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Combined electrochemical and biological treatment for pesticide degradation – Application to phosmet

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ABSTRACT: The aim of this study was to determine the feasibility of coupling an electrochemical process with a biological treatment in order to degrade phosmet, an organophosphorous pesticide. The absence of biodegradability of phosmet by *Pseudomonas fluorescens* and activated sludge was verified in our operational conditions. So, a conventional biological treatment is not appropriate for phosmet polluted effluents. Electrochemical behavior of phosmet was studied by cyclic voltammetry and the feasibility of an electrochemical pre-treatment was thus demonstrated. Preliminary results with activated sludge showed a diminution of 26% for COD (chemical oxygen demand) measured when the electrolyzed solution was used as the sole carbon and nitrogen sources. When glucose and ammonium were added as supplementary carbon and nitrogen sources, the COD diminution reached 34% after 79 h of culture. This study demonstrates the feasibility of an electrochemical pre-treatment prior to biotreatment.

Key words: Electrochemical process, Biological degradation, Hybrid process, Recalcitrant compounds, Organophosphorous pesticides

INTRODUCTION

In intensive agricultural practice, repeated use of pesticides may result in more frequent occurrence of agrochemicals in raw water resources. Some effluents of agricultural activities (unused treatment solutions, spray, machine and pesticide container washing) contribute to water resource pollution. Pollution of water with biorecalcitrant organic compounds is becoming increasingly worrying and pesticides removal from environment is now a great challenge for the scientific community. Physical techniques such adsorption, flocculation, electro-flocculation, membrane processes can be applied for the removal of recalcitrant pollutants (Auriol *et al.*, 2006; Gong *et al.*, 2010; Hassani *et al.*, 2008; Robinson *et al.*, 2001; Vandevivere *et al.*, 1998). These processes are not destructive; they only allow to transfer pollution to another phase and then the main drawback is the need to quite costly regeneration and post-treatment processes (Arslan *et al.*, 2000; Chaudhuri & Sur, 2000; Stock *et al.*, 2000).

Another way to remove this pollution is to consider physico-chemical processes which are destructive. Ozonation (Chelme-Ayala *et al.*, 2010; Chiron *et al.*, 2000) and advanced oxidation processes (AOP) (Badawy *et al.*, 2006; Chiron *et al.*, 2000; He, 2008; Oppenländer, 2003) are widely documented but the production of ozone or free hydroxyl radicals are expensive in comparison with biological treatments. However, in case of recalcitrant compounds such as pesticides, conventional treatment involving activated sludge appears inefficient (Badawy *et al.*, 2006; Chiron *et al.*, 2000). To reduce operational cost, the coupling of a physico-chemical process and a biological treatment could be a solution. Several studies dealt with this kind of integrated processes (Pulgarin *et al.*, 1999; Scott & Ollis, 1995).Most of the integrated processes involved activated sludge (Muñoz *et al.*, 2006; Oller *et al.*, 2007; Sarria *et al.*, 2002). Pure cultures have also been considered (Ballesteros Martin *et al.*, 2008; Basha *et al.*, 2009; Chan *et al.*, 2004). These physico-chemical processes constitute a pre-treatment in order to increase the biodegradability of the effluent and/or to reduce toxicity (De La Rochebrochard d'Auzay *et al.*, 2007). In this study, the degradation of phosmet, an

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organophosphorous fungicide used for the treatment of foliar soil and seed-borne diseases, was studied.

