Clopyralid degradation by AOPs enhanced with zero valent iron

M.B. Ferreira¹+, F.L. Souza², M. Muñoz-Morales²+, C. Sáez², P. Cañizares², C.A. Martínez-Huitle¹, M.A. Rodrigo²,*

¹ Institute of Chemistry, Federal University of Rio Grande do Norte, Campus Universitario 3000, 59078-970 Natal-RN, Brazil

² Department of Chemical Engineering, Faculty of Chemical Sciences and Technologies, University of Castilla-La Mancha, Campus Universitario s/n. 13071 Ciudad Real, Spain

Abstract

Four different technologies have been compared (photolysis, ZVI+ photolysis, electrolysis and ZVI + electrolysis) regarding the: (1) degradation of clopyralid, (2) extent of its mineralization, (3) formation of by-products and main reaction pathways. Results show that photolysis is the less efficient treatment and it only attains 5% removal of the pollutant, much less than ZVI, which reaches 45% removal and that electrolysis, which attains complete removal and 78% mineralization within 4h. When ZVI is used as pre-treatment of electrolysis, it was obtained the most efficient technology. The identification of transformation products was carried out for each treatment by LC-MS. In total, ten products were identified. Tentative pathways for preferential clopyralid degradation for all processes were proposed. This work draws attention of the synergisms caused by the coupling of techniques involving the treatment of chlorinated compound and sheds light on how the preferential mechanisms of each treatment evaluated occurred.

Keywords

Clopyralid degradation; ZVI; photolysis; electrolysis; coupled processes; reaction pathways
Highlights

- Oxidation capacity increases in the sequence: UV < ZVI + UV < EO < ZVI + EO.
- Higher rates of herbicides and mineralization were obtained in hybrid processes.
- High synergistic coefficients were obtained for the coupling of ZVI to UV and EO.
- Transformation products of clopyralid for each treatment have been identified.
- Degradation pathways of clopyralid for each treatment evaluated were proposed.

*author to whom all correspondence should be addressed: manuel.rodrigo@uclm.es
+both authors contribute equally
1. Introduction

In the recent years, it has emerged an increasing interest in the application of advanced oxidation processes (AOPs) to deplete chlorinated hydrocarbons that have been widespread in the environment, because of their use as pesticides, degreasing agents or solvents. Among the technologies evaluated, it is important to highlight the electrochemical advanced oxidation processes (EAOPs), which have experienced great advances with the development of new electrodes materials, different processes and cells configurations, always searching for in order to increasing the cost-effectiveness of the treatments [1-4]. However, general drawbacks of these technologies are still those related to the high costs of electrode materials and the high power consumption required.

To face these drawbacks, special design of cells looking for narrower electrode gaps and higher turbulences are looked for. Alternatively in some cases, it is proposed the addition of salts to the supporting electrolyte in order to increase the ionic conductivity, although this is not a sustainable choice because it leads to another type of pollution less hazardous but more persistent [5]. For these reasons, lots of studies have shown different alternatives to develop highly efficient electrochemical processes, based on the use of a combination of technologies that can improve the treatments in terms of economy. The coupling of electro-oxidation with concentration technologies reduces the negative impact of the mass transfer limitations on the efficiency. Among the novel technologies, it is worth to mention the electrocoagulation process (EC) that concentrates the colloid pollutants into flocs, reducing the volume of waste to be treated and making more efficient the later electrolysis [6, 7]. It is also important the novel electrochemical cells that integrate the concentration of ionic organics by electrodialysis with their electrooxidation [8, 9]. Finally regarding these novel concentration technologies, it has
also been proposed an adsorption process with granular active carbon (GAC) that allows to concentrate the pollutants in methanol, from which they can be efficiently electrolyzed [10]. This later technology has also been successfully applied to remove gaseous pollutants, such as perchloroethylene [11].

