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Treatment of Wastewaters Containing Sulfonylurea Herbicides by Electroflotation: Chemical and Ecotoxicological Efficacy

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Abstract: Electroflotation (EF) is an electroanalytical technique applied for separating substances suspended in phase and is reported to be efficient for effluents and wastewater treatments. To date, there are no studies employing EF for the treatment of wastewater containing toxic organic compounds. In the present study, EF was tested as an electro-oxidative process to decontaminate wastewater containing the sulfonylurea herbicide chlorimuron-ethyl. To evaluate the value of EF, both the chemical and ecotoxicological efficacies were assessed in non-treated and treated wastewaters; the former monitored the herbicide and degradation products in the reaction medium through analytical chromatographic methods, whereas the latter comprised testing the 72 h growth of the green microalga *Raphidocelis subcapitata* and the 48 h lethality of the cladoceran *Daphnia magna*. Analysis by HPLC-UV allowed the monitoring of the electrochemical reaction, and a degradation mechanism based on gas chromatography–mass spectrometry was proposed. Despite the compromised herbicide structure, non-treated and treated wastewaters were similarly toxic for the microalgae *Raphidocelis subcapitata* and the invertebrate *Daphnia magna*. Even though EF did not remove the wastewater toxicity, the results indicate that toxic organic compounds are potentially oxidized by EF while signaling the need to combine chemical and ecotoxicological approaches to gauge the environmental sustainability of EF.

Keywords: electroflotation; chlorimuron-ethyl; *Raphidocelis subcapitata*; *Daphnia magna*; green chemistry

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1. Introduction

Sulfonylureas are a large group of herbicides applied primarily on cereal crops that represent approximately 10% of the world herbicide market, totaling USD 3.3 billion in sales per year [1,2]. Due to their low application rates (10–50 g ha^{-1}), high herbicidal activity, and low mammalian toxicity, sulfonylureas are of considerable interest to farmers all over the world [1,3]. In general, a sulfonylurea presents the molecular structure R_1-SO_2-NH-CO-NH-R_2, in which the radical R_1 can be an aromatic, an aliphatic, or a heterocyclic chain connected to a heterocyclic triazine or pyrimidine (the radical R_2) through a sulfonylurea bridge [1,4]. Of the formulations on the market, one of the most commercialized is chlorimuron-ethyl (CAS 90982-32-4), which is widely applied for weed control in grain and cereal crops [2,5].

Pesticide overuse is a worldwide cause of high environmental concern that is paralleled by the high industrial production of herbicides, which consequently increases the volume of contaminated wastewater. In order to protect the natural environment, such wastewaters require adequate treatment before their disposal in natural watercourses [6,7]. Effluents contaminated with herbicides generally present concentrations that are both above those allowable in legislation and toxic for the efficient application of biological treatments [8]. Consequently, there is an urgent need to reduce the environmental impacts caused by these

wastewaters; in past decades, this has been a key driver of the development of effective methods for the degradation of pesticides in effluents [6,7,9,10].

Electroflotation (EF) has been reported to be an advanced effective treatment to reduce the toxic effects of effluents due to the presence of contaminants, such as metals and organic compounds, which are present, for instance, in wastewaters from agricultural and industrial activities [11–14]. Moreover, EF has proven very effective in treating oily wastewater and oil–water emulsions, mining effluents, groundwater, food processing wastewater, industrial sewage, effluents containing metals, and many other types of water and wastewater [15]. The EF methodology is flotation using hydrogen and oxygen bubbles generated for separating suspended substances from aqueous phases. Compared with other conventional dissolved air flotation methods, EF has many advantages, including high flotation efficiency, compact units, easy operation, and low maintenance costs [16–18]. An EF reactor has aluminum passive electrodes of sacrifice, which generate Al^{+3} ions due to the application of an electric potential, although this step is part of the anode process wherein the Al metal is oxidized to Al^{+3}. The cation generated in the anode step is hydrolyzed to $Al(H_2O)_6{}^{3+}$, forming the coagulating agent $Al(OH)_3$, which is responsible for the coagulation and formation of colloidal particles $Al(OH)_{3\,(s)}$, that is, for the removal of contaminants [15,16]. In the case of the herbicide chlorimuron-ethyl, the large Al clots produced (mainly $Al(H_2O)_6{}^{3+}$ and $Al(OH)_3$) are responsible for the adsorption of the particles resulting from the incomplete mineralization of the herbicide or from its oxidation into degradation products, thus allowing for the removal of these molecules from the system [15,16]. Ideally, a compromise between a satisfactory yield and the selected electroanalytical process will make the pesticide industry treat contaminated effluents so that they are free of toxic contamination loads or degradation products and can be safely discharged into water bodies [16].

