Int. J. Electrochem. Sci., 9 **(2014)** 342 - 351

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Biological, Ionizing and Ultraviolet Radiation and Electrochemical Degradation of Chlorpyrifos Pesticide in Aqueous Solutions

I. Ben Salem[1], *M.Errami*[2], *M. Mezni*[3], *R. Salghi*[2,*], *Eno. E. Ebenso*[4,*], *B. Hammouti*[5], *S. Fattouch*[6], *F. Raboudi*[7]

[1]Research Unit, Application of Nuclear Techniques in the Fields of Health, Agriculture, and Environment, National Centre for Nuclear Science and Technology (CNSTN), Sidi Thabet Technopark, 2020 Ariana, Tunisia
[2] Ecole Nationale des Sciences Appliquées, Equipe Génie de l'Environnement et de Biotechnologie, B.P 1136, 80000 Agadir, Morocco.
[3] NMR-laboratory, National Institute of Research and Physical-Chemical Analysis, Technopark Sidi-Thabet, INRAP
[4]Material Science Innovation & Modelling (MaSIM) Focus Area, Faculty of Agriculture, Science and Technology, North-West University (Mafikeng Campus), Private Bag X2046, Mmabatho 2735, South Africa
[5] LCAE-URAC18, Faculté des Sciences, Université Mohammed Premier, BP 4808, Oujda,Morocco.
[6] LIP-MB, INSAT, University of Carthage, Tunisia.
[7] UR - GIRC, Faculté des Sciences de Tunis, University El Manar, Tunisia
[*]E-mail: r.salghi@uiz.ac.ma; Eno.Ebenso@nwu.ac.za

Received: 22 September 2013 / *Accepted:* 7 November 2013 / *Published:* 15 November 2013

Laboratory experiments were carried out on the effect of the electrochemical degradation, biological removal, ultraviolet and ionizing radiation of chlorpyrifos (O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate) as model compounds of insecticides in aqueous solutions. The percentage removal of chemical oxygen demand (COD) measurement during the different treatment showed a significant increase of 64%, 15.66%, 11.84% and 19.12% respectively by electrochemical oxidation at optimized conditions (70 mA.cm^{-2} at 25°C), biological removal by C.metallidurans, ionizing radiation (50 kGy) and UV radiation. The, liquid state 13C-nuclear magnetic-resonance (NMR) confirmed the hydrolysis of the chloropyrifos aliphatic chain after ionizing radiation and UV treatment. The same trend was observed and confirmed by the 1H-NMR. The biological degradation of chloropyrifos by C.metallidurans showed a completely removal of aliphatic chain. This result can be a potential for combined use of electrochemical technique to degrade the toxic compound to intermediate compound as first step to be next bio-assimilated and eliminated by bacteria.

Keywords: Electrochemical treatment, chloropyrifos; biological removal, C.metallidurans.

1. INTRODUCTION

The use of acutely toxic pesticides associated with a weak or absent legislative framework regulating pesticide and mycotoxins use is one of the major reasons for the high incidence of poisoning in some developing countries [3]. Additional factors such as lack of information, low literacy, and education levels of the rural population, poor and inadequate working conditions, inadequate protection during pesticide application, and inappropriate spraying technology have also been shown to play important roles in the intoxication scenario [2-3]. A pesticide may be a chemical substance or biological agent used against pests including insects, plant pathogens, weeds, mollusks, bird, mammals, fish, nematodes (roundworms) and microbes that compete with humans for food, destroy property, spread disease or are a nuisance. Many pesticides are poisonous to humans [1]. Organophosphorus and carbamate compounds are rapidly absorbed through the respiratory tract and through the digestive route, and to a lesser extent through the skin. After absorption, these compounds act by inhibiting the action of esterases, especially of acetylcholine esterase's, following the interaction with the hydroxyl group of serine, which may determine: accumulation of acetylcholine which stimulates muscarinic and nicotinic receptors, increase cholinergic activity, and induce paralysis and death [4]. Chloropyrofos herbicides O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate are used worlwide on a large scale for weed control on fruit, cereal crops, grasslands and lawns. These compounds resist biodegradation and have potential toxicity towards humans and animals [4]. Various innovative technologies have been proposed for the removal of pesticides namely electrochemical oxidation, ionizing and ultraviolet radiation, bioremediation and thermal desorption. They are neither cost effective, nor ecofriendly, nor involving low concentrations. Among these technologies, the electrochemical processe, constitute the emergent methods for the degradation of pesticides [5–7]. These methods are environmentally friendly and they do not form new toxic wastes. In anodic oxidation, organic pollutants are directly destroyed by reaction with hydroxyl radical (HO•) formed at the anode surface from water oxidation [5–10]:

