Analytica Chimica Acta 745 (2012) 64–69

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Detoxification of organophosphate residues using phosphotriesterase and their evaluation using flow based biosensor

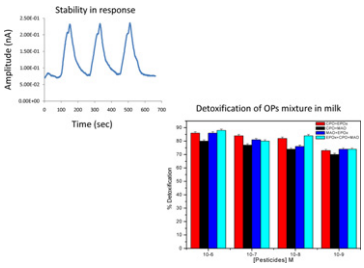
Rupesh K. Mishra[a,b], George Istamboulie[a], Sunil Bhand[b], Jean-Louis Marty[a,*]

[a] Université de Perpignan via Domitia, IMAGES EA4218, Building S 52 Av. Paul Alduy, 66860 Perpignan Cedex, France
[b] Biosensor Lab., Department of Chemistry, BITS, Pilani - K. K. Birla Goa Campus, Goa 403726, India

HIGHLIGHTS

- Present work demonstrates the detoxification of OPs at low level.
- This is the first report of OPs detoxification in milk using PTE.
- PTE reacts rapidly with OPs and hydrolyzes within few minutes.
- Detoxification of OPs using PTE was evaluated using flow based biosensor.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 April 2012
Received in revised form 28 June 2012
Accepted 12 July 2012
Available online 8 August 2012

Keywords:
Organophosphate
Detoxification
Phosphotriesterase
Water
Milk
Flow based biosensor

ABSTRACT

Among known pesticide groups, organophosphates (OPs) have grasped attention due to their hazardous nature and their applications as pesticides and chemical weapons. This work presents the development of cost-effective column based biosensor for detoxification of OPs in water and milk. Enzyme phosphotriesterase (PTE) was immobilized on an activated Sepharose 4B via covalent coupling using an Omnifit glass column. Three different OPs, ethyl paraoxon (EPOx), malaoxon (MAO) and chlorpyriphos-oxon (CPO) were spiked in water and milk to test the detoxification of OPs. Mixtures of these pesticides were also tested to check the cumulative detoxification in the real samples. The efficiency of detoxification was evaluated using a highly sensitive acetylcholinesterase (AChE) B394 biosensor based flow system. The column conditions were optimized for the detoxification studied. The method was shown to be promising when we tested real milk samples spiked with OPs. Detoxification obtained in milk was up to 86% whereas in water, 100% detoxification was obtained.

1. Introduction

Organophosphate (OP) compounds are the most widely used group of insecticides in the world [1,2]. Since the removal of organochlorine insecticides from use, OPs have become the most widely used insecticides available today [3–5]. More than forty of them are currently registered for use and all run the risk of acute and sub-acute toxicity. OPs are used in agriculture, in the home, in gardens, and in veterinary practice. The toxicity of these compounds is due primarily to the practically irreversible inhibition of acetylcholinesterase (AChE), the enzyme responsible for hydrolysis of the neurotransmitter acetylcholine [6]. The hazardous nature of OPs and their wide usage have led to concerted efforts for developing highly sensitive detection techniques as well as efficient destruction methods for these compounds [7].

The World Health Organization reported that exposure to such pesticides results in roughly 3 million cases of severe poisoning and 220,000 deaths annually worldwide. Also, more than 0.7 million cases of poisoning worldwide could be traced to occupational and incidental exposure to OPs [7]. Due to their high acute toxicity and risk towards the population, some directives have been established to limit the presence of pesticides in water and food

* Corresponding author. Tel.: +33 468662254; fax: +33 468662223.
E-mail address: jlmarty@univ-perp.fr (J.-L. Marty).

0003-2670/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2012.07.018

resources. Concerning the quality of water for human consumption, the European Council Directive 98/83/EC [8] has set a maximum admissible concentration of 0.1 $\mu g\,L^{-1}$ per pesticide and 0.5 $\mu g\,L^{-1}$ for the total amount of pesticides. CODEX ALIMENTARIUS has set different–different maximum residual limit (MRL) for food items. For milk the MRL of OPs is 0.1 $mg\,kg^{-1}$ [9].

Recently, our group has developed a flow based biosensor using a genetically modified AChE B394 based probe for OPs determination in milk [5]. That work motivated us to extend the analysis for detoxification of OPs in milk using enzyme phosphotriesterase (PTE). Nowadays, milk is being contaminated by various means. OPs can become throughout the food chain. When dairy cows are fed with OPs polluted forages or drink polluted water, bovine milk and dairy product could be tainted with pesticide residues [10,11].

