Analytical Letters, 38: 1071–1083, 2005
Copyright © Taylor & Francis, Inc.
ISSN: 0003-2719 print/1532-236X online
DOI: 10.1081/AL-200057192

BIOSENSORS

Microbial Biosensor for the Analysis of 2,4-Dichlorophenol

Jongjit Jantra
Department of Biotechnology, Lund University, Lund, Sweden, Biophysics Research Unit: Biosensors and Biocurrents, Prince of Songkla University, Hat Yai, Songkhla, Thailand, and Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Hamid Zilouei, Jing Liu, and Benoit Guieysse
Department of Biotechnology, Lund University, Lund, Sweden

Panote Thavarungkul
Biophysics Research Unit: Biosensors and Biocurrents, Prince of Songkla University, Hat Yai, Songkhla, Thailand and Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Proespichaya Kanatharana
Biophysics Research Unit: Biosensors and Biocurrents, Prince of Songkla University, Hat Yai, Songkhla, Thailand and Department of Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Bo Mattiasson
Department of Biotechnology, Lund University, Lund, Sweden

Received 7 December 2004; accepted 27 January 2005

Supported by a grant from VR (The Swedish Research Council)-SIDA (The Swedish International Development and Cooperation Agency) research links. The Royal Thai Government is also acknowledged for the financial support of Ms. Jongjit Jantra Msc. studies under the Higher Education Development Project: Postgraduate Education and Research Program in Chemistry (PERCH), Thailand.

Address correspondence to Bo Mattiasson, Department of Biotechnology, Lund University, P.O. Box 124, S-221 00, Lund, Sweden. E-mail: bo.mattiasson@biotek.lu.se

Abstract: A flow injection cell-based biosensor was constructed for 2,4-dichlorophenol (DCP) analysis by using a Clark-type oxygen electrode as a transducer. A mixed bacterial culture capable to biodegrade DCP was immobilized between a Teflon membrane and a dialysis membrane and attached to the oxygen electrode. Optimization of the flow rate, the injection volume, the carrier buffer concentration, and pH was carried out. Under optimum conditions (100 mM phosphate buffer, pH 7.50; flow rate 0.10 mL min^{-1}; sample volume 100 μL), the sensor response was linear between 0.01 and 0.30 mM DCP. The detection limit was 0.02 mM DCP, and the sensor was quite stable during 5 days of operation.

Keywords: Biosensor, chlorophenol, DCP, environmental pollution

INTRODUCTION

Chlorophenols are common environmental pollutants as a result of being widely used in various industrial processes (Annachhatre and Gheewala 1996; Antonopoulos et al. 2001; Czaplicka 2004; Abd-El-Haleem et al. 2003). Hence, because of their toxic properties and ubiquity in the environment, the U.S. EPA has classified 2,4-dichlorophenol (DCP) and many other chlorophenols as priority pollutants in water (Czaplicka 2004;Yee and Wood 1997). Likewise, the International Agency for Research on Cancers (IARC) has listed various chlorophenols as possible human carcinogens. Hence, it is important to develop fast, reliable, sensitive, and cheap analytical methods suitable for their detection from contaminated streams (Timur et al. 2003).

A number of sensitive and specific analytical methods have been developed (Timur et al. 2003). The most conventional is based on spectrophotometric detection monitoring the colored derivative formed when reactants are coupling with 4-aminoantipyrine (4-AAP). An inherent problem is, however, that the enol-keto equilibrium disturbs phenol analysis. In addition, certain *p*-substituted phenols show negligible response or do not react at all with 4-AAP. Finally, the method is sensitive to pH variations and it can be difficulty to find suitable buffers for the reaction (Kang et al. 2000). Other analytical techniques for the determination of phenolic compounds are based on gas or liquid chromatography combined with various detection methods. However, these analytical methods require extensive sample preparations, which often include a preconcentration step that is relatively time-consuming, requires skilled operators, and remains expensive with regards to the costs for equipments and the use and disposal of high-grade solvents (Puig and Barceló 1996; Lee et al. 1998; Bagheri, Mohammadi, and Selemi 2004; Kojima, Tsunoi, and Tanaka 2004).

The development of bioassays, mainly immunoassay and biosensors, has been considerable in the last few years because of the need to develop fast and cost-effective technologies suitable for on-site applications or for pollutant

screening prior to chromatographic analysis. Several immunoassays are commercially available for the detection of organic contaminants, but biosensors remain highly promising because of their faster response, ease to operate, and lower cost (Timur et al. 2003; Puig and Barceló 1996). Enzymes may be used for construction of phenol bioelectrodes (Anh et al. 2002; Dzyadevych et al. 2002; Rajesh, Takashima, and Kaneto 2004; Nandakumar and Mattiasson 1999), but these biosensors are often unstable because of fouling of electrodes by accumulation of polymerization products formed by the resulting quinines. Another reason may be inactivation of enzyme by these reactive products. Alternatively, whole cells and plant tissue from various fruits and vegetables have been combined with suitable electrodes. Microbial whole cell sensors are inexpensive and easily produced, possess sensitivity and stability comparable with those of enzyme sensors, and no additional efforts are needed for purification of enzyme. However, they usually lack the high selectivity that purified enzyme preparations offer and must be carefully optimized (Timur et al. 2003; Mattiasson 1983; Skládal, Morozova, and Reshetilov 2002).

