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Relevance of an electrochemical process prior to a biological treatment for the removal of an organophosphorous pesticide, phosmet

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ABSTRACT

The aim of this study was to examine the feasibility of coupling an electrochemical pre-treatment with a biological step in order to degrade phosmet, an organophosphorous pesticide. Preliminary biodegradation experiments showed that the target molecule was not assimilated by activated sludge. The pre-treatment consisted of potentiostatic electrolysis (−1.3 V/SCE) in a flow cell. After only one pass (1 mL min^{-1}), cyclic voltammetry with a vitreous carbon electrode showed a total phosmet reduction in neutral medium confirmed by thin layer chromatography, which also highlighted the presence of several by-products. H NMR spectra of the main by-product showed the absence of the aromatic ring, only the phosphorus part of phosmet has been identified and phosmet-oxon, a very toxic derivative, was not formed. Lower toxicity and higher biodegradability characterized the electrolyzed solution, EC_{50} value increased from 7% to 58% and BOD_5 value increased from 4 to 9 mg O_2 L^{-1} after electrolysis leading to an increase of the BOD_5 on COD ratio from 0.19 to 0.42 (limit of biodegradability, 0.4). These encouraging results were confirmed during activated sludge culture since an almost total mineralization of the electrolyzed solution was recorded (97%), confirming the feasibility of the proposed coupled process.

1. 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. This situation is becoming increasingly worrying so pesticides removal from environment is now a great challenge for the scientific community.

Some agricultural effluents can reach high levels of pesticides, almost 500 mg L^{-1} [1], like bottom tank farm; while the limit given by the French legislation is 0.5 μg L^{-1} in drinking water. Therefore, these effluents should be treated before their release into the environment.

Physical techniques can be used to remove recalcitrant pollutants [2–4]. Among them: adsorption, flocculation, electroflocculation, reverse osmosis, ultrafiltration, coagulation have been applied. The main drawback is the need to quite costly regeneration and post-treatment processes, owing to the non-destructive characteristic of these processes. Indeed, the pollutant is only transferred to another phase [5–7].

Contrarily, physico-chemical processes are destructive and are also widely used to remove recalcitrant compounds. Among them, ozone was taken into account [1,8], a powerful oxidative compound (E = +2.07 V/SHE) which is generally produced by electric chock in the presence of air or oxygen. However, the production of ozone is expensive and its oxidative potential is lower than that of free hydroxyl radicals •OH (E = +3.06 V/SHE), which are therefore the most widely used to remove pesticides. These Advanced Oxidative Processes [1,9,10] can include a chemical activation, a photochemical and/or a catalytic activation.

Processes with photochemical and/or catalytic activations include heterogeneous and homogeneous processes. Homogeneous processes included H_2O_2/UV, photo-Fenton and electro-Fenton; this last process involves the electrochemical generation of H_2O_2 and Fe^{2+} catalyst [11]. Heterogeneous photocatalysis appears as an interesting technique for the treatment of endogenous organic pollution [12–14]. TiO_2, the most used catalyst [13,15–17] was activated under UV irradiation (λ < 390 nm), allowing the generation from water or hydroxide ions of free radicals •OH, highly reactive.

Direct and indirect electro-oxidative processes can also be used to remove xenobiotics [18]. In case of a direct oxidation, the com-

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pounds directly react at the surface of the electrode [19], while in case of a indirect oxidation, pollutant removal can be mediated by the couples ClO^-/Cl^- or Ag^{2+}/Ag^+. The level of the redox potential standard of the couple Ag^{2+}/Ag^+ (1.96 V/SHE) allows Ag^{2+} to oxide some organic compounds, such as tributylphosphate (TBP), tetraphenylborate (TPB) or benzene [18].

The cost of physico-chemical treatments is high in comparison with biological treatments, which are the most cost-effective and the most environmental friendly ones. However, in case of recalcitrant compounds like pesticides, conventional treatment involving activated sludge appears inefficient [1,9]. Thus, in order to degrade recalcitrant compounds and reduce operational costs, several studies recommend integrating processes, more especially the coupling of advanced oxidative processes and biological treatment [20,21]. Most of the integrated processes involved activated sludge [22–24]; however, pure cultures have also been considered [19,25,26]. The advanced oxidative processes constitute a pre-treatment in order to increase the biodegradability of the effluent degrading recalcitrant pollutants and/or to reduce toxicity [27]. Among the oxidative processes, UV/Fe^{2+}/H_2O_2 [24,25], UV/TiO_2 [23,24,26], UV/Fe^{2+}/O_2 [22], ozonation [28] or electro-oxidation [19] can be found.

If the feasibility of a physico-chemical pre-treatment is confirmed, a possible application could be the removal of concentrated pesticide effluents, for instance wastewater from the washing of agricultural tank and pesticide containers. For this purpose, the degradation of phosmet, an organophosphorous insecticide used for the treatment of foliar soil, was examined in this work.

