Sensors **2006**, *6*, 466-472

sensors

ISSN 1424-8220

http://www.mdpi.org/sensors

Biosensor for Direct Determination of Fenitrothion and EPN Using Recombinant *Pseudomonas putida* JS444 with Surface Expressed Organophosphorus Hydrolase. 1. Modified Clark Oxygen Electrode

Yu Lei [1], Priti Mulchandani [2], Wilfred Chen [2] and Ashok Mulchandani [2,*]

[1] Department of Chemical and Biomolecular Engineering, Nanyang Technological University, 639798, Singapore

[2] Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA

[*] Author to whom correspondence should be addressed. Email address: adani@engr.ucr.edu

Received: 29 July 2005 / Accepted: 9 March 2006 / Published: 11 April 2006

Abstract: This paper reports a first microbial biosensor for rapid and cost-effective determination of organophosphorus pesticides fenitrothion and EPN. The biosensor consisted of recombinant PNP-degrading/oxidizing bacteria *Pseudomonas putida* JS444 anchoring and displaying organophosphorus hydrolase (OPH) on its cell surface as biological sensing element and a dissolved oxygen electrode as the transducer. Surface-expressed OPH catalyzed the hydrolysis of fenitrothion and EPN to release 3-methyl-4-nitrophenol and *p*-nitrophenol, respectively, which were oxidized by the enzymatic machinery of *Pseudomonas putida* JS444 to carbon dioxide while consuming oxygen, which was measured and correlated to the concentration of organophosphates. Under the optimum operating conditions, the biosensor was able to measure as low as 277 ppb of fenitrothion and 1.6 ppm of EPN without interference from phenolic compounds and other commonly used pesticides such as carbamate pesticides, triazine herbicides and organophosphate pesticides without nitrophenyl substituent. The applicability of the biosensor to lake water was also demonstrated.

Keywords: Organophosphorus, fenitrothion, EPN, biosensor, *Pseudomonas putida*.

Introduction

Organophosphate pesticides fenitrothion and EPN are widely used for agricultural purpose in many countries, such as China, Brazil, Japan and Australia, due to their low cost and broad spectrum activity. Like other organophosphates, they are neurotoxic, inhibiting the activity of cholinesterase and over-stimulating the nervous system causing nausea at lower level exposure, and even death at higher and prolonged exposure [1,2]. Because of increasing concerns over agricultural worker health and its potential environmental impacts, there is a growing interest to develop novel analytical methods capable of performing rapid detection of these compounds in the field.

Many analytical methods, including gas and liquid chromatographic technologies [3-7], immunoassays [8-11], and biosensors based on cholinesterase or alkaline phosphatase inhibition [12-15], have been reported for the determination of organophosphorus pesticides. Although very sensitive, these methods are not satisfactory for rapid and on-line and in-field monitoring.

With the discovery of organophosphorus hydrolase (OPH), an enzyme that can hydrolyze a wide range of organophosphate pesticides releasing detectable products [16,17], several enzyme and microbial biosensors based on OPH for rapid, simple, selective monitoring of these neurotoxic pesticides with the potential for in the field analysis have been reported [18-29].

Recently, OPH was functionally expressed onto the surface of a natural *p*-nitrophenol (PNP) degrader, *Pseudomonas putida* JS444, using an ice nucleation protein (INP) anchor, creating a single microorganism with the capability to rapidly degrade organophosphate pesticides and PNP simultaneously while consuming oxygen [30]. Using this genetically engineered *Pseudomonas putida* we modified the Clark dissolved oxygen electrode, to construct a simple microbial biosensor for rapid, cost-effective, selective, precise and accurate determination of organophosphates with PNP substituent and demonstrated the application to paraoxon, parathion and methyl parathion monitoring [31]. In this communication, we extend the application of this biosensor to two other OPs with nitrophenyl substituent, fenitrothion and EPN under the optimum operating conditions reported before and demonstrate the applicability of the microbial biosensor for the rapid, cost-effective, selective, precise and accurate detection of fenitrothion and EPN.