In this study, an amperometric biosensor using bacterial cells was developed for the detection of 2,4-dichlorophenol (DCP). A mixed bacterial culture was obtained from a biofilm reactor used for chlorophenols biodegradation, and the sensor signals were based on the measurement of respiratory activity of the cells.

METHODS AND MATERIALS

All chemicals were commercially available and of reagent grade. All solutions were prepared with ultrapure water (Mill-Q water purification system, Millipore). All experiments were conducted at room temperature (23 ± 2°C)

Inoculum Preparation and Culture Conditions

A mixed bacterial culture taken from an aerobic biofilm reactor used for chlorophenol biodegradation and able to biodegrade DCP as a sole source of carbon and energy was used as inoculum. It was first enriched in mineral salt medium (MSM) supplied with DCP ($50\,mg\,L^{-1}$) as sole source of carbon and energy. This mixture was maintained by transferring the culture to 500 mL of fresh MSM every 10 days and was used as inoculum and for biosensor preparation in the following experiment. The MSM contained ($mg\,L^{-1}$): K_2HPO_4 4000, Na_2HPO_4 5200, KNO_3 3000, $CaCl_2 \cdot 7H_2O$ 10, $MgSO_4 \cdot 7H_2O$ 500, $FeSO_4 \cdot 7H_2O$ 10, $MnCl_2 \cdot 4H_2O$ 5.5, $ZnCl_2$ 0.68, $CoCl_2 \cdot 6H_2O$ 1.2, $NiCl_2 \cdot 6H_2O$ 1.2, $CuCl_2 \cdot 2H_2O$ 0.85, H_3BO_3 0.0031, $NaMoO_4 \cdot 2H_2O$ 0.012, $NaSeO_3 \cdot 5H_2O$ 0.013, and $NaWO_4 \cdot 2H_2O$ 0.0165.

Preliminary Tests

Serum flasks of 120 mL were filled with 65 mL of liquid growth medium containing DCP at 20, 50, 80, 110, and 150 mg L^{-1} and inoculated with 5 mL of bacterial mixture. The flasks were then sealed with rubber septa to allow gas sampling from the flasks' headspaces and agitated at 120 rpm on a rotary shaker. Gas samples were thereby periodically taken to follow the microbial activity. When necessary, liquid samples were taken for chemical analysis (DCP analysis by HPLC).

Biosensor Construction

Microbial culture (75 mL) was centrifuged for 5 min. After discharging the supernatant, the cells were washed once with 5 mL of 100 mM potassium phosphate buffer (KH_2PO_4-K_2HPO_4, pH 7.50) during 2–3 min before being centrifuged again. The supernatant was discharged, and the cells were suspended in phosphate buffer and gently stirred during 12 h. The suspended cells were then centrifuged, and the supernatant was discarded. The cell pellet was used as a cell paste for construction of a biomembrane.

A sample of cell pellet was placed on a Teflon membrane (PTFE, thickness 10 μm, Goodfellow, Cambridge, UK) and soaked with 100 mM of potassium phosphate buffer. A dialysis membrane (Spectra/por® 2-dialysis membrane, MWCO 12,000–14,000, Spectrum Laboratories, Inc., Houston, TX) was added to cover the cell preparation in such a way that no air bubbles were trapped. The Teflon side of the synthetic bilayered membrane containing microbial cells was placed toward the cathode of an oxygen electrode. Electrolyte was filled in the space between the synthetic biomembrane and the Clark electrode. The synthetic biomembrane was then fixed on the surface of the Clark electrode by means of a rubber O-ring. The sensor was placed in the flow cell, and 100 mM of potassium phosphate buffer (pH 7.50) was passed through the flow cell to stabilize the sensor during 12 h prior to use for 2,4-dichlorophenol analysis. The sensor was placed in a wall-jet flow cell with a modified cover to hold the sensor. The working and reference electrodes of the sensor were connected to a potentiostat (Zäta-Elektronik, Höör, Sweden), and the output was recorded on a chart recorder. An applied potential of −600 mV vs. Ag/AgCl was delivered to the Pt-working electrode during all measurements. The flow cell was integrated into a flow injection system equipped with an injection valve (VICI; Valco, Europe, Schenkon, Switzerland), an injection loop (100 μL; Valco), a peristaltic pump (U4-MIDI; ALITEA®AB, Stockholm, Sweden), and equipment to aerate the buffer in the buffer reservoir. The flow rate through the system was 0.10 mL min^{-1}, and an aerated 100 mM of potassium phosphate buffer (pH 7.50) was used as a carrier stream (Liu, Björnsson, and Mattis 2000; Håkansson and Mattiasson 2004).

Measurement and Optimization

Dