

Article

# Ecotoxicological Evaluation of Methiocarb Electrochemical Oxidation

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**Abstract:** The ecotoxicity of methiocarb aqueous solutions treated by electrochemical oxidation was evaluated utilizing the model organism *Daphnia magna*. The electrodegradation experiments were performed using a boron-doped diamond anode and the influence of the applied current density and the supporting electrolyte (NaCl or Na<sub>2</sub>SO<sub>4</sub>) on methiocarb degradation and toxicity reduction were assessed. Electrooxidation treatment presented a remarkable efficiency in methiocarb complete degradation and a high potential for reducing the undesirable ecological effects of this priority substance. The reaction rate followed first-order kinetics in both electrolytes, being more favorable in a chloride medium. In fact, the presence of chloride increased the methiocarb removal rate and toxicity reduction and favored nitrogen removal. A 200× reduction in the acute toxicity towards *D. magna*, from 370.9 to 1.6 toxic units, was observed for the solutions prepared with NaCl after 5 h treatment at 100 A m<sup>-2</sup>. An increase in the applied current density led to an increase in toxicity towards *D. magna* of the treated solutions. At optimized experimental conditions, electrooxidation offers a suitable solution for the treatment and elimination of undesirable ecological effects of methiocarb contaminated industrial or agricultural wastewaters, ensuring that this highly hazardous pesticide is not transferred to the aquatic environment.

**Keywords:** acute toxicity; *Daphnia magna*; electrochemical oxidation; emerging contaminants; methiocarb

## 1. Introduction

Emerging contaminants including some pharmaceuticals, personal care products and pesticides are a cause of concern due to their ubiquity in the aquatic environment and their potential for causing deleterious ecological effects [1–4]. This has led the European Union to establish a surveillance mechanism for the monitoring of substances that show a possible risk to the environment. In 2018, the Commission Implementing Decision (EU) 2018/840 was published, establishing a watch list of substances for Union-wide monitoring in the field of water policy, which includes hormones, antibiotics and pesticides [5].

One of the priority substances identified by the Commission Implementing Decision (EU) 2018/840 watch list is methiocarb (MC),  $C_{11}H_{15}NO_2S$ , a carbamate pesticide employed in agriculture worldwide, which has been detected in natural waters and in wastewaters [5–9]. Due to MC and some of its metabolites' high toxicity, the World Health Organization classified MC as a highly hazardous pesticide, being also strictly regulated by the European Union [10–12]. Although the presence of methiocarb in waters and wastewaters at concentrations ranging from  $ng\ L^{-1}$  to  $\mu g\ L^{-1}$  is usually associated with its use, the industrial production of pesticides and herbicides generates a large volume of wastewaters with a high concentration of these pollutants that, if not properly treated, will also contaminate the water bodies [13,14].