Few studies focused on the biodegradation of phosmet in aqueous media. Biodegradation of a 100 ppm solution of phosmet at 30°C has been studied (Crowe *et al.,* 2007) in shake flasks with or without glucose, an additional carbon source and in presence of trace elements, $(NH_4)_2SO_4$, K_2HPO_4 and Na_2HPO_4 (Bano & Mussarat, 2004). When *Pseudomonas fluorescens* and *Enterobacter agglomerus* adapted to phosmet and isolated from lowbush blueberries (*vaccinum angustifolum)* were added to growth culture, results showed a preferential use of phosmet as energy source instead of glucose with a biodegradation yield less than 40 % after 72 h of culture. Moreover, the authors noticed hydrolysis of phosmet at neutral pH during the biotreatment. These data did not permit to conclude on a total mineralization of this organophosphorous pesticide after a biological treatment. Chemical oxidation and photochemical processes (Crowe *et al.,* 2006) have been carried out for the degradation of the residual phosmet on lowbush blueberries. Photochemical processes included UV/H_2O_2, chlorine/UV and $O_3/H_2O_2/UV$ while chemical processes also involved hydrogen peroxide, ozone and chlorine. Solution of ozone (1 ppm), hydrogen peroxide (1 %) and chlorine (100 mg/L) were pulverized on fruits with a contact time of 60 s prior to blast freezing at –25°C for 10 min to simulate industrial conditions. Chemical processes involving chlorine and ozone showed the best results, namely 57.7% and 46% of phosmet degradation for an initial concentration of 45 mg/L. The presence of phosmet-oxon, a toxic by-product was not highlighted during these treatments. The electrochemical behavior of phosmet was examined using differential pulse polarography for concentrations between $1.2\ 10^{-5}$ and $1.89\ 10^{-9}$ mol/L in acidic medium and results showed that this compound can be reduced on mercury (Sreedhar *et al.,* 1997). These encouraging results let to investigate the electrochemical degradation of phosmet as a pre-treatment prior to the biodegradation. Indeed, one way to minimize the operational cost is to develop an efficient pre-treatment process that reduces the toxicity and/or increases the biodegradability of the effluent containing the target compound before a low cost biological treatment. In this work, we report that the electrolyzed solution of phosmet can be used as a substrate for a microbial culture with the objective of a total mineralization. Electrolysis were carried out using a graphite felt working electrode with a high specific area in a flow electrochemical cell. Biological treatment is performed with activated sludge.

MATERIALS & METHODS

Phosmet (Imidan, $C_{11}H_{12}NO_4PS_2$) (Fig. 1) was supplied by Sigma Aldrich, glucose by Merck. Since phosmet was not soluble in aqueous media, ethanol (33 % v/v) was added to the solution to solubilize the organic compound.

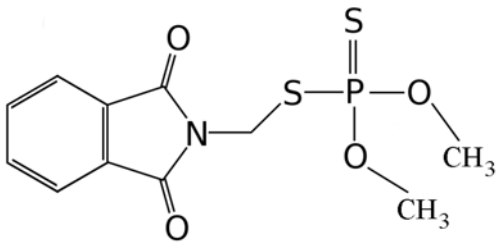

Fig.1. Chemical structure of phosmet

Graphite felt used as working electrode was purchased from Le Carbone Lorraine (RVG 4000). Its specific area, measured by the BET method is 0.7 m^2/g. Electrochemical pre-treatment was performed in a flow cell presented in Fig.2. The compartment containing the working electrode (graphite felt) was separated from the two interconnected stainless steel counter-electrode compartments by cationic exchange membranes (Ionac 3470). A good homogeneity of the potential distribution in the three dimensional working electrode was obtained when the felt was located between two counter-electrodes (Moinet, 1994). The reference electrode (Saturated Calomel electrode- SCE) was positioned in the middle of the felt. The potential control was performed using an e-daq potenstiostat linked to e-corder 401 converter. The electrolyte solution (0.05 mol/L Na_2SO_4 + 100 mg/L phosmet) percolated the porous electrode with a constant flow rate monitored by a Gilson minipuls 2 peristaltic pump (1.5 mL/min).

Stock cultures of *Pseudomonas fluorescens* were maintained at -18°C in the following medium (g/L): glycerol, 200, yeast extract, 15 and glucose, 10. Before culture, bacteria were reactivated by propagation in Petri dishes containing the following agarose medium (g/L): casein pancreatic peptone, 5, yeast extract, 3 and bacteriological agar type E, 15. The pH of the propagation medium was adjusted to 7.0. Bacteria were cultivated during 24 h at 25°C. Before use and to avoid any residual nutrient from the propagation medium, bacteria were collected at the surface of the agarose gel and resuspended in a saline solution (9 g/L NaCl); after less than 4 h this suspension was used to inoculate culture media.