Materials and methods

Materials, bacterial strains and growth conditions

All chemical reagents and the details of the recombinant PNP-degrader *Pseudomonas putida* JS444 anchoring and displaying OPH on the cell surface and its growth conditions used in this study have been described elsewhere [30].

Microbial electrode assembly

A predetermined amount of the cell suspension, based on the desired cell loading, was slowly dropped on a 25 mm diameter 0.4 μm pore size Nucleopore polycarbonate membrane (Whatman, NJ, USA) with slight suction. The cell retaining membrane was then placed on the top of the Teflon

membrane of the oxygen electrode (Model YSI 5331, Yellow Springs, OH, USA) and fixed in place by a rubber O-ring. The cells were thus immobilized (entrapped) between the two membranes.

Experiment set-up and measurement

The experimental set-up used in this research was identical to the one reported earlier [32]. All measurements were made at our reported optimized conditions with 0.086 mg dry weight of cell loading, operating in 50 mM pH 7.5 citrate-phosphate buffer with 50 μM $CoCl_2$ at room temperature [31]. The steady-state output of the oxygen electrode (after 5 minutes) was measured using a digital biological oxygen monitor (model YSI 5300, Yellow springs, Ohio, USA) connected to a chart recorder.

Results and discussion

Analytical characteristics

Figure 1A shows the dependence of the biosensor response on fenitrothion and EPN concentrations in operating buffer. As shown, the biosensor had a wide dynamic range with the oxygen consumption and a linear function of the pesticide concentrations up to 0.05 mM. The sensitivity (slope) of the microbial biosensor was 0.20% oxygen consumed per μM and 0.10% oxygen consumed per μM for fenitrothion and EPN, respectively.