The presence of OPs residues in milk has already been reported by many research groups worldwide [4,12–16]. Since consumption of dairy products can be a significant dietary source of contaminants, it is important to update or develop analytical methods for milk monitoring, as new substances and new regulatory limits are introduced. These works demonstrated that the potential existence of OPs pesticides in the milk or dairy products cannot be ignored. Hence, the detoxification of OPs in milk is indispensable.

Degradation of OPs is very mandatory for the detoxification of waters and milk devoted to human consumption. Conventional methods generally involve burning and chemical hydrolysis of these products [17]. However these techniques are hindered by their high environmental impact as well as logistics and operational safety issues [7]. Efforts have thus been made to find safer technologies, mainly based on biotechnological degradation techniques. New methods of environmentally friendly and safe OPs detoxification are urgently needed. Enzymes that are capable of hydrolyzing and detoxifying such agents are of significant utility.

The current methods for the detoxification of OP compounds are harmful and possess serious environmental consequences. They are classically based on extraction, cleanup and analysis using gas chromatography or liquid chromatography coupled to sensitive and specific detectors [18–21]. These methods are highly sensitive and allow the determination of a large number of compounds but expensive and time consuming with complex procedure. Alternative methods are mainly based on the biosensor technology. There are plentiful reports have been described in the literature for OPs determination in milk and water [1,2,4,5,11,14,22].

Bacterial enzymes capable of hydrolyzing the lethal OP nerve agents are of special interest. The use of OPs, though very important to the success of the agricultural industry, affects the environment. PTE displays a significant rate enhancement and substrate promiscuity for the hydrolysis of OPs, including chemical warfare agents like sarin or soman [23,24]. Various researchers have studied detoxification of toxic analytes such as paraoxon, parathion and chlorpyriphos exploiting various biomolecules such as enzymes and whole cells. The insecticides paraoxon and parathion were successfully hydrolyzed by PTE that was immobilized on a trityl agarose matrix [25,26]. The application of PTE based detoxification column for chlorpyriphos and chlorfenvinfos was demonstrated recently in flow [27]. Organophosphorus hydrolase enzyme was also used for detoxification of OPs such as coumaphos with 99% degradation using prolongs incubation time (2–3 h) [28]. PTE has been also described as a potential catalytic scavenger for the treatment of OP poisoning [29]. The matrix reported in the literature is mostly water. In present work, along with water we used complex matrix milk for OPs detoxification. Our study also focused on detoxification of mixture of OPs. The PTE column exhibited a stability of 3 weeks (for water). This effort is among a few reports where detoxification of OPs mixture is successfully reported with higher stability of PTE based column.

Compared to the disadvantage of conventional methods for detoxification of pesticides, biodegradation using enzymes has been considered as a potentially convenient, effective, low cost and environmentally friendly method. Herein, we report the PTE catalyzed degradation of chlorpyriphos-oxon (CPO), ethyl paraoxon (EPOx) and malaoxon (MAO) in water and milk which is an important and widely consumed matrix by all age group. The efficiency of the detoxification process has been monitored using a highly sensitive flow based biosensor based on recombinant AChE B394.

2. Materials and methods

2.1. Chemicals and stock solutions

PTE recombinant enzyme was kindly provided by Prof. D. Fournier (Toulouse, France). This enzyme was first isolated from *Flavobacterium* sp. and cloned in *Escherichia coli* [30]. The pesticides ethyl paraoxon and malaoxon were obtained from Fluka, Sigma–Aldrich (Germany). Chlorpyriphos-oxon was procured from Dr. Ehrenstorfer (Augsburg, Germany). A 0.1 M stock solution was prepared in acetonitrile and stored at 4 °C.

Genetically modified AChE from Drosophila melanogaster type B394 was produced by our laboratory (IMAGES, France). The substrate acetylthiocholine chloride (ATChCl) was purchased from Sigma. A 0.1 M ATChCl stock solution was daily prepared in 0.9% sodium chloride solution and stored at 4 °C. Sepharose 4B activated by cyanogens bromide was purchased from Amersham (Buckinghamshire, UK).