Activated sludge issued from a local wastewater treatment plant was used in this study. It was washed at least five times with water and centrifuged to remove any residual carbon and mineral source. The mineral supplementation used in this work contained:
Inorganic phosphates: 25 mmol/L of KH_2PO_4 and 25 mmol/L of $NaH_2PO_4.H_2O$, and a solution of EDTA (EthyleneDiamineTetraAcetate) (584 mg/L) chelated trace elements (mg/L) (Trinci, 1969): Mg, 25; Fe, 20; Ca, 18; Zn, 4.5; Mo, 2; Cu, 1.3.

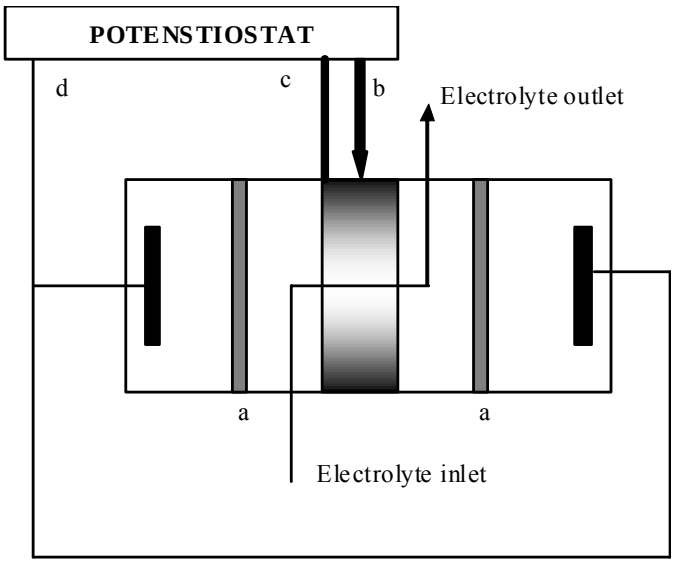

Fig. 2. Schematic diagram of the percolation cell: a: cationic membranes; b: saturated calomel electrode (SCE); c: working electrode (disc of graphite felt: 10 mm diameter, 10 mm thickness); d: auxiliary counter electrodes (carbon-graphite plates)

Batch cultures were carried out in 250 mL Erlenmeyer flask containing 100 mL of mineral medium with a commercial strain of *Pseudomonas fluorescens* Migula 1895AL or activated sludge (1 g/L). All flasks were incubated at 30°C on a rotary shaker at 350 rpm agitated speed. All tests were conducted in duplicates.

Electrochemical analyses of phosmet were performed using a conventional three electrodes cell with a vitreous carbon electrode (7 mm^2) as working electrode and a platinum wire as counter electrode. All the electrode potentials were measured with respect to a saturated calomel electrode (SCE) located near the working electrode. The experiments were performed under a nitrogen atmosphere and ambient temperature. Voltammograms were obtained by cyclic voltammetry (100 $mV.s^{-1}$) using an e-daq potenstiostat linked to e-corder 401 converter.

When electrolysis was carried out in presence of ethanol, products were extracted from the solution with dichloromethane, after saturation with NaCl. Organic phase was dried with $MgSO_4$ and dichloromethane was then evaporated.

Solid phase was analyzed by Thin Layer Chromatography on silica plate with a dichloromethane / ethanol mixture (90/10 %) as mobile phase. UV and $KMnO_4$ were used as developers at 254 nm.

Silica column (about 50 g) was used to separate and purify the various by-products from the electrolytic reaction. The mobile phase was a mixture of dichloromethane / ethanol (90/10 %).

The by-products from electrolysis, isolated on silica column, were analyzed by NMR proton (Brucker 200 MHz).

Chemical Oxygen Demand (COD) was measured by means of Test Nanocolor® CSB 160 from Macherey-Nagel (Düren, Germany).

