Effect of temperature on Imidacloprid oxidation by homogeneous photo-Fenton processes

C. Zaror, C. Segura, H. Mansilla, M. A. Mondaca and P. González

ABSTRACT
This paper presents experimental results on the effect of temperature on the rate of Imidacloprid removal from waste water using homogeneous photo-Fenton processes. Experiments were conducted in a 2 L photo reactor set at 15–42 °C, initial concentrations in the range of 10 to 40 mg L⁻¹ Fe(II) and 100–450 mg L⁻¹ H₂O₂; 30–150 min processing times. Initial H₂O₂ concentration determined the extent of the oxidation process, whereas iron concentration played a key role in the process kinetics. Homogeneous photo-Fenton showed a fast initial reaction leading to 50% Imidacloprid degradation after less than 1 min of treatment, followed by a slower process until full removal was achieved. Rapid Fe(II) oxidation to Fe(III) seems responsible for the initial Imidacloprid removal. Imidacloprid removal fitted well a pseudo-first order kinetic scheme, with apparent activation energy of approximately 31.6 kJ/mole. Untreated Imidacloprid samples showed significant acute toxicity to Daphnia magna and genotoxic effects on Bacillus subtilis. Acute toxicity and genotoxicity remained detectable even after complete pesticide removal, showing that toxic by-products were present. The design and operation of photo Fenton processes should focus on toxicity removal rather than on specific target pollutants.

Key words | B. subtilis rec, Daphnia magna, Imidacloprid, kinetics, photo-Fenton

INTRODUCTION
Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine) is a relatively stable chloronicotinic insecticide, classified as Category I due to its high leaching potential. It could easily contaminate water sources, leading to negative environmental and health effects. Indeed, acute exposure to Imidacloprid may cause apathy, spasms and thyroid lesions (Mizell & Sconyers 1992; Scam et al. 1998; Smith & Krischik 1999). Although no chronic toxicity tests have been made available to the public, Imidacloprid is known to affect mammalian reproduction (Luo et al. 1999). This is of major concern because the absence of proof by no means indicates the absence of harm. Moreover, little attention has been paid to the removal of potential toxic effects due to this insecticide from contaminated effluents and drinking water sources. Because of its shape, Imidacloprid fits into the receptors meant to receive acetylcholine, which carries nerve impulses from one nerve cell to another. By blocking these acetylcholine receptors an excess of acetylcholine accumulates causing paralysis and eventual death.

Natural water bodies in Central Chile are heavily contaminated with Imidacloprid, reaching concentrations in the order of 100 mg/L, and appropriate treatment processes ought to be implemented. In this context, advanced oxidation processes, such as those based on photo-Fenton (Fe(II)/H₂O₂/UV) reactions, have already been identified as a potential alternative to remove pesticides from contaminated wastewater, since conventional biological treatment systems cannot efficiently degrade such highly toxic pollutants (Pignatello 1992; Chiron et al. 2000; doi: 10.2166/wst.2008.661}
Acero et al. 2002; Malato et al. 2002; Paterlini & Nogueira 2005). The best option seems to be the use of chemical oxidation before biological treatment, preferably applied to highly toxic and low volume segregated effluents (González et al. 2003). However, advanced oxidation processes may lead to the formation of toxic intermediate compounds (González et al. 2003), and no published information exists on the possible generation of toxic by-products as a result of Imidacloprid oxidation by photo-Fenton processes. Moreover, ambient temperature in Central Chile varies over a wide range, both daily and seasonally, and its effect on photo-Fenton oxidation kinetics is not well understood. Indeed, few papers on temperature effects have been published, particularly, on hydroxyl radical formation (Lee & Yoon 2004), and oxidation of phenolic compounds (Lopez et al. 2005; Santos et al. 2007). In this paper, experimental results are presented on the effect of temperature on Imidacloprid oxidation, and toxicity removal by homogeneous photo-Fenton treatment.