According to the literature, conventional wastewater treatments are incapable of removing pollutants such as MC [6,11]. Thus, new treatment technologies have been studied to achieve the complete degradation of these contaminants to environmentally benign, non-toxic and biodegradable products and prevent their discharge in the aquatic environment [15–19]. Methiocarb oxidation through  $ClO_2$ ,  $NaOCl$  and  $NH_2Cl$  has been studied by Qiang et al. [20] and Tian et al. [21,22]. The authors found that, despite the effective elimination of MC, a significant increase in toxicity due to the formation of more toxic degradation products occurred. By applying a vacuum ultraviolet/ultraviolet (VUV/UV) process in the degradation of several pesticides including MC, Yang et al. [23] observed a reduction in the toxicity of the solutions although the pesticides' mineralization rates were considerably lower than their degradation rates. Cruz-Alcalde et al. [11] studied the removal of MC by ozonation and observed that during the degradation experiments, direct ozone reaction was effective to remove MC but not its formed intermediates whereas hydroxyl radicals could oxidize all species. In fact, treatment technologies based on oxidation through hydroxyl radicals have been widely studied because hydroxyl radicals are one of the most powerful oxidizing species that are able to react unselectively and instantaneously with the surrounding organic pollutants [24]. Among these technologies, electrochemical processes have received great attention due to their versatility, ease in operation and, specially, their high efficiency in the degradation of recalcitrant compounds [17,25]. Several authors have reported the removal of pesticides and herbicides using different electrochemical methods including electrochemical oxidation (EO), electro-Fenton (EF), photoelectro-Fenton (PEF) and electrochemical peroxidation [13,14,26–28]. In the degradation of solutions containing  $100\ mg\ L^{-1}$  of the herbicide clopyralid, Santos et al. [14] found that, among EO with electrogenerated hydrogen peroxide, EF and PEF processes, PEF led to the most attractive results with almost a complete transformation of clopyralid in inorganic ions. In a different study, Silva et al. [26] applied an EO process utilizing dimensionally stable anodes (DSA) to degrade the insecticide imidacloprid at a concentration of approximately  $25\ mg\ L^{-1}$  and achieved its complete oxidation (100% mineralization). According to these authors, the imidacloprid degradation occurred via direct anodic oxidation and  $Cl_2$ -mediated oxidation and the breakdown of the insecticide's molecular structure and formation of short chain compounds occurred before its complete mineralization. In a study performed by Martínez-Huitle et al. [28], the performance of different anode materials,  $Pb/PbO_2$ ,  $Ti/SnO_2$  and a boron-doped diamond anode (BDD), was evaluated during the electrochemical oxidation of the pesticide methamidophos at a concentration of  $50\ mg\ L^{-1}$ . A few authors have found that, using  $Pb/PbO_2$  and  $Ti/SnO_2$ , formaldehyde appeared as a product of the reaction, giving evidence of an indirect mineralization mechanism. However, when BDD was used, formaldehyde production was not detected and the formation of phosphate was observed instead, indicating the complete mineralization of methamidophos. In fact, EO using a BDD anode has been regarded as the electrochemical process of choice with outstanding degradation results that are due to the higher oxidation ability of BDD compared with other anode materials [29–33].

Although the application of the EO process in the treatment of waters and wastewaters appears as an attractive alternative for the degradation of biorefractory or priority pollutants such as pesticides and herbicides, the influence of the EO treatment in the toxicity of the treated solutions is not well known because the removal of the contaminants might not always correspond to a quantitative reduction of the toxic effects. The physicochemical and biological parameters usually determined

to evaluate the quality of wastewaters are not sufficient to assess the toxicity to organisms where ecotoxicological tests with aquatic organisms are required to better characterize the toxicity removal and prevent undesirable ecological effects in the aquatic environment caused by wastewater discharge [34]. Hence, the present work aims to fill this gap by evaluating the ecotoxicity of methiocarb solutions treated by EO using the freshwater crustacean *Daphnia magna* as the model species, as endorsed by international organizations including the American Society for Testing and Materials (ASTM, USA), the Organization for Economic Cooperation and Development (OECD, France) and the International Organization for Standardization (ISO, Switzerland). In the particular case of the highly toxic pesticide methiocarb, there are no studies addressing its degradation by electrochemical oxidation with a subsequential ecotoxicological evaluation. Thus, electrochemical oxidation experiments utilizing a BDD anode were performed with MC aqueous solutions at a concentration of 20 mg L<sup>-1</sup>. In order to assess the influence in the toxicity results of two major experimental conditions that greatly affect the EO performance, MC degradation was carried out at two different applied current densities ( $j$ ), 100 and 300 A m<sup>-2</sup>, utilizing two different supporting electrolytes, NaCl and Na<sub>2</sub>SO<sub>4</sub>. Although the MC concentrations reported for waters and wastewaters are in the range of ng L<sup>-1</sup> to µg L<sup>-1</sup>, the concentration of 20 mg L<sup>-1</sup> was selected for this study with the aim of evaluating the ability of the EO process to reduce the high toxicity of this priority substance in an extreme-case scenario and attending to the concentrations found in the literature for electrochemical studies involving pesticides and herbicides. In addition, in the cases where membrane filtration processes are utilized to purify the effluents, the concentrations in the permeate may reach values much higher than those usually found at the entrance of the effluent treatment plants.