Aiming to protect watercourses that are receptors of wastewater, national and international environmental agencies regulate wastewater discharges through the establishment of the maximum allowed concentrations of specific contaminants (e.g., metals and organic compounds), thus assessing their environmental risks according to physico-chemical parameters [19–21]; in the case of EF treatments, the quantification of residual Al^{+3} is particularly relevant, with limit values across the United States [21], Brazil [19], and Europe [20] lower or equal to 0.20 mg L^{-1}. However, evaluating the ecotoxicity potential of these hazardous wastes is not mandatory, even though determining the effective bioavailability of such contaminants and their damage to biological systems and the environment is only possible by conducting bioassays [22–24]. Toxicity bioassays are usually conducted under laboratory conditions by exposing selected representative organisms to a concentration range of a chemical or sample to measure, after a specific period of time, a biological response and calculate a toxicity parameter (e.g., the EC50, i.e., the effective concentration eliciting a 50% response of the measured endpoint) [23]. On the basis of the ecotoxicity results, decisions can be made regarding the appropriateness of the effluent discharge to the environment [22,23]. Regarding the herbicide chlorimuron-ethyl, there is no legislation on the maximum allowed concentration in waters or effluents. Moreover, there are few studies concerning its concentration in waterbodies and effluents (reported to be at the μg L^{-1} level; [25]) or its environmental adverse effects (14 d growth EC50 for duckweed *Lemna gibba* of 0.26 μg L^{-1}; 48 h lethal EC50 and 21 d reproduction lowest observed effect concentration for *Daphnia magna* of >10 and 211 mg L^{-1}, respectively; and 96 h lethal EC50 for *Oncorhynchus mykiss* (rainbow trout) and *Cyprinodon variegatus* (sheepshead minnow) of 16 and 120 mg L^{-1}, respectively [26]).

In this context, the aim of the present study was to assess the chemical and ecotoxicological efficacy of implementing EF as an innovative electro-oxidative process to decontaminate wastewater containing the sulfonylurea herbicide chlorimuron-ethyl. Chemical efficacy was evaluated by monitoring the presence of the herbicide and degradation products in the reaction medium through analytical chromatographic methods (high-performance liquid chromatography, HPLC, and gas chromatography, GC). The ecotoxicological effi-

cacy was assessed by conducting standard ecotoxicity tests, namely the 72 h growth of the phytoplanktonic green microalga *Raphidocelis subcapitata* and the 48 h lethality of the planktonic zooplanktonic cladoceran *D. magna*, on non-treated and treated samples of wastewater containing chlorimuron-ethyl. The latter organisms were selected to represent two key functional groups potentially sensitive to the parental and degradation products (particularly the microalgae) and because they are widely used and recommended at the regulatory level [24,27,28].

2. Materials and Methods

2.1. Electroflotation

The EF process was carried out in an electroflotation reactor (Figure 1) developed in the Laboratory of Environmental Chemistry (LQA) at the University of São Paulo (Brazil) [16]. The electroflotation reactor had a 0.50 L capacity and was equipped with six aluminum electrodes (cathodes) with dimensions of 7.00 × 4.00 × 0.25 cm (length × width × wall thickness) and a 1.0 cm distance between them. The agitation system using an aluminum bar was a special feature of this reactor. Agitation ensured the homogeneity of the solution during EF, and furthermore, the aluminum bar acted as an electrode (anode) of the electroanalytical process.

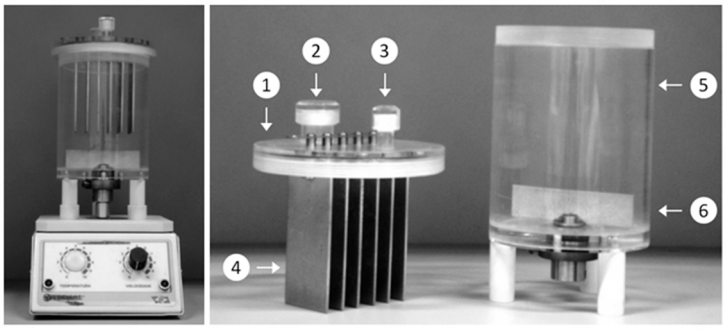

Figure 1. Electroflotation reactor used in the chemical treatment of the wastewater contaminated with the herbicide chlorimuron-ethyl under different conditions and electrochemical parameters. (1) Acrylic cover; (2) sample collection point; (3) control of air and temperature; (4) aluminum electrodes (cathode); (5) acrylic container; (6) aluminum electrode used for agitation (anode).

