegradability significantly improved upon Fenton oxidation at increasing H_2O_2 doses being this more accused for MCPA, in spite of the aforementioned higher relative amount of non-identified byproducts. With H_2O_2 at 60% of the stoichiometric, Fenton oxidation of MCPA led to an important fraction of biodegradable products according to the BOD_5/COD ratio, as high as 60%. Then, looking to the subsequent biological treatment, the Fenton oxidation step does not need more than 60% of the stoichiometric H_2O_2 . In

the case of 2,4-D, the BOD₅/COD ratio reaches only around 30% at that H₂O₂ dose and around 40% with H₂O₂ at 80% of the stoichiometric.

The biodegradability of the effluents from Fenton oxidation was further analyzed by the fast biodegradability respirometric assay (Polo et al., 2011). The evolution of TOC and SOUR observed for the effluents resulting from Fenton oxidation at 60-100% of the stoichiometric are shown in Figure 6. SOUR profiles revealed the presence of different fractions of organic matter. The easily biodegradable TOC corresponds to short-chain organic acids, oxidized in the 5 first hours of the respirometric assay. The medium-term biodegradable fraction was removed after 24 h, and the refractory fraction left a residual TOC.

Attending to the biodegradability of the effluents from Fenton oxidation of 2,4-D, an improvement of the biodegradability was achieved when using the two most oxidant conditions. Thus, the effluent from Fenton oxidation at 80% of the stoichiometric H₂O₂ dose was selected for further biological treatment. In the case of the effluents from Fenton oxidation of MCPA, a slightly higher microbial activity and TOC conversion was observed at 60% of the stoichiometric H₂O₂ dose.

3.4. Biological treatment of Fenton effluents

The effluents resulting from Fenton oxidation of MCPA and 2,4-D at 60 and 80% of the stoichiometric H₂O₂, respectively, were subjected to biological treatment in a SBR. No additional carbon sources were used (0.21 kg COD kg⁻¹ VSS d⁻¹ in the case of the 2,4-D effluent and 0.13 kg COD kg⁻¹ VSS d⁻¹ for the MCPA one). The SBR were operated for several cycles in order to ensure steady performance. The biodegradation was evaluated through the evolution of SOUR, COD, TOC, short-chain organic acids and inorganic

nitrogen species within a cycle. The results corresponding to an intermediate cycle (7th cycle) are shown in Figure 7.

A significant decrease of COD and TOC took place during the first hour of the cycle (corresponding to the anoxic filling), as a consequence of the biodegradation of part of the main short-chain organic acids present in the effluents, especially oxalic acid. At the same time, it can be observed a remarkable reduction of nitrate concentration. Thus, the removal of organic matter in absence of oxygen can be attributed to heterotrophic denitrification, where organic compounds are used as external electron donors for nitrate reduction (Carini et al., 2003; Monsalvo et al., 2009). The alternation of aerobic and anoxic stages is an important advantage of SBR versus other biological systems (Tobajas et al., 2013) and appears particularly useful in general for effluents from Fenton oxidation with oxalic acid, taking into account the poor aerobic biodegradability of this compound (Molina et al., 2010). The removal of organic matter continued in the aerobic reaction step of the SBR cycle, corresponding with high SOUR values, especially during the first hours, where the degradation of easily biodegradable compounds, the short-chain organic acids, is occurring. Oxalic and formic acids were preferentially degraded once acetic acid was almost completely removed. In all the experiments a certain fraction of TOC remained at the end of each cycle, related to the presence of unidentified non-biodegradable oxidation byproducts and refractory soluble microbial products released by cell lysis (Monsalvo et al., 2012).

The evolution of COD and TOC along the earliest 7 cycles of the biological step showed the adaptation of the activated sludge to the effluent from Fenton oxidation of 2,4-D. Nearly 90% removal of organic matter was achieved upon the combined treatment (See Figure S2.a in the Supplementary data for the SBR cycles). The SOUR profiles along the cycles (data not shown) confirmed biomass acclimation. The COD

and TOC conversion values were higher than the obtained in the fast biodegradability test, also supporting the biomass acclimation along the successive SBR cycles. In the case of MCPA the Fenton effluent showed a more homogeneous response in the SBR along the whole experiment, and 85-90% reduction of COD and TOC was achieved upon the combined treatment (See Figure S2.b in the Supplementary data for the SBR cycles).