Few studies focused on the biodegradation of phosmet in aqueous media. Biodegradation of phosmet was shown in the presence of *Pseudomonas fluorescens* adapted to phosmet and *Enterobacter agglomerus* isolated from lowbush blueberries (*Vaccinium angustifolium*) [29]. However, the collected data did not allow to conclude a total mineralization of this organophosphorous pesticide after a biological treatment. Chemical oxidation including hydrogen peroxide, ozone and chlorine, as well as photochemical processes including UV/H_2O_2, chlorine/UV and O_3/H_2O_2/UV were carried out for the degradation of residual phosmet on lowbush blueberries [30]. 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^{-1} in acidic medium and results showed that this compound can be reduced on mercury [31]. These encouraging results led to investigate the electrochemical degradation of phosmet as a pre-treatment prior to biodegradation. In this aim, electrochemical behavior of phosmet was studied by cyclic voltammetry with a vitreous carbon electrode. Phosmet was reduced in neutral medium; the feasibility of an electrochemical pre-treatment was thus demonstrated. After potentiostatic electrolysis (−1.3 V/SCE), phosmet was not detected by cyclic voltammetry; a by-product was pointed out by thin layer chromatography and NMR spectra, but its structure was not similar to phosmet-oxon, a toxic derivative of phosmet. Preliminary biodegradation assays showed that pure cultures of *P. fluorescens* did not allow degrading the electrolyzed solution, while 34% decrease of the chemical oxygen demand (COD) was recorded with activated sludge. This study demonstrates the utility of an electrochemical pre-treatment prior to bio-treatment and the feasibility of coupling electrochemical and biological treatment [32].

However, these results were preliminaries and should be confirmed, especially the electrochemical pre-treatment. It was the main purpose of this work. Electrolyses were carried out using a graphite felt working electrode with a high specific area in a flow electrochemical cell, and biological treatment was performed with activated sludge.

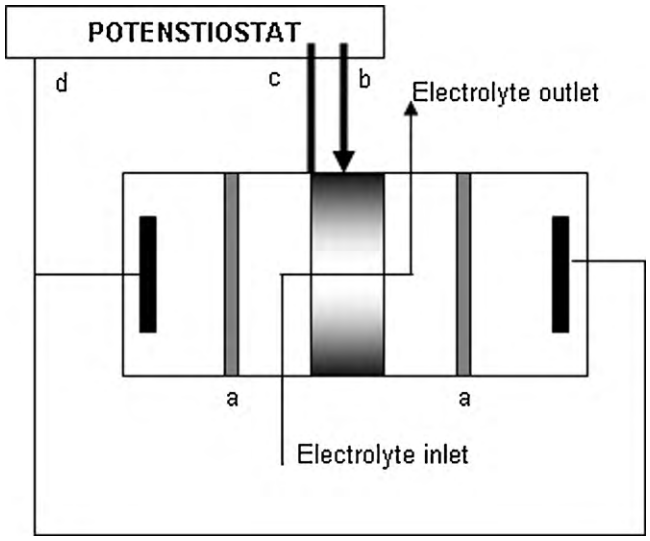

Fig. 1. Schematic diagram of the percolation cell: a: cationic membranes; b: saturated calomel electrode (SCE); c: working electrode (disc of graphite felt: 10 mm diameter, 10 mm thickness); d: auxiliary counter electrodes (carbon-graphite plates).

2. Experimental

Phosmet (Imidan, $C_{11}H_{12}NO_4PS_2$) was supplied by Sigma–Aldrich (Lyon, France).

2.1. Materials for the electrochemical pre-treatment

Graphite felt used as working electrode was purchased from Le Carbone Lorraine (RVG 4000). Its specific area, measured by the BET method was 0.7 $m^2\ g^{-1}$; its density was 0.088 g cm^{-3} and its carbon yield was 99.9%.

Electrochemical pre-treatment was performed in a flow cell presented in Fig. 1. The compartment containing the working electrode (graphite felt) was separated from the two interconnected carbon-graphite plate 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 [33] 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 (AD Instruments Pty Ltd., Castle Hill, Australia). The electrolyte solution (0.05 M Na_2SO_4 + phosmet) percolated the porous electrode with a constant flow rate monitored by a Gilson minipuls 2 peristaltic pump (1 mL min^{-1}).

2.2. Biological treatment

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.

Cultures were carried out at least in duplicates at 30 °C in 250 mL erlenmeyer flasks containing 100 mL of medium with 0.5 g L^{-1} of activated sludge. The basic culture medium contained 0.5 g NH_4Cl and the following mineral supplementation (mg L^{-1}): KH_2PO_4, 85; K_2HPO_4, 208; $Na_2HPO_4{\cdot}2H_2O$, 334, $CaCl_2$, 27.6; $MgSO_4{\cdot}7H_20$, 22.6; $FeCl_3{\cdot}6H_2O$, 0.26.

The target compound, phosmet, was added either at a concentration of 25 mg L^{-1} or added after electrolysis, which was carried out on a pure solution of 20 mg L^{-1} phosmet or on a solution of 1 g L^{-1} phosmet in 50% ethanol; in this latter case, ethanol evapo-

ration was carried out before biological treatment. The pH was then adjusted to 7.0 with 1 mol L^{-1} NaOH.

Samples (5 mL) were taken on a daily basis. After pH and turbidimetric measurements, the samples were centrifuged (4000 rpm at 2700 × *g* for 20 min – Jouan, Thermo Fisher Scientifics, Saint Herblain, France). 211 pHmeter Hanna with combined microelectrode probe (Thermo Spectronic, Rochester, USA) was used for pH measurement and bacterial growth [34] was turbidimetrically followed at a wavelength of 600 nm, using a thermospectronic Helios λ Spectrophotometer (Bioblock, Illkirch, France).