Search for by-product was performed using a high performance liquid chromatography (HPLC) system involving a gradient pump WATERS™ 600 controller, an automatic injector WATERS™ 717 Plus and UV detector (230 nm) separation was performed by a WATERS™ C_{18} symmetry column (4.6 mm × 250 mm) and a methanol/water mixture (60% / 40%) as the mobile phase. Flow rate was set at 1 mL/min.

Bacterial growth (Cook, 1987)was turbidimetrically followed at a wavelength of 600 nm using a thermospectronic Helios γ Spectrophotometer (Bioblock, Illkirch, France).

RESULTS & DISCUSSION

Electrochemical behavior of phosmet was studied in three different media: acidic (H_3PO_4, NaH_2PO_4 0.25 mol/L and ethanol, pH of 2.2), basic (NaOH and ethanol) and neutral (Na_2PO_4 0.05 M and ethanol) media. In acidic medium, voltammograms obtained by cyclic voltammetry did not show a signal for the electrochemical reduction of phosmet. In basic medium, the reduction wave was not significant. However, in neutral medium, a distinct signal at –0.9 V/ECS was observed on the voltammogram as seen in Fig. 3. Advantageously, a pH of 6.25 for the neutral medium appears therefore adapted to a further utilization of the electrolyzed solution as growth medium for biodegradation experiments. Consequently, all the electrochemical experiments were performed in neutral medium.

Phosmet was first reduced at –1.3 V/SCE in a batch cell to check the feasibility of the electrochemical pre-

treatment. For a volume of 50 mL and after 4 hours of reaction, the peak at –0.9 V/SCE disappeared on cyclic voltammograms, showing that phosmet was entirely reduced on the working electrode. To treat higher volumes of effluent (for example 700 mL), a second technique was used: flow electrolysis. The high surface area of the porous electrode increases the contact between the working electrode and the electrolyte, decreasing the electrolysis time of the reaction. (8 hours for 700 mL). The phosmet was totally reduced without the need for solution recycling through the electrode, as shown by cyclic voltammetry. This result was confirmed by Thin Layer Chromatography and several products of degradation were highlighted. Proton NMR spectra of phosmet and the degradation products were performed after extraction by dichloromethane of the electrolyte medium. The comparison of both spectra showed clear differences. A diminution of the peak of the $-OCH_3$ group present in phosmet and proton-phosphorus coupling were noticed. The analysis of the crude product seems to show that phosmet-oxon, a very toxic derivative of phosmet, was not formed. Cyclic voltammogram of the crude product on vitreous carbon showed a signal in oxidation (Fig.4).If the reduction product was not metabolized during microorganisms culture, an alternative would be to perform a second electrolysis in oxidation to form new compounds, since the oxidation wave was not reversible. Electrolysis in oxidation could be carried out and oxidation products could be tested for bacterial growth.

A first study was carried out with pure culture of *Pseudomonas fluorescens*, a non pathogenic bacterium, largely used in xenobiotic bioremediation. A preliminary study was performed on the biodegradation of phosmet, namely in presence of additional carbon and nitrogen sources: ethanol, glucose (100 mg/L) and NH_4Cl (75 mg/L) respectively. Bacterial growth was followed by means of turbidimetric measurements (600 nm). During culture, pH values remained constant, turbidity values initially very low remained constant showing no bacteria growth, moreover no consumption of ammonium was noticed. Results showed that a pure solution of phosmet can not be biodegraded by *Pseudomonas Fluorescens* in our operating conditions.Similar study was carried out with *Pseudomonas fluorescens* using the electrolyzed solution and in presence of additional carbon and nitrogen sources or without any additional carbon and nitrogen source. For both culture media, a slight pH decrease was observed during the first 40 hours of culture. This diminution coincided with an increase of the turbidity (Fig.5). revealing a slight bacterial growth. After 40 hours of culture, a stationary phase was reached and no decline phase was observed at the end of the culture. The mean peak area noticed for the crude products using HPLC did not decrease during microbial culture. The products were not biodegraded by *P. fluorescens*. Glucose and ammonium consumption by bacteria would explain the slight bacterial growth. From this, an absence of biodegradability of the products by *P. fluorescens* was shown, but also an absence of toxicity revealed by the slight bacterial growth.