$$H_2O \rightarrow HO\bullet + H^+ + e- \quad (1)$$

In addition, the use of ionizing and ultraviolet radiation has great ecological and technological advantages. Ionizing radiation degrades aromatic organic compounds, generating substances that are easily biodegraded [25]. Thereafter, one promising treatment method is to exploit the ability of microorganisms to remove pollutants from contaminated sites, an alternative treatment strategy that is effective, minimally hazardous, economical, versatile and environment-friendly, is the process known as bioremediation [26]. The aim of this study is to degrade the organophosphorus insecticide chloropyrifos by electrochemical oxidation, ionising UV radiation and biological degradation

2. EXPERIMENTAL METHODS

2.1. Electrolytic system

Electrochemical measurements were performed using a computer controlled by Potentiostat/Galvanostat model PGZ 100 associated to "Volta-Master 4" software. A conventional

three electrodes cell (100 cm^3) thermo regulated glass cell was used (Tacussel Standard CEC/TH). The anode was a square plate of BDD electrode with effective surface area of 1 cm^2, whereas the cathode was a platinum electrode, and the gap between electrodes was 1.5 cm. A saturated calomel electrode (SCE) was used as a reference. Galvanostatic electrolysis was carried out with a volume of 75 cm^3 aqueous solution of initial COD_0 (2498 mg/L). The range of applied current density was 30 to 70 mA/cm^2 and samples were taken, at predetermined intervals during the experiment, and submitted for analysis. All tests have been performed at different temperature in magnetically stirred and aerated solutions. In all cases sodium chloride was added to the electrolytic cell, at different concentrations. The chemical oxygen demand (COD) is measured according to the standard methods for examination of wastewater [8]. The Chemical Oxygen Demand (COD) values were determined by open reflux, a dichromate titration method. All chemicals used in the experiments were of analytical pure grade and used without further purification. The sodium chloride used was of analytical-reagent grade and was obtained from Aldrich (Spain).

2.2. Chemicals

Chlorpyrifos-ethyl (Fig.1) is an active ingredient of plant protection product which has an insecticidal effect, which belongs to the chemical family of organophosphates. Chlorpyrifos-ethyl formulation is commercially available in the Duracid 5G.

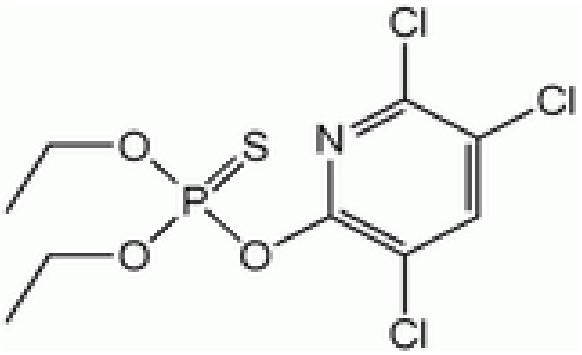

Figure 1. Chemical structure of Chlorpyrifos(O,O-Diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate)

2.3 Chlorpyriphos ethyl toxicity for Cupriavidus metallidurans CH_{34}.

Three separate cultures (3 biological replicates) of Cupriavidus metallidurans CH34 in mid exponential growth phase (OD600 = 0.5 or ~ 5.108 colonies forming units per milliliter (CFU/ml)) were used. The bacterial cells were harvested and the pellets were diluted in 100 ml of liquid broth (LB) supplemented with 6 mg of chloropyrifos. After 2 days of incubation at 30 °C, the solutions were centrifuged 1 min at 9000 rpm to remove cells, and supernatant of each solution was collected for NMR analysis. These supernatant samples were filtered (filter with 45 μm).