2.2. Experimental Design

To optimize the EF procedures to treat wastewater contaminated with chlorimuron-ethyl (using the commercial formulation Clorimuron Master Nortox, Arapongas–Brazil), the herbicide was dissolved in water, and the nominal concentration was adjusted to 1 mg L^{-1}. Thus, four different treatment tests (A1, A2, A3, and A4) were performed, varying the electrolyte concentration (NaCl in mg L^{-1}) in the solution and the direct electric current (I in A) applied to the electrodes (Table 1), as these are considered the two main parameters determining the efficiency of the EF process [16,17]. NaCl was selected as a suitable electrolyte due to its efficacy and because, at these concentrations (0.25–0.50 mg/L), no toxicity is expected for freshwater species [29]. The four combinations of I and NaCl were based on optimization procedures in previous studies [11,30,31]. The solutions A1, A2, A3, and A4 derived from the different EF treatments were submitted to chemical and ecotoxicological efficacy evaluations. In addition, a negative control (A0) containing a wastewater sample untreated by EF but contaminated with the herbicide was also chemically and ecotoxicologically characterized. The ecotoxicological efficacy was only evaluated in samples from A0- and A3-treated solutions, as they both fulfilled the legislated criteria regarding Al^{+3} residual levels ($\leq$0.20 mg L^{-1}; see also Results and Discussion section). Furthermore, preliminary tests with *D. magna* indicated high toxicity of all treated solutions contaminated with chlorimuron-ethyl (A1–A4). The A0 sample (not EF-treated) presented 100% mortality after 24 h. As for the EF-treated samples, A1 and

A2 presented 75% mortality, while A3 and A4 resulted in 37.5% mortality. On the basis of these results, a definitive battery of bioassays with the microalgae and the cladoceran was conducted with two samples, i.e., the sample with the least toxicity and the lowest aluminum content (A3) and the (negative control) sample with the maximum toxicity (A0) but without aluminum (see Results and Discussion section).

Table 1. Values of the tested combinations of the electrochemical parameters electric current (I) and electrolyte (NaCl) concentration selected to optimize the procedure of electroflotation to treat wastewater contaminated with the herbicide chlorimuron-ethyl (at a nominal concentration of 1 mg L^{-1}).

Treatment Test	Electric Current I (A)	Electrolyte Concentration (mg L^{-1} NaCl)
A1	1.50	0.25
A2	1.50	0.50
A3	1.00	0.25
A4	1.00	0.50

2.3. Chemical Efficacy

2.3.1. Residual Aluminum (Al^{+3})

To determine the amount of aluminum Al^{+3} present in the solution not treated by EF (A0) and released during EF (solutions A1–A4), atomic adsorption spectroscopy by Inductively Coupled Plasma (ICP; Perkin Elmer/Optima 3000DV, Wellesley, MA, USA) operating in the standard mode of analysis was used (equipment limit of quantitation (LOQ) of 0.01 mg L^{-1}). To perform this analysis, 250 µL of the sample was collected online during a 3 h reaction.

2.3.2. Herbicide Degradation

High-performance liquid chromatography with an ultraviolet detector (HPLC-UV) (Shimadzu model SCL-10A and UV-Vis SPD-20A, Kyoto, Japan) was used to assess the herbicide degradation on the basis of the areas under the chromatographic peaks related to chlorimuron-ethyl; the HPLC-UV was equipped with the chromatography column RP18 (5 µm × 250 mm × 4.6 mm) for the determinations. The chromatography parameters utilized were a mobile phase composition equal to 60% acetonitrile plus 40% H_2O + 1% H_3PO_4, a mobile phase flow of 1.4 mL min^{-1} (isocratic elution), a temperature of 25 °C, and a duration of 5 min. To monitor the chemical efficacy, 10 mL of each solution was collected and immediately injected at the following times: 0, 30, 60, 120, and 180 min. A chlorimuron-ethyl analytical standard (CAS 90982-32-4, Sigma-Aldrich®, Munich–Germany) was applied to the method to verify the herbicide quantification during the EF procedures.

2.3.3. Degradation Products

Gas chromatography coupled with mass spectrometry (GC–MS) (Shimadzu GC-2010 MS QP2010 Plus model, Kyoto, Japan) was used to identify degradation products generated during the EF processes. The GC–MS had a high-resolution HP5 capillary column (dimensions of 0.25 µm × 30 m × 0.25 mm). One microliter of the sample was immediately injected using CTC PAL (autosampler) into the GC injection port at 250 °C in splitless mode. The GC parameters utilized were as follows: a mobile phase of helium gas, a total mobile phase flow of 15 mL min^{-1}, a column flow of 2 mL min^{-1}, and a heating ramp-up to 270 °C with a heating rate of 28.8 °C min^{-1}.

2.4. Ecotoxicological Efficacy

2.4.1. Growth Test (72 h) with *Raphidocelis subcapitata*

The green microalga *R. subcapitata* was acquired from the Carolina Biological Supply Company (Burlington, NC, USA), cultured for at least one year prior to the present study in non-axenic cotton-stoppered 250 mL Erlenmeyer flasks, filled with 100 mL of Woods Hole MBL growth medium [32] supplemented with vitamins (0.1 mg L^{-1} B_1, 0.5 µg L^{-1} B_{12}, and 0.5 µg L^{-1} biotin), and incubated at 19 to 21 °C under continuous cool white

fluorescent illumination (lateral disposition; 8000 lx). New cultures were started from an inoculum obtained by harvesting algae while they were still in the exponential growth phase (between days 5 and 7).