Thereby, electrochemical technologies can be integrated in other processes that promote the indirect oxidation mechanisms, including UV light irradiation [12-14] or the applications of ultrasounds [15] to stimulate the formation of large amount of oxidants and free radicals, increasing the active substances that can react with the pollutants [16-18]. Additionally, to increase the efficiency, other studies have considered the use of reductive pathways using carbonaceous cathodes to produce hydrogen peroxide [19]. These systems increase their efficiency by using pressurized systems or combining with catalysts (electro-Fenton (EF) processes) [20]. Besides, they have demonstrated to be successful even in the integral treatment of anaerobic sludge [21] and in the treatment of soil. With these novelties, it was developed a prototype for the efficient treatment of soil-washing wastes [22]. Considering other reductive pathways, many authors have studied the hydro-dechlorination, that appears as an efficient technology under mild reaction conditions (room temperature and atmospheric pressure). This non-electrochemical process does not remove the pollutant but reduces its hazardousness and toxicity [23]. It requires expensive catalysts (including ZVI) and continuous bubbling of hydrogen. Because of that, in order to obtain a cheaper method and more easily integrated with other technologies, the use of zero valent iron (ZVI) in combination with electrolysis has emerged as a promising alternative. Successful results in the dechlorination were reported for short chain chlorinated paraffins [24], lindane [25] or trichloroethene [26], with studies included the evaluation of the intermediates generated and the possible degradation pathways. Regarding electrochemical processes, in previous
works of our group about the combination of this technology with EAOPs, it was demonstrated that, from the viewpoint of electrochemical treatment, a pre-treatment with ZVI does not show important advantages regardless of the electrode material used and the size of the ZVI particles [27]. However, it was confirmed great improvements in biological treatability and toxicity of effluents after the application of these technologies [28]. In order to clarify the mechanism of the removal of chlorinated hydrocarbons, various authors have detailed reaction product formation using a photocatalytic degradation in TiO$_2$ suspensions [29] and low pressure UV/H$_2$O$_2$ treatment [30]. Recent studies report excellent results in the removal of persistent organic pollutants following the use of combined systems with the use of ZVI and electro-oxidation technology, as regards discharging standards, [31] biodegradability and toxicity [32] Furthermore, a system using a Fe foam (Fe-F) was used as catalyst in the presence of tripolyphosphate electrolyte (TPP) for electro-Fenton (EF) at neutral pH, allowed an 8.55-fold increase in the rate of phenol degradation [33]. Nevertheless, to the best of our knowledge, there are no fundamental studies of the different degradation pathways which can result from the combination of ZVI dechlorination with AOPs technologies, such as photolysis or electro-oxidation.

In this work, we compared the removal efficiency of a well-known herbicide, clopyralid (CLP), a polar organochlorinated compound effectively used to control annual and perennial broadleaf weeds, with two AOPs technologies, photolysis and diamond electrolysis, operated alone or coupled with a previous dehalogenation process with micro particles of ZVI. In this line, some of the most recent works related to the removal of CLP are summarized in Table 1 for the sake of comparison with the results that will be presented in this work.

Table 1. Different alternatives to the Treatment of Clopyralid wastes using a coupled process.
<table>
<thead>
<tr>
<th>Method</th>
<th>Contaminant Concentration</th>
<th>Experimental Conditions</th>
<th>Removal</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZVI and soil-washing electrolysis</td>
<td>30 mg L(^{-1})</td>
<td>ZVI = 48 g and 72 g; BDD anode and stainless steel cathode; T: 40º C; V: 2 L; J = 25 mA cm(^{-2}); Complete.</td>
<td>Complete.</td>
<td>[34]</td>
</tr>
<tr>
<td>Electro-Fenton</td>
<td>180 mg L(^{-1})</td>
<td>Fe(^{2+}) = 0.1; 0.5; 1.2 and 5 mM; Platinum anode and carbon felt cathode; T = 20ºC; V = 0.8 L, J = 50; 100, 200 and 300 mA; 80% of removal.</td>
<td>80% of removal.</td>
<td>[35]</td>
</tr>
<tr>
<td>Low pressure UV/H(_2)O(_2) oxidation</td>
<td>20 mg L(^{-1})</td>
<td>(\lambda = 254 \text{ nm; } [\text{H}_2\text{O}_2] = 60 \text{ mg L}^{-1}; V = 55 \text{ mL}; \text{ Room temperature.})</td>
<td>56% of removal.</td>
<td>[36]</td>
</tr>
<tr>
<td>UV/H(_2)O(_2) and ozone oxidation</td>
<td>0.078, 0.260, 0.391 and 0.521 mmol L(^{-1})</td>
<td>( [\text{H}_2\text{O}_2] = 1 \text{ g L}^{-1} \text{ and } 2 \text{ g L}^{-1} \text{ V = 200 mL; Room temperature.})</td>
<td>Complete removal with UV/TiO(_2).</td>
<td>[37]</td>
</tr>
<tr>
<td>Photoelectrochemical oxidation and Sono electrochemical oxidation</td>
<td>0.02 mg g(^{-1})</td>
<td>BDD anode and stainless steel cathode; ultrasound source of low (200 W) and high frequency (450 W); uv lamp (254 nm); T = 25 ºC; J = 12.8 mA cm(^{-2}); Complete.</td>
<td>Complete.</td>
<td>[12]</td>
</tr>
<tr>
<td>Electrochemical oxidation with GAC adsorption</td>
<td>120 mg L(^{-1})</td>
<td>BDD anode and stainless steel cathode; V = 1 L; T = 25 ± 1 ºC; J = 20-30 mA cm(^{-2}); Complete.</td>
<td>Complete.</td>
<td>[38]</td>
</tr>
<tr>
<td>ZVI, electrolysis and photolysis</td>
<td>30 mg L(^{-1})</td>
<td>[ZVI] = 45 g L(^{-1}); BDD and DSA anode; UV lamp (11 W); V = 1 L; Room temperature; J = 50 mA cm(^{-2}); Complete with BDD anode.</td>
<td>Complete with BDD anode.</td>
<td>[28]</td>
</tr>
<tr>
<td>Electrodialysis and electro-oxidation</td>
<td>100 mg dm(^{3})</td>
<td>BDD and MMO anodes; NaCl and Na(_2)SO(_4) as supporting electrolytes; J = 177.7 A m(^{-2}); V = 1 dm(^{3}); Higher removal rates for the BDD anode.</td>
<td>Higher removal rates for the BDD anode.</td>
<td>[39]</td>
</tr>
<tr>
<td>Photoelectrolysis</td>
<td>100 mg dm(^{3})</td>
<td>Novel laser-prepared mixed metal oxide (MMO RuO(_2)TiO(_2)) NaCl (3.7 g L(^{-1})) and Na(_2)SO(_4) (3 g L(^{-1})) as supporting electrolytes j=30 A m(^{-2}); V = 150 mL; Non total removal. MMO more effective in NaCl media.</td>
<td>Non total removal. MMO more effective in NaCl media.</td>
<td>[40]</td>
</tr>
</tbody>
</table>