2.4. Effect of process irradiation on chlorpyriphos ethyl degradation

The Tunisian gamma irradiation facility (at Sidi Thabet) is designed for the preservation of foodstuff and sterilisation of medical devices. The source consists of eight encapsulated 60Co pencils with a diameter of 9.7 mm and an overall length of 452 mm. The starting activity of the source was 99.162 kCi. The installation is equipped with a stainless steel telescopic source rack that allows obtaining a linear source of approximately 900 mm height. The source pencils are distributed circularly on a diameter of 140 mm for the upper source rack and of 80 mm for a lower one. The source rack comprises 20 housings allowing sources loading for several years. These sources are stored in dry condition in a cylindrical shield container in which they were transported. Three independent solution of chloropyrifos ethyl (6 mg / 100 ml) were exposed to gamma radiation dose of 50 kGy at a dose rate of 22.21 Gy/min and at room temperature (27±2 °C).

2.5. UV-treatment

Three independent solutions of chloropyrifos (6 mg / 100 ml) were incubated for two hours at 27°C under UV treatment at 366 nm using a UV lamp (CAMAG, Wilmington, NC, USA). The tubes were placed 5 mm from the UV lamp.

2.6 Nuclear Magnetic Resonance (NMR)

Nuclear Magnetic Resonance (NMR) spectra were recorded using CDCl3 as a solvent on Bruker DPX-300 spectrometer capable of producing 300 MHz Radio Frequency (RF). The chemical shifts in δ (ppm) were recorded from Tetramethyl silane (TMS) for ^{13}CNMR. FTIR spectrum of the compound was recorded using Perkin Elmer Spectrum 100 instrument.

3. RESULTS AND DISCUSSION

3.1. Effect of supporting electrolytes

The investigation of the mediator concentration effect has been performed in the range 0.5–2 g /L for NaCl. As shown in Fig. 2, the electrochemical degradation of the pesticide is achieved at reasonable rates only in the presence of the mediator and is higher at higher NaCl concentrations, up to values around 2 g/L. Further increase, above this limit, causes and inversion of the trend. Possibly, when the chloride concentration becomes sufficiently high, a decrease of the anode potential takes place, due to the potentiostatic buffering by the chlorine evolution reaction.

According to the literature, the increased rate of pesticide removal is due to the formation of chlorine gas (Cl_2) and hypochlorite (ClO^-), which are both powerful oxidising agents. Chlorine gas is formed at the anode according to Vlyssides [6].

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (2)$$

The subsequent reaction of Cl_2 with HO^- formed at the cathode results in the formation of hypochlorite (ClO^-):

$$Cl_2 + 2HO^- \rightarrow H_2O + ClO^- + Cl^- \quad (3)$$

Subsequently Cl_2 and ClO^- react to oxidise the organic in solution, resulting in an indirect oxidation mechanism.

Obviously, the uncertainties inherent in the experimental data do not allow to distinguish between results obtained for slightly different sodium chloride concentrations: a more detailed discussion could be carried out only on the basis of repeated data, which would require a huge experimental work.