The toxicity tests for samples A0 and A3 were conducted according to the guidelines in [28,33] using 24-wells plates with all edge wells filled with distilled water to minimize water evaporation during exposure. MBL growth medium diluted 2.5 times according to the N/P specifications in the guidelines was used as the standard control medium and to perform the following wastewater dilutions: 3.12, 6.25, 25, 50, and 100%; all dilutions were supplemented with nutrients in the same amounts as the control to discriminate potential toxic effects from those due to differences in nutrient levels. Six and three replicates were set up for the control and wastewater dilutions, respectively, each containing 900 µL of the test solution and 100 µL of an algal suspension ensuring an initial algal concentration of 10^4 cell mL^{-1}; the latter was prepared by centrifuging (10 min at 2040× *g*) an aliquot of an exponentially growing culture. Both tests were incubated simultaneously under the same conditions used for batch culturing, and during testing, the well contents were mixed each day by repetitive pipetting to promote gas exchange and prevent the clumping of cells. After the 72 h exposure, cell counts were conducted with well-mixed aliquots of each replicate under a microscope (at 400× magnification) using a Neubauer chamber to estimate the mean specific growth rate per day calculated from the initial and final cell densities.

2.4.2. Lethality Test (48 h) with *Daphnia magna*

The *Daphnia magna* used in both tests (samples A0 and A3) were third to fifth brood neonates (less than 24 h old) obtained from cultures maintained in reconstituted [34] hard water supplemented with vitamins (7.5 µg L^{-1} of B_1, 1 µg L^{-1} of B_{12}, and 0.75 µg L^{-1} of biotin) and Marinure extract (7.5 mL L^{-1} of a suspension with an absorbance of 620 units at 400 nm) at 19 to 21 °C under a 14:10 h light:dark photoperiod. Cultures (25 and 12 daphnids L^{-1} up to the first brood and from then onward, respectively) were renewed every other day and fed daily with *R. subcapitata* (3×10^5 cells mL^{-1}).

The lethal tests were performed following the guidelines in [27]. The same medium used for culturing, but not Marinure extract or vitamin supplement, was used as the control and for the following wastewater dilutions: 5, 10, 20, 40, and 80% (preliminary tests indicated 100% mortality for less than 1 h exposure at the 100% concentration). Four replicates were set up per treatment in 60 mL glass vials filled with 50 mL of solution and five juveniles and were incubated under the same conditions used for stock culturing. Measurements of pH (WTW 537 pH meter, Wissenschaftlich Technische Werkstätten, Weilheim, Germany), conductivity (WTW LF 92 conductivity meter), and dissolved oxygen (WTW OXI 92 oxygen meter) were taken at the start and end of each test. After the first 24 h, the dead organisms were counted and removed, and after the 48 h exposure, the dead organisms were counted to estimate the total lethal percentage per treatment; mortality was assessed as immobility for 15 s.

2.5. Data Analysis

For the chemical monitoring, each procedure was carried out in triplicate (EF, chromatographic analysis, and residual Al^{+3} quantification), and Al^{+3} content data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's test to evaluate differences between means (A1 to A4). For the cladoceran lethal tests, the lethal concentrations inducing 20 and 50% mortality (LC20 and LC50) and the respective 95% confidence limits (CLs) were calculated using the software PriProbit 1.63 (http://ars.usda.gov/Services/docs.htm?docid=11284; accessed 13 June 2022, applying probit transformation to mortality and logarithmic transformation to concentration. Comparisons of LC20/LC50 values between EF treated solutions A0 and A3 were made by Behrens–Fisher tests (which estimate the t-value as a function of the degrees of freedom; Excel Office 2010, Microsoft Corporation, Redmond, WA, USA, was used). Regarding the microalga tests, effective concentrations inducing 20 and 50% inhibition in growth (EC20 and EC50) could not be determined because the growth was inhibited by more than 50% at the lowest tested con-

centration of 3.13% for both wastewater samples. Thus, growth responses for each sample were compared via one-way ANOVA followed by Dunnett's test to identify significant differences between each tested concentration and the respective control. Furthermore, given that both algal tests were conducted simultaneously and thus had the same control treatment, one-way ANOVA was conducted to compare the growth between samples A0 and A3 within each tested concentration. Prior to the univariate analysis of variance, using the software STATISTICA 7.0 (StatSoft, Inc., Tulsa, OK, USA), data were verified for the assumptions of normality (Shapiro–Wilk's test) and homoscedasticity (Bartlett's test). Results were considered statistically significant at the 5% level ($p < 0.05$).