In this case, it has also evaluated the kinetics of the combined process, mineralization decay, identification of transformation products to conclude with the discussion of tentative degradation pathways proposed for each technology studied, in order to shed light on the treatability of such wastes, which allows a more efficient design of future applications.
Chemical reagents. All chemicals were purchased of reagent grade and used as received. Clopyralid - C₆H₃Cl₂NO₂, 99 % purity, solubility > 1 g L⁻¹ at 20°C, Kow = 2.34⁻³ at pH 7 and 20°C (a.r., Sigma-Aldrich) was selected as a model of organic compound. Iron zero valent, ≥ 99%, granular, 10-40 mesh, average size is 568 μm. Methanol HPLC grade and formic acid were used as mobile phase. Sodium sulphate anhydrous used as supporting electrolyte. These chemicals were purchased from Sigma-Aldrich, Spain. Sulfuric acid (98%), sodium hydroxide pellets (97%) were obtained (a.r., Scharlab, Spain) and used to control pH. Double deionized water (Millipore Milli-Q system, resistivity: 18.2 MΩ cm at 25°C) was used to prepare all solutions.

Experimental set-up. In order to evaluate the efficiency of the combined technologies, four strategies were developed. The first one consists of applying single electrochemical treatment employing a single flow cell (SFC) using boron-doped diamond electrodes with 78 cm² of area as anode and cathode (WaterDiam, France). A 1.5 L glass reactor was filled with 1 L of water containing 30 mg L⁻¹ of clopyralid and 7.04 mM of Na₂SO₄. The electro-oxidation was carried out galvanostatically by applying a current density of 50 mA cm⁻² as it was explained elsewhere [27]. The second strategy consists of a photochemical treatment using the same set up described early coupled to UV lamp of 11W and wavelength of 254 nm. UV lamp is a cylinder tube of 15 cm of length and 10 mm of diameter and it was 80% covered by water in a vertical position. UV light was irradiated directly to the tank without applying any electrical current. The combined strategies consist of the pre-treatment of the waste for 72 h with 45 g L⁻¹ of ZVI (stirring rate 300 rpm) followed by application of photolysis or electrolysis process as described above. Optimum experimental conditions for ZVI dehalogenation were determined in preliminary experiments as described elsewhere [34]. Before the application of AOPs processes, clopyralid solutions were filtered with 0.45 μm Nylon Supelco filters to
avoid the possible iron suspended particles generated in the dehalogenation process. All experiments were conducted for 240 minutes and samples were taken at defined times along the experiments. Temperature of the system was kept constant (25°C) by means of a thermostatic bath (JP Selecta, Digiterm 100) and a heat exchanger. The monitored parameters were pH and conductivity of the solution, total organic carbon (TOC), clopyralid concentration and intermediates generated.