## 2. Materials and Methods

### 2.1. Methiocarb Aqueous Solutions Composition and Characterization

The composition and characterization of the methiocarb aqueous solutions used in this study are described in Table 1. MC solutions were prepared in ultrapure water obtained with a Milli-Q system using the methiocarb PESTANAL<sup>®</sup>, analytical standard (CAS Number 2032-65-7), purchased from Sigma-Aldrich. As the MC aqueous solution did not have enough electrical conductivity to pursue with the electrochemical experiments at the applied current densities, the addition of a background electrolyte was required. Two different salts were studied as background electrolytes, sodium sulfate anhydrous (CAS Number 7757-82-6) of 99.7% purity and sodium chloride ACS reagent (CAS Number 7647-14-5), purity ≥ 99.0%. Both were purchased from Sigma-Aldrich. The concentration of added salt was set as the minimum required to perform the EO assays at the highest applied current density studied (300 A m<sup>-2</sup>), which was found to be 250 mg L<sup>-1</sup>, corresponding to an increase of the solution's electrical conductivity to approximately 500 µS cm<sup>-1</sup>.

**Table 1.** Composition and characterization of the methiocarb aqueous solutions used in the experiments.

Solution		MC + Na <sub>2</sub> SO <sub>4</sub>	MC + NaCl
Composition mg L <sup>-1</sup>	Methiocarb	20	20
	Na <sub>2</sub> SO <sub>4</sub>	250	
	NaCl	–	250
Chemical oxygen demand/mg L <sup>-1</sup>		43 ± 1	43 ± 1
Total organic carbon/mg L <sup>-1</sup>		11.6 ± 0.4	11.8 ± 0.5
Total nitrogen/mg L <sup>-1</sup>		1.23 ± 0.03	1.24 ± 0.04
pH		5.45 ± 0.03	5.28 ± 0.03
Conductivity/µS cm <sup>-1</sup>		437 ± 5	514 ± 5
Acute toxicity/	EC <sub>50</sub> (48 h)	0.21%	0.27%
	TU <sup>1</sup>	470.4	370.9

<sup>1</sup> TU—toxic units (TU = (1/EC<sub>50</sub>) × 100, TU > 100: highly toxic, 10 < TU < 100: very toxic, 1 < TU < 10: toxic, TU < 1 non-toxic [35]).

## 2.2. Electrodegradation Experiments

The EO experiments aimed to study the influence of the composition of the supporting electrolyte, of the applied current density and of the electrolysis duration/applied electric charge on the toxicity reduction towards *Daphnia magna* and on the removal rate of methiocarb, total organic carbon (TOC) and total nitrogen (TN). Thus, the EO assays were conducted at room temperature (22–25 °C) in batch mode with stirring (250 rpm), using an open, undivided and cylindrical glass cell (250 mL of capacity) and 200 mL of solution. A commercial Si/BDD anode purchased from Neocoat and a stainless-steel cathode, each one with an immersed area of 10 cm<sup>2</sup>, were utilized as electrodes. They were placed in parallel with an inter-electrode gap of 0.3 cm and were centered in the electrochemical cell. The experiments, utilizing the solutions described in Table 1, MC + Na<sub>2</sub>SO<sub>4</sub> and MC + NaCl, were performed at two different applied current densities, 100 and 300 A m<sup>-2</sup>, which was the electric current supplied by a GW, Lab DC, model GPS-3030D (0–30 V, 0–3 A) power source. For the applied current density of 100 A m<sup>-2</sup>, 5 h and 6 h duration assays were performed, corresponding to applied charges of 1.80 and 2.16 kC, respectively. For the experiments run at 300 A m<sup>-2</sup>, the assays had 3 h duration, corresponding to 3.24 kC of applied charge. Samples were collected each hour for physicochemical determinations to monitor the experiments. Additionally, in the experiments run at 300 A m<sup>-2</sup>, a sample was collected after 20 min of starting the assay corresponding to an applied charge of 0.36 kC, which was the same reached after 1 h by the experiments run at 100 A m<sup>-2</sup>. Toxicological assays were only performed for the initial (MC + Na<sub>2</sub>SO<sub>4</sub> and MC + NaCl) and final samples (MC + Na<sub>2</sub>SO<sub>4</sub>–100 A m<sup>-2</sup>–1.8 kC (5 h), MC + Na<sub>2</sub>SO<sub>4</sub>–100 A m<sup>-2</sup>–2.16 kC (6 h), MC + Na<sub>2</sub>SO<sub>4</sub>–300 A m<sup>-2</sup>–3.24 kC (3 h), MC + NaCl–100 A m<sup>-2</sup>–1.8 kC (5 h), MC + NaCl–100 A m<sup>-2</sup>–2.16 kC (6 h) and MC + NaCl–300 A m<sup>-2</sup>–3.24 kC (3 h)). For the kinetic study, assays with 1 h duration were run with samples being collected every 15 min.