3. Results and Discussion

3.1. Chemical Efficacy

3.1.1. Residual Aluminum (Al^{+3})

As previously mentioned, the monitoring of Al^{+3} release is mandatory since the electrodes are passive and the Al^{+3} concentration in wastewater is regulated by worldwide environmental laws ($\leq$0.20 mg L^{-1}; [19–21]). The results of the ICP analysis to detect residual Al^{+3} in the final solutions indicated that in all four EF test treatments (solutions A1 to A4), Al^{+3} was released into the wastewater, although in statistically significantly different amounts (Duncan's test, $p < 0.05$) (Table 2). Such differences in aluminum values were probably due to the differences in the reaction conditions of the electric current (I) and electrolyte (NaCl) concentration applied in each test. The A3 test resulted in the lowest mean residual Al^{+3} content (0.19 mg L^{-1}), probably due to the softer reaction conditions (1.00 A and 0.25 mg L^{-1} NaCl); the final A3 wastewater presented 56% less Al^{+3} than the final A2 wastewater, which presented the significantly highest residual Al^{+3} content (0.43 mg L^{-1}). Although samples A1 and A3 exhibited statistically similar Al^{+3} contents, the level of Al^{+3} in A1 did not comply with the environmental legislation ($\leq$0.20 mg L^{-1}). The mean values of Al^{+3} in the control sample A0 (untreated by EF) were below the LOQ of the equipment (0.01 mg L^{-1}).

Table 2. Mean ($\pm$ standard deviation; $n = 3$) residual aluminum (Al^{+3}) content in wastewater contaminated with the herbicide chlorimuron-ethyl (at 1 mg L^{-1}) and untreated (A0) or treated by electroflotation (A1 to A4; see Table 1 for specification of treatment conditions) and comparison with important international environmental legislation in Brazil, the European Union (EU), and the United States (US).

Treatment Test	**Al^{+3} (mg L^{-1}) [a]**	
A0	<LOQ [b]	
A1	0.22 $\pm$ 0.01 [a]	
A2	0.43 $\pm$ 0.04 [c]	
A3	0.19 $\pm$ 0.01 [a]	
A4	0.32 $\pm$ 0.02 [b]	
Maximum Al^{+3} permitted in international legislation (mg L^{-1}) [c]		
Country/Region	**Al^{+3} (mg L^{-1})**	**Reference**
Brazil	0.20	[19]
EU	0.20	[20]
US	0.05–0.20	[21]

[a] Common letters beside means indicate values are not statistically different from each other according to Duncan's test ($p < 0.05$); [b] LOQ = limit of quantitation (0.01 mg L^{-1}); [c] refers to legislation for drinking water indicated for human consumption.

The comparison between the residual aluminum obtained in the four EF tests and the most important international environmental legislation in Brazil [19], the European Union [20], and the United States [21] is shown in Table 2. Test A3 was the only EF treatment in agreement with all three legislations, although the result for the A1 EF-treated solution was only 0.02 mg L^{-1} above the permitted Al^{+3} value of $\leq$0.20 mg L^{-1}. Given that the residual aluminum contents in wastewaters from EF test treatments A1, A2, and A4 were

higher than the values permitted in all three legislations, further chemical efficacy and ecotoxicological efficacy tests were performed only for the final A3 wastewater relative to the A0 wastewater.

3.1.2. Herbicide Degradation

In the present study, EF treatment occurred without the observation of the formation of structures in suspension or in colloidal form during the electric current application, indicating that the herbicide remained completely dissolved in the aqueous phase during the process. What is more, this means that the electrons transferred between electrodes were allowed to affect the herbicide structure, breaking (some of) the chemical bonds. Although direct electro-transfer was the most probable mechanism of degradation, since the electrolyte intensifies the interaction between the analyte and the free electrons, the possible concurrent occurrence of chain reactions (electro-triggered) involving some radicals formed in small concentrations (not detected in the GCMS analysis) during EF cannot be excluded.

The chromatograms obtained by HPLC-UV (Figure 2) to assess the degradation of the chlorimuron-ethyl herbicide in the EF test of A3 indicate that EF changed the reaction medium (i.e., the herbicide concentration in the solution after the EF treatment). The results obtained during EF (up to 180 min) showed a displacement of the herbicide chromatographic peak paralleled by the appearance of bands without defined structure, certainly related to the generated degradation products, over time. Initially, the peak of the herbicide chlorimuron-ethyl was detected at 1.98 min during the analysis (retention time), whereas during EF, the herbicide retention time increased by a few seconds, up to 2.19 min after 60 min of EF. After 60 min of EF, the chromatogram baseline lost stability, with the herbicide peak appearing in the middle of bands without definition. This lack of stability could be related to the quantities of products released into the solution from the herbicide molecule or the electrodes (aluminum residue). The decreasing height of the chromatographic peak after 60 min of the EF process strongly suggests that herbicide oxidation had started. Concerning the chromatographic results obtained after a retention time of 180 min, the herbicide peak did not appear or was superimposed by other bands. The analytical signal was only one band without a defined structure of a chromatographic peak. Due to these results, it was not possible to monitor or quantify the concentration of herbicide during EF. In order to study the presence of products, their structure, and the breaking of chemical bonds, it was necessary to analyze the GC–MS results (see below).