**Analytical techniques**

Selected samples were collected and filtered with 0.22 µm Nylon Supelco filters before analysis. The quantification of the clopyralid and some dehalogenated intermediates were carried out by HPLC (Agilent 1200 series) using a ZORBAX Eclipse Plus5 C18 analytical column. The mobile phase consisted of 30% methanol / 70% water with 0.1% of formic acid (flow rate: 0.8 mL min⁻¹). The DAD detection wavelength was 280 nm, the retention time around 2.6 min, the temperature was maintained 25°C and the injection volume was 20 µL. The TOC concentration was monitored using a Multi N/C 3100 Analytic Jena Analyser. The evolution of the pH and conductivity were determined by a pH meter Crison pH25+ and a conductometer Crison CM35+ (Crison Instruments, Spain), respectively. Both instruments were calibrated regularly with standard solutions and all the samples were measured in duplicate. The reactions intermediates of clopyralid were identified by Liquid Chromatography-Mass Selective Detector (LC-MSD) using a Zorbax SB-C18 (150mm×4.6 mm, 3.5 μm particle size) column (Agilent Technologies CA, USA). Detection was carried out with a UV–Vis diode array detector (DAD) at 230 and 280 nm (Agilent, 1260 infinity model) equipped with a flow cell coupled in series to an Agilent 6110 series MS detector (Waldbronn, Germany) equipped with an atmospheric pressure ionization source electrospray (API-ES). Selected samples were analyzed using the ESI interface in positive ionization (PI) mode. For the analysis in PI
mode a gradient elution was performed by a binary gradient, composed of solvent A (water/0.1% HCOOH) and solvent B (acetonitrile/0.1% HCOOH) according to the following program: initial conditions 80% A, kept constant for 1 min, decreased to 50% in 4 min, decreased to 20% in 6 min, kept constant for 8 min, returned to the initial conditions after 2 min. Re-equilibration time was set at 2 min, while the total run analysis lasted 10 min. Column temperature was set at 25°C, injection volume and flow rate were 1000 μL and 1 mL min⁻¹, respectively. To optimize the MS detection of each analyte, the drying gas flow was investigated within the 1.0–13.0 L min⁻¹. The temperature of the drying gas was also studied in a range from 50 to 350°C. The capillary voltage ranged from 2000 to 6000 V. Additionally, the nebulizer pressure, ranged from 5.0 to 60 psi, and the fragmentor from 50 to 200.

3. Results and discussion

Fig. 1 shows the evolution of clopyralid and TOC as a function of time in the four treatment technologies evaluated in this work. Regarding herbicides decay (Fig. 1a), the photolytic process exhibited very low removal attaining only 10%. Although the pesticide absorbs UV light, the efficiency of this kind of treatment depends on many factors like UV fluence, irradiation time, molecule structure, etc. In the case of clopyralid structure, as discussed by Wilzbach and Rausch, 1970 [41], even though there is a presence of a heteroatom and an aromatic system in its structure, the photochemical dissociation mechanism of the pyridine ring is through of the n/p* excitation, leading to a bicyclic valence isomer, Dewar pyridine, which re-aromatizes completely to pyridine in a few minutes at room temperature. This important phenomena was also discussed by Semitsoglou-Tsiapou et al, that showed that clopyralid (55 mL containing 20 mg L⁻¹) was hardly susceptible to low-pressure
photolysis, achieving only 1.2% of removal, although it follows the same mechanism \cite{36}. Similarly, the treatment with ZVI particles for 72 h led to a slight decrease in the clopyralid removal (around 5%). To understand this value, it is important to consider that the pesticide concentration in this work is higher than that used in others works in the literature. Thus, Correia et al. \textit{2013} \cite{42}, studied the 2,4-D degradation by ZVI and showed around 50% degradation was achieved in the presence 2% w/v of ZVI after 150 min. However, when photolysis was applied after the ZVI process, the degradation of pesticide was accelerated, increasing the rate of removal from 5% to 58%. This behavior can be explained in terms of the presence of oxygen (mixing kept for 72 h) during the ZVI treatment, which allows to transform the ZVI reducing conditions into oxidative reactions. Thus, oxygen reacts with ZVI generating $O_2^-$, which can react further through two routes. The reduction of $O_2$ through a $4e^-$ pathway with production of $H_2O_2$ as an intermediate and a $2e^-$ pathway, with formation of $H_2O_2$ as product. From this point of view, the efficiency of oxidative degradation of clopyralid depends on the extent to which the branching process favors hydroxyl radical formation by reduction of $H_2O_2$ through $2e^-$pathway\cite{42}. Thus, the photo-assistance in the UV promotes the Fenton reaction, yielding $\cdot OH$. Furthermore, studies previous \cite{43,44} suggest that the photo radiation in the presence of ZVI can promote ZVI/$O_2$ reaction to produce $HO\cdot$ as shown following Eqs. (1) and (2), enhancing the process efficiency

\begin{align*}
Fe^0 + O_2 + 2H^+ & \rightarrow Fe^{II} + H_2O_2 \quad (1) \\
Fe^{II} + H_2O_2 & \rightarrow Fe^{III} + OH^- + HO\cdot \quad (2)
\end{align*}

On the other hand, results demonstrate that the single EO process is a suitable technology for the clopyralid removal, being able to oxidize completely the pesticide
after 180 min of treatment (current charge of 1.6 Ah dm$^{-3}$). Considering the cell voltage, this means that the energy consumed in the process was only 11.46 kWh m$^{-3}$. These results are expected considering the formation of hydroxyl radical on the surface of the anode, which will be discussed later. In addition, other oxidants are expected to be formed with this technology and as reported in the literature, when sulfate radicals are present in the medium, they can be transformed into persulfate [45-47]. These electrogenerated oxidant species may produce mediated oxidation in the bulk solution, complementing the mechanisms of oxidation and contributing to an increase in global oxidation efficiency. In order to expedite the electro-oxidation, previous treatment with ZVI particles was added to this technique. The addition led to slight improvement of the process performance, decreasing the time to attain the complete herbicide removal.