All of the EO assays were performed at least in duplicate and results presented are mean values of all of the determinations performed.

## 2.3. Toxicological Assays

The freshwater crustacean *Daphnia magna* Strauss, Clone K6, was used in the toxicological assays. The daphnids were obtained from a stock culture maintained in the laboratory under standardized conditions. The animals were reared at a constant temperature (20 ± 1 °C) and photoperiod (16:8 h light/dark) in ASTM (American Society of Testing Materials) hard water. The culture medium was changed every other day during weekdays and was supplemented with seaweed extract. The daphnids were fed daily with the green algae *Raphidocelis subcapitata* (3.0 × 10<sup>5</sup> cells mL<sup>-1</sup>). From this stock culture, individual daphnids were transferred to 100 mL beakers prior to the beginning of the tests. Neonates with less than 24 h from the 3rd to the 6th broods were used. The evaluation of daphnia fitness and the validation of the test were achieved by performing the 24 h acute toxicity test with K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> as recommended by the OECD Guideline 202 and fully described by Fernandes et al. [36,37].

The acute toxicity tests were performed following the OECD Guideline 202 [36]. For each treatment and respective controls, five replicates with five neonates each were tested. Daphnids were exposed to different dilutions of the treated and untreated solutions in a six well plate. For each replicate, 10 mL of the test solutions were used, corresponding to 2 mL per daphnid as recommended by the OECD Guideline [36]. The number of immobilized daphnia was registered after 24 and 48 h and the median effective concentration (EC<sub>50</sub>) was calculated using the software GraphPad Prism8. All of the toxicity tests were performed within 1 month of the EO treatment and the samples were preserved under dark conditions at –20 °C. Prior to the beginning of the tests, the samples were left to unfreeze at 4 °C.

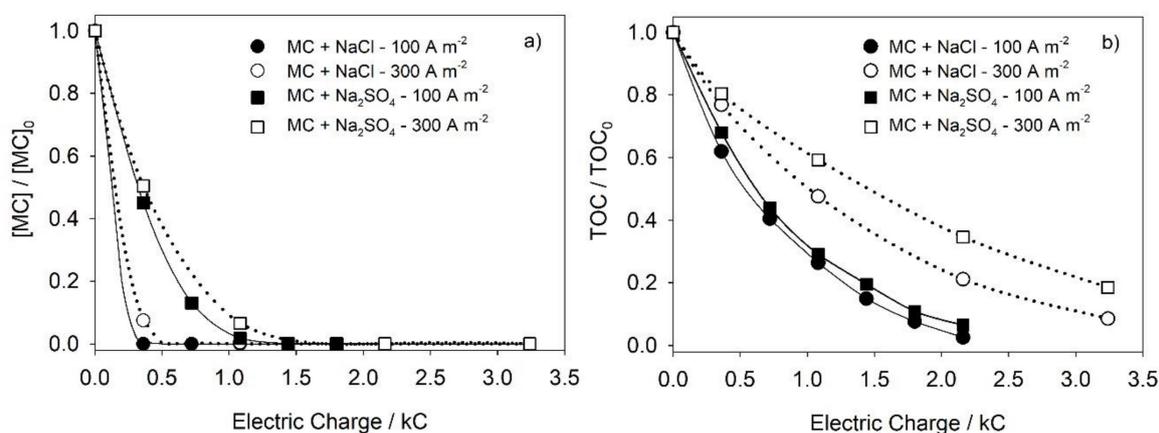
### 2.4. Physicochemical Determinations

The characterization of samples was performed by measuring TOC and TN measured in a Shimadzu TOC-V CPH analyzer combined with a TNM-1 unit and by the determination of chemical oxygen demand (COD) using a closed reflux and titrimetric method according to standard procedures [38]. High performance liquid chromatography (HPLC) was also performed for MC determinations using a Shimadzu 20A Prominence HPLC system equipped with a SPD-M20A diode array detector, a CTO-20AC column oven and a LC-20AD pump and using a Purospher STAR RP18 endcapped column (250 × 4 mm (i.d.), 5 μm). The elution was performed isocratically with a mixture of formic acid aqueous solution (0.05%) and acetonitrile, 50:50 (v/v), at a flow rate of 1 mL min<sup>-1</sup> and 35 °C. The injection volume was 20 μL and the detection wavelength was 262 nm. The reagents were Sigma-Aldrich HPLC grade and ultrapure water (Milli-Q system) was utilized to prepare the solutions.

pH (HANNA, HI 931400) and conductivity (Mettler Toledo, SevenEasy S30K) were measured along the assays.