METHODS

Materials
Imidacloprid was extracted from commercial Confidor® SC (Bayer) using Soxhlet extraction with methylene chloride (Merck) and dried under vacuum at 40°C. Analytical grade Imidacloprid, purchased from Riedien-de Haën, was used for HPLC analysis. Heptahydrated iron sulphate 99.5%, hydrogen peroxide 30%, and sulphuric acid (97–98%) were purchased from Merck. Hydrogen peroxide consumption was monitored using Merckoquant® peroxide analytical test strip (range 0.5–100 mg L⁻¹ H₂O₂) from Merck. Ultrapure water (18 μS cm⁻¹), acetonitrile HPLC grade, ortho-phosphoric and di-sodium hydrogen phosphatedodecahydrate (analytical grade) were used in HPLC determinations. All solutions were prepared using bi-distilled water. All HPLC chemicals were provided by Merck.

Experimental scheme
A 2 L jacketed Pyrex glass jacketed photo reactor featuring magnetic stirring, and three encapsulated 6W Philips black light fluorescent lamps (λmax 365 nm) arranged in parallel to the reactor axis, were used in this study. The incident light intensity in the reactor, as determined by potassium ferrioxalate, was 7 × 10⁻⁶ Einstein s⁻¹ (Muroy 1973). Temperature was kept at 25 ± 1°C by water circulation through the reactor jacket. Initial Imidacloprid concentration was set at 100 mg L⁻¹ in all cases, and initial pH was adjusted to 2.8–3 using 9 N sulphuric acid. Such Imidacloprid concentration levels represent maximum concentrations found in contaminated effluents. Typically, FeSO₄·7H₂O was added to the reactor to achieve the required initial Fe(II) concentration. Then, lamps were switched on, and a set amount of H₂O₂ was added. Samples were collected at different intervals, placed into test tubes containing 30 μL sodium disulphite 40% w/w to stop the reaction, and then stored at 5°C for further analysis.

Analytical methods
Imidacloprid insecticide was assayed using reverse phase HPLC-UV (Merck Hitachi, L-7100 pump, and UV L-7400 detector), equipped with a RP-18e column (Purosfer® Star RP-18e 5 μm, 4.6 × 150 mm, Merck). The mobile phase was composed of ultrapure water buffered at pH 3, containing 75/25 v/v acetonitrile, and run at 1 mL min⁻¹, 20°C, 20 μL injection volume. Detection was set at 270 nm. Chemical oxygen demand (COD) was determined by closed reflux colourimetric methods, previous removal of residual hydrogen peroxide by sodium hydrogen sulphite. In order to prevent hydrogen sulphite presence during COD determinations, an excess of 2 mg L⁻¹ H₂O₂ was allowed. A Shimadzu UV-1603 photometer was used to determine absorbance at 585 nm. Fe(II) concentration was measured by colourimetric determination with 1,10-phenanthroline. Total organic carbon (TOC) was determined by direct injection in a Shimadzu TOC-VCPN analyser.

Toxicity analysis
Acute toxicity was assessed by bioassay using Daphnia magna, according to the procedure described by standard methods (USEPA 1991; NCh 1999). Cultures were conducted under natural light conditions, including 16 h and 8 h under darkness, at 20°C ± 2°C; dissolved oxygen was
kept over 80% saturation level. Samples were diluted with distilled water, at different dilutions, and cultures were conducted under batch conditions throughout the test. The mean lethal concentration 50% (LC50) was determined after 24 and 48 h culture.

Preliminary assessment of genotoxicity was carried out using the Bacillus subtilis method described by Mazza (1982). This analysis has been widely used as a quantitative measure of the extent of DNA damage due to the presence of a contaminant. Two isogenic Bacillus subtilis strains were used here. One strain, the 1652 rec(+), presents the ability to self-repair any DNA damage and, therefore, the presence of genotoxic pollutants should have no affect on growth. On the other hand, the 1791 rec(−), strain is incapable of repairing any DNA damage and, growth should be significantly affected by genotoxic compounds. Strains were cultured for 24 h at 37°C, on agar plates containing samples at various dilutions. DNA damage is expressed as plaque efficiency, i.e. the ratio between the viable bacterial counts in the presence (N) and absence (No) of the contaminant. The relative affinity (A) provides a measure of the extent of the contaminant’s genotoxic potential, and is estimated as the ratio between the 1791 rec(−) and the 1652 rec(+) strains plaque efficiencies. Samples featuring a relative affinity below 0.9 could be considered genotoxic.