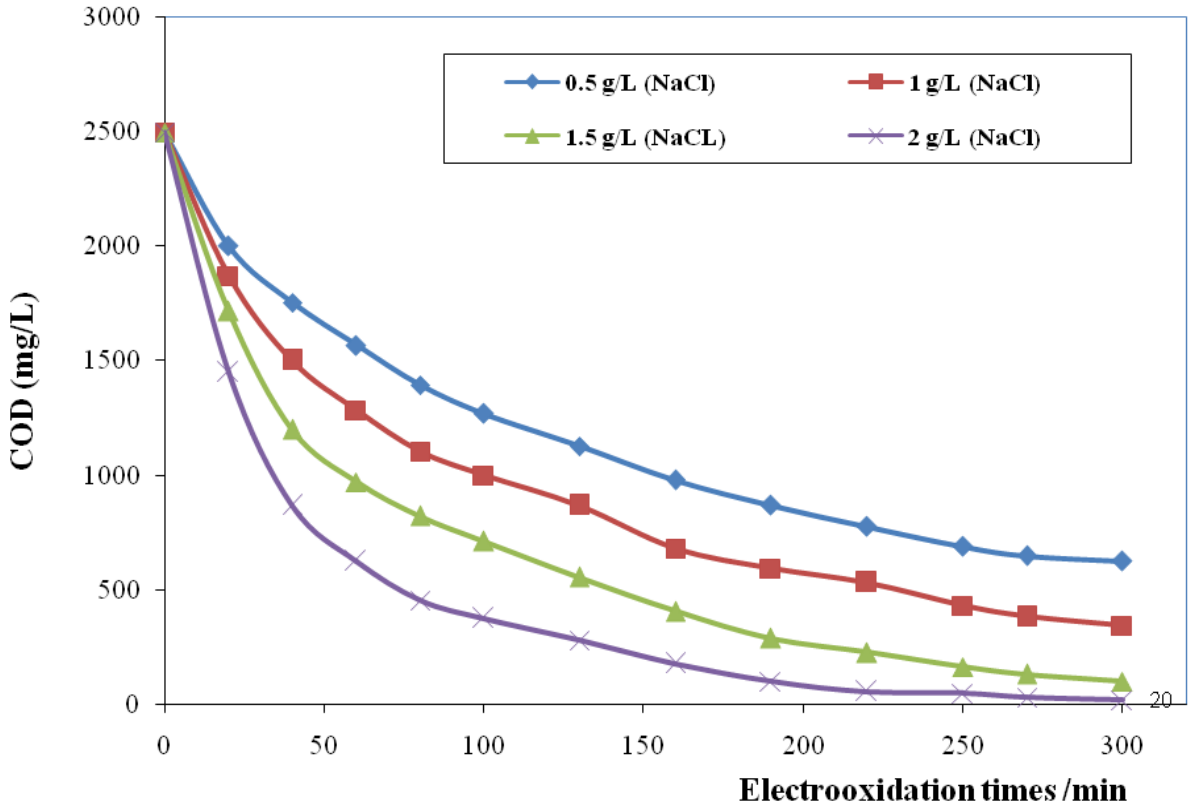

Figure 2. Evolution of COD during the galvanostatic electrolyses of wastes polluted with 100 mg L^{-1} of chlorpyrifos pesticide under effect of NaCl concentration. Operating conditions: T=25°C; I=70 mA. cm^{-2}.

3.2. Effect of current density

The influence of the current density on the COD removal during the electrochemical oxidation of chloropyrifos at the anode is shown in Fig. 3. chlorpyrifos degradation and COD removal increase with increasing the applied current density up to 70 mA cm-2 by using BDD electrodes. After 2 h time of electrolysis, the COD percent removal increased from 45% to 64% when the current density increased from 30 to 70 $mA.cm^{-2}$. This behavior indicates that in these experimental conditions, the oxidation of chloropyrifos is completely under mass transport control and an increase of the applied

current favors only the secondary reaction of oxygen evolution [11, 12]. This was confirmed by the fact that the COD (mg/L of O_2) decreased with the current density. The decay of COD concentration exhibits an exponential behavior with all the applied current indicating first-order reaction kinetics for the oxidation reaction [7-9, 12]. For all the concentrations the COD removal follows pseudo first-order kinetics and the apparent rate constants were 114×10^{-4}, 83×10^{-4} and 45×10^{-4} min^{-1} for the chloropyrifos concentrations of 30, 50 and 70 mA/cm^2, respectively.

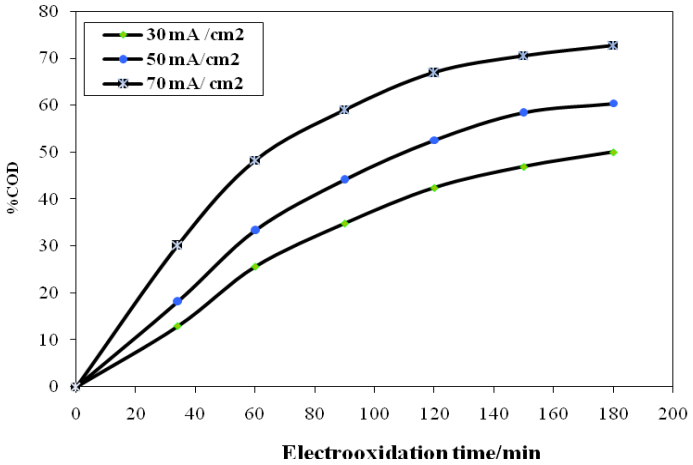

Figure 3. Influence of the applied current density on the trends of % COD electrolysis of chloropyrifos (C_0 = 100 $mg.L^{-1}$) using a $1cm^2$ BDD anode.

The effect of ionizing, ultraviolet radiation and biological degradation of chloropyrifos was investigated. The COD after two hours of treatment was measured on the same conditions. The results obtained in Fig. 4 showed a significant decrease of DCO comparing to the control. The percentage of COD decrease was respectively 15.66%, 11.84% and 19.12% for the biological removal by *C.metallidurans*, the ionizing radiation (50 kGy) and the unltraviolet radiation.