These results were not satisfactory for the combination of an electrochemical pre-treatment and a biological treatment, owing to the absence of products consumption by bacteria and hence the absence of mineralization, even if the products from electrolysis were shown to be not inhibitory for *Pseudomonas fluorescens* growth. A second set of experiments were

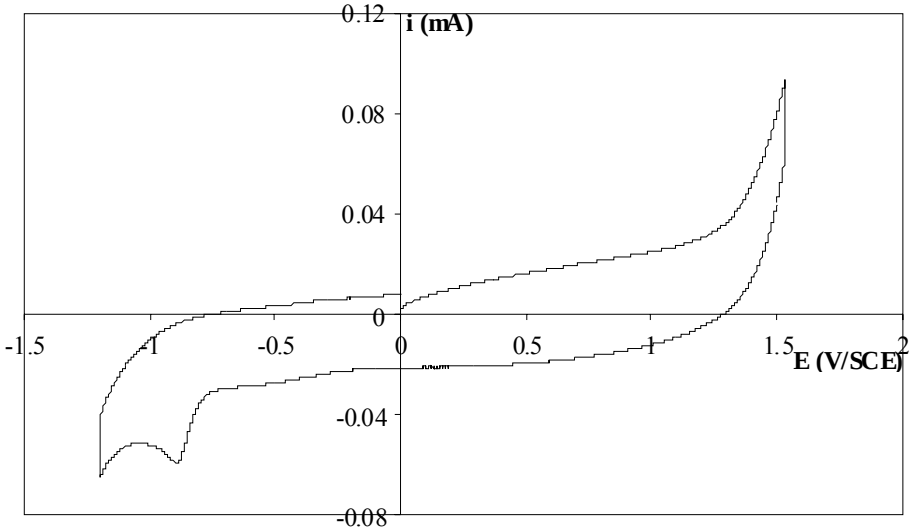

Fig. 3. Current-potential curve obtained by cyclic voltammetry (100 $mV.s^{-1}$) with a vitreous carbon electrode ($S = 3.2\ 10^{-6}\ m^2$), under nitrogen atmosphere and T = 298 K, of phosmet (100 $mg\ L^{-1}$) in neutral media (Na_2PO_4 0.05 mol/L and ethanol)

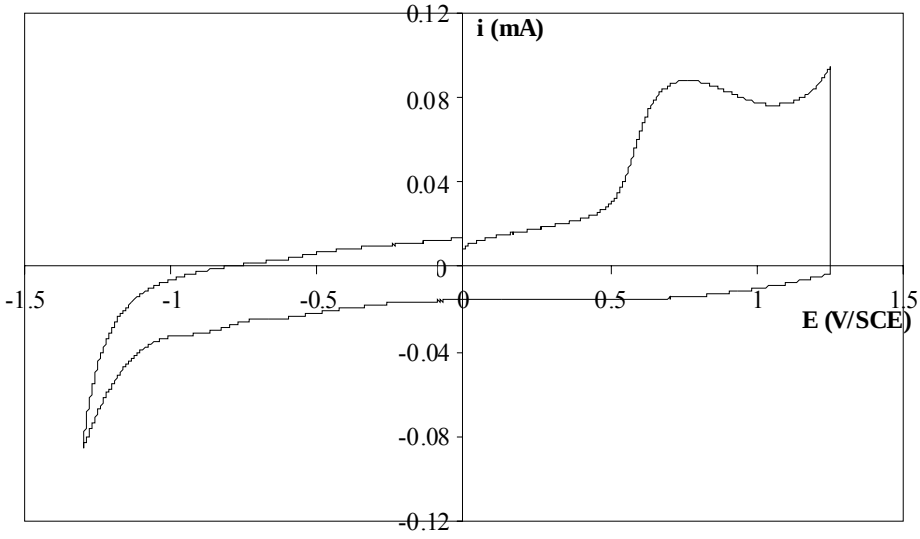

Fig. 4. Current-potential curve obtained by cyclic voltammetry (100 mV/s) with a vitreous carbon electrode (S = 3.2 10^{-6} m^2), under nitrogen atmosphere and T = 298 K, with electrolyzed solution in neutral media (Na_2PO_4 0.05 mol/L and ethanol)