### 3. Results and Discussion

Figure 1 presents the normalized variation of MC and TOC concentrations with applied charge during the EO assays accomplished with different supporting electrolyte and applied current densities. When the influence of the supporting electrolyte was analyzed, the MC and TOC removal rates were higher for the solutions containing NaCl. In fact, in the presence of chloride, MC was completely degraded during the initial period of the assays for both *j* whereas in the presence of Na<sub>2</sub>SO<sub>4</sub>, the MC removal rate was lower and decreased along the assays. This faster degradation in the presence of chloride has been described in the literature for other contaminants and, according to the authors, is due to the additional indirect oxidation in the bulk of the solution by active chlorine species generated from the oxidation of chloride ions at the BDD [33,39].



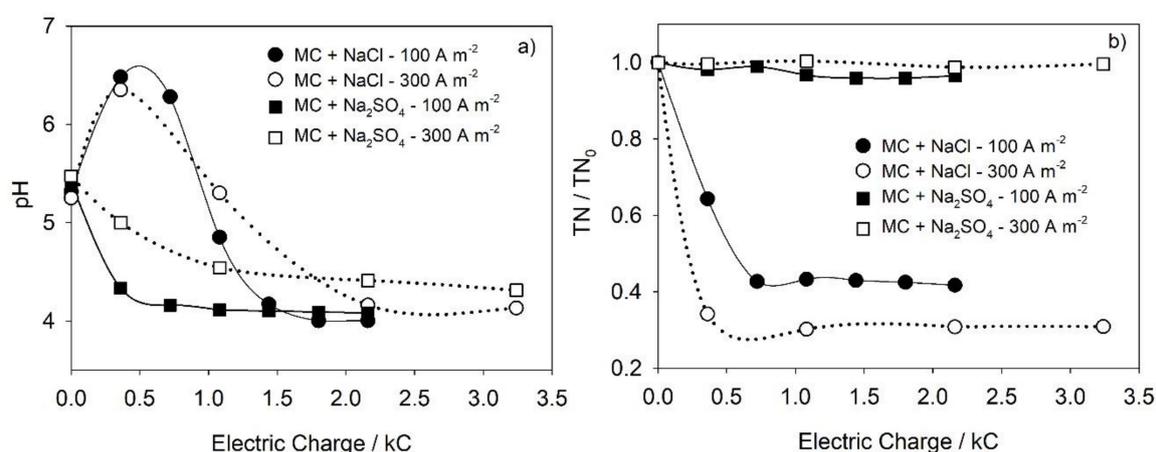
**Figure 1.** Variation with applied charge of the normalized (a) methiocarb (MC) concentration and (b) total organic carbon (TOC) for the electrochemical oxidation (EO) experiments performed at 100 and 300 A m<sup>-2</sup> using Na<sub>2</sub>SO<sub>4</sub> or NaCl as the supporting electrolyte.

To elucidate the reaction kinetics in both electrolytes, the decay in time of the MC concentration determined by HPLC for the first hour of the assays was analyzed. The MC degradation showed a first-order reaction mechanism with the following kinetic constants:  $k = 0.28 \times 10^{-3} \text{ s}^{-1}$  (Na<sub>2</sub>SO<sub>4</sub>\_100 A m<sup>-2</sup>);  $k = 0.77 \times 10^{-3} \text{ s}^{-1}$  (Na<sub>2</sub>SO<sub>4</sub>\_300 A m<sup>-2</sup>);  $k = 1.6 \times 10^{-3} \text{ s}^{-1}$  (NaCl\_100 A m<sup>-2</sup>);  $k = 1.7 \times 10^{-3} \text{ s}^{-1}$  (NaCl\_300 A m<sup>-2</sup>). This showed that, in the chloride medium, the oxidation happened mainly in the bulk of the solution and, because the MC concentration was low, even at the lowest applied current density the chlorine active species formed were enough to oxidize MC at a very good reaction rate. Regarding oxidation in the presence of

sulfate, the oxidation mechanism must be either by hydroxyl or sulfate radicals with a lower lifetime, thus giving more importance to MC diffusion towards the anode's surface.