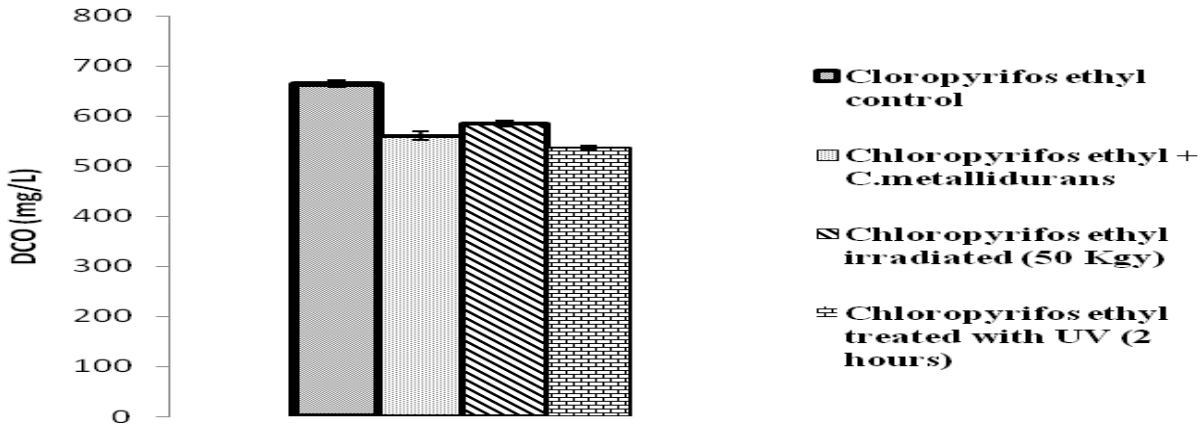

Figure 4. COD measurement of 6 mg /L of chloropyrifos after ionizing radiation, UV treatement, and biological treatment with *C.metallidurans* NMR analysis of chloropyrifos hydrolysis

The liquid state of ^{13}C-NMR chloropyrifos was shown in Fig. 5. Only one peak was recorded at 50 ppm in control sample. Using ionizing radiation at 50 kGy and UV, the ^{13}C-NMR irradiated chloropyrifos showed a new spectra in aliphatic region at approximatively 65 ppm and 30 ppm for ionizing radiation and UV treatment. This result confirms the hydrolysis of aliphatic chain of the chloropyrifos.