### Experimental design

A central composite factorial experimental design was used here. The effect of Fe(II) and H2O2 initial concentrations on Imidacloprid oxidation was assessed following the multi-variate surface-response analysis described elsewhere (Torrades et al. 2003; Paterlini & Nogueira 2005). Such a technique is based on a central composite circumscribed design, consisting of a factorial design and star points. Variables were coded on two levels and normalised as unit values: +1 as the highest and −1 as the lowest value of a variable. Central points, coded as 0, were obtained from such extreme values and assayed in triplicate for statistical consistency. Star points were distributed at 1.414 distance from the central point. The influence of Fe(II) and H2O2 initial concentrations in the range of 15 to 35 mg L\(^{-1}\), and 150 to 400 mg L\(^{-1}\), respectively, was assessed. Measured responses were the time required to remove 80% Imidacloprid (\(T_{80}\)), total organic carbon removal yield (Y), and the time required to achieve total hydrogen peroxide consumption (\(T_F\)). Imidacloprid initial concentration (100 mg L\(^{-1}\)), temperature (25°C) and initial pH (2.8) were kept as constant parameters. A total of 11 runs were conducted, and real and codified variables are presented in Table 1, together with experimental results. Data analysis, determination of empirical models and response surfaces, and optimisation were carried out using Modde 7.0 \(^{\text{TM}}\) commercial software. Statistical validation was determined by ANOVA test at 95% confidence level. The model was used to select optimal initial reagents concentrations used in this study.

### RESULTS AND DISCUSSIONS

#### Effect of reagents concentrations

Experimental design results are summarised in Table 1. The treatment time to remove 80% of initial Imidacloprid, depicted as \(T_{80}\), ranged from 5 to 28 min, with greater \(T_{80}\) obtained at lower Fe(II) initial concentrations. Hydrogen peroxide initial concentrations seem to present less influence on \(T_{80}\). Since \(T_{80}\) was directly related to the rate of

<table>
<thead>
<tr>
<th>Run</th>
<th>([\text{H}_2\text{O}_2]) (x_1) (mg L(^{-1}))</th>
<th>([\text{Fe(II)}]) (x_2) (mg L(^{-1}))</th>
<th>(T_{80}) (min)</th>
<th>Y (%)</th>
<th>(T_F) (min)</th>
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<td>3</td>
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<td>8</td>
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<td>4</td>
<td>400 (+1)</td>
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<td>11</td>
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<td>102</td>
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<td>5</td>
<td>100 (−1.4)</td>
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\(T_{80}\) = Reaction time to eliminate 80% initial imidacloprid (min) = 12.1 + 1.2x_1 - 7.3x_2 + 2.2x_1^2 + 2.0x_2^2.

\(T_F\) = Reaction time to total \(\text{H}_2\text{O}_2\) consumption (min) = 60.0 + 33.9x_1 + 17.6x_2 - 19.1x_1^2 + 6.8x_2^2 - 1.5x_1x_2.

Y = Final TOC removal (%) = 66.1 + 22.8x_1 - 5.0x_2 - 11.7x_1^2 - 4.4x_2^2 + 3.5x_1x_2.
Imidacloprid oxidation, such results would show that initial Fe(II) concentration had a much greater effect on the rate of free radical generation, than the initial peroxide concentration.

The time required to achieve total consumption of hydrogen peroxide (\(T_F\)) ranged from 38 to 145 min, under conditions tested here. \(T_F\) increased with the initial peroxide concentration, and tend to decrease as the Fe(II) concentration increased. The extent of TOC reduction was less sensitive to Fe(II) concentration within the range tested here. TOC reduction is a direct measure of the extent of mineralisation, which is highly dependent on the availability of oxidising agents, in this case, \(\text{H}_2\text{O}_2\).