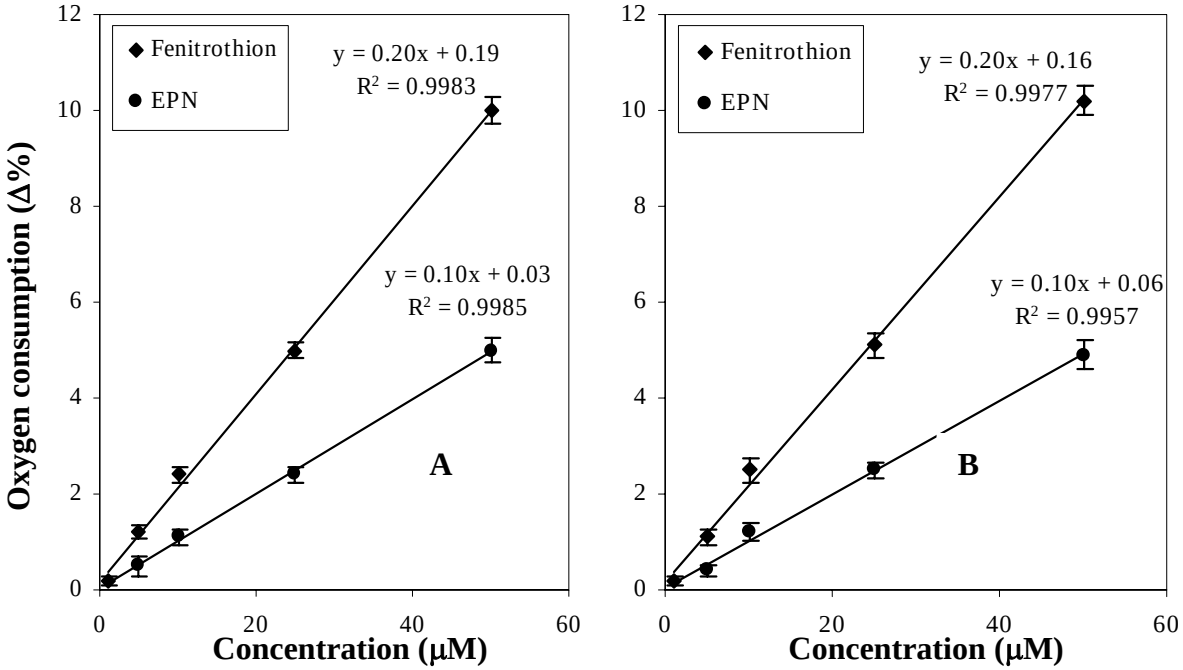

Figure 1. Calibration plots for fenitrothion and EPN. A) in 50 mM pH 7.5 citrate-phosphate buffer with 50 μM $CoCl_2$ and B) in Lake Elsinore water filtered and adjusted to pH 7.5 and 50 μM $CoCl_2$, at room temperature, with 0.086 mg cell loading. Data are given as mean ± 1 standard deviation for three experiments.

The lower limit of detection (LOD), determined as 3 times the standard deviation of the signal for buffer (blank), were 277 ppb of fenitrothion and 1.6 ppm of EPN, respectively. While comparable to the lower detection limit for biosensors based on cholinesterase and alkaline phosphatase inhibition [12-15], they were 2~3 orders of magnitude higher than those for immunoassays [8-11] and gas and liquid chromatography methods [3-7]. This will therefore limit the applicability of the present sensor for environmental monitoring. For any such application of the present biosensor, off-line sample preparation involving solid-phase extraction and sample concentration will be necessary. The present microbial electrode, however, will be ideal for selective on-line monitoring wastewater generated during production and consumption of the organophosphate pesticides (OP) and chemical or biological methods for treatment of OP-contaminated wastewaters.

In order to evaluate the matrix effect of naturally occurring compounds in real samples, the microbial biosensor was applied to measure target compounds spiked in lake water from Lake Elsinore, CA, after the lake water filtered through a 0.22 μm membrane and adjusted to pH 7.5 and 50 μM $CoCl_2$. The sensitivity of the biosensor response was similar to that observed in synthetic samples (Fig. 1B), thus demonstrating there was no interference from the components such as chlorophyll, phosphorus, nitrogen and several metal ions in the water from Lake Elsinore, CA, and the applicability of the microbial biosensor for organophosphate pesticides contaminated wastewaters. Additionally, as reported previously, the biosensor response was not interfered by 20-fold higher concentrations of sugars such as sucrose, fructose and galactose and other organic compounds such as glycerol and sodium acetate. However, high concentrations of glucose and lactic acid interfered. This interference is, however, not a concern as these compounds are not expected to be present in samples of interest [31].

Reproducibility is an important parameter for the sensor performance. The microbial biosensor demonstrated a very good reproducibility as evidenced by the low relative standard deviations (n=6) of 4.4% and 4.8% for 50 μM fenitrothion and EPN, respectively. Additionally, there was an excellent electrode-to-electrode reproducibility as characterized by the low relative standard deviations of 5.0% and 5.4% in the response of six microbial biosensors prepared at different times using different batches of cells to 50 μM fenitrothion and EPN, respectively.

In order to validate the microbial sensor, simulated samples (prepared by addition of OP compounds into buffer) representing OP-contaminated water were analyzed using the microbial biosensor and compared to a spectrophotometric assay based on the measurement of PNP (at 412 nm) formed by the OPH-catalyzed hydrolysis of OPs. As shown in Figure 2, there was an excellent agreement between the two methods confirming the accuracy and reliability of the developed microbial biosensor.

The detection of fenitrothion and EPN with the novel microbial biosensor is simple, direct, single step and rapid. The analysis time for each sample was less than 5 min, which is significantly shorter than the hours required for gas and liquid chromatography and immunoassays. The biosensor also shows excellent precision and selectivity described in our previous report [31]. When stored at operating buffer at 4 °C, the sensor response was stable for a period of 5 days. The rapid drop of the respiratory activity after 5 days is hypothesized to be a result of the depletion of the NAD(P)H in the resting/non-growing cells [33,34].

