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Combined process for 2,4-Dichlorophenoxyacetic acid treatment – Coupling of an electrochemical system with a biological treatment.

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Abstract

A coupled process was studied for the removal of a chlorinated pesticide: 2,4-Dichlorophenoxyacetic acid (2,4-D). A home-made electrochemical flow cell was used for the pre-treatment and a biological treatment was then carried out using activated sludge supplied by a local wastewater treatment plant. 2,4-D was used as a target compound for the study. Several parameters were monitored during the biological treatment, like Dissolved Organic Carbon (DOC), the target compound and the major by-product. Pretreatment led to a quick decrease of DOC during the biological process, since a 66% mineralization yield was measured after the second day, and 79% after the seventh day of culture. After two days of treatment, HPLC results revealed a total degradation of Chlorohydroquinone, the major by-product. The electrochemical pretreatment

shortened the length of the biological treatment, since DOC measurements showed that in the case of non-pretreated 2,4-D, no mineralization was observed before day 7. These promising results should be subsequently confirmed on commercial 2,4-D-containing solutions and then on real effluents.

Keywords: 2,4-D, carbon felt electrode, electrochemical pre-treatment, biodegradability, activated sludge, biological treatment

1. Introduction

The usage of pesticides in intensive agricultural practice has brought out environmental and health concerns since it negatively impacts water and soil quality. Removing pesticides to protect water resources has thus become a great challenge in current research [1]. Wastewater from agricultural industries like pesticides formulating plants or bottom farm tanks can contain high levels of pesticides (up to 500 mg L⁻¹) [2]. These effluents need therefore to be treated by specific physical, chemical or biological processes.

2,4-Dichlorophenoxyacetic acid (2,4-D, Figure 1) is one of the most used phenoxyalkanoic herbicides in the world [3,4], and more than 1500 pesticides contain 2,4-D as the main active substance [5]. Phenoxyalkanoic herbicides are considered as serious contaminants of streams, lakes and rivers [6], especially because of their high solubility in water and low tendency to accumulate in organic matter [7]. As a matter of fact, 2,4-D has frequently been detected in surface and ground waters in Europe and North America [8,9]. Moreover, 2,4-D is known to present serious ecological impact, like toxic effects on birds, beneficial insects, non-target plants but also on algae, small invertebrates, amphibians, fishes and more generally on aquatic life [10,11].

Over the past few decades, the literature has disclosed a plethora of studies focusing on 2,4-D degradation. Many bacterial strains capable of degrading 2,4-D have been isolated, such as several strains of *Pseudomonas*, *Arthrobacter* and *Alcaligenes* [12–16]. However, in many cases, the mineralization yield was low or even not measured [17]. The feasibility of a biological treatment based on pure strains of bacteria remains therefore limited, and 2,4-D removal was thus also studied with consortium of bacteria, isolated from soil samples or activated sludge from wastewater treatment plant. A 250 mg L⁻¹ 2,4-D concentration as a sole carbon source was degraded in 8 days using different enrichment cultures based on soil samples from a fertilizer manufacturing plant site [18]. Activated sludge from municipal wastewater treatment plant has also shown the ability to partially degrade a waste stream containing 10 mg L⁻¹ of the herbicide [19]. Experiments carried out in Sequencing Batch Reactors (SBRs) led to the removal of a 100 mg L⁻¹ 2,4-D concentration. However, a 4 months acclimation period of the activated sludge was necessary [8]. More recently, this acclimation period was reduced to 30 days to degrade up to 500 mg L⁻¹ of 2,4-D, but this results were obtained with a biomass that was previously exposed to the pesticide [20]. Despite the economical advantage of the activated sludge, the efficiency of the biological treatment can be limited with recalcitrant compound and also be time-consuming when a long acclimation period is necessary.