4. Conclusions

The combination of Fenton and biological oxidation in a SBR allowed an effective removal of the chlorophenoxy herbicides 2,4-D and MCPA at high concentrations, with an important reagent saving in the chemical treatment. The use of around 60 and 80% of the stoichiometric H_2O_2 dose for MCPA and 2,4-D, respectively, led to chlorine-free effluents mainly formed by short-chain acids, which were easily degraded in the biological step, achieving around 90% reduction of organic matter upon the combined treatment. Biodegradability and toxicity assays are a useful tool in order to select the optimal dose of oxidizing reagent in the chemical step looking at the subsequent biological treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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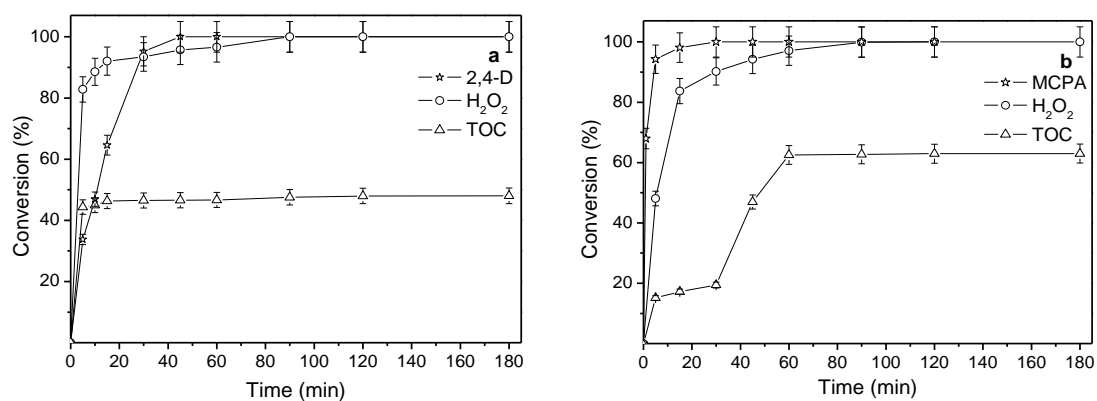


Fig. 1. Time-evolution of H_2O_2 , TOC and herbicide conversion upon Fenton oxidation of 2,4-D (a) and MCPA (b) using the stoichiometric dose of hydrogen peroxide.

Figure 2

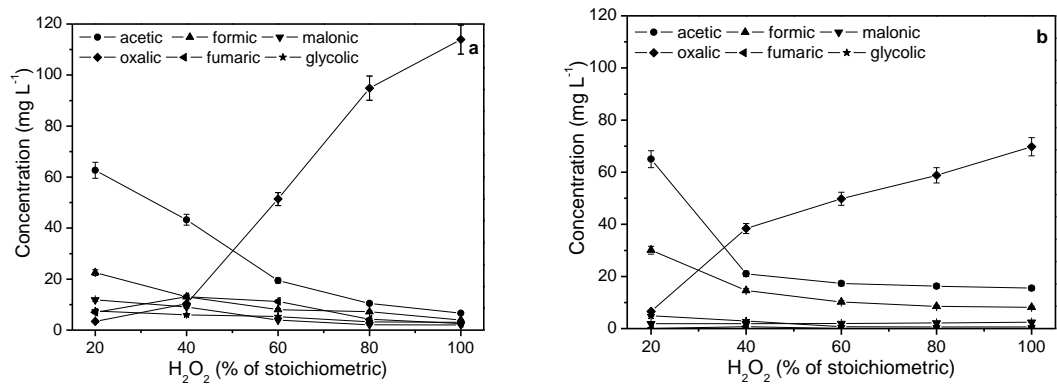


Fig. 2. Main oxidation byproducts in the effluents from Fenton treatment of 2,4-D (a) and MCPA (b) at different H₂O₂ doses after 3 h reaction time.