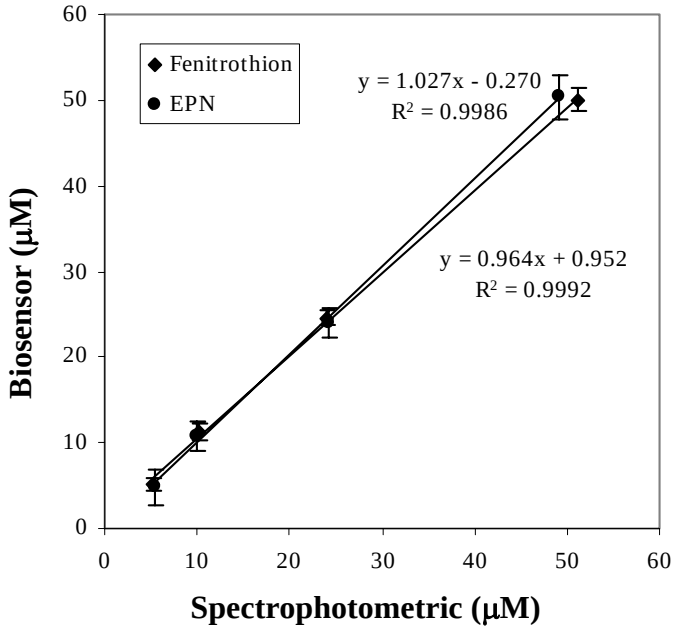

Figure 2. Accuracy of microbial biosensor. Fenitrothion and EPN as target compounds and operating in 50 mM pH 7.5 citrate-phosphate buffer with 50 μM $CoCl_2$ at room temperature, with 0.086 mg cell loading. Data are given as mean ± 1 standard deviation for three experiments.

Conclusions

In conclusion, a novel microbial biosensor using recombinant *Pseudomonas putida* JS444 and a Clark dissolved oxygen electrode for the direct, rapid and selective measurement of fenitrothion and EPN was reported. As a first reported microbial biosensor for fenitrothion and EPN, this very simple and low cost sensor showed excellent precision, accuracy, short response time and selectivity. These features make it a potentially attractive analytical tool for on-line monitoring of fenitrothion and EPN.

Acknowledgments

This work was supported by grants R82816001-0 from U.S. EPA, 99-35102-8600 from USDA, and BIO6-004-002 from U.S.-Egypt Joint Science and Technology Board. We thank Dr. J.C. Spain of the Air Force Engineering and Service Center, Tyndall Air Force Base, FL, for providing *Pseudomonas putida* JS444.

References

1. Kuriharai, N.; Paulson, G.D.; Otto, S.; Miyamot, J.; Hollingworth, R.M. Use of isolated cells to study the metabolism of agrochemicals in animals. Pure Appl. Chem., 1993, 65, 2299.
2. Wang, J.; Chen, G.; Muck, A.; Chatrathi, M.P.; Mulchandani, A.; Chen, W. Microchip enzymatic assay of organophosphate nerve agents. Analytica Chimica Acta, 2004, 505, 183.
3. Kim, D.H.; Heo, G.S.; Lee, D.W. Determination of organophosphorus pesticides in wheat flour by supercritical fluid extraction and gas chromatography with nitrogen-phosphorus detection. Journal of Chromatography A, 1998, 824, 63.