2,4-D removal has been thoroughly studied using ozonation processes [21–23] or Advanced Oxidation Processes (AOPs) like anodic oxidation, Fenton and Fenton-like processes [24–27], photocatalysis [28,29]. Despite their efficiency, the total mineralization of the effluent to treat can be time and energy-consuming, thus increasing operational costs. Moreover, these processes showed other drawbacks such as the disposal of iron waste in case of Fenton and Fenton-like processes. The combination of AOP with biological treatments can therefore

appear as a good alternative to reduce operating costs. The aim of the pre-treatment step is to increase the biodegradability of the initial biorecalcitrant solution; the intermediates formed should therefore be more biodegradable than the target compound, while the mineralization yield should remain limited to ensure significant residual organic carbon content for the subsequent biological treatment and to limit the energy costs. The literature reports numerous studies dealing with AOPs as pre-treatment steps prior to biological processes. For example, isoproturon was mineralized by combining heterogeneous photocatalysis with a bioreactor containing bacteria supported on biolite [30]. The herbicides diuron and linuron treated by photo-Fenton led to non-toxic and biodegradable intermediates that were degraded in a biological Sequencing Batch Reactor [31].

As an alternative to Advanced Oxidative Processes, electrochemical systems can also be considered. Electrochemical systems were already combined with biological processes to remove heavy metals or nitrates from different wastewaters [32,33]. Regarding organic recalcitrant compounds, the biodegradability of a 100 mg L^{-1} tetracycline solution was found to increase after electrolysis in a home-made flow cell involving a carbon felt electrode [34], as well as the biodegradability of a 1000 mg L^{-1} acetamipirid solution after pretreatment using a boron-doped diamond electrode [35]. In both cases, the Biological Oxygen Demand after 5 days (BOD_5) on Chemical Oxygen Demand (COD) ratio (BOD_5/COD) increased from negligible values for the target compounds to above 0.4, namely the limit of biodegradability, after the electrochemical pretreatment, showing that the produced intermediates can be most likely biodegraded during biological treatment. However and to our knowledge, the implementation of an electrochemical pretreatment and a biological process seems to be only performed for the treatment of an herbicide, phosmet, leading to 97 % of mineralization [36].

The feasibility of the electrochemical pre-treatment to increase the biodegradability of 2,4-D was previously shown in the laboratory [37]. The electrochemical step was based on a home-made flow cell using bare graphite felt as a working electrode. The graphite felt presents several advantages, like its moderate cost and its high specific area. The electrochemical behavior of the target compound was investigated and major intermediates were detected and quantified. To complete this previous work, the relevance of the combined process for 2,4-D treatment should be assessed. This task was the main purpose of this work. The aerobic biological treatment was carried out using activated sludge purchased from a local wastewater treatment plant. Assessment of the efficiency of the combined process was based on the monitoring of specific parameters namely the target compound and the major by-products (HPLC measurements) on the one hand, and global parameters namely mineralization and oxidation through the determination of DOC and COD values on the other hand.

2. Experimental

2.1 Chemicals

2,4-Dichlorophenoxyacetic acid (98%) was purchased from Alfa Aesar (Schiltigheim, France). Acetonitrile (ACN) and formic acid were LC/MS grade from JT Baker (Deventer, Netherlands). All standards were prepared with ultra pure water (PurelabOptions-Q7/15, Elga, 18.2 M Ω .cm).

2.2 Materials for the electrochemical pre-treatment

The electrochemical pre-treatment has been carried out as previously described [37]. This electrochemical step was based on a home-made flow cell using a graphite felt as a working electrode supplied by Mersen (RVG 4000 – Mersen, Paris La Défense, France). The dimensions of the graphite felt were 48 mm diameter and 12 mm width.

A potential of 1.6 V/SCE was applied using a VersaSTAT 3 potentiostat from Ametek/Princeton Applied (Elancourt, France). The cell was thoroughly rinsed with distilled water before and after each experiment. The electrolyte solution (500 mg L⁻¹ 2,4-D in 0.1 M Na₂SO₄) percolated the porous electrode at a constant flow rate of 1 mL min⁻¹ monitored by a Gilson minipuls 2 peristaltic pump (Middleton, WI, USA).

2.3 Biological treatment

After only one pass through the electrochemical flow-cell, the effluent was collected for the subsequent biological treatment, which was carried out in aerobic conditions, using activated sludge purchased from the local wastewater treatment plant (Station de Beaurade, Rennes, France). Before use, activated sludge was washed four times with tap water and once with distilled water. After each washing, activated sludge was centrifuged at 3000 rpm for five minutes (Jouan, Thermo Fisher Scientifics, Saint Herblain, France). The supernatant was then separated from the sludge to remove any residual carbon or mineral source.