2.3. Analysis

2.3.1. Electrochemical analysis

Electrochemical analysis of phosmet was performed using a conventional three electrode-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. Experiments were performed at ambient temperature under nitrogen atmosphere to avoid dissolved oxygen. Voltammograms were obtained by cyclic voltammetry (100 mV s^{-1}) using an e-daq potenstiostat linked to e-corder 401 converter.

2.3.2. Thin layer chromatography

When electrolysis was carried out in the 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.

2.3.3. NMR analysis

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). Main fraction (11 mg): ^{1}H NMR ($CDCl_3$, 200 MHz, δppm): 4.8 (s); 3.63 (d, 14 Hz); 3.33–3.31 (m).

2.3.4. Dissolved organic carbon (DOC) measurements

DOC was measured by means of a 1010 O.I. Analytical TOC analyzer. $Na_2S_2O_8$ (100 g L^{-1}) and H_3PO_4 (5%) were considered for CO_2 production, which was quantified by infra-red spectroscopy (1700 cm^{-1}).

2.3.5. Chemical oxygen demand (COD) measurements

Chemical oxygen demand (COD) was measured by means of a Test Nanocolor® CSB 40 and 160 from Macherey-Nagel (Düren, Germany). The amount of oxygen required for the oxidation of the organic and mineral matter at 148 °C for 2 h was quantified after oxidation with $K_2Cr_2O_7$ at acidic pH and heating.

2.3.6. Biological oxygen demand (BOD_5) measurements [35]

BOD_5 measurements were carried out in Oxitop IS6 (from WTW) in order to check the non-biodegradability of the azo dye.

The following procedure was applied to inoculate samples, the blank solution and the control solution: 100 g of ground was mixed in distilled water; after agitation the solution was left stand for 10 min; 20 mL of supernatant was then harvested and mixed to distilled water to obtain a total volume of 2 L.

The following mineral basis was used for all experiments (g L^{-1}): $MgSO_4{\cdot}7H_2O$, 22.5; $CaCl_2$, 27.5; $FeCl_3$, 0.15; NH_4Cl, 2.0; Na_2HPO_4, 6.80; KH_2PO_4, 2.80.

The BOD_5 value was initially estimated based on the COD value experimentally measured or calculated, BOD_5 = COD/1.46. The range of expected BOD_5 measurement was then deduced and hence led to the volumes of sample, of ground solution and nitrification inhibitor (10 mg L^{-1} solution of N-allylthiourea) which have to be added in the shake flask of the Oxitop apparatus.

Similar protocol was applied for the control sample except that it was replaced by a solution of easily biodegradable compounds, namely glutamic acid (150 mg L^{-1}) and glucose (150 mg L^{-1}). Before use, KOH was added to achieve neutral pH (7.0 ± 0.2). Similar protocol was also considered for the blank solution, for which the sample was replaced by water to have a negligible BOD_5 value.

2.3.7. Toxicity

It was measured by means of the Microtox test (Microtox 500 analyser), which is a laboratory-based temperature controlled photometer (15–27 °C) that maintains the luminescent bacteria reagent and test samples at the appropriate test temperature. This self-calibrating instrument measures the light production from a luminescent marine bacterial strain, *Vibrio fischeri* NRRL B-11177 (standard ISO 11348-3). The sample toxicity is determined by measuring the effective concentration at which 50% of the light is lost due to compound toxicity (EC50) [22].

2.3.8. Ethanol determination

It was measured by back-titration. In a first step, ethanol reacts with dichromate in acidic solution as follows:

$$3CH_3CH_2OH + 2Cr_2O_7^{2-} + 16H_3O^+ \rightarrow 3CH_3COOH + 4Cr^{3+} + 27H_2O \quad (1)$$

Back-titration of the dichromate, which has not react with ethanol was carried out by potentiometric analysis at zero intensity in acidic conditions using Fe(II) solution for titration and a system involving a platinum working electrode and a reference electrode (saturated calomel electrode – SCE). The titration reaction was:

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H_3O^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 21H_2O \quad (2)$$

The ethanol concentration was then deduced from the excess dichromate.

3. Results and discussion

3.1. Electrochemical pre-treatment

3.1.1. Electrochemical behavior of phosmet

It was previously shown that in acidic medium voltammograms obtained by cyclic voltammetry did not show a signal for the electrochemical reduction of phosmet, while in basic medium, the reduction wave was not significant [32]. Contrarily, in neutral medium (Na_2SO_4 0.05 mol L^{-1} and ethanol) a distinct signal at −1.3 V/SCE was observed on the voltammogram (Fig. 2). It should be noted that a neutral pH was advantageous in view of the use of the electrolyzed solution as growth medium for subsequent biodegradation experiments, since a supplementary step of medium neutralization is avoided.