4. Lee, X.P.; Kumazawa, T.; Sato, K.; Suzuki, O. Detection of organophosphate pesticides in human body fluids by headspace solid-phase microextraction (SPME) and capillary gas chromatography with nitrogen-phosphorus detection. Chromatographia, 1996, 42, 135.
5. Schellin, M.; Hauser, B.; Popp, P. Determination of organophosphorus pesticides using membrane-assisted solvent extraction combined with large volume injection-gas chromatography-mass spectrometric detection. Journal of Chromatography A, 2004, 1040, 251.
6. Sanchez, M.E.; Mendez, R.; Gomez, X.; Martin-Villacorta, J. Determination of diazinon and fenitrothion in environmental water and soil samples by HPLC. Journal Of Liquid Chromatography & Related Technologies, 2003, 26, 483.
7. Cho, Y.; Matsuoka, N.; Kamiya, A. Determination of organophosphorus pesticides in biological samples of acute poisoning by HPLC with diode-array detector. Chemical & Pharmaceutical Bulletin, 1997, 45, 737.
8. Kolosova, A.Y.; Park, J.H.; Eremin, S.A.; Park, S.J.; Kang, S.J.; Shim, W.B.; Lee, H.S.; Lee, Y.T.; Chung, D.H. Synthesis of haptens of organophosphorus pesticides and development of enzyme-linked immunosorbent assays for parathion-methyl. Analytica Chimica Acta, 2004, 511, 323.
9. Kim, Y.J.; Cho, Y.A.; Lee, H.S.; Lee, Y.T. Synthesis of haptens of organophosphorus pesticides and development of enzyme-linked immunosorbent assays for parathion-methyl. Analytica Chimica Acta, 2003, 494, 29.
10. Watanabe, E.; Kanzaki, Y.; Tokumoto, H.; Hoshino, R.; Kubo, H.; Nakazawa, H. New approach to immunochemical determinations for triclopyr and 3,5,6-trichloro-2-pyridinol by using a bifunctional hapten, and evaluation of polyclonal antiserum. J. Agric. Food Chem., 2002, 50, 53.
11. Watanabe, E.; Kubo, H.; Nakazawa, H. Group-specific enzyme-linked immunosorbent assay for fenitrooxon and 3-methyl-4-nitrophenol in water samples based on a polyclonal antibody. Analytica Chimica Acta, 2002, 460, 99.
12. Kumaran, S.; Morita, M. Application of a cholinesterase biosensor to screen for organophosphorus pesticides extracted from soil. Talanta, 1995, 42, 649.
13. Diaz, A.N.; Peinado, M.C.R. Sol-gel cholinesterase biosensor for organophosphorus pesticide fluorimetric analysis. Sensors and Actuators B, 1997, 39, 426.
14. Diaz, A.N.; Sanchez, F.G.; Bracho, V.; Lovillo, J.; Aguilar, A. Enzymatic determination of fenitrothion by cholinesterase and acetylcholinesterase on fluorogenic substrates. Fresenius Journal of Analytical Chemistry, 1997, 357, 958.
15. Sanchez, F.G.; Diaz, A.N.; Peinado, M.C.R.; Belledone, C. Free and sol-gel immobilized alkaline phosphatase-based biosensor for the determination of pesticides and inorganic compounds. Analytica Chimica Acta, 2003, 484, 45.
16. Dumas, D.P.; Caldwell, S.R.; Wild, J.R.; Raushel, F.M. Purification and properties of the phosphotriesterase from pseudomonas-diminuta. J. Biol. Chem., 1989, 33, 19659.
17. Dumas, D.P.; Wild, J.R.; Raushel, F.M. Diisopropylfluorophosphate hydrolysis by a phosphotriesterase from pseudomonas-diminuta. Biotechnol. Appl. Biochem., 1989, 11, 235.
18. Mulchandani, A.; Kaneva, I.; Chen, W. Biosensor for direct determination of organophosphate nerve agents using recombinant Escherichia coli with surface-expressed organophosphorus hydrolase. 2. Fiber optic microbial biosensor. Anal. Chem., 1998, 70, 5042.