Regarding the TOC, for the highest  $j$ , the difference between the removal rates when NaCl or Na<sub>2</sub>SO<sub>4</sub> were used was more pronounced than at 100 A m<sup>-2</sup>. Regardless of that, MC and TOC removals were energetically more efficient at the lowest  $j$ . These results indicated that at 300 A m<sup>-2</sup>, MC and by-product oxidation through hydroxyl radicals were reduced and the oxidation by active chlorine species was enhanced, which was due to the higher amount of active chlorine species available at this  $j$  and to the lower amount of hydroxyl radicals available, caused by the augmentation of secondary reactions such as the oxidation of the electrolyte or oxygen and hydrogen evolution [40]. Although the mentioned reactions were always present, their rate increased with applied current densities.

The different degradation mechanisms occurring when NaCl or Na<sub>2</sub>SO<sub>4</sub> were used were also evidenced in pH variation along the assays (Figure 2a). When NaCl was used, an increase in pH during the initial period of the assays was found, followed by a decrease to below the initial value and ending in a plateau. The initial increase in pH observed was probably due to the chloride oxidation/water reduction and the subsequent decrease could be a result of the formation of by-products such as short chain carboxylic acids [17]. In the presence of Na<sub>2</sub>SO<sub>4</sub>, a decrease in pH during the initial period of the assays was observed with a subsequent tendency to a plateau.

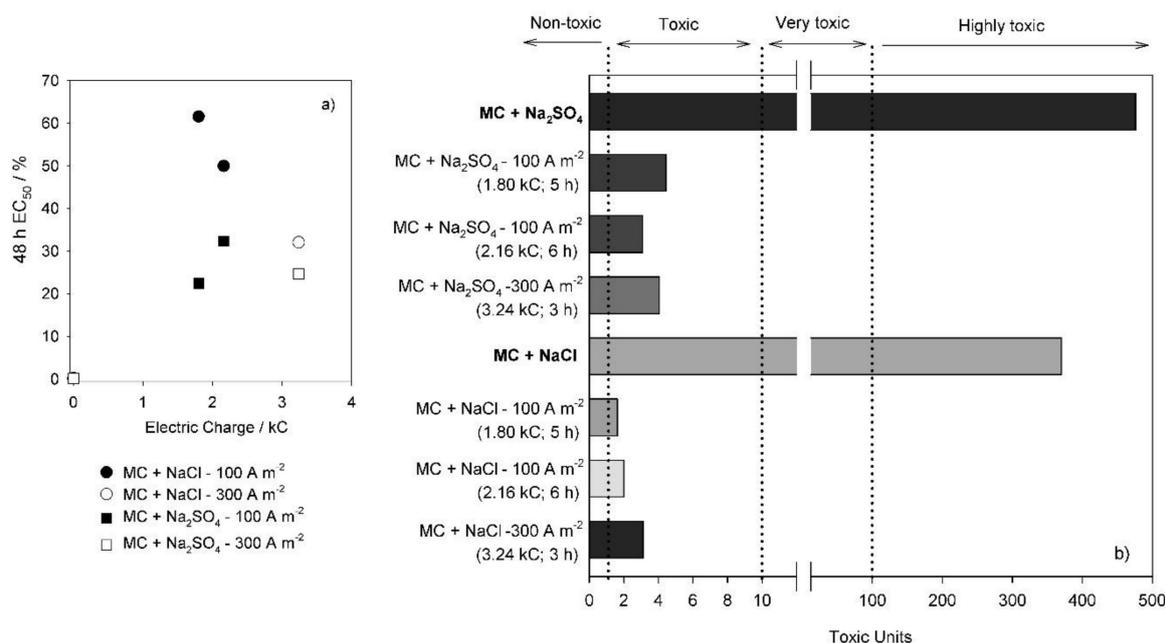
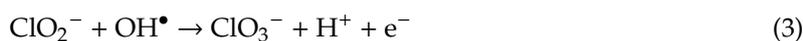
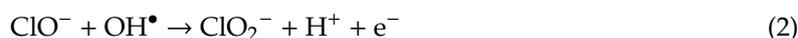


**Figure 2.** Variation with applied charge of the (a) pH and (b) normalized total nitrogen (TN) for the EO experiments performed at 100 and 300 A m<sup>-2</sup> using Na<sub>2</sub>SO<sub>4</sub> or NaCl as the supporting electrolyte.