3.1.2. Phosmet electrolysis

Owing to the electrochemical reduction of phosmet, electrolysis was carried out in a percolation cell (Fig. 1). A flow rate of 1 mL min^{-1} was chosen to avoid the need for recycling the solution through the electrode. The phosmet solution (1 g L^{-1} and 20 mg L^{-1}) percolated through the working electrode at a flow rate of 1 mL min^{-1}. The high surface area of the porous electrode increased the contact between the working electrode and the electrolyte, decreasing the electrolysis time of the reaction, and hence

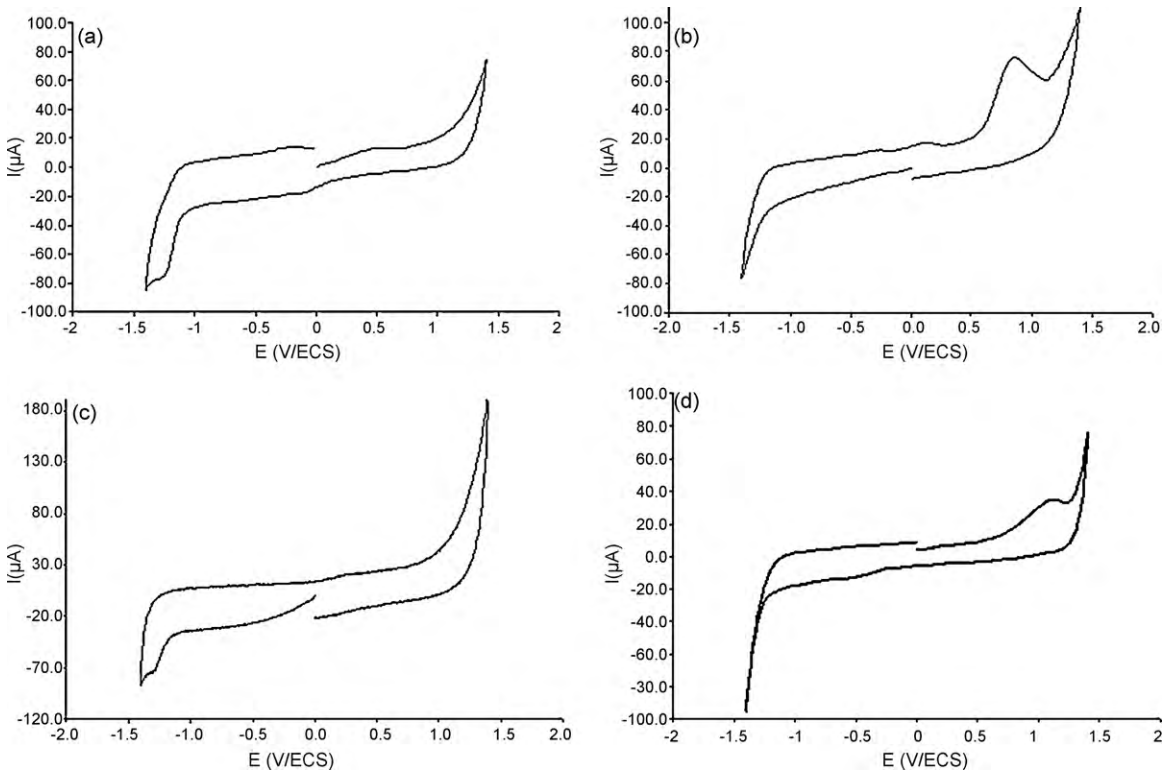

Fig. 2. Current–potential curve obtained by cyclic voltammetry (100 mV s^{-1}) with a vitreous carbon electrode ($S = 3.2 \times 10^{-6}$ m^2), under nitrogen atmosphere and $T = 298$ K, of phosmet (1 g L^{-1}) (a) and after electrolysis in neutral medium (Na_2PO_4 0.05 mol L^{-1} and ethanol) (b), or of pure phosmet (20 mg L^{-1}) (c) and after electrolysis in neutral medium (Na_2SO_4 0.1 mol L^{-1}) (d).

phosmet should be totally reduced without any solution recycling. This high phosmet concentration of 1 g L^{-1} was first chosen to allow an easier identification of the electrolysis by-products.

After phosmet reduction in the percolation cell, a current–potential curve was obtained by cyclic voltammetry analysis (Fig. 2b). The reduction wave had disappeared, showing a complete phosmet reduction, confirming that the recycling of the solution through the electrode was not necessary.

The cyclic voltammogram of the electrolyzed solution also showed a signal in oxidation (Fig. 2b). This result may appear useful in case of a non-biodegradability of the products obtained after phosmet reduction. The oxidation products of a second electrolysis in oxidation would be tested as a substrate for growth microorganisms.

To avoid phosmet precipitation when its concentration was 1 g L^{-1}, a high ethanol amount should be added, at least 33%. However, the use of ethanol can prevent the biological treatment, since ethanol can be inhibitory at this high concentration. Moreover in the event of bacterial growth high ethanol concentrations could hide the degradation of electrolysis by-products. To avoid ethanol addition, electrolysis was therefore carried out with 20 mg L^{-1} phosmet, its limit of aqueous solubility, in the presence of Na_2SO_4 0.1 mol L^{-1} as an electrolyte medium. A current–potential curve obtained by cyclic voltammetry with a vitreous carbon electrode confirmed the presence of a reduction wave at the same potential value (−1.3 V/SCE) (Fig. 2c).

After a single pass through the percolation cell, the voltammogram of the electrolyzed solution confirmed the total phosmet reduction and the presence of an electroactive by-product in oxidation (Fig. 2d).