19. Mulchandani, A.; Mulchandani, P.; Kaneva, I.; Chen, W. Biosensor for direct determination of organophosphate nerve agents using recombinant Escherichia coli with surface-expressed organophosphorus hydrolase. 1. Potentiometric microbial electrode. Anal. Chem., 1998, 70, 4140.
20. Mulchandani, A.; Mulchandani, P.; Chen, W.; Wang, J.; Chen, L. Amperometric thick film strip electrodes for monitoring organophosphate nerve agents based on immobilized organophosphorus hydrolase. Anal. Chem., 1999, 71, 2246.
21. Mulchandani, A.; Pan, S.; Chen, W. Fiber-optic enzyme biosensor for direct determination of organophosphate nerve agents. Biotechnol. Progress, 1999, 15, 130.
22. Mulchandani, P.; Mulchandani, A.; Kaneva, I.; Chen, W. Biosensor for direct determination of organophosphate nerve agents. 1. Potentiometric enzyme electrode. Biosens. Bioelectron., 1999, 14, 77.
23. Mulchandani, A.; Chen, W.; Mulchandani, P.; Wang, J.; Rogers, K.R. Biosensors for direct determination of organophosphate pesticides. Biosens. Bioelectron., 2001, 16, 225.
24. Mulchandani, P.; Chen, W.; Mulchandani, A. Flow injection amperometric enzyme biosensor for direct determination of organophosphate nerve agents. Env. Sci. Technol., 2001, 35, 2562.
25. Mulchandani, P.; Chen, W.; Mulchandani, A.; Wang, J.; Chen, L. Amperometric microbial biosensor for direct determination of organophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. Biosens. Bioelectron., 2001, 16, 433.
26. Rogers, K.R.; Wang, Y.; Mulchandani, A.; Mulchandani, P.; Chen, W. Organophosphorus hydrolase-based assay for organophosphate pesticides. Biotech. Prog., 1999, 15, 517.
27. Wang, J.; Chen, L.; Mulchandani, A.; Mulchandani, P.; Chen, W. Remote biosensor for in-situ monitoring of organophosphate nerve agents. Electroanalysis, 1999, 11, 866.
28. Chough, S.H.; Mulchandani, A.; Mulchandani, P.; Chen, W.; Wang, J.; Rogers, K.R. Organophosphorus hydrolase-based amperometric sensor: Modulation of sensitivity and substrate selectivity. Electroanalysis, 2002, 14, 273.
29. Wang, J.; Chatrathi, M.P.; Mulchandani, A.; Chen, W. Capillary electrophoresis microchips for separation and detection of organophosphate nerve agents. Analytical Chemistry, 2001, 73, 1804.
30. Lei, Y.; Mulchandani, P.; Chen, W.; Mulchandani, A. Highly sensitive and selective amperometric microbial biosensor for direct determination of p-nitrophenyl-substituted organophosphate nerve agents. Env. Sci. Technol., 2005, 39, 8853.
31. Lei, Y.; Mulchandani, P.; Chen, W.; Mulchandani, A. Direct determination of p-nitrophenyl substituent organophosphorus nerve agents using a recombinant Pseudomonas putida JS444-modified Clark oxygen electrode. J. Agric. Food Chem., 2005, 53, 524.
32. Lei, Y.; Mulchandani, P.; Chen, W.; Wang, J.; Mulchandani, A. A microbial biosensor for p-nitrophenol using Arthrobacter sp. Electroanalysis, 2003, 15, 1160.
33. Han, T.S.; Kim, Y.C.; Karube, I. Microbial sensor for trichloroethylene determination. Anal. Chima. Acta, 2001, 431, 225.
34. Lei, Y.; Mulchandani, P.; Chen, W.; Wang, J.; Mulchandani, A. Whole cell-enzyme hybrid amperometric biosensor for direct determination of organophosphorous nerve agents with p-nitrophenyl substituent. Biotechnology and Bioengineering, 2004, 15, 706.