The pastes used for screen-printing, i.e. Electrodag PE-410, 423SS and 6037SS were obtained from Acheson (Plymouth, UK). Cobalt-phtalocyanine-modified carbon paste was purchased from Gwent Electronic Materials, Ltd. (Gwent, UK). A glycerophthalic paint (Astral, France) was used as insulating layer. Transparent PVC sheets (200 mm–100 mm–80.5 mm) were used as printing supports. Slide-A-Lyzer Dialysis Cassettes were purchased from Thermo Scientific (Rockford, USA) for purification of enzyme powder in liquid phase.

2.2. Instrumentation

Amperometric measurements were carried out with an automated flow based system. The flow system is comprised by a modular syringe pump coupled with a multiport valve (Cavro XLP 6000), a custom flow through cell, a 12 bits data acquisition card (National Instruments) and a 641VA potentiostat (Metrohm, Switzerland). Screen printed electrodes (SPEs) were fabricated using a semi-automatic DEK248 printing machine according to a procedure previously described [31], but in a three-electrode configuration. The working electrode was a 4 mm-diameter disk, the auxiliary 2 electrode was a 16 mm × 1.5 mm curved line and the Ag/AgCl pseudo-reference electrode was a 5 mm × 1.5 mm straight line. A graphical user interface was developed in Lab-View 8.5 to control the whole system.

2.3. Apparatus

Spectrophotometric measurements were performed at 25 °C using a Hewlett Packard diode array 8451A spectrophotometer. Amperometric measurements were carried out at 25 °C with a 641VA potentiostat (Metrohm, Switzerland), connected to a BD40 (Kipp & Zonen, The Netherlands) flatbed recorder. SPEs were produced using a semi-automatic DEK 248 printing machine according to a procedure previously [32]. The working electrode was a 4-mm diameter disk, the auxiliary electrode was a 16 mm × 1.5 mm curved line and the Ag/AgCl pseudo-reference electrode was a

5 mm × 1.5 mm straight line. Peristaltic pump (Gilson, France) was used to dispense liquid in PTE based Omnifit column.

2.4. Determination of enzymatic activities

Before immobilisation, enzymatic activity of B394 was measured spectrophotometrically at 412 nm using 5, 50-dithiobis (2-nitrobenzoic acid) according to Ellman's method [33]. PTE activity was measured in 10 mM phosphate buffer (PB), pH 8 by monitoring the formation of p-nitrophenol at 405 nm using 1 mM of the reference substrate paraoxon [34].

2.5. Principle of OP insecticides detoxification by PTE

The activity of PTE for different OP substrates was already investigated and described in a previous work [34]. PTE was shown to exhibit a high affinity for all the tested OPs EPOx, CPO and MAO but with differences in detoxifications. Paraoxon is the main substrate for PTE activity and the product of hydrolysis is *p*-nitro phenol. It is important to mention that these detoxification products are not inhibitors of AChE and are not pointed out as harmful chemicals by European regulation and WHO recommendations on water quality [35].

2.6. Immobilisation of PTE on Sepharose Gel 4B

The gel was prepared by mixing 1 g of Sepharose 4B in 10 mL of 1 mM, HCl, this suspension was then gently casted in an Omnifit column (internal volume 10 mL) (Cambridge, England). The column was cleaned with 200 mL of 1 mM HCl and then equilibrated using 50 mL of 0.1 M PB, pH 8. After the determination of enzyme activity, PTE solutions of different concentrations were gently mixed with the Sepharose gel for 2 h at room temperature (25 °C). The saturation step was then performed by passing 10 mL of 1 M ethanolamine through the gel for another 2 h. Finally, the gel was cleaned using 20 mL of 0.1 M acetate buffer pH 4 and equilibrated with 20 mL of 0.1 M PB, pH 8. The final bed volume was 4 mL, before use the column was stored at 4 °C.