The time to remove 80% Imidacloprid decreased as the catalyst (Fe(II), \(x_2\)) concentration increased. On the other hand, the initial peroxide concentration (\(x_1\)) seems to have little effect on this response, particularly at low iron concentrations. As the iron concentration increased so did the sensitivity of the response to increases in \(\text{H}_2\text{O}_2\).

The extent of mineralisation was greatly affected by the initial \(\text{H}_2\text{O}_2\) concentration (\(x_1\)). Within the range of initial hydrogen peroxide concentrations used here (100–450 mg L\(^{-1}\)), the Fe(II) concentration (\(x_2\)) shows little effect on TOC reduction. The time (\(T_F\)) required for complete \(\text{H}_2\text{O}_2\) consumption increased at higher \(\text{H}_2\text{O}_2\) doses; moreover, \(T_F\) decreased as the initial Fe(II) concentration increased, at a given peroxide concentration.

Extreme values of \(\text{H}_2\text{O}_2\) and/or Fe(II) concentrations seem to reduce the efficiency of organic pollutant removal (Torrades et al. 2003; Pera-Titus et al. 2004). Indeed, both \(\text{H}_2\text{O}_2\) and Fe(II) could act as HO’ radical scavengers. Such behaviour was not experimentally observed within the range of conditions used here.

According to model predictions, a minimum \(T_{80}\) around 5.5 min was obtained at 43 mg L\(^{-1}\) Fe(II) and 234 mg L\(^{-1}\) \(\text{H}_2\text{O}_2\) initial concentrations. Moreover, maximum TOC reduction, around 77%, was obtained at 23 mg L\(^{-1}\) Fe(II) and 393 mg L\(^{-1}\) \(\text{H}_2\text{O}_2\) initial concentrations. Finally, minimum overall process time 35 min could be obtained at 37 mg L\(^{-1}\) Fe(II) and 170 mg L\(^{-1}\) \(\text{H}_2\text{O}_2\) initial concentrations. It could be concluded that high oxidant doses led to a greater extent of mineralisation, but higher catalyst concentrations were required to increase the oxidation rate and reduce reaction times.

### Imidacloprid degradation kinetics

The process kinetics were assessed from experiments using 100 mg L\(^{-1}\) Imidacloprid, 35 mg L\(^{-1}\) Fe(II) and 350 mg L\(^{-1}\) \(\text{H}_2\text{O}_2\) initial concentrations. These conditions were selected on the basis of findings discussed above, taking into consideration the requirement for short treatment time, low reagent consumption, and high pollutant removal rate. Preliminary runs using UV light in the absence of reagents in combination with \(\text{H}_2\text{O}_2\) showed that the contribution of UV photolysis, either in the absence or presence of \(\text{H}_2\text{O}_2\), on Imidacloprid degradation was negligible under conditions used here. It must be mentioned that the experimental system used in this study features a negligible radiation fraction below 350 nm. It must be mentioned that \(\text{H}_2\text{O}_2\) presents an absorption peak at 254 nm.

**Figure 1** illustrates Imidacloprid removal as a function of time and temperature. As temperature increases, the reaction time to fully remove Imidacloprid decreases. At 42°C, Imidacloprid is completely removed after 16 min reaction time, as compared with 45 min at 15°C. In all cases, two distinctive kinetic regimes could be observed. Indeed, there was a very fast, almost instantaneous, initial phase where nearly 50% of initial Imidacloprid was degraded, followed by a slower phase featuring a monotonic oxidation until full degradation was achieved at 25 min. The second phase of Imidacloprid removal fitted well a pseudo-first
order kinetic scheme \((R^2 > 0.98)\), with an apparent activation energy of approximately 31.6 kJ/mole (plot not shown here).