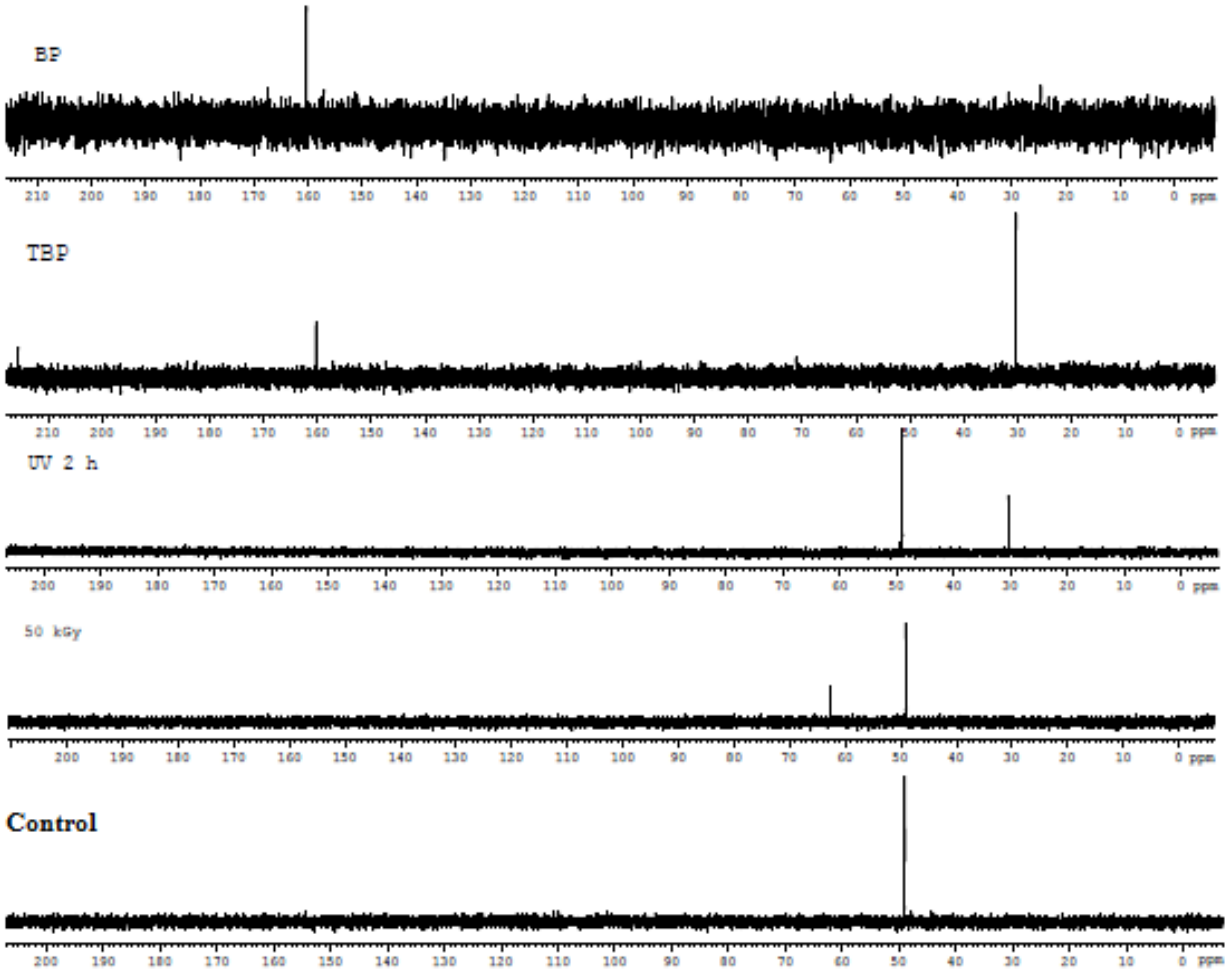

Figure 5. ^{13}C NMR spectra of chlorpyrifos ethyl. TBP : Control with *C.metallidurans* and chloropyrifos at 4°C. BP : *C.metallidurans* and chloropyrifos ethy incubated at 30°C.

In addition, the analysis liquid state ^{1}H-NMR spectra in Fig. 6 confirmed the hydrolysis of the chloropyrifos aliphatic chain after ionizing radiation and UV treatment. Using *C.metallidurans* bacterium to degrade chloropyrifos, the result obtained in Fig. 5 showed a completely disappear of 30 ppm peak corresponding to the aliphatic chain. This result is in accordance with the results obtained with ^{1}H-NMR (Fig. 6). The disappear of this peak is probably due to the growth of the bacterium *C.metallidurans* and it's using as carbon source.