2.7. Amperometric measurements of AChE activity

The activity of AChE immobilized on the electrodes was determined by electrochemically monitoring the thio-choline formed upon enzymatic hydrolysis of ATChCl. The amperometric measurements were carried out using a 641VA potentiostat (Metrohm, Switzerland), connected to a BD40 (Kipp & Zonen, The Netherlands) flatbed recorder. The applied potential was 100 mV vs. a printed Ag/AgCl reference electrode. In order to make amperometric measurements, the biosensor strip was vertically inserted into flow cell of the automated flow system and finally integrated to the potentiostat. A working potential was applied to 100 mV to reference electrode and 500 μL substrate was dispensed three times using automated pump. The produced current (Amplitude) was recorded using data acquisition card. The pesticide analysis completed in three-step procedure as follows: first, the initial response of the biosensor to the substrate ATChCl (1 mM) was recorded three times, then 500 $\mu L\,min^{-1}$ of each pesticides were dispensed through flow cell to inhibit biosensors for 10 min. The stock pesticide solution (1×10^{-3} M) was diluted to 1000 times to 1×10^{-6} M, and then further diluted. Each pesticide was tested against each biosensor. And finally the residual response of the biosensor was recorded again. Electrodes were cleaned between each measurement. The percentage of inhibition was correlated with the insecticide concentration. The limit of detection was calculated as the insecticide concentration inducing a 10% decrease of the biosensor response.

Table 1
Intra reproducibility of electrode performance for evaluation of OPs detoxification.

S.N.	Electrodes	Value obtained (nA)	% CV
1	SPE-1	252 ± 4	1.08
2	SPE-2	249 ± 6	1.12
3	SPE-3	255 ± 2	1.08

SPE: screen printed electrode.

3. Results and discussion

The aim of the proposed work was to investigate the degradation of the OPs (MPOx, CPO and MAO) in water and milk to determine optimum conditions for detoxification using PTE based biosensor. As reported in the literature, PTE is a very good candidate to detoxify the hazardous OPs in water [27]. Since the OPs are also determined in milk, it is also important to see the possibility of detoxification of OPs in milk. Initially, tests were performed in water as a reference then tested in milk at same concentration. The mixtures of OPs were also tested to see the effect of detoxification in additive form and evaluated the % detoxification using AChE B394 based biosensor. The experimental set up used for detoxification studies is shown in supplementary Figure S1.

3.1. Characterization and performance of biosensor B394

The operational stability and performance of the developed biosensors needs to be tested in order to make certain that the matrix show negligible effect on the signal. The operational stability of immobilized B394 was evaluated by measuring the response of the same sensor to 1 mM ATChCl, at 100 mV vs. Ag/AgCl. The sensors were washed between the tests with PB. The sensors were shown to be stable for atleast 8–10 consecutive measurements. The reproducibility of electrode was evaluated by the repetitive measurements ($n = 8$) of acetylthiocholine at a fixed concentration of 1 mM. The corresponding % coefficient of variation (% CV) is 1.08 confirms that results are satisfactorily reproducible. Intra reproducibility of electrode performance is presented in Table 1. In order to examine intraday (repeatability) and interday (reproducibility) response, signals were recorded for a fixed concentration of substrate ATChCl (1 mM) using SPE. The experimental results show that the current intraday responses deviate by 2.73% and interday responses by 2.40%, suggesting thereby that SPE possesses adequate reproducibility for the determination of OPs as well as evaluate the detoxified product by PTE. Thus, the SPE electrodes exhibit a good reproducibility and stability. The biosensor showed a good reproducibility with maximum signal variation of 5%. The real peaks are shown in Fig. 1a obtained using biosensor B394. The inhibitory effect of the three OPs in water was evaluated with constructed biosensor B394. The I% was obtained by calculating the activity of AChE, B394 before and after the incubation with pesticide. Each experimental point was the mean of three measurements. Fig. 1b represents a real inhibition peak obtained during the analysis of MAO using flow based biosensor. The inhibition experiments showed a high level of intra-laboratory reproducibility with a CV of 3.43%. The dynamic range for all the pesticides was kept similar to observe the affinity of the biosensors to the pesticides. The wide dynamic range tested in water and milk for EPOx, MAO and CPO is varied from 1×10^{-6} to 1×10^{-11} M. The LODs obtained for EPOx, MAO and CPO is 1×10^{-9}, 1×10^{-10}, and 1×10^{-11} M, respectively with an average % RSD of 2.82. The inhibition curve obtained for B394 using three pesticides is shown in Fig. 2.