Figure 3

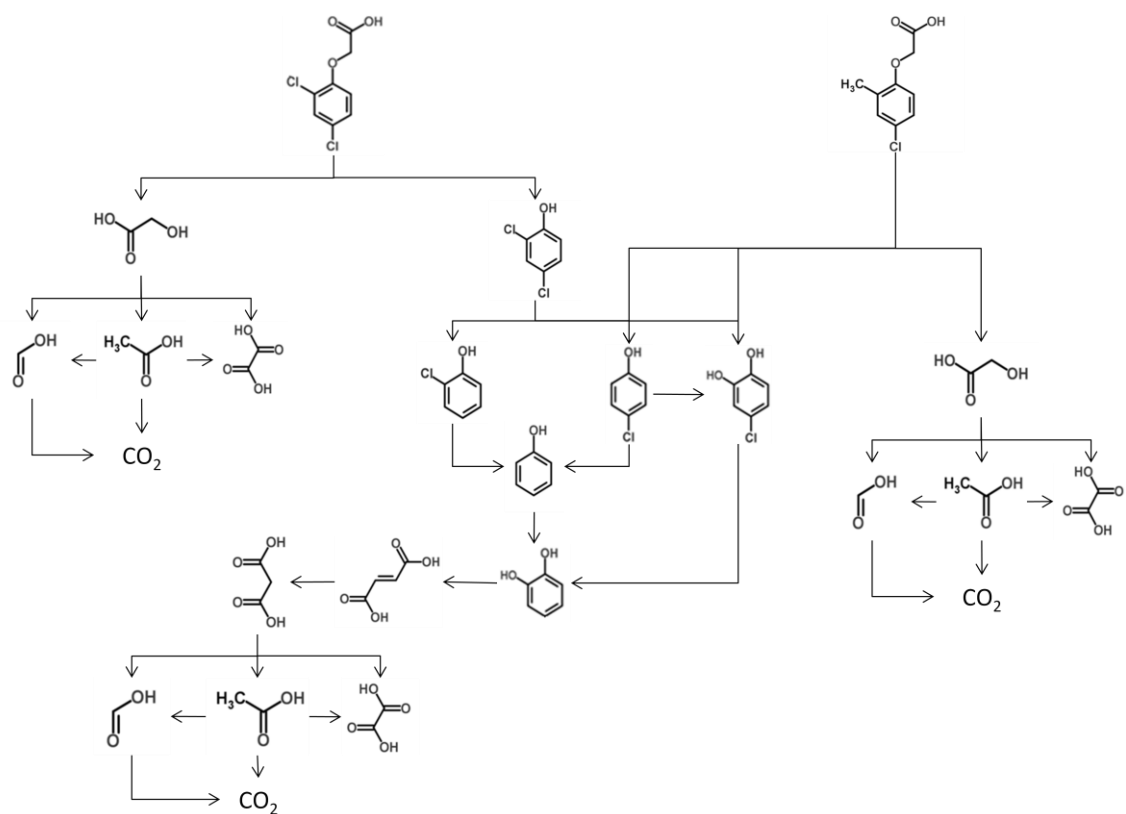


Fig. 3. Proposed pathways for 2,4-D and MCPA oxidation by Fenton’s reagent.