3.1.3. By-product analysis

To characterize the by-products of phosmet degradation, they were extracted from the electrolyte medium using dichloromethane. The recovered organic phase contained 44% of the by-products (84 mg of products for 191 mg of phosmet initially present in the solution were collected). Therefore, the main part of the by-products remained in the aqueous phase, which however did not show an oxidation signal in cyclic voltammetry; thus, the corresponding by-product(s) have been recovered in the organic phase. The total phosmet disappearance during electrolysis was confirmed by thin layer chromatography, which also highlighted the presence of many by-products of phosmet degradation. The by-product, corresponding to the more intense spot in UV, was isolated (11 mg) on a silica column. Its ^{1}H NMR spectrum showed the absence of the aromatic ring; only the phosphorus surrounded part has been identified (Fig. 3). The acidification of the remaining aqueous part (pH = 1) to extract the residual by-products allowed to recover a mass of 42 mg of by-products. ^{1}H NMR spectra of this fraction, as well as that of the organic fraction containing the minor by-products (36 mg) showed phosmet cleavages, leading to the presence of several electrolysis by-products. Moreover, the comparison with the ^{1}H NMR spectrum of phosmet showed a decrease of the peak of the $-OCH_3$ group present in phosmet as well as a modification of the signal of the SCH_2 group. The analysis of the crude product seems to show that phosmet-oxon, a very toxic derivative of phosmet, was not formed.

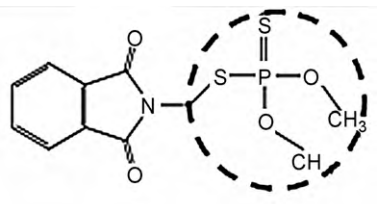

Fig. 3. Identification of the phosphorus by-product of phosmet.

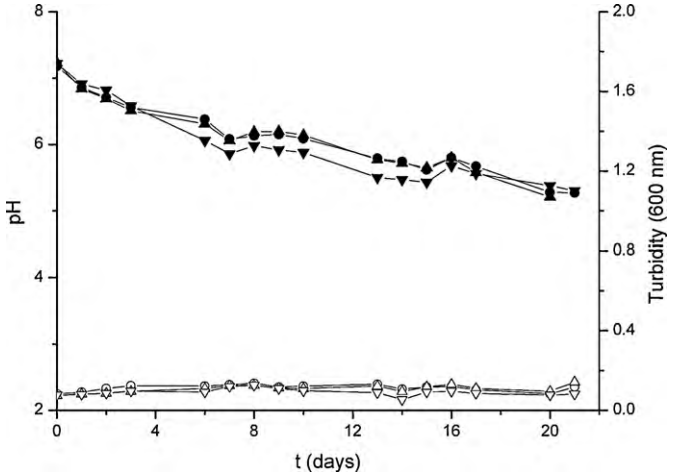

Fig. 4. Turbidity (open symbols) and pH (closed symbols) time-courses during activated sludge culture on the basic culture medium containing pure phosmet (25 mg L^{-1}).

3.2. Biodegradation experiments

3.2.1. Phosmet biodegradation

Biodegradation tests (made in triplicates) of pure phosmet solution (25 mg L^{-1}) near the solubility limit were carried out with activated sludge taken from a local municipal wastewater treatment plant. Fig. 4 shows that turbidity remained constant throughout culture, indicating an absence of growth on phosmet, confirming a previous report [32]. TOC time-course confirmed the absence of growth, since it remained constant throughout culture (not shown). Acute toxicity measurement (Microtox test) showed a high toxicity of the phosmet, since the EC_{50} value was 7% after 15 min time-contact. The biological oxygen demand value was 4 mg O_2 L^{-1} leading to a BOD_5 on COD value of 0.14, namely below 0.4 (the COD value of phosmet was 28 mg O_2 L^{-1}), which characterize the limit of biodegradability [21,22]. A low pH decrease was however observed which could be most likely attributed to phosmet hydrolysis in aqueous phase.

The absence of phosmet biodegradation justified the test of a hybrid process involving an electrochemical process prior to a biological treatment.

3.2.2. Ethanol biodegradation

Owing to a possible presence of ethanol due to the low aqueous solubility of phosmet, ethanol biodegradation by activated sludge was studied to examine if its presence could be a limitation to the biological treatment and thus three volumetric ethanol ratios were tested, 7.5%, 15% and 30%.

Fig. 5a shows activated sludge growth for the lowest ethanol amount (7.5%), owing to the increase of the turbidity and the medium acidification recorded; while these parameters did not vary for the two other ethanol ratios (15% and 30% – not shown). It should be observed a sharp decrease of the growth rate after 6 days most likely due to the inhibitory acidic pH (about 2.5). Ethanol and TOC time-courses confirmed these observations, since no variations were recorded for 15% and 30% (not shown), while for 7.5% ethanol in the medium, 68.4% and 61.0% of the ethanol and total organic carbon were assimilated after 17 days activated sludge culture (Fig. 5b). The nearly similar rate of ethanol assimilation and mineralization were in agreement with the obvious high ethanol biodegradability.