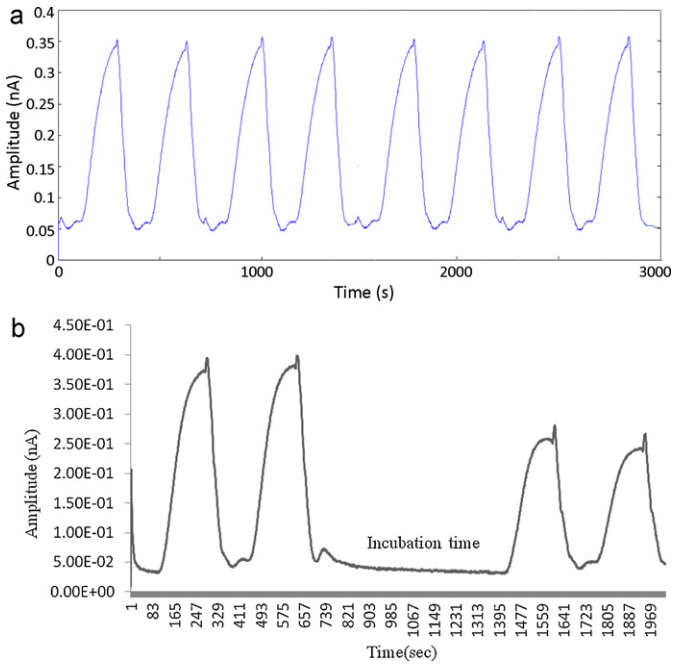


Fig. 1. (a) Stability of the peaks obtained using flow based biosensor using B394 enzyme with consecutive 8 injections. (b) The real peaks obtained before and after incubation with malaoxon (1×10^{-9} M) spiked in milk using biosensor B394 under optimized experimental conditions.

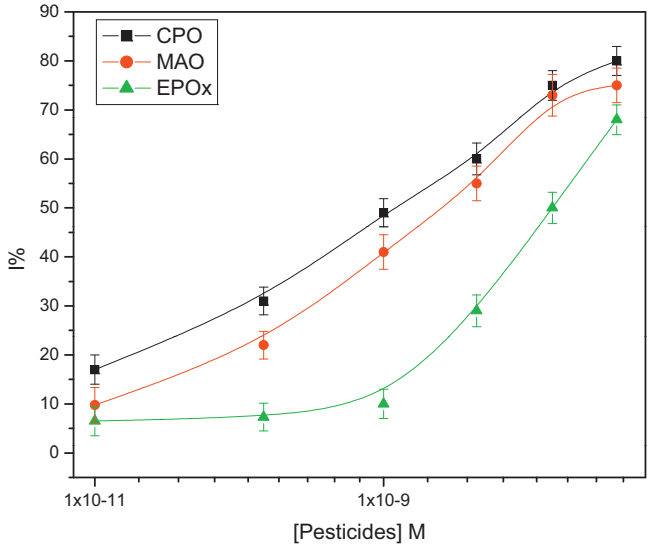


Fig. 2. Calibration curve of OPs (CPO, MAO and EPOx) in milk using B394 flow based biosensor.

3.2. Influence of flow rate on OPs degradation

The effect of contact time on the hydrolysis of a 1×10^{-6} M EPOx solution was studied by changing the flow rate (0.5 mL min^{-1} and 1.0 mL min^{-1}). The results revealed the relationship between OPs degradation and flow rate as a feed solution through an Omnifit column. Three EPOx concentration levels (1×10^{-4} to 1×10^{-4} M) were tested. For all experiments, the feed solution was prepared in 0.1 M PB, pH 8. Degradation of OPs by the PTE reached almost 100% with the flow rate 0.5 mL min^{-1}. The time required to reach the maximum degradation percent was found to be inversely proportional to the flow rate at each pesticide concentration. An optimum flow rate of 0.5 mL min^{-1} was selected, corresponding to a contact time of 6 min.

3.3. Effect of storage on column efficiency

The stability of the Omnifit-PTE based column over the 30 days following immobilization on Sepharose 4B was examined by measuring the capacity for degradation of OPs. The stability with time of the detoxification column was studied using different concentrations of EPOx (1×10^{-6} to 1×10^{-9} M). The column exhibited excellent stability in water matrix over the milk matrix. For first 10 days, no significant loss is activity was observed in water. When Omnifit column immobilized PTE was used for detoxification of OPs in water over 3 weeks, the column retained about 75% activity. An intra-assay coefficient of variation (% CV) of 2.2% was observed. In case of milk, the column retained about 50% activity after 7days of operation.