Fig. 1b shows the performance of the treatment techniques regarding to the mineralization of the solution. As can be seen, the single ZVI treatment does not provide significant mineralization (only 5%). Photolysis, as well, yields only 10% mineralization after 180 min. Despite ZVI + photolysis improved the degradation of CLP, the mineralization only reached 22%. This behavior can be attributed to the dechlorination of the CLP, which makes the structure more vulnerable to photolysis. On the other hand, despite EO and ZVI + EO were able to attain complete CLP degradation, around 20% of initial TOC still remained in the solution. This means that organic content corresponds to the degradation by-products of CLP. However, the ZVI + EO process was found to give a remarkable improvement in the mineralization, increasing the efficiency from 4.3 to 4.9 mg TOC (Ah)$^{-1}$.
Figure 1. Relative removal of Clopyralid and b) Relative TOC decay as a function of time during the (■) photolysis; (●) ZVI + photolysis, (▲) EO and (▼) ZVI + EO.

Concentration and TOC decay were fitted to a pseudo first-order kinetics, and values are reported in the Fig. 2a. As expected, values obtained for ZVI process were very low (in the range of $10^{-7}$ min$^{-1}$) for both CLP and TOC removal. Photolytic process also presents low reaction rate ($10^{-4}$ min$^{-1}$), albeit higher as compared to ZVI process. When light is applied, a significant improvement is observed ($10^{-4}$ to $10^{-2}$ min$^{-1}$), explained in terms of an increase in hydroxyl radical production stemming from the photolysis of H$_2$O$_2$, which accelerates the oxidation rate of all organic compounds present in the medium and contributes to the optimization of the process performance. Besides, ZVI + EO process shows a higher increasing of the amount of hydroxyl radicals and other oxidants formed on the BDD surface and in the bulk (such as persulfates, ozone and peroxide species). On the other hand, analyzing the mentioned combined process ZVI+
it is possible to see that k values for CLP oxidation and mineralization did not increase with the pre-treatment with ZVI. Nevertheless, a significant improvement is observed in the mineralization when electrooxidation using diamond electrodes is assessed in comparison with photolytic processes. This behavior suggests a constant electrolytic production of *OH radicals from reaction (3), which acts mainly in the oxidation of byproducts in the bulk.

\[
\text{H}_2\text{O} \rightarrow \cdot \text{OH} + \text{H}^+ + \text{e}^- \quad (3)
\]

In order to evaluate quantitatively the synergistic effect when two or more processes are combined, the *synergistic* index was calculated from kinetics values according to eq. 4.

\[
\text{Synergistic index } (S) = \frac{k_{ZVI+\text{PHOTO}}}{k_{ZVI} + k_{\text{PHOTO}}} \quad (4)
\]

Figure 2b shows the synergistic index of the combined system obtained for 2,4-D CLP and TOC removal. The first combined process (ZVI + photolysis) is called S1 while the combination ZVI+ EO is called S2. Synergy index values >1 indicate that there is a synergism effect when process is combined. Considering the clopyralid removal, the values obtained from Eq. (4) yields a synergistic index of 5.1 for S1 and 10.1 for S2, indicating a strong synergistic effect for 2,4-D CLP removal and both hybrid process. However, S values equal to 0.93 and 1.2 were obtained for S1 and S2 for TOC decay indicating an almost nil synergistic effect for mineralization. These results indicate that ZVI is acting in the transformation of CLP via reductive dechlorination reactions. In this way their concentration decreases significantly. However, after successive elimination of Cl atoms, the ZVI is not able to degrade the more recalcitrant molecule formed to CO\(_2\) and water and neither the EO process is able to get a total removal. Then, results indicate that the application of combined process ZVI + EO is not an excellent mechanism for the mineralization of organic compound once the target
molecule fails to undergo mineralization, which agrees with our previous results published elsewhere [27].

![Graph](image)

**Figure 2.** a) Kinetic constants obtained after fitting the clopyralid and TOC decay results to a first-order kinetic reaction model. b) Synergistic effect calculated for (■) compound decay and (●) TOC.