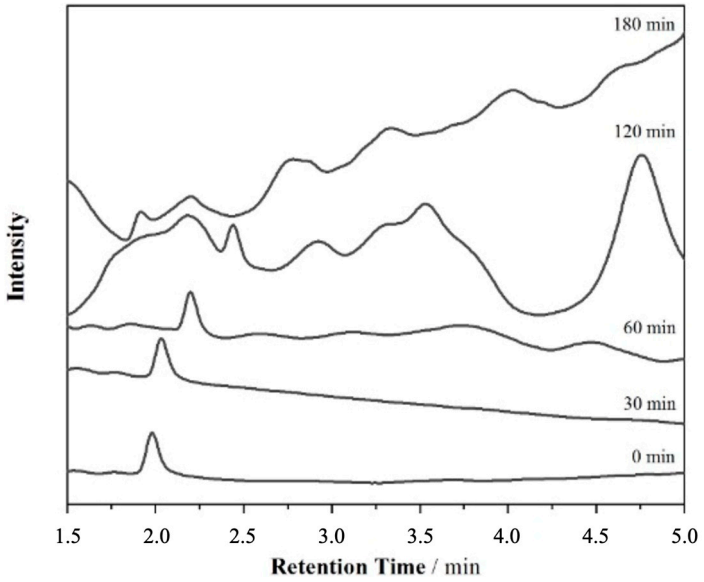

Figure 2. Chromatograms obtained by HPLC-UV indicating the degradation of chlorimuron-ethyl (initial retention time = 1.98 min) during the electroflotation test treatment of A3 at the retention times of 0, 30, 60, 120, and 180 min of reaction.

3.1.3. Degradation Products

The outcome of GC–MS indicated that the EF test treatment of A3 changed the molecular structure of chlorimuron-ethyl but was not enough to perform the complete oxidation of the herbicide. Indeed, it was not possible to detect the herbicide in the mass spectra obtained at 0 min (before the reaction), most likely due to its volatility. Between 30 and 60 min, the mass spectrum of a detected ion (m/z = 399) indicated a breakage of only one chemical bond in the herbicide structure, corresponding to the chlorimuron-ethyl molecule (MM = 414.821 g mol^{-1}) lacking one methyl group (molecular mass (MM) = 15.034 g mol^{-1}). After 2 h of reaction, the ion detected indicated an additional loss of 14.026 g mol^{-1} of MM. The sum of these lacking MMs led to a value corresponding to an ethyl group (29.039 g mol^{-1}). Finally, after 3 h, the mass spectrum indicated the breakage of a chemical bond in the middle of the molecular structure separating the urea and sulfonyl groups. Only one fragment (m/z = 281) corresponding to the urea group and a residue of the sulfonyl molecule (-SO_2^-) were detected.

On the basis of the GC–MS results, a mechanism of degradation was put forward and is presented in Figure 3. The small differences between the detected ions and the suggested molecules are related to the rearrangements that may have occurred within the molecule and its products (fragments). Although the results indicated incomplete oxidation until 3 h of analysis, the treated solution did not present the same level of ecotoxicity in comparison with the starting effluent (before treatment) (see below).