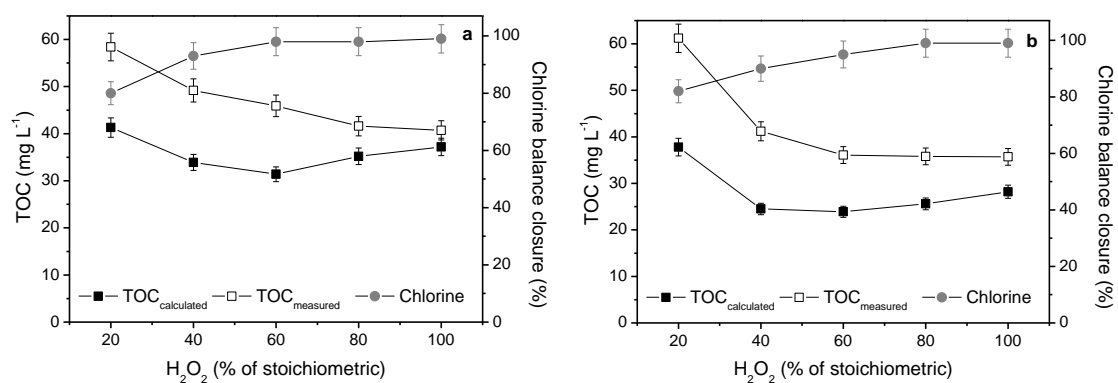


Fig. 4. Measured and calculated TOC and chlorine balance from Fenton oxidation of 2,4-D (a) and MCPA (b) at different H₂O₂ doses.

Figure 5

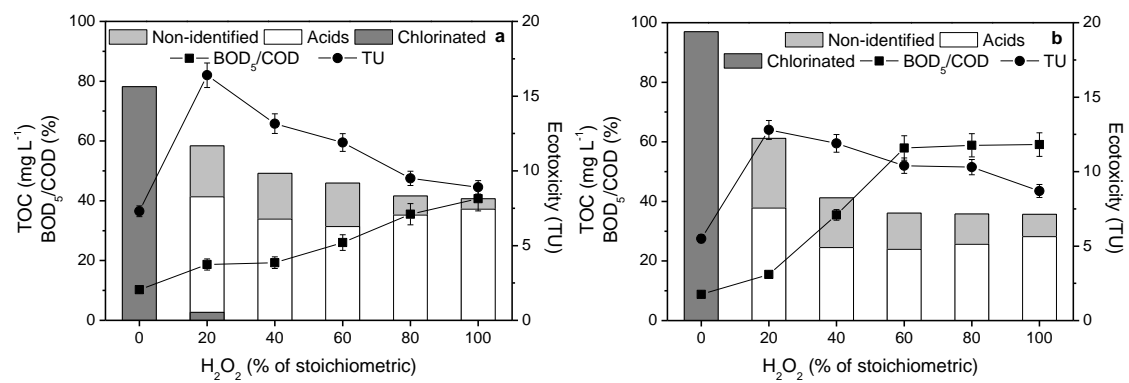


Fig. 5. Ecotoxicity, BOD₅/COD ratio and composition in organic acids, chlorinated compounds and non-identified species of the effluents from Fenton treatment of 2,4-D (a) and MCPA (b) using different initial doses of H_2O_2 .

Figure 6

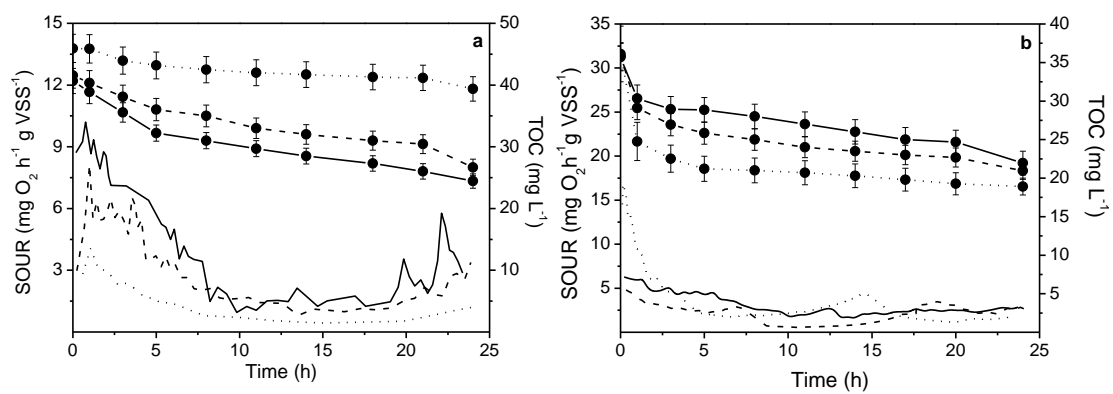


Fig. 6. SOUR (lines) and TOC (line-symbols) evolution during the respirometric assay with the effluents from Fenton oxidation of 2,4-D (a) and MCPA (b) at 100% (solid line), 80% (dash line) and 60% (dot line) of the stoichiometric H₂O₂.