In Fig. 3, it can be seen the evolution of pH and conductivity during the treatments evaluated. These parameters give an information about the species generated during the treatments. As seen, after 72 h of the ZVI pre-treatment, there is a slight decrease in the solution pH. However, this parameter remained unchanged during the coupled processes (e.g ZVI + photolysis and ZVI+EO). In addition, it was not observed the formation of iron hydroxides and just low concentration of iron (II) ions and chloride were obtained according with eq (5) because of the low hydroxyl ions released during
the whole pre-treatment proposed. However, further research should be necessary to know more about these iron products.

\[ \text{Fe}^0 + 2\text{H}_2\text{O} \rightarrow \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \]  

(5)

Then, this indicates that reduction reactions can promote the generation of mildly acid species. During the photolytic process, the pH remained stable. This behavior was expected since considerable changes were observed in the treatment process (as observed in the Fig.1ab). The remarkable change in the pH was observed after the electro-oxidation process, for which there is an increase of pH from 5.2 to 9.5. In terms of conductivity, their changes were negligible over the whole reaction period in the photolysis and ZVI + photolysis processes. However, the values significantly increased during the electro-oxidation processes. This can be explained not only by the changes in the pH but also in terms of the larger generation of oxidant species, initially produced through a low concentration of \( \text{H}_2\text{O}_2 \) generated prior to the efficient radicals promoted by EO processes as it was suggested by Minella et al.[48] at slightly acid pH.
Figure 3. a) pH and b) Conductivity evolution as a function of time during the (■) photolysis, (●) ZVI + photolysis, (▲) EO and (▼) ZVI + EO.

Fig. 4 shows the HPLC chromatograms of the processed samples in the four evaluated processes. It can be observed the higher peak is related to Clopyralid at retention time 2.8 min. Besides, a very short number of byproducts appear in chromatograms. This may be a reflection of the low concentration of the byproducts generated. It is possible to see two important reaction products in retention times at 1.0 and 1.4 min. Both peaks appear during the photolytic process, being the most relevant at 1.4 min. However, in the ZVI + photolysis (fig.4b) this peak is lower than in photolysis and quickly disappears. Fig. c shows that during the EO technology, CLP was destroyed within few minutes of reaction. The byproduct at 1.4 min was not found and the peak at 1.0 min is negligible. In the presence of ZVI particles as pre-treatment of the EO process, it can be
observed (Fig.4d) that the compound at 1.0 min is continually removed, decreasing their concentration with the time.

Figure 4. Chromatograms obtained from HPLC analysis for each sample taking during a) photolysis process; b) ZVI + photolysis; c) EO process and d) ZVI + EO. (Eluent: water with 0.1% formic acid and acetonitrile 30: 70 v/v; flow rate: 1 cm³ min⁻¹, Vol. inj.: 20 μL; Column: Zorbax Eclipse Plus 5 C18, 30 cm; λ = 280 nm; Temp. 25 ºC)

These two important by-products were identified based on the literature and throughout of internal standard method. Peaks were quantified with external standard calibration based on areas from the standard reagents. The by-product at 1.0 min was identified as pyridine- 2-carboxylic acid and the product at 1.4 min as 6-chloro-3-hydroxypyridine-2-carboxylic acid. As it can be seen in the Fig.5ab, both concentrations are very low. One
important point to be considered is the fact that the both products mainly appears after using the ZVI as pre-treatment. The pyridine-2-carboxilic acid concentration (Fig.5a) decreases with time when UV irradiation is coupled to ZVI process. Besides, it is completely oxidized within 50 minutes of electro-oxidation (ZVI+EO). Regarding to 6-chloro-3-hydroxypyridine-2-carboxylic acid (Fig.5b), its concentration remains unchanged over time when ZVI + photolysis process was accomplished. This behavior means that this product is more refractory, and it does not undergo photolytic cleavage easily. However, it is completely degraded with ZVI+EO process.

**Figure 5.** Intermediates evolution as a function of time during the (■) photolysis; (●) ZVI + photolysis, (▲) EO and (▼) ZVI + EO. a) pyridine-2-carboxylic acid and b) 6-chloropyridine-2-carboxylic acid. Formation of these dehalogenated species is known to be carried out by reductive chemical processes catalyzed by the iron particles (heterogeneous catalytic reaction) as
shown in eqs. 6 and 7 or by cathodic hydro-dehalogenation reactions, which should lead to the formation of the same intermediates but without production of iron (II) ions (eqs. 8 and 9) [34]

\[
\begin{align*}
\text{Fe}^0 + \text{C}_6\text{H}_5\text{Cl}_2\text{NO}_2 + \text{H}^+ &\rightarrow \text{Fe}^{2+} + \text{C}_6\text{H}_4\text{ClNO}_2 + \text{Cl}^- \\
\text{Fe}^0 + \text{C}_6\text{H}_4\text{ClNO}_2 + \text{H}^+ &\rightarrow \text{Fe}^{2+} + \text{C}_6\text{H}_5\text{NO}_2 + \text{Cl}^- \\
\text{C}_6\text{H}_5\text{Cl}_2\text{NO}_2 + 2\text{e}^- + \text{H}^+ &\rightarrow \text{C}_6\text{H}_4\text{ClNO}_2 + \text{Cl}^- \\
\text{C}_6\text{H}_4\text{ClNO}_2 + 2\text{e}^- + \text{H}^+ &\rightarrow \text{C}_6\text{H}_5\text{NO}_2 + \text{Cl}^-
\end{align*}
\]