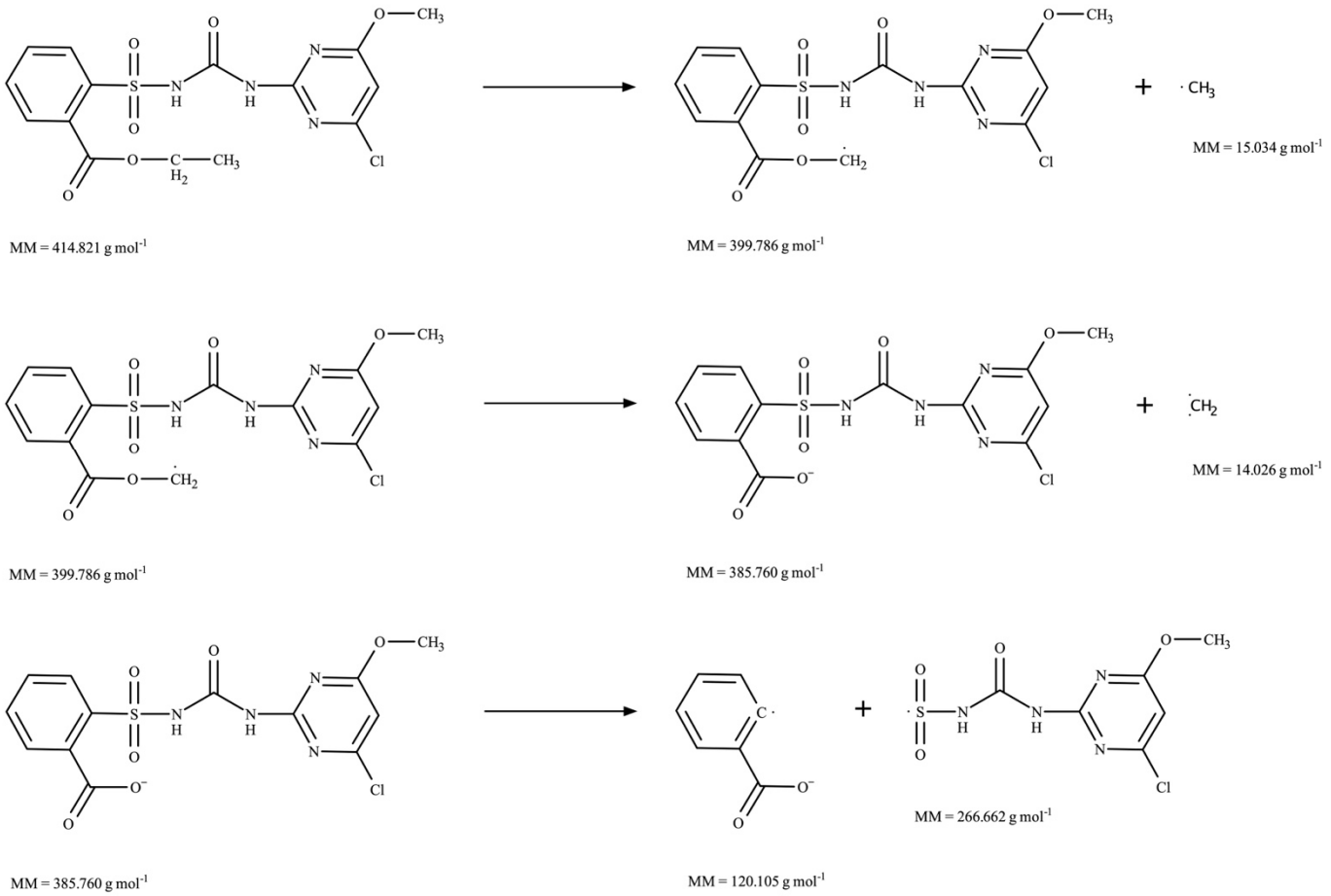

Figure 3. Mechanism of degradation proposed for the chlorimuron-ethyl herbicide to elucidate the chemical reactions during the electroflotation treatment and the degradation products formed. MM indicates molecular mass.

3.2. Ecotoxicological Efficacy

All toxicity tests fulfilled the validity criteria for control performance required in the respective guidelines (for microalgae tests [28], the cell density increase is at least 16-fold (corresponding to a specific growth rate of 0.92 day^{-1}) and the coefficient of variation of the mean specific growth rate is equal to or lower than 7%; for the cladoceran tests [27], survival is at least 90%). In addition, the overall range (minimum–maximum) of the pH (7.33–8.06), conductivity (138–568 µS cm^{-1}), and dissolved oxygen (>60% saturation) levels measured at the start and end of the *D. magna* tests indicated that these parameters were within the optimal range for this species (Table S1 in Supplementary Materials) [27].

The results for the EC/LC20 and EC/LC50 values estimated for both test organisms for samples A0 and A3 are presented in Table 3 and Tables S2 and S3 in the Supplementary Materials. Effective concentrations (EC20 and EC50) could not be estimated for the microalgae tests since the lowest tested concentration of both samples (A0 and A3, 3.13%) caused an inhibition of growth as high as 69% (Table S2 in Supplementary Materials). Indeed, in both the A0 and A3 wastewaters, growth was significantly inhibited ($p < 0.001$) at all tested concentrations relative to the control (with a specific growth rate per day of 1.67 for both samples)—by 69 to 97% for A0 (ranging from 0.52 to 0.0447 d^{-1}) and by 72 to 85% for A3 (ranging from 0.46 to 0.24 d^{-1})—in agreement with the increase in the tested concentrations (3.13 to 100%) (Tables S2 and S4 in Supplementary Materials). However, when comparing the growth between samples A0 and A3 at each tested concentration, significant differences were observed at 100% concentration, with the growth in A0 lower than that in A3 ($p < 0.01$); for all other tested concentrations, the growth in both samples was similar ($p > 0.15$) (Tables S2 and S4 in Supplementary Materials). For the cladoceran tests, no significant differences ($p > 0.05$) were found between samples A0 and A3 relative to either the LC20 or LC50 values; in effect, they differed by less than 1.2 times with a strong overlap of the 95% CL.