The presence of ethanol prevented the biological treatment owing to the ratio (30%) needed to solubilize 1 g L^{-1} of phosmet

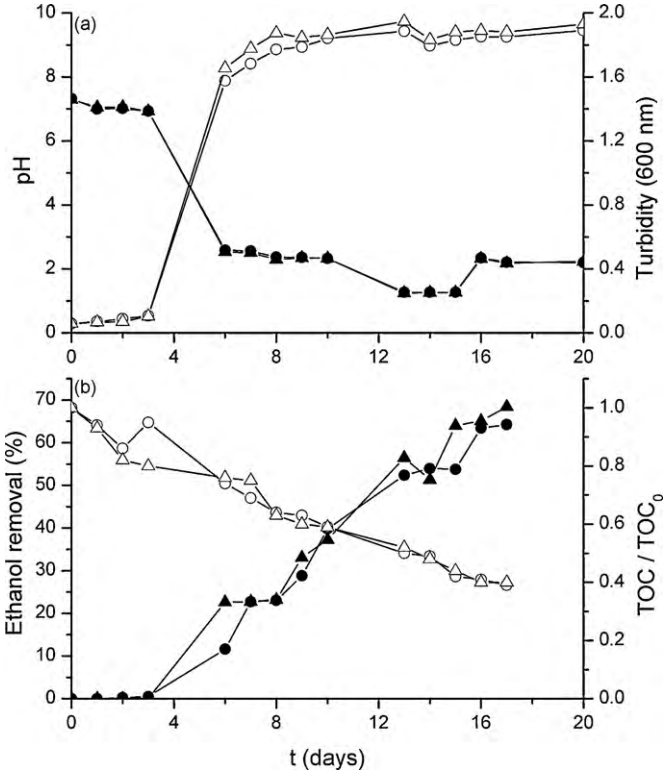

Fig. 5. pH (closed symbols) and turbidity (open symbols) time-courses (a) as well as ethanol (closed symbols) and TOC (open symbols) concentration time-courses (b) during activated sludge cultures on the basic culture medium supplemented with 7.5% ethanol.

and since its assimilation led to a rapid inhibitory pH. It should be therefore evaporated before biological treatment.

3.2.3. Biodegradation of the electrolyzed solution

Pure 20 mg L^{-1} phosmet or 1 g L^{-1} phosmet in the presence of 50% ethanol in neutral medium were considered for electrolysis prior to the biological treatment. Ethanol was evaporated before activated sludge culture.

As observed in Fig. 6a and b, during the first day of culture and concomitantly to medium acidification, turbidity increased indicating cellular growth. Then, cessation of growth was recorded with a weak decline phase, while pH increased until remaining constant close to its initial value (6.3).

TOC time-courses confirmed the initial growth, since a sharp decrease (in the range 57–63% of its initial amount) was recorded during the first days of culture (Fig. 6c and d). TOC remained then constant after nearly 18 days of culture, before a second sharp decreased until an almost nearly complete mineralization after 19 days of culture (Fig. 6c and d).

It can be assumed that at the beginning of culture, microorganisms assimilated the more readily biodegradable by-products from electrolysis. The nearly stationary growth phase until day 18 corresponded most likely to an acclimatization of the sludge to the remaining by-products leading to a constant TOC value owing to a possible assimilation by microorganisms of the intracellular material released by the autolyzed cells. Then after acclimatization, bacteria assimilated the remaining by-products until their exhaustion from the medium.

Acute toxicity measurement of the electrolyzed solution of 20 mg L^{-1} initially of phosmet led to an EC_{50} value of 58% after 15-min time-contact, showing a sharp decrease of toxicity after electrochemical pre-treatment. The BOD_5 value of the same electrolyzed solution was 9 mg O_2 L^{-1}, leading to a BOD_5 on COD ratio of