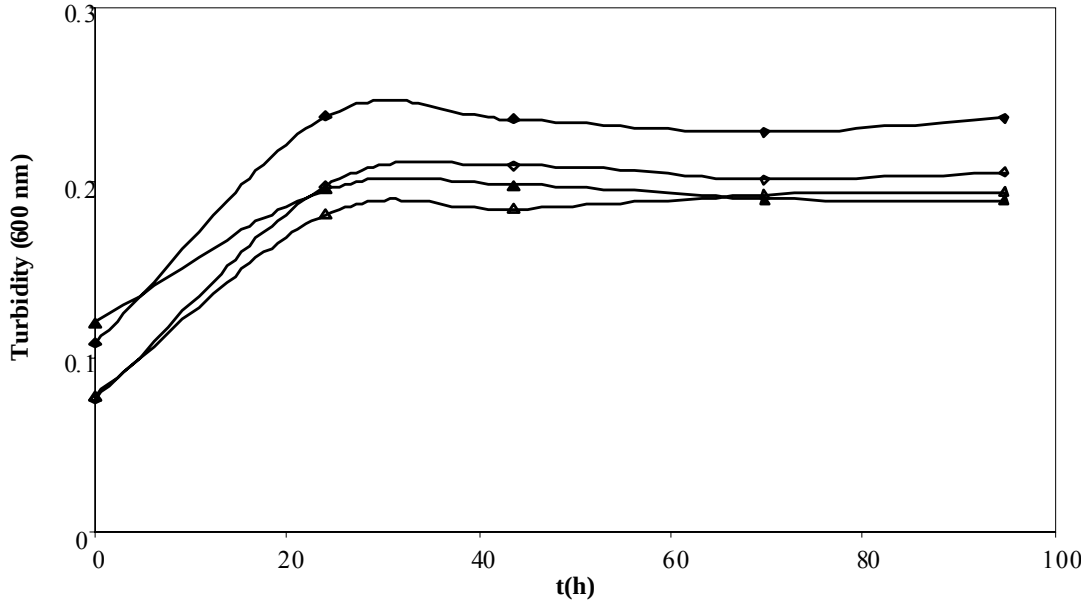

Fig. 5. Evolution of turbidity during culture of *Pseudomonas fluorescens* with electrolyzed solution; (◆ , —◇—): with glucose (100 mg/L) and NH_4Cl (75 mg/L); (▲ ,--△--): without additional carbon and nitrogen sources

carried out with activated sludge taken from a local municipal wastewater treatment plant. Without any additional carbon and nitrogen sources, COD values remained constant during culture on a solution of phosmet (30 mg/L), showing the absence of oxidation of the organic compound by activated sludge. In presence or not of additional carbon and nitrogen sources, a decrease of the pH was recorded during growth on the electrolyzed solution. Preliminary results showed a diminution of 26% for COD (chemical oxygen demand) measured after 79 h of culture when the electrolyzed solution was used as the sole carbon and nitrogen source. When glucose and ammonium were added as supplementary nutritional sources, the COD diminution reached 34 % in a similar culture time. These results confirmed an oxidation of the products of electrolysis by microorganisms, showing their consumption. These preliminary results are encouraging for the coupling of electrochemical and biological processes.

CONCLUSION

Electrochemical pre-treatment using a flow cell led to a total reduction of phosmet in neutral medium. Phosmet-oxon, a toxic derivative of the target compound seems not to be produced. The degradation of the electrolyzed solution with a pure culture of *Pseudomonas fluorescens* showed that the crude product can not be

used as substrate for bacterial growth. However, encouraging results were recorded with activated sludge. COD decrease throughout culture indicated an oxidation of the products and thus their assimilation by the microorganisms. Complementary studies about biodegradation of the electrolyzed solution are needed.

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