3.4. Optimization of Omnifit-PTE column for detoxification of OPs in water and milk

To check the performance of the prepared PTE based Omnifit column, 1×10^{-6} M solution of EPOx was first spiked in water and checked as a reference to test the column and efficiency of PTE, then spiked in milk and passed through the column. For each samples, weather it is water or milk, the pH was adjusted to 8 because PTE works optimum at this point. All experiments were done in 0.1 M PB, pH 8, at 25 °C. Each experiment was repeated at least three times. In first attempts, the control of the pesticide solution, flow rate was checked using a peristaltic pump fitted with polytetrafluorethylene (Teflon) tubes. The efficiency of the developed detoxification column was studied using EPOx, CPO and MAO. The effect of contact time on the hydrolysis of a 1×10^{-6} M EPOx solution was studied by varying the flow rate (0.5 mL min^{-1} and 1.0 mL min^{-1}). The detoxification efficiency was controlled using an AChE biosensor B394, which allows detecting pesticide lower down to 1×10^{-11} M. As expected, decreasing the flow rate allows increasing the contact time thus the percentage of detoxification. An optimum flow rate of 0.5 mL min^{-1} was selected, corresponding to a contact time of 6 min. In these conditions, PTE based column showed consistency in the performance and allow us to assume that the EPOx was completely hydrolyzed.

3.5. Bio-detoxification of CPO, EPOx and MAO spiked in water and milk

A detoxification column was prepared using a 10 mL Omnifit column filled with 4 mL of Sepharose Gel 4B previously loaded with 335 IU of PTE. The column was connected via peristaltic pump fitted with Teflon tubes and the flow rate was set to 0.5 mL min^{-1}. Because of the high activity of PTE for EPOx, the biodetoxification of EPOx was more as compared with other two analytes CPO and MAO. Different concentrations of EPOx, CPO and MAO ranging from 1×10^{-6} to 1×10^{-9} M were tested in water and milk. PTE can hydrolyze EPOx and CPO completely (100%) whereas MAO hydrolysis is 88% in water. Significant detoxification of these pesticides was obtained in milk. EPOx was found to be the best substrate for PTE as it degraded EPOx by 86% in milk whereas PTE showed 76% and 70% degradation for CPO and MAO respectively. Table 2 presents the brief summary of the detoxification of OPs in milk. To ensure the detoxification of OPs in the milk, the end products were passed through the flow based biosensor and compared with the calibration of the original solution.

Table 2
Summary of % detoxification of individual OPs spiked in milk measured using PTE biosensor (CPO: chlorpyriphos oxon, EPOx: ethyl paraoxon, MAO: malaoxon).

Before treatment		After treatment-flow rate 0.5 mL min^{-1}		
Actual conc. (M)	Inhibition (%)	Inhibition (%)	Calculated conc. (M)	% detoxification
CPO				
1×10^{-6}	80	12	$<1 \times 10^{-12}$	85.00
1×10^{-7}	78	14	$<1 \times 10^{-12}$	82.05
1×10^{-8}	61	12	$<1 \times 10^{-12}$	80.32
1×10^{-9}	48	6.5	$<1 \times 10^{-12}$	86.45
EPOx				
1×10^{-6}	68	16.0	$\leq 5 \times 10^{-9}$	76.47
1×10^{-7}	50	13.6	$<5 \times 10^{-10}$	72.80
1×10^{-8}	29	7.5	$<5 \times 10^{-11}$	74.13
1×10^{-9}	10	2.6	$<5 \times 10^{-12}$	74.00
MAO				
1×10^{-6}	75	23.7	$\leq 5 \times 10^{-10}$	68.40
1×10^{-7}	73	21.9	$<5 \times 10^{-10}$	70.54
1×10^{-8}	55	18.0	$<5 \times 10^{-10}$	67.27
1×10^{-9}	41	12.3	$<5 \times 10^{-10}$	70.00