Figure 7

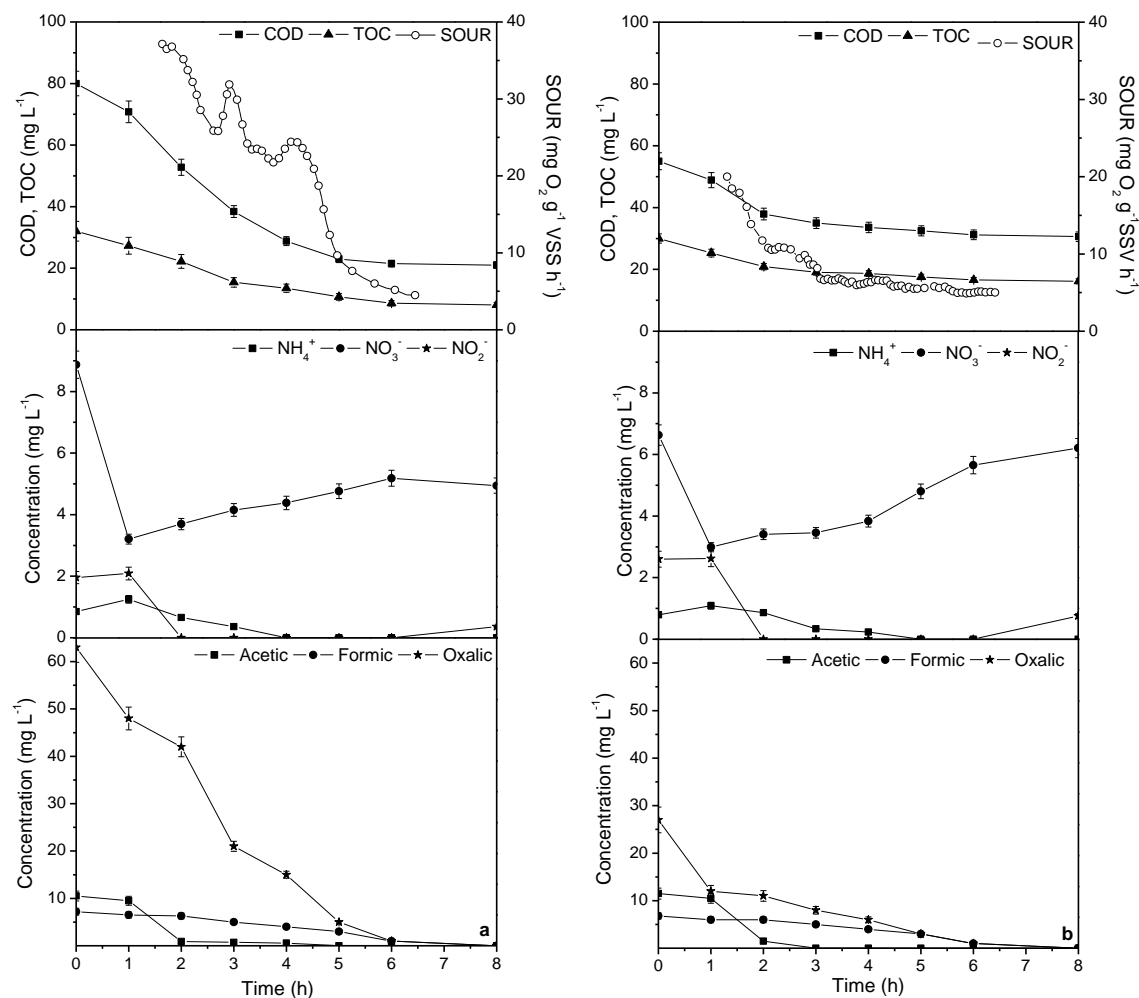


Fig. 7. Time-evolution of SOUR, COD, TOC, nitrogen inorganic species and short-chain organic acids along the 7th SBR cycle with the effluents from Fenton oxidation of 2,4-D (a) and MCPA (b).