Experiments were carried out in 250 mL erlenmeyer flasks containing 100 mL of medium, stirred at 250 rpm, kept at 30°C and triplicated to ensure the reproducibility of the results. The following mineral basis was added to each experiment (mg L⁻¹): KH₂PO₄: 85, K₂HPO₄: 208, Na₂HPO₄·2H₂O: 334, CaCl₂: 27.6, MgSO₄·7H₂O: 22.6, FeCl₃·6H₂O: 0.26, NH₄Cl: 75. Erlenmeyer flasks were inoculated with 0.5 g L⁻¹ of activated sludge. The initial target compound, 2,4-D, concentration was 100 mg L⁻¹ and the electrolyzed solution (initially 500 mg L⁻¹ 2,4-D) was diluted (five times dilution) to allow a direct comparison to non-treated 2,4-D. The sole carbon source for microorganisms was therefore 2,4-D on the one hand or the electrolyzed solution on the other hand. A biological treatment carried out in similar conditions but lacking of any carbon source was used as a “blank” test. The pH of each assay was then adjusted to 7.0 ± 0.2 using 1 mol L⁻¹ NaOH.

Samples (5 mL) were taken every 2 or 3 days and filtered on 0.45 μm . pH measurements were carried out using a pHmeter Hanna with a combined micro-electrode probe (Thermo Spectronic, Rochester, USA). Samples were then diluted for DOC, COD and HPLC measurements.

2.4 Analysis

2.4.1 HPLC

Measurement of the residual 2,4-D and Chlorohydroquinone concentration were performed by HPLC (High Pressure Liquid Chromatography) (Milford, USA) system involving a pump Waters 600, fitted with a Phenomenex Kinetex® C18 2.6 μm column (4.6 mm x 100 mm), along with a Waters 996 Photodiode array detector, a Waters 717 ph Autosampler and controlled through an Empower® 2 program. The mobile phase consisted of acetonitrile and trifluoroacetic acid (TFA) 0.1% in ultra-pure water with a ratio of 30/70 at 1 ml.min⁻¹.

2.4.2 Dissolved Organic Carbon (DOC) measurements

After electrolysis, the solutions were filtered on a Satorius Stedim Minisart 0.40 μm GF prefilters (Goettingen, Germany). DOC was measured by means of a TOC-V_{CPH/CPN} Total Organic Analyzer Shimadzu. Organic carbon compounds were combusted and converted to CO₂, which was detected and measured by a non-dispersive infrared detector (NDIR). Reproducible DOC values were always obtained using the standard NPOC (Non Purgeable Organic Carbon) method. For DOC, COD and BOD₅ measurements, a 2,4-D solution (100 mg L⁻¹, Na₂SO₄ 0.02 M) was considered as the reference. The electrolyzed solution (initially 2,4-D 500 mg L⁻¹ Na₂SO₄ 0.1M) was always diluted by 5 so the results obtained can be directly compared to the reference.

2.4.3 Chemical Oxygen Demand (COD) measurements

Chemical Oxygen Demand (COD) was measured by means of a Test Nanocolor® CSB 160 from Macherey-Nagel (Düren, Germany). The amount of oxygen required for the oxidation of

the organic and mineral matter at 164°C for 30 min was quantified after oxidation with $K_2Cr_2O_7$ at acidic pH and heating.

3. Results and discussion

3.1 Electrolysis on carbon felt electrode

The electrochemical behavior and electrolysis of 2,4-D were investigated in a previous study, and promising results were obtained, showing the improvement of the biodegradability of the target compound [37]. As explained in this previous study, electrolysis was carried out in oxidation at 1.6 V/SCE, at a flow rate of 1 mL min⁻¹. After a single pass through the electrochemical flow cell, the effluent was collected for analysis, and was first analyzed by cyclic voltammetry (Fig. 2). The initial oxidation signal of 2,4-D decreased and a quasi-reversible signal appeared at about 0.3 V/SCE. The 2,4-D degradation was confirmed by HPLC since high removal and mineralization (deduced from DOC measurements) yields were obtained, 96 ± 3% and 34 ± 4% [37].