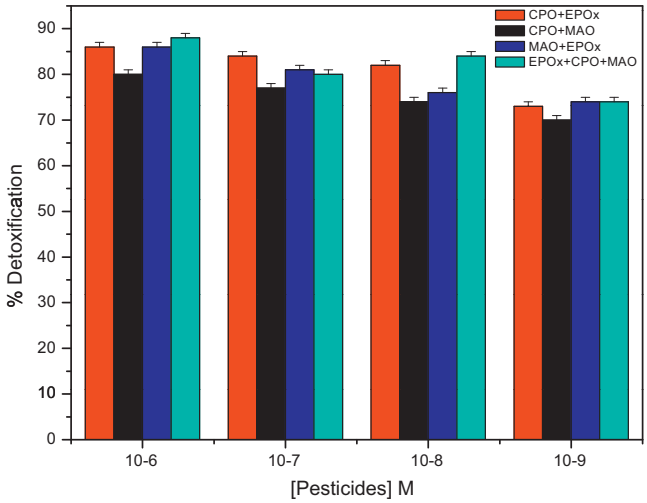


Fig. 3. Analysis of mixture of OPs (EPOx, MAO and CPO) in milk using PTE based detoxification column. Error bars represent mean and standard deviation of 4 values ($n = 4$).

3.6. Bio-detoxification of mixtures of OPs (EPOx, MAO and CPO)

After individual detoxification of three OPs, mixtures of these pesticides in milk were also tested using constructed detoxification column. Combination of pesticides was degraded using PTE enzyme. The pesticide concentration from 1×10^{-6} to 1×10^{-9} M was used for degradation studies. The compiled results are depicted in figure using bar graph between % detoxification and concentration of OPs mixture. As known to us, with increase in concentration, % detoxification is observed to be more for mixture of pesticides.

Fig. 3 represents detoxification of mixture of pesticides at four different concentrations in milk. When mixture of two pesticides is degraded, maximum degradation was observed for CPO + EPOx at each concentration. The observed trend is as follows: EPOx + CPO > EPOx + MAO > CPO + MAO. The mixture which contains EPOx, showed always higher detoxification as compare to other mixtures. When mixture of 3 pesticides (EPOx + MAO + CPO) was analyzed, cumulative/or additive detoxification effect was observed. Hence, obviously the degradation was observed to be more than binary mixture composition. It is also observed from the obtained data that the decrease in concentration also causes the reduction in % detoxification. The analytical figures of merit for detoxification of OPs are shown in Table 3.

Table 3
Analytical figures of merit obtained for detoxification of OPs using PTE column.

S.N.	Analytical features	Results obtained
1	Matrix tested	Water and milk
2	Temperature	25 °C
3	Response time	5 min
4	Analysis time	15 min
5	Range of OPs for detoxification	1×10^{-6} to 1×10^{-9} M
6	Minimum detoxification concentration	1×10^{-9} M
7	Maximum % detoxification achieved	Water 100% and milk 86%
8	% coefficient of variation	1.20

4. Conclusion

The present investigation showed the potential of PTE as a detoxification tool for OPs in water and milk. Three most toxic insecticides (EPOx, MAO and CPO) were tested in water and milk because of their presence and to check the cumulative detoxification effect in the presence of PTE. Under optimum conditions, a column containing 335 IU of PTE was shown to immediately detoxify EPOx, MAO and CPO in the concentration down to 1×10^{-9} M. In the case of MAO, the detoxification was lower, due to the very slow hydrolysis of this pesticide by PTE. In case of EPOx and CPO, the detoxification was found 86% and 76%, when the detoxified product was evaluated using flow based biosensor. OPs mixtures were also significantly detoxified in milk up to 86%. The method was shown to be adapted for the detoxification of OPs containing water and milk.

Acknowledgment

Rupesh K Mishra is highly thankful to The French Embassy in India; New Delhi for providing fellowship for 6 months through sandwich Ph D. program.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2012.07.018.

References

[1] G. Valdés-Ramírez, M. Gutiérrez, M. Del Valle, M.T. Ramírez-Silva, D. Fournier, J.L. Marty, Biosens. Bioelectron. 24 (2009) 1103–1108.
[2] R.K. Mishra, K. Deshpande, S. Bhand, Sensors 12 (2010) 11274–11286.
[3] G. Jeanty, A. Wojciechowska, J.-L. Marty, Anal. Bioanal. Chem. 373 (2002) 691–695.
[4] J.H. Salas, M.M. Gonzàles, M. Noa, N.A. Pèrez, G. Dìaz, R. Gutièrrez, R.H. Zazueta, I. Osuna, J. Agric. Food Chem. 51 (2003) 4468–4471.