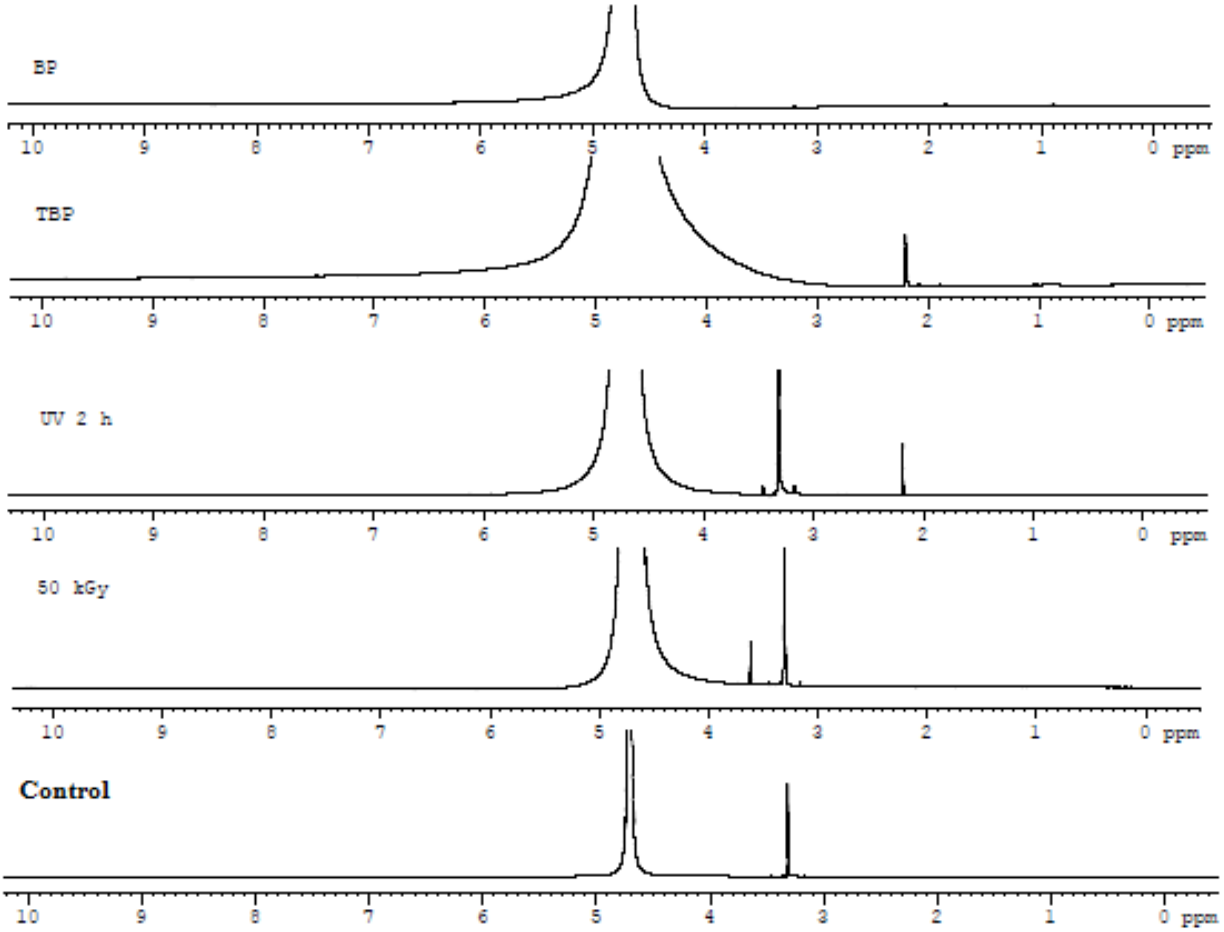

Figure 6. [1]H NMR spectra of chlorpyrifos. TBP : Control with *C.metallidurans* and chloropyrifos at 4°C. BP : *C.metallidurans* and chloropyrifos ethyl incubated at 30°C

4. CONCLUSION

Various research groups around the world have developed physical-chemical and biological techniques to eliminate or degrade pollutants. In this work, we compare the efficiency of electrochemical oxidation, the UV and ionizing radiation and the biological removal by *C.metallidurans* technics to degrade chloropyrifos. The COD of chloropyrifos sample presented a significant reduction of 64% when electrochemical oxidation was applied, 15.66% by biological removal by *C.metallidurans*, 11.84% ionizing radiation (50 kGy) and 19.12% by UV radiation. Thus, the electrochemical oxidation is considered the most efficient method for the degradation of this toxic comparing to the other treatment method. However, the decomposition products were still present in the medium. Several research groups have studied the possibility of using combined technology which gave excellent results for the removal of these toxic species present in treated water. One of promising technique is biological degradation. *C.metallidurans* is the best bacterium to deal with heavy metals and organic toxic. The results obtained by [13]C NMR and confirmed by [1]H-NMR showed a completely removal of aliphatic chain of chloropyrifos by *C.metallidurans*. This result can be a potential for

combined use of electrochemical technique to degrade the toxic compound to intermediate compound as first step to be next bio-assimilated and eliminated by bacteria.

ACKNOWLEDGEMENTS
The authors wish to thank the Tunisian-Moroccan Cooperation for Scientific Research and Technology 12/TM/24 for supporting this work.