As seen in Figure 2, Fe(II) underwent very fast oxidation to Fe(III), coinciding with extensive Imidacloprid initial degradation. Within the first few seconds of starting the experiments, Fe(II) decreased to around 23% of its initial value, and remained around that level for the rest of the experiment. Massive free radicals generation at that stage could account for such initial Imidacloprid oxidation. This effect has not been reported in the literature to the extent seen in this work. The relatively large Fe(II) concentrations used here may have allowed detection of such behaviour.

Figure 3 shows the extent of the oxidation process during homogeneous photo-Fenton, in terms of total organic carbon (TOC) and chemical oxygen demand (COD) reductions. Both, TOC and COD steadily decreased, following similar trends at both temperatures. Final TOC and COD removals of around 67 and 80%, respectively, were reached when all hydrogen peroxide was consumed.

Greater reductions in TOC and COD could be achieved when higher peroxide concentrations were used. More stable low molecular weight final by-products slowed down reductions in TOC and COD during long exposure. Identified final organic by-products were mainly oxalic, formic and acetic acids, coinciding with other reported work (Malato et al. 2001).

Toxicity assessment

Acute and chronic toxicity results are summarised in Table 2, for untreated Imidacloprid, and treated by homogeneous photo-Fenton using 35 mg L\(^{-1}\) Fe(II) and 350 mg L\(^{-1}\) H\(_2\)O\(_2\) during 2, 40 and 60 min, at 15 and 42 \(^{\circ}\)C. As seen in the table, untreated Imidacloprid samples presented significant acute and chronic toxicities. Indeed, *Daphnia magna* LC\(_{50}\) within 40–60% and 10–20% v/v, after 24 and 48 culture, respectively, were recorded. Untreated Imidacloprid also presented genotoxic effect since *Bacillus subtillis* since *B. subtillis* 1791, rec \((-\) ) N/N\(_0\) was 0.57, as compared with 0.99 in the case of *B. subtillis* 1652, rec \(+(+)\), corresponding to a relative affinity of around 0.58.

After the first 2 min of photo-Fenton treatment, nearly 60% of the initial Imidacloprid was removed at both temperature levels. However, both acute and chronic toxicity were still significant at levels similar to untreated samples. After 40 min treatment, most Imidacloprid was removed accompanied by significant mineralisation of organic compounds. At 15\(^{\circ}\)C, both acute and chronic toxicity were still significant, indicating that some partially oxidised intermediates negatively affected biological activity. On the other hand, neither acute nor chronic toxicity were detected after 40 min treatment at 42\(^{\circ}\)C. Finally, at both temperature levels, samples treated for 60 min featured extensive mineralisation and low concentration of organic compounds, mostly low molecular weight molecules (e.g. oxalic, formic and acetic acids).
acids). Such samples did not show any measurable toxic affect to *Daphnia magna*. Moreover, *Bacillus subtilis* relative affinity was close to unity, i.e. both rec(+) and rec(−) strains featured similar plaque efficiencies when cultured in the presence of treated samples, indicating that no genotoxic effect could be detected in those cases. Results obtained here confirm that the removal of toxic molecules using advanced oxidation processes could lead to secondary toxic by-products, and treatment systems should be designed and operated in order to achieve complete detoxification, rather than focusing on the removal of specific target pollutants.

**CONCLUSIONS**

This study has contributed with experimental data to support the use of homogeneous photo-Fenton in the removal of residual pesticides from waste water. The initial hydrogen peroxide concentration determines the extent of the oxidation process, whereas iron concentration plays a key role in the process kinetics. Fe(II) undergoes fast oxidation to Fe(III) leading to a massive formation of free radicals and fast Imidacloprid removal. Once Fe oxidation-reduction reactions reach equilibrium, the contaminant oxidation rate slows down until full degradation is achieved. Temperature plays an important role in process kinetics and the overall rate of imidacloprid degradation presents apparent activation energy around 31.6 kJ/mole. Untreated Imidacloprid samples show significant acute toxicity to *Daphnia magna* and genotoxic effects on *Bacillus subtilis*. Acute toxicity and genotoxicity remain detectable even after significant removal of the pesticide, due to the presence of toxic by-products. Such toxic effects only disappear after considerable mineralisation resulting in final low molecular weight by-products.

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