In order to assess the feasibility of the biological treatment, the biodegradability of both the 2,4-D and the electrolyzed 2,4-D solution were determined, considering that an effluent can be considered as easily biodegradable if its BOD₅/COD ratio exceeds 0.4 [38,39]. As presented in table 1, this ratio increased from 0.04 before electrolysis to 0.25 after electrolysis, showing a significant increase of the effluent's biodegradability; hence and even if the limit of biodegradability was not achieved a biological treatment could be promisingly considered. Finally, the major degradation by-products were identified by LC/MS/MS and GC/MS, and quantified by HPLC. Chlorohydroquinone (Figure 1) was identified as the major by-product since it represented 35% of the residual organic carbon [37].

3.2 Biological treatment

Prior to the biological treatment step, possible biosorption on activated sludge of 2,4-D on the one hand and the major by-product, Chlorohydroquinone, for the electrolyzed effluent on the other hand was assessed. Results are depicted in Figure 4. 250 mL Erlenmeyer flasks containing 100 mL of medium were inoculated with 0.5 g L^{-1} of activated sludge, which was washed in the same conditions as the biological treatment. The flasks were stirred at 250 rpm and the experiments were triplicated to ensure the reproducibility of the results. 2,4-D concentration in the untreated effluent on the one hand, and Chlorohydroquinone in the oxidized effluent on the other hand, were monitored by HPLC throughout biosorption experiments. Samples were regularly taken over a 3 hours period, filtered on $0.45 \mu\text{m}$ and then analyzed by HPLC. As displayed in Figure 3, no significant decrease of 2,4-D or Chlorohydroquinone concentrations was detected after 3 hours, showing that neither 2,4-D nor Chlorohydroquinone were biosorbed on activated sludge. Indeed, as biosorption is a rapid phenomenon, it would have been noticed within the 3 hours of experiment.

The biological treatment was then run in aerobic conditions, and several parameters were monitored over a 21 days period. Samples were regularly taken to measure pH, DOC, 2,4-D and Chlorohydroquinone concentrations.

Evolution of DOC during the biological treatment is presented in Figure 4a. The initial DOC concentration was higher for the 2,4-D solution than for the electrolyzed solution, since the electrochemical step led to a 34% mineralization. No decrease of DOC was observed in 2,4-D samples until day 7. This steady phase corresponded most likely to a lag phase or a short acclimation period of microorganisms to 2,4-D, since the pesticide was the sole available carbon source. A similar lag phase was already observed in other studies using soil samples instead of activated sludge [18]. A decrease of the DOC was then observed between day 7 and day 12. During this lapse of time, 87% of the organic carbon were mineralized, the DOC

concentration measured in the blank test being considered as the reference (100% mineralization). This decrease of DOC corresponded to a mineralization rate of $14.1 \text{ mg L}^{-1} \text{ day}^{-1}$. From day 12 and until day 14, a low mineralization yield of 8% was observed; DOC values remained then constant until the end of culture. It should also be noted that the final concentration of dissolved organic carbon during non-pretreated 2,4-D experiments was as low as the concentration of dissolved organic carbon in the blank test (15.9 mg L^{-1} compared to 17.1 mg L^{-1}). At a concentration of 100 mg L^{-1} , 2,4-D and its intermediates were thus fully biodegraded after 21 days. The HPLC monitoring of the 2,4-D concentration confirmed DOC results, as displayed in Figure 4b. No significant 2,4-D degradation was observed until day 7, in agreement with the observation drawn from DOC measurements (Figure 4a), and hence the period of acclimation of activated sludge to the pesticide was confirmed. 2,4-D was then fully degraded in between day 7 and day 14, at a removal rate of $13.7 \text{ mg L}^{-1} \text{ day}^{-1}$. Since 2,4-D and DOC time-courses showed similar trends at nearly similar rates with a complete removal after 14 days in both cases, it can be assumed that the degradation products resulting from 2,4-D biodegradation were readily assimilable by microorganisms, leading to a total mineralization. Chemical Oxygen Demand was also measured at the end of the biological treatment. As presented in Table 1, the COD of 2,4-D samples decreased from $182 \text{ mg O}_2 \cdot \text{L}^{-1}$ to $38 \text{ mg O}_2 \cdot \text{L}^{-1}$ (namely 79% decrease). This result was thus in agreement with the DOC and 2,4-D concentrations tendencies previously described.