Table 3. Effective concentrations (in %) inducing 20 (EC20) and 50% (EC50) inhibition of growth for the toxicity tests performed with *Raphidocelis subcapitata* (72 h growth) and lethal concentrations (in %) provoking 20 (EC20) and 50% (EC50) lethality for the toxicity tests performed with *Daphnia magna* (48 h lethality), along with respective 95% confidence limits in brackets, on the non-treated (A0) and treated (A3) electroflotation wastewater contaminated with the sulfonylurea herbicide chlorimuron-ethyl.

Toxicity Test	Parameter	A0 (%)	A3 (%)
R. subcapitata	EC20	<3.13 [a]	<3.13 [b]
	EC50	<3.13 [a]	<3.13 [b]
D. magna	LC20	12.0 (7.94–15.4)	12.3 (9.80–14.8)
	LC50	20.5 (16.0–25.6)	17.1 (14.2–26.0)

[a] The concentration of 3.13% was the lowest tested and caused 69% growth inhibition; [b] 3.13% was the lowest concentration tested and caused 72% growth inhibition.

The results of the present study on the toxicity toward a microalga and a cladoceran of wastewater contaminated with the sulfonylurea herbicide chlorimuron-ethyl and then treated and not treated by EF (A0 and A3, respectively) revealed high toxicity of both samples. However, the results of the *R. subcapitata* growth test strongly suggests that there was a decrease in toxicity after EF, as wastewater A3 was slightly less toxic than A0 at a 100% concentration. The low ecotoxicological efficacy of the selected EF treatment was somewhat unexpected given that the chemical monitoring indicated that EF had the capacity to break the main chemical structure of the herbicide, although the quantification of chlorimuron-ethyl was not possible. Moreover, aluminum toxicity is not expected to have occurred since Al^{+3} levels in wastewater A0 were below the LOQ, whereas in wastewater A3, Al^{+3} levels were within ranges known not to cause toxic effects on microalgae [35] and *Daphnia* sp. [36]. It should also be emphasized that the NaCl electrolyte was added at concentrations known to be non-toxic to both the microalgae and the invertebrates; this fact was corroborated by the

conductivity values of the tested solutions, which were always below 600 µS/cm (Table S1 in Supplementary Materials); salt toxicity for freshwater species is rarely observed for values below 1 mS/cm [29]. Nevertheless, the possibility that the resulting products maintain the high toxicity of the wastewater should not be ruled out, even though wastewater A0 was not treated by EF, and its toxicity toward the microalgae was higher than that of A3. Overall, the obtained results strongly suggest that the main factor responsible for the observed toxic levels after EF was the incomplete degradation of the herbicide.

4. Conclusions

The present study confirms the expectation that EF (electroflotation) can be applied to decontaminate effluents contaminated with sulfonylurea herbicides. The results obtained by HPLC-UV indicate that EF can break some of the main chemical bonds of the chlorimuron-ethyl herbicide, followed by the generation of products, as indicated in the mechanism of degradation (built by interpreting GC–MS chromatograms). Even with a compromised herbicide structure, the effluent maintained its high toxicity toward the green algae *Raphidocelis subcapitata* and the crustacean *Daphnia magna*, with low effective dilutions causing 20 and 50% inhibition of microalga growth (all values below 3.13%) and cladoceran mortality (values between approximately 12 and 20%, respectively). Although EF did not completely eliminate the toxicity of chlorimuron-ethyl-contaminated wastewater, the observed results indicate that toxic organic compounds are potentially oxidized by EF, and above all, they signal the need to combine both the chemical and ecotoxicological approaches not only to fully evaluate environmental risks but also to appraise the sustainability of decontamination methods and pave the way for green chemistry.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14172723/s1, Table S1: Results of pH, conductivity, and dissolved oxygen measured at the start and at the end of the 48 h lethal test with the cladoceran Daphnia magna conducted on the untreated (A0) and electroflotation-treated (A3) wastewater solutions; Table S2: Results of the cell density and specific growth rate per day of the microalgae Raphidocelis subcapitata, which was used as test organism to conduct 72 h growth tests on the untreated (A0) and electroflotation-treated (A3) wastewater solutions; Table S3: Results of the cumulative number of dead organisms of the cladoceran Daphnia magna, which was used as test organism to conduct 48 h lethal tests on the untreated (A0) and electroflotation-treated (A3) wastewater solutions; Table S4: Results of the statistical analysis conducted to evaluate the effects of the of untreated (A0) and electroflotation-treated (A3) solutions on the growth rate responses of the microalgae *Raphidocelis subcapitata*.

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