The LC-MS analyses carried out at a negative ion mode was used for the identification of the degradation products. Firstly, the conditions of the mass detector were optimized. The best results obtained when the drying gas was operated at 12 L min\(^{-1}\) flow at 340°C. The nebulizing pressure at 60 psi, capillary voltage at 5000 V and the fragmentation voltage was set at 70 V (for PI ionization). Then, samples collected at regular time intervals were injected into the MS analysis in mode scan at different ranges, being set at two channels 1 and 2. The products identified, as well their structure, retention time and sampling time of the process and % relative abundance for each technology are showed in Table 2. As it can be observed, the analyzed samples during the four investigated treatments indicated the formation of several intermediates. During the photolytic process, four reaction products were identified. This means that the UV irradiation was able to break chemicals bonds: the photonic energy absorbed exceeded the bond energy as radiation at 254 nm which even has more energy than the necessary to break the C–Cl bond (dissociation energy is 330 kJ mol\(^{-1}\)) and which may promote further reactivity. Those compounds were 2,5-dichloropyridine (m/z = 148), 2’,3,5’,6-tetrachloro[2,3’-bipyridine]-6’-carboxylic acid (m/z = 337), 3,6-dichloro-
pyridine-2-ol (m/z = 163) and 6-chloropyridine-2-carboxylic acid (m/z = 157). These intermediates were also found during the ZVI + photolysis process. However, two more products were identified: pyridine-2-carboxylic acid (m/z = 122) and 3-chloropyridine (m/z = 113). Considering that these molecules have lower molecular weight than those obtained during the photolytic treatment, this means that the oxidations steps achieved superior levels, confirming the improvement obtained for the ZVI + photolysis process in the oxidation of the solution.

The largest number of intermediates was found for the ZVI+ EO process. As it can be observed, two hydroxylated species were observed during the EO and ZVI+ EO processes. These byproducts are 3,6-dichloro-4,5-dihydroxypyridine-2-carboxylic acid, m/z = 224 at retention time tr= 11.0 min and 3,6-dichloro-5-hydroxypyridine-2-carboxylic acid, m/z = 207 at tr 9.4 min (set at channel#2). At this point, it is worthy of note that during the electro-oxidation processes using BDD anodes are expected to be produced an enormous amount highly active and non-selective oxidizing species, the *OH radicals (HRs) [49, 50]. Also, it is known that one of the major mechanistic steps of *OH radicals (HRs) treatment is the hydroxylation of organic compounds, especially of unsaturated bonds, through hydrogen substitution or hydroxyl addition [51-53]. However, as discussed above, not only *OH radicals (HRs), but others powerful oxidants species also acts in the oxidation process. Then, smaller molecules are formed.

In the case of EO process, the products were slightly different than those obtained when ZVI was applied as pre-treatment. As can be seen in the Table 2, the main products identified during the EO process were 6-chloro-3-hydroxypyridine-2-carboxylic acid (m/z = 174) and 6-chloropyridin-3-ol (m/z = 128). Conversely, for ZVI + EO process, four more products were found, 3,6-dichloro-pyridine-2-ol (m/z = 164), 6-chloropyridine-2-carboxylic acid (m/z = 157), pyridine-2-carboxylic acid (m/z = 122)
and 3-chloropyridine (m/z = 113). These two last products appeared to be more hydrophilic than the other products, because its retention time in the C18 column was lower than all other compounds, being \( t_R = 3.6 \text{ min} \) and \( t_R = 2.8 \text{ min} \), respectively.

Table 2. Analytes identified, structure, retention time and sampling time of electrolysis and % relative abundance for each technology.
Fig. 6 shows a plausible mechanism proposed for CLP degradation based on the peaks observed by LC-MS. The main pathway for each process is marked by dotted coloured lines. The primary pathway of clopyralid degradation by UV irradiation (marked by blue points-via 1), takes place by transfer of one electron to generated radical anion.
followed by decarboxylation (-COOH) and attack of the •OH radical leading to the formation of 3,6-dichloropyridin-2-ol (m/z=164). Another important reaction route is the generation of the dimer 2,3,3,6-tetrachloro(2,3-bipyridine)-6-carboxylic acid. This formation is caused because the clopyralid loses an electron generating a radical cationic species, followed by the loss of a H⁺ to give a radical species, which reacts with the radical 2,5-dichloropyridine to form the dimer. The dimers generation is related in the literature as result of the fact that the radiation at 254 nm has more energy than needed to break the C–Cl bond, as it has previously commented. This rupture leads to the formation of a chlorine radical and an unpaired electron on the carbon of the organic molecule [54]. Then, the combinations lead to form more stable molecules (e.g dimers). Similar proposals were also obtained by some other authors applying photocatalysis in the CLP treatments [29, 55].