In the case of the electrolyzed effluent, no significant lag phase was observed since a 39% decrease of the initial DOC was measured after only one day of culture. This observation illustrated a readily assimilation of some of the degradation products. Mineralization continued until day 5 of culture, since 77% mineralization yield was measured, corresponding to a mineralization rate of $10 \text{ mg L}^{-1} \text{ day}^{-1}$. After day 5, no really significant decrease of the initial dissolved organic carbon was measured until day 21 since only 8% of the DOC were

mineralized during this period, corresponding to a mineralization rate of 0.3 mg L^{-1} per day. Residual 2,4-D concentration was also monitored in the oxidized effluent (Figure 4b). 2,4-D concentration was stable until day 12 and fully degraded at day 14, probably corresponding to the acclimation of the microorganisms. However, no decrease of the Dissolved Organic Carbon was measured between day 14 and day 21, and this concentration remains slightly higher than the DOC concentration measured in both the blank test and the 2,4-D essays (25 mg L^{-1} compared to 17 mg L^{-1} and 16 mg L^{-1}); the final mineralization yield was thus 85%. Chlorohydroquinone, the main degradation by-product (35% of the residual DOC [37]) was also monitored by HPLC and analysis showed its total removal within the first two days of biological treatment. It can be therefore assumed the presence of refractory degradation products in the oxidized effluent, which could not be assimilated by microorganisms even after 21 days of activated sludge culture. As presented in Table 1, Chemical Oxygen Demand confirmed the removal of degradation by-products, since a decrease from $130 \text{ mg O}_2 \cdot \text{L}^{-1}$ to $40 \text{ mg O}_2 \cdot \text{L}^{-1}$ was measured (corresponding to a 69% decrease). Even though final COD of 2,4-D samples and oxidized 2,4-D samples were similar, the decrease percentage was lower in case of oxidized 2,4-D samples, as also observed for DOC values.

4 Conclusions

A 2,4-D solution was electrochemically pre-treated in order to increase the biodegradability of the solution. After a single pass through the electrochemical flow cell, the solution was collected and subsequently aerobically treated using activated sludge. DOC measurements showed that in the oxidized solution, a high mineralization was observed during the first two days of culture (about 66%), probably including the mineralization of Chlorohydroquinone. This rapid mineralization step occurred 8 days before the mineralization observed in the 2,4-D samples. Then mineralization only increased to 79 and 85% after 7 and 21 days, respectively.

Even though Chlorohydroquinone has been quickly removed, the Dissolved Organic Carbon concentration in the oxidized solution samples at the end of the experiment remained slightly higher than in the blank sample, showing that a small part of the degradation products could not be easily assimilated by the considered sludge. This result could be improved by optimizing the pre-treatment step conditions, such as flow rate or even by recycling the effluent through the cell. At the end of the biological treatment, DOC concentrations in the non-pretreated 2,4-D trials and in the blank test were similar, showing that 2,4-D was fully removed; however, no mineralization was detected before day 7 of the biological treatment of 2,4-D solutions, confirming the relevance of the proposed combined process. Indeed, the electrochemical pretreatment improved significantly the mineralization rate and thus significantly shortened the length of the biological treatment. These positive results should be subsequently confirmed on commercial 2,4-D-containing solutions, and then on real effluents such as bottom farm tanks to fully demonstrate the relevance of this coupled processes.

Nomenclature

2,4-D: 2,4-Dichlorophenoxyacetic acid

SBRs: Sequencing Batch Reactors

AOPs: Advanced Oxidation Processes

SCE: Saturated Calomel Electrode

HPLC: High Pressure Liquid Chromatography

LC/MS/MS: Liquid Chromatography/Mass Spectroscopy/Mass Spectroscopy

GC/MS: Gas Chromatography/Mass Spectroscopy

DOC: Dissolved Organic Carbon

NPOC: Non Purgeable Organic Carbon

COD: Chemical Oxygen Demand

BOD₅; Biological Oxygen Demand after 5 days

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Table 1: DOC, COD and biodegradability determination of 2,4-D (100 mg.L^{-1}) and electrolyzed solution (5 times diluted).