As an MC molecule has nitrogen in its structure, TN variation during the assays was also assessed. A normalized TN variation with an applied charge, presented in Figure 2b, showed that in the presence of Na<sub>2</sub>SO<sub>4</sub> no nitrogen removal occurred. However, when NaCl was used, TN concentration decreased by more than 50%. According to the literature, organic nitrogen is converted to NH<sub>4</sub><sup>+</sup> in the EO using a BDD anode, which in turn is oxidized mainly to nitrate and nitrogen gas as NH<sub>4</sub><sup>+</sup> oxidation takes place by indirect oxidation through active chlorine species [41,42]. It was also observed that, for the solutions containing chloride, TN removal was energetically more efficient at the highest current density. These results agreed with the enhancement of the electrolyte oxidation reaction at a higher  $j$  because more active chlorine species available lead to a higher TN removal rate.

As MC is a highly toxic compound, the ecotoxicological evaluation of the treatment methods applied to its degradation is mandatory. Thus, the ecotoxicity of the treated samples obtained in this study as well of the initial samples was evaluated towards *Daphnia magna*. As it can be perceived from Figure 3 and Figure S1 (Supplementary Material), the electrochemical oxidation treatment of methiocarb solutions led to a remarkable decrease in the acute toxicity towards *Daphnia magna*. In both MC solutions containing Na<sub>2</sub>SO<sub>4</sub> or NaCl, the initial toxicity was very high although solutions prepared with NaCl were slightly less toxic (EC<sub>50</sub> = 0.21%, 470.4 TUs against EC<sub>50</sub> = 0.27%, 370.9 TUs for solutions prepared

with Na<sub>2</sub>SO<sub>4</sub> and NaCl, respectively). Methiocarb is an acetylcholinesterase inhibitor pesticide widely used in agriculture as a molluscicide, acaricide and avicide and its toxicity towards aquatic invertebrates has already been reported [43]. Figure 3a presents the EC<sub>50</sub> results after 48 h of exposure for the different experimental conditions studied. The higher EC<sub>50</sub> values were attained for the experiments performed at 100 A m<sup>-2</sup> using NaCl as the supporting electrolyte. At these experimental conditions, an increase in EC<sub>50</sub> from 0.27% to 62% was achieved after a 5 h assay (1.8 kC). However, when the assay duration was extended for one more hour to 6 h (2.16 kC) in total, the EC<sub>50</sub> decreased to 50%. In solutions containing chloride, the increase in toxicity during the electrochemical treatment is usually attributed to the formation of chlorinated compounds [44,45], which exhibit a high octanol/water coefficient (K<sub>ow</sub>), thus affecting biological membranes and presenting higher toxicity for living organisms [37]. Furthermore, the formation of perchlorate at the final stage of the electrochemical process when using a BDD anode is well described in the literature [46–48]. According to Lacasa et al. [45], the hydroxyl radicals formed in large quantities during the electrolysis of aqueous solutions when a BDD anode is used in the presence of chlorides can be oxidized successively to different oxochlorinated compounds according to Equations (1)–(4).



**Figure 3.** (a) EC<sub>50</sub> results obtained for the treated solutions at 100 and 300 A m<sup>-2</sup> using Na<sub>2</sub>SO<sub>4</sub> or NaCl as the supporting electrolyte. (b) Comparison of the toxicity of the different methiocarb solutions in terms of toxic units before and after treatment at the different experimental conditions.

Attending that, in the last hour of the assay, the organic load in the solution is low and its oxidation is under mass transport control, the occurrence of the reactions described by Equations (1)–(4) will be enhanced and the perchlorate concentration in the solution, as the final oxidation product, will increase, explaining the increase in ecotoxicity found. The possible formation of organochlorinated compounds

in the last hour of the assay, although in low concentration because the TOC was less than  $1 \text{ mg L}^{-1}$  at this point, could also be a possible explanation for the increased ecotoxicity.

Both perchlorate and organochlorinated compound formation can also explain the lower ecotoxicity reduction presented by the chloride-containing solutions treated at  $300 \text{ A m}^{-2}$ . At the applied charge of  $3.24 \text{ kC}$  (3 h assay at  $300 \text{ A m}^{-2}$ ),  $\text{EC}_{50}$  increased only to 32%. At higher applied current densities, chloride oxidation and hydroxyl radical formation were enhanced, augmenting the occurrence of the reactions described by Equations (1)–(4) and the possible formation of organochlorinated compounds.