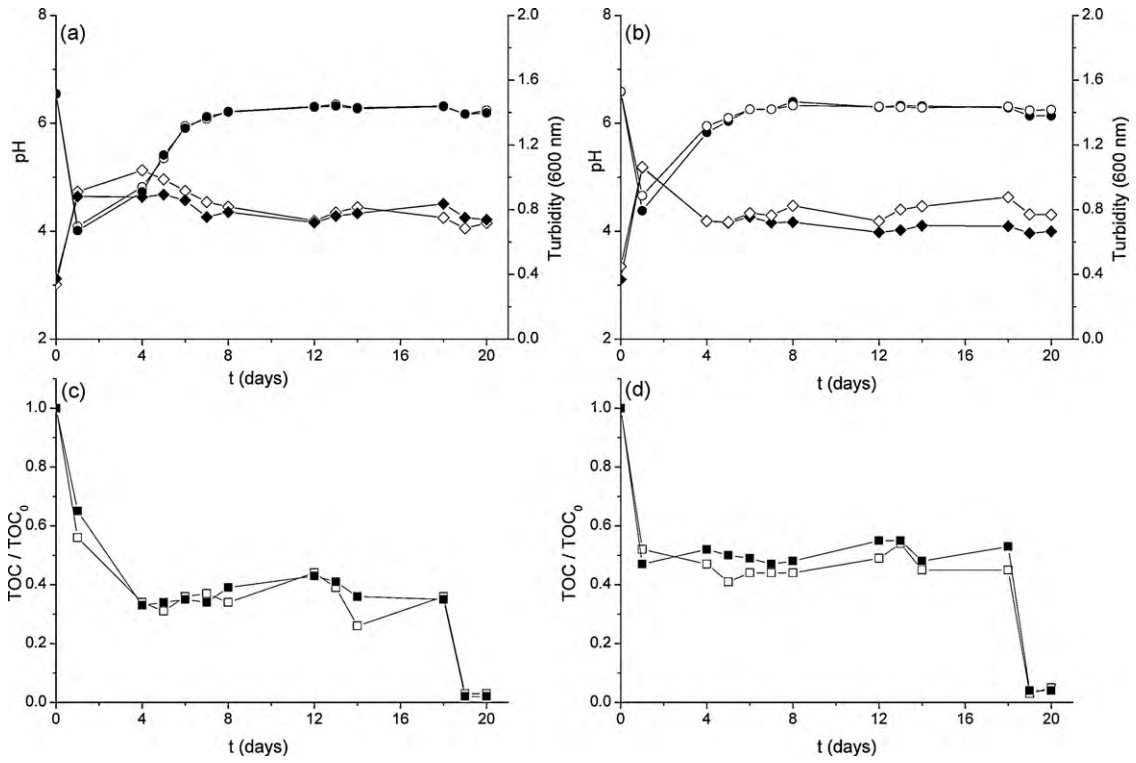

Fig. 6. Turbidity (●, ○) and pH (♦, ◊) time-courses duplicated (a) and (b) as well as TOC (■, □) concentration time-courses duplicated (c) and (d) during activated sludge cultures on the basic culture medium containing an electrolyzed solution of pure phosmet (20 mg L^{-1}) in Na_2SO_4 0.1 mol L^{-1} (a) and (c) or an electrolyzed solution of phosmet (1 g L^{-1}) in ethanol and Na_2PO_4 0.05 mol L^{-1} (b) and (d).

0.42 (the COD value after electrolysis was 21.4 mg O_2 L^{-1}), namely slightly above the limit of biodegradability of 0.4 [21,22].

4. Conclusion

The non-biodegradability of the target compound was beforehand confirmed since it was not assimilated by activated sludge. Electrochemical pre-treatment using a flow cell led to a total reduction of phosmet in neutral medium without the need for solution recycling through the electrode. Electrolysis led to the following results:

- A decrease of the toxicity, characterized by the increase of EC_{50} value from 7% to 58% for phosmet and the electrolyzed solution respectively.
- An increase of the biodegradability, since the BOD_5 value increased from 4 initially to 9 mg O_2 L^{-1} after electrolysis leading to an increase of the BOD_5 on COD ratio from 0.19 to 0.42.
- These encouraging results were confirmed during activated sludge culture since an almost total mineralization of the electrolyzed solution was recorded (97%). Mineralization showed two steps, during the first days of culture at the expense of the most readily biodegradable by-products, and after an acclimatization phase after 18 days of culture at the expense of the less readily biodegradable by-products.

The feasibility of the proposed integrated process was therefore shown.

It should be noticed that at the high cathodic potential used during electrolysis (−1.3 V/ECS), another reduction can occur; the reduction of water leading to hydrogen production. The faradic yield was therefore very low (below 10%) and can be less cost-effective. For this purpose, other electrolytes or material for electrodes could be tested, especially those leading to a higher hydrogen overvoltage. To complete this work, the flow rate in the percolation cell as well as the thickness of the graphite felt could also be optimized.

The reuse of the acclimated activated sludge for successive experiments could also be helpfully considered to reduce the process duration. This process should also be subsequently validated on polluted wastewater (real effluent).

References

[1] S. Chiron, A.R. Fernandez-Alba, A. Rodriguez, E. Garcia-Calvo, Pesticide chemical oxidation: state of the art, Water Res. 34 (2000) 366–377.
[2] T. Robinson, G. McMullan, R. Marchand, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.
[3] P.C. Vandevivere, R. Bianchi, W. Verstraete, Treatment and reuse of wastewater from the textile wet-processing industry: review of emerging technologies, J. Chem. Technol. Biotechnol. 72 (1998) 289–302.
[4] M. Auriol, Y. Filali-Meknassi, R.D. Tyagi, C.D. Adams, R.Y. Surampalli, Endocrine disrupting compounds removal from wastewater, a new challenge, Process Biochem. 41 (2006) 525–539.
[5] I Arslan, I.A. Bacioglou, T. Tuhkanen, D. Bahnemann, H_2O_2/UV-C and Fe^{2+}/H_2O_2/UV-C versus TiO_2/UV-A treatment for reactive dye wastewater, J. Environ. Eng. 126 (2000) 903–911.
[6] S.K. Chaudhuri, B. Sur, Oxidative decolorization of reactive dye solution using fly ash as catalyst, J. Environ. Eng. 126 (2000) 583–594.
[7] N.L. Stock, J. Peller, K. Vinodgopal, P.V. Kamat, Combinative sonolysis and photocatalysis for textile dye degradation, Environ. Sci. Technol. 34 (2000) 1747–1750.
[8] A. Laplanche, G. Martin, F. Tonnard, Ozonation schemes of organo phosphorus pesticides application in drinking water treatment, Ozone: Sci. Eng. 6 (1984) 207–219.
[9] M.I. Badawy, M.Y. Ghaly, T.A. Gad-Allah, Advanced oxidation processes for the removal of organo phosphorus pesticides from wastewater, Desalination 194 (2006) 166–175.
[10] T. Oppenländer, Photochemical Purification of Water and Air. Advanced Oxidation Processes (AOPs): Principles, Reaction Mechanisms, Reactor Concepts, Wiley-VCH, 2003.
[11] E. Guivarch, N. Oturan, A.M. Oturan, Removal of organophosphorus pesticides from water by electrogenerated Fenton's reagent, Environ. Chem. Lett. 1 (2003) 165–168.