	COD before the biological treatment (mg.L^{-1})	BOD₅/COD ratio before the biological treatment [37]	COD after the biological treatment (21 days) (mg.L^{-1})
2,4-D solution	182 ± 4	0.04 [37]	38 ± 3
Electrolyzed 2,4-D solution	130	0.25[37]	40 ± 9

Captions:

Figure 1: Chemical structures of 2,4-Dichlorophenoxyacetic acid and Chlorohydroquinone.

Figure 2 : Cyclic voltammetry of 2,4-D solution before (--) and after electrolysis (—) at pH 3, and that of Na₂SO₄ 0.1 M (dot/dash) . Voltammograms recorded at 100 mV.s⁻¹ in Na₂SO₄ on a GC electrode.

Figure 3: Biosorption on activated sludge (0.5 g L⁻¹) of 2,4-D (100 mg L⁻¹, Na₂SO₄ 0.1 M) (▲) and Chlorohydroquinone in the oxidized 2,4-D solution (●).

Figure 4a: Time-courses of the Dissolved Organic Carbon concentration during activated sludge culture on non-pretreated 2,4-D (▲) and oxidized 2,4-D (●) solutions, as well as during the “blank” test, namely activated sludge culture in the absence of any carbon source (—).

Figure 4b: Time-courses of the 2,4-D concentration during activated sludge culture on non-pretreated 2,4-D (▲) and oxidized 2,4-D (●) solutions.

Figures :

Figure 1 :

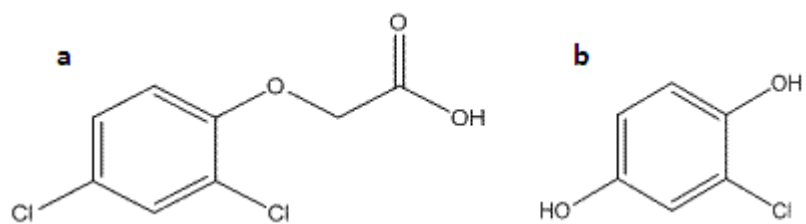


Figure 2 :

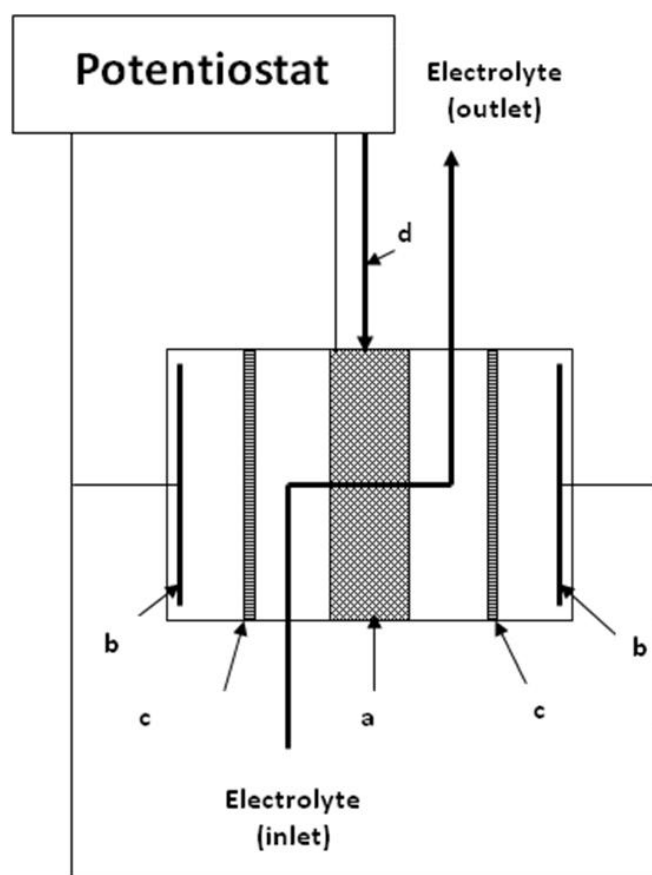


Figure 3 :