For the solutions prepared with sulfate at  $100 \text{ A m}^{-2}$ ,  $\text{EC}_{50}$  increased from 0.21% to 22% after a 5 h assay ( $1.8 \text{ kC}$ ) and to 32% after 6 h ( $2.16 \text{ kC}$ ). These results agreed with the lower MC degradation rate observed for the solutions containing sulfate and indicated that at  $1.8 \text{ kC}$  there were in the solution toxic MC degradation products that were being further oxidized to less or non-toxic compounds, decreasing the solution toxicity at  $2.16 \text{ kC}$ . Nevertheless, the increase in applied current density and charge did not result in a further decrease in toxicity. At the applied charge of  $3.24 \text{ kC}$  (3 h assay at  $300 \text{ A m}^{-2}$ ),  $\text{EC}_{50}$  increased only to 25% in the sulfate-containing solution. This result can be explained by the energy efficiency loss when  $j$  was increased as observed for the TOC removal, mainly due to mass transport limitations.

In terms of toxic units (Figure 3b), it can be seen that, using NaCl as the supporting electrolyte, it was possible to reduce the toxicity more than  $200\times$  from 370.9 TUs to 1.6 TUs with 5 h treatment at  $100 \text{ A m}^{-2}$ , whereas for the same conditions but using  $\text{Na}_2\text{SO}_4$ , the reduction was only of  $107\times$  from 470.4 TUs to 4.4 TUs. At the higher applied current density of  $300 \text{ A m}^{-2}$ , the difference between the supporting electrolytes was less pronounced, with a similar reduction in toxicity of  $120\times$  versus  $115\times$  for NaCl and  $\text{Na}_2\text{SO}_4$  containing solutions, respectively.

Overall, the results obtained clearly demonstrated the remarkable efficiency of EO treatment for MC complete degradation and its high potential for reducing the toxic ecological effects of this highly hazardous pesticide even when present in concentrations far above those currently reported for waters and wastewaters. According to Pablos et al. [35], the MC solutions went from being classified as highly toxic before treatment to toxic and very close to be considered non-toxic after EO treatment. Although the present study only addressed experiments at a laboratory scale, it offers a useful contribution to further develop the application of the electrochemical oxidation process at an industrial level. In fact, according to the results achieved, EO can be considered suitable for the remediation of contaminated industrial or agricultural wastewaters, enabling the elimination of MC and its adverse effects before they reach the aquatic environment.

#### 4. Conclusions

Electrochemical oxidation with a BDD anode can effectively degrade methiocarb and its transformation by-products, reducing drastically the acute toxicity towards *Daphnia magna*. The MC electrodegradation mechanism depends on the species present in the solution and on the applied current density:

- In the presence of chloride, MC is rapidly transformed as result of its additional indirect oxidation by active chlorine species. These active chlorine species are responsible for the nitrogen elimination, above 50%.
- In the presence of sulfate, the electrooxidation mechanism involves mainly hydroxyl radicals formed at the BDD surface leading to a slow MC elimination because the oxidation rate depends on the MC diffusion to the anode's surface. No nitrogen removal was observed using this supporting electrolyte.
- An increase in current density enhances secondary reaction rates, thus decreasing the energy efficiency. An exception is nitrogen removal in chloride-containing solutions, which is promoted by the increased amount of active chlorine species.

- Chloride-containing solutions lead to higher toxicity reductions than sulfate-containing solutions most likely due to the faster MC degradation and of its transformation products when NaCl is used.
- In chloride-containing solutions, the increase in applied electric charge due to the increase in assay duration or applied current density leads to a lower decrease in ecotoxicity probably due to the formation of perchlorate and organochlorinated compounds.

At optimized experimental conditions, the EO process offers a suitable solution for the treatment and elimination of undesirable ecological effects of MC contaminated industrial or agricultural wastewaters, ensuring that this priority substance is not transferred to the aquatic environment.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2076-3417/10/21/7435/s1>, Figure S1: Number of immobilized daphnids after 24 and 48h of exposure to the different treatments. Immobilization refers to the inability of animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel [1].

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