References

1. G. H. Coman, M. Pop, N. Taus, A. Brezina, M. Florescu, *Revista De Terapeutica Farmacologie Si Toxicologie Clinica*. 4(4) (2000) 48.
2. A.K. Hurtig, M. San Sebastian, A. Soto, A. Shingre, D. Zambrano, W. Guerrero, *Environ. Health*. 58 (2003) 223.
3. S. I. Karlsson, *Environment*. 46 (2004) 22.
4. M. Mijanovic, A. Zaciragic, *Bosn. J. Basic Med. Sci*. 6(3) (2006)54.
5. S.K. Hoar., A. Blair, F.F. Holmes, J.R. B. Robel, R. Hoover, H. Fraumen, *J. Am. Med. Assoc*. 256 (1986)1141.
6. A. Vlyssides, E. M. Barampouti, S. Mai, D. Arapoglou, A. Kotronarou, *Environ. Sci. Technol.* 38 (2004) 6125.
7. R. Salghi, M. Errami, B. Hammouti, L. Bazzi. Pesticides in the Modern World-Trends in Pesticides Analysis. Edited by Margarfita Stoytcheva . Publisheb by In Tech (2011) 71.
8. H. Bouya, M. Errami, R. Salghi, A. Zarrouk, A. M. Assouag, H. Zarrok, A. Chakir, B. Hammouti, S. S. Al-Deyab, *J. Chem. Pharm. Res.* 4(7) (2012) 3468.
9. M. Erram, R. Salghi, A. Zarrouk, M. Assouag, H. Zarrok, O. Benali, El. Bazzi, B. Hammouti S. S. Al-Deyab, *J. Chem. Pharm. Res.* 4(7) (2012) 3518.
10. M. Errami, O. ID El Mouden, R. Salghi, M. Zougagh, A. Zarrouk, B. Hammouti, A. Chakir, S.S. Al-Deyab, M. Bouri, *Der Pharma Chemica*. 4(1) (2012) 297.
11. O. Id El Mouden, M. Errami, R. Salghi, A. Zarrouk, H. Zarrok, B. Hammouti, *Journal of Chemica Acta*. 1(1) (2012) 44.
12. M. Errami,R. Salghi, A. Zarrouk, M. Assouag, H. Zarrok, O. Benali, El. Bazzi, B. Hammouti, S. S. Al-Deyab, *J. Chem. Pharm. Res.* 4 (2012) 3518.
13. O. ID El Mouden, M. Errami, R. Salghi, A. Zarrouk, M. Assouag, H. Zarrok, S.S. Al-Deyab, B. Hammouti, *J. Chem. Pharm. Res.* 4 (2012) 3437.
14. M. Errami, R. Salghi, M. Zougagh, A. Zarrouk, El. Bazzi, A. Chakir, H. Zarrouk, B. Hammouti, L. Bazzi, *Res Chem Intermed*. 39 (2013) 505.
15. A.Vlyssides, D. Arapoglou, S. Mai, E. M. Barampouti, *Int. J. Environ. Pollut*. 23 (2005) 289.
16. A. Vlyssides, D. Arapoglou, S. Mai, E. M. Barampouti, *Chemosphere*. 58 (2005) 439.
17. M. Mamián, W. Torres, F. E. Larmat, *Port. Electrochim. Acta*. 27 (2009) 371.
18. D. Arapoglou, A, Vlyssides, C. Israilides, A. Zorpas, P. Karlis, *J. Hazard. Mater*. 98 (2003) 191.
19. E. Brillas, M. A. Banos, M. Skoumal, P. L. Cabot, J. A. Garrido. R. M. Rodriguez, *Chemosphere*. 68 (2007) 199.
20. G. R. P. Malpass, D.W. Miwa, S.A.S. Machado, A. J. Motheo, *J. Hazard. Mater*. 137(2006) 565.
21. C. A. Martínez-Huitle, A. Battisti, S. Ferro, S. Reyna, M. Cerro-López, M. A Quiro, *Environ. Sci.Technol.* 42 (2008) 6929.
22. M. Panizza, I. Sirés, G. Cerisola, *J. Appl. Electrochem*, 38 (2008) 923.
23. I. Sirés, E. Brillas, G. Cerisola, M Panizza, *J. Electroanal. Chem.* 613 (2008) 151.
24. E. Brillas, B. Boye, I. Sirés, J. A. Garrido, R. M. Rodriguez, C. Arias, P. L. Cabot., C. H. Comninellis, *Electrochim. Acta*. 49 (2004) 4487.

25. W. J. Cooper, M. G. Nickelsen, D. E. Meacham, T. D Waite, C. N Kurucz, *J. Environ. Sci. Health. A*. 27(1) (1992) 219.
26. S. D. Finley, L. J. Broadbelt, V. Hatzimanikatis, *BMC Systems Biology*. 4 (7) (2010) 4.

© 2014 by ESG (www.electrochemsci.org)