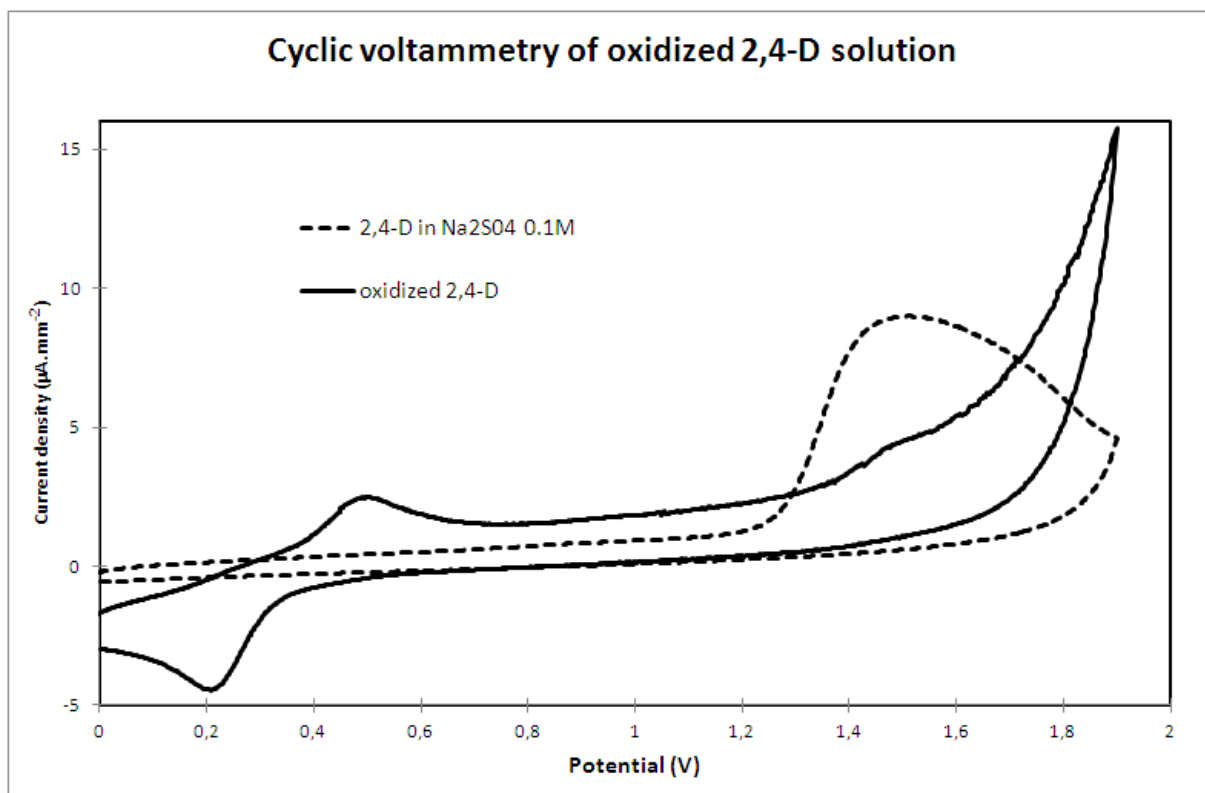


Figure 4 :