[5] R.K. Mishra, R.B. Dominguez, S. Bhand, R. Muñoz, J.-L. Marty, Biosens. Bioelectron. 32 (2012) 56–61.
[6] D.J. Ecobichon, Toxic effect of pesticides, in: C.D. Klaassen (Ed.), Casaren & Daull's Toxicology: The basic science of Poisons, 6th ed., McGraw-Hill, New York, 2001, pp. 763–810.
[7] I. Gill, A. Ballesteros, Biotechnol. Bioeng. 70 (2000) 400–410.
[8] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption, Off. J. Eur. Comm. L 330 (1998) 32–54.
[9] Codex Alimentarius Pesticide Residues in Food, Joint FAO/WHO food standards programme, vol. 2, 1993, ISSN0259-2916.
[10] H.G. Bolles, H.E. Dixon-White, R.K. Peterson, J.R. Tomerlin, E.W. Day Jr., G.R. Oliver, J. Agric. Food Chem. 47 (1999) 1817–1822.
[11] T. Gazzotti, P. Sticca, E. Zironi, B. Lugoboni, A. Serraino, G. Pagliuca, Bull. Environ. Contam. Toxicol. 82 (2009) 251–254.
[12] R.K. Juhler, J. Chromatogr. A 786 (1997) 145–153.
[13] H. Mallatou, C.P. Pappas, E. Kondyli, T.A. Albanis, Sci. Total Environ. 196 (1997) 111–117.
[14] Y. Zhang, S.B. Muench, H. Schulze, R. Perz, B. Yang, R.D. Schmid, T.T. Bachmann, J. Agric. Food Chem. 53 (2005) 5110–5115.
[15] Zde.L. Cardeal, C.M. Dias Paes, J. Environ. Sci. Health Part B 41 (2006) 369–375.
[16] G. Pagliuca, A. Serraino, T. Gazzotti, E. Zironi, A. Borsari, R. Rosmini, J. Dairy Res. 73 (2006) 340–344.
[17] F.W. Holm, NATO ASI Series, Disarmament Technol, 6th ed., Kluwer Academic, Berlin, 1996.
[18] S. Lacorte, C. Molina, D. Barcelo, Anal. Chim. Acta 281 (1993) 71–84.
[19] R.B. Geerdink, W.M.A. Niessen, U.A.T. Brinkman, J. Chromatogr. A 970 (2002) 65–93.
[20] E. Ballesteros, M.J. Parrado, J. Chromatogr. A 1029 (2004) 267–273.
[21] M. Kuster, L.M. De Alda, D. Barcelo, Mass Spectrom. Rev. 25 (2006) 900–916.
[22] K. Deshpande, R.K. Mishra, S. Bhand, Appl. Biochem. Biotechnol. 164 (2011) 906–917.
[23] F.M. Raushel, Curr. Opin. Microbiol. 5 (2002) 288–295.
[24] E. Ghanem, F.M. Raushel, Toxicol. Appl. Pharmacol. 207 (2005) 459–470.
[25] S.R. Caldwell, F.M. Raushel, Appl. Biochem. Biotechnol. 31 (1991) 59–63.
[26] S.R. Caldwell, F.M. Raushel, Biotechnol. Bioeng. 37 (1991) 103–109.
[27] G. Istamboulie, R. Durbiano, D. Fournier, J.-L. Marty, T. Noguer, Chemosphere 78 (2010) 1–6.
[28] A.H. Mansee, W. Chen, A. Mulchandani, J. Ind. Microbiol. Biotechnol. 32 (2005) 554–560.
[29] P. Masson, D. Josse, O. Lockridge, N. Viguie, C. Taupin, C. Buhler, J. Physiol. 92 (1998) 357–362.
[30] S. Dennis-Quanquin, L. Lamouroux, A. Lougarre, S. Maheo, I. Saves, L. Paquereau, P. Demange, D. Fournier, J. Biotechnol. 131 (2007) 223–230.
[31] S. Andreescu, L. Barthelmebs, J.-L. Marty, Anal. Chim. Acta 464 (2002) 171–180.
[32] G. Istamboulie, S. Andreescu, J.-L. Marty, T. Noguer, Biosens. Bioelectron. 23 (2007) 506–512.
[33] G.L. Ellman, S.M. Coutney, V. Andres, R.M. Featherstone, Biochem. Pharm. 7 (1961) 88–92.
[34] G. Istamboulie, D. Fournier, J.-L. Marty, T. Noguer, Talanta 77 (2009) 1627–1631.
[35] M.A. Sogorb, E. Vilanova, Toxicol. Lett. 128 (2002) 215–228.