[12] S. Malato, J. Blanco, J. Càceres, A.R. Fernandez-Alba, A. Agüera, A. Rodriguez, Photocatalytic treatment of water-soluble pesticides by photo-Fenton and TiO2 using solar energy, Catal. Today 76 (2002) 209–220.
[13] K. Tanaka, K. Padermpole, T. Hisanaga, Photocatalytic degradation of commercial azo-dyes, Water Res. 34 (2000) 327–333.
[14] C. Guillard, H. Lacheb, A. Houas, M. Ksibi, E. Elaloui, J.M. Herrmann, Influence of chemical structure of dyes, of pH and of inorganic salts on their photocatalytic degradation by TiO_2 comparison of the efficiency of powder and supported TiO_2, J. Photochem. Photobiol. A: Chem. 158 (2003) 27–36.
[15] C. Hachem, F. Bocquillon, O. Zahraa, M. Bouchy, Decolourization of textile industry wastewater by the photocatalytic degradation process, Dyes Pigments 49 (2001) 117–125.
[16] C. Galindo, P. Jacques, A. Kalt, Photooxidation of the phenylazonaphthol AO20 on TiO_2: kinetic and mechanistic investigations, Chemosphere 45 (2001) 997–1005.
[17] M.M.H. Khalil, A.A. Abdel-Shafi, M.S.A. Abdel-Mottaleb, Photcatalytic degradation of some toxic analytical reagents with TiO_2, Int. J. Photoenergy 1 (1999) 1–4.
[18] J. Grimm, D. Bessarabov, R. Sanderson, Review of electro-assisted methods for water purification, Desalination 115 (1998) 285–294.
[19] C.A. Basha, E. Chithra, N.K. Sripriyalakshmi, Electro-degradation and biological oxidation of non-biodegradable organic contaminants, Chem. Eng. J. 149 (2009) 25–34.
[20] J.P. Scott, D.F. Ollis, Integration of chemical and biological processes for water treatment: Review and recommendations, Environ. Progress. 14 (1995) 88–103.
[21] C. Pulgarin, M. Invernizzi, S. Parra, V. Sarria, R. Polania, P. Péringer, Strategy for the coupling of photochemical and biological flow reactors useful in mineralization of biorecalcitrant industrial pollutants, Catal. Today 54 (1999) 341–352.
[22] V. Sarria, S. Parra, N. Adler, P. Péringer, N. Benitez, C. Pulgarin, Recent developments in the coupling of photoassisted and aerobic biological processes for the treatment of biorecalcitrant compounds, Catal. Today 76 (2002) 301–315.
[23] I. Muñoz, J. Peral, J.A. Ayllon, S. Malato, P. Passarinho, X. Domènech, Life cycle assessment of a coupled solar photocatalytic-biological process for wastewater treatment, Water Res. 40 (2006) 3533–3540.
[24] I. Oller, S. Malato, J.A. Sanchez-Perez, M.I. Maldonado, R. Gasso, Detoxification of wastewater containing five common pesticides by solar AOPs-biological coupled system, Catal. Today 129 (2007) 69–78.
[25] M.M. Ballesteros Martin, J.A. Sanchez Perez, F.G. Acien Fernandez, J.L. Casas Lopez, A.M. Garcia Ripoll, A. Arques, I. Oller, S. Malato Rodriguez, Combined photo-Fenton and biological oxidation for pesticide degradation. Effect of photo-treated intermediates on biodegradation kinetics, Chemosphere 70 (2008) 1476–1483.
[26] C.Y. Chan, S. Tao, R. Dawson, P.K. Wong, Treatment of atrazine by integrating photocatalytic and biological processes, Environ. Pollut. 131 (2004) 45–54.
[27] S. De La Rochebrochard d'Auzay, S. Brosillon, F. Fourcade, A. Amrane, Integrated process for degradation of amitrole in wastewaters: photocatalysis/biodegradation, Int. J. Chem. Reactor Eng. 5 (2007) A51.
[28] F.J. Rivas, F.J. Beltran, O. Gimeno, Joint treatment of wastewater from table olive processing and urban wastewater. Integrated ozonation–aerobic oxidation, Chem. Eng. J. 23 (2000) 177–181.
[29] K.M. Crowe, A.A. Bushway, R.J. Bushway, K. Davis-Dentici, Microbial degradation of phosmet on blueberry fruit and in aqueous systems by indigenous bacterial flora on lowbush blueberry (*Vaccinium angustifolium*), J. Food Sci. 72 (2007) 293–299.
[30] K.M. Crowe, A.A. Bushway, R.J. Bushway, R.A. Hazen, Evaluation of chemical and photochemical oxidation process for degradation of phosmet on lowbush (*Vaccinium angustifolium*), J. Agric. Food Chem. 54 (2006) 9608–9613.
[31] N.Y. Sreedhar, P.R. Kumar Reddy, G.R.V. Subba Reddy, S.R. Jayarama Reddy, Electroanalytical determination of the fungicides folfet, phosmet, and dialifos in grains and soils, Bull. Chem Soc. Jpn. 70 (1997) 2425–2427.
[32] M. Assassi, F. Fourcade, F. Geneste, D. Floner, R. Maachi, A. Amrane, 2nd International Congress on Green Process Engineering, Venice, 2009, p. 6.
[33] C. Moinet, Electrosynthèse organique en continu. Méthodes directes et indirectes, J. Phys. IV 4 (1994), C1 175-C1 184.
[34] A.M. Cook, Biodegradation of s-triazine xenobiotics, FEMS Microbiol. Rev. 46 (1987) 93–116.
[35] PNE In Métodos de anàlisis de agua, Determinación de la demanda bioquímica de oxígeno, Método de dilución y siembra, Madrid, Spain, 77-003, 1978.