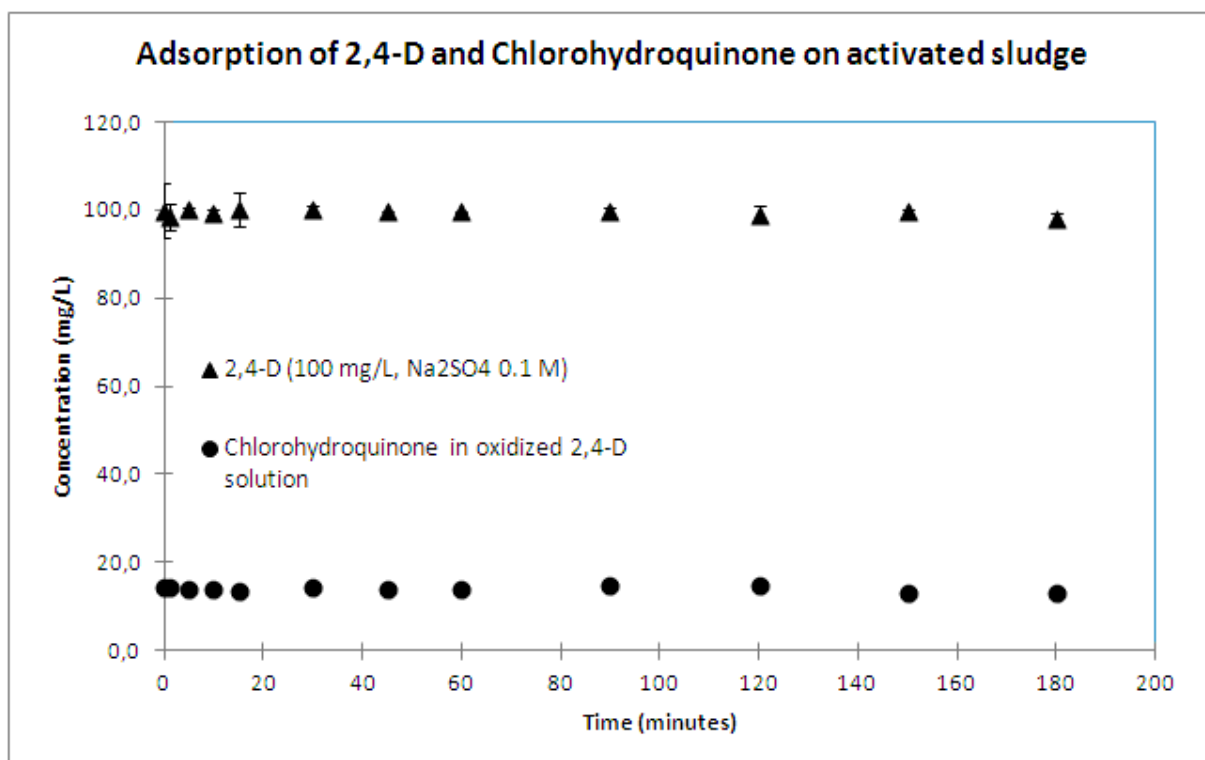


Figure 5a :

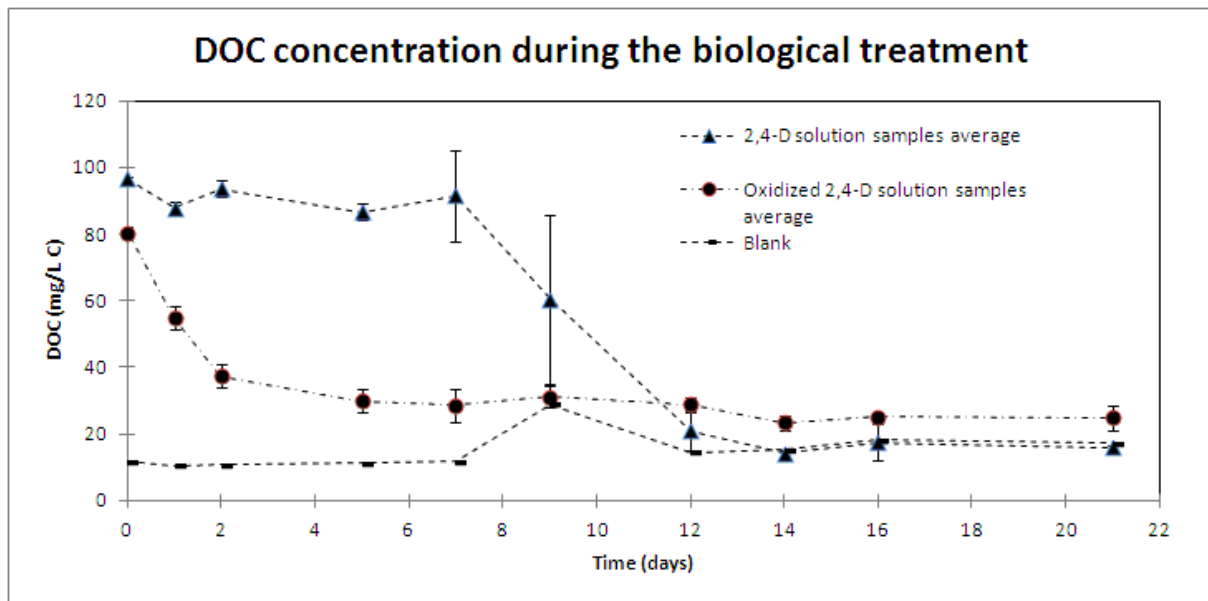


Figure 5b :