A similar trend in reactivity with ZVI coupled to photolysis was observed as those obtained with only photolysis. However, in this case, more products were identified with higher abundance during the first minutes of reactions. In this way, the main transformation of CLP is proposed to have occurred via sequential reductive dechlorination reactions, forming the monochlorinated product (m/z = 157) that ultimately generated the m/z = 122, which did not contain any Cl atom. In addition, the 6-chloropyridine-2-carboxylic acid is attacked by •OH radical followed by its decarboxylation (-COOH). Once it is formed, it can be reduced to lead to 3-chloropyridine.

During the EO process (pathway 3-red line), the primary reaction route, as commented above, is the hydroxylation of the CLP on unsaturated bond of the pyridine ring, through attack of hydroxyl radicals generated on BDD surface. The sequential hydroxylation forms the products of m/z = 207 and with less abundance the m/z = 224.
Then, the 3,6-dichloro-5-hydroxypyridine-2-carboxylic acid (m/z = 207) suffers
decarboxylation to form the m/z = 164. Another reaction route that can occur is the
attack of hydroxyl radical on CLP, which there is the substitution of chlorine specie by
hydroxyl radical to form the molecule m/z = 174. From this point, oxidation reaction
occurs by attack of hydroxyl radical and other oxidants such as persulfates to generate
the decarboxylated product m/z = 128). The oxidative ring-opening reactions follow,
possibly to form short-chain carboxylic acids and inorganic ions.

The pathway marked by green dotted points is related to ZVI as pre-treatment of the
electro-oxidation. As seen, the main products observed at high abundance are those
which there is a substitution of Cl species by ·OH, forming the product 6-chloro-3-
hydroxypyridine-2-carboxylic acid, followed by decarboxylation via attack of powerful
oxidants species. Besides, the products obtained via 4 (EO-red lines) were also
identified, meaning that both routes are probable to occur. However, it is worthy to note
that albeit both pathways can occur (because products of both pathways were
identified), the transformation based on hydroxylation of CLP (via 4) is less important
than that of via reaction 3. Finally, these intermediates are degraded to CO₂, H₂O, NH₄⁺
as it can be postulated on the basis TOC measurement where around 80% of
mineralization was attained after about 240 min of electrooxidation. Non-mineralized
organics can be explained in terms of the formation of iron-carboxylic acid complexes,
which are known to be very refractory. However, these potential intermediates were not
identified with the analytical techniques used in this work.
**Figure 6:** Tentative pathways for preferential clopyralid degradation by photolysis (blue), ZVI + photolysis (Orange), electrooxidation (red) and ZVI + EO (green).

**Conclusions**

From this work, the following conclusions can be drawn:

- Single photolysis is not very efficient, and it only attains 5% of removal of the pollutant. An improvement up to 45% in the removal is obtained when ZVI was employed as pre-treatment, albeit almost 80% of organic carbon still remain in the solution after the treatment. Electro-oxidation is an excellent technology to treat clopyralid attaining 100% of removal and 78% of mineralization within 4
h of reaction. ZVI as pre-treatment of EO was found to be the most efficient technique evaluated for leading **more than 80% of total mineralization, slightly improving the results of the single process.**

- Higher rates of mineralization were obtained in this order: ZVI + EO > EO > ZVI > photolysis, increasing from a minimum of $10^{-4} \text{ min}^{-1}$ in single photolysis to $10^{-2} \text{ min}^{-1}$ in the coupled ZVI + EO process. The synergistic index ($S$) obtained for the coupled techniques were 5.1 for ZVI+ photolysis and 10.1 for ZVI+EO that indicated a strong synergistic effect for CLP removal. However, the synergy coefficient for the mineralization were very closed to unity for both coupled processes indicating no synergism.

- The identification of transformation products was carried out for each treatment. In total, ten transformation products were identified. Tentative pathways for preferential clopyralid degradation for all processes were proposed shedding light on how occurs the preferential mechanism of each treatment evaluated.

**Acknowledgements**

Financial support from the Spanish Agencia Estatal de Investigación through project CTM2016-76197-R (AEI/FEDER, UE) and grant FPU16/00067 is gratefully acknowledged. This work contains also first results of the Project PID2019-107271RB-I00, continuation of the CTM2016-76197-R. Coordenação de Aperfeiçoamento de Pessoal de nível Superior (CAPES-Brazil) through process 88881.171154/2018-01 for the scholarship awarded to Fernanda L. Souza are gratefully acknowledged.

**References**


A. Correia de Velosa, R. Nogueira, 2,4-Dichlorophenoxyacetic acid (2,4-D) degradation promoted by nanoparticulate zerovalent iron (nZVI) in aerobic suspensions, Journal of environmental management, 121C (2013) 72-79.


L. Krumina, A. Tunlid, P. Persson, Generation of hydroxyl radicals from reactions between a dimethoxyhydroquinone and iron oxide nanoparticles, Scientific Reports, 8 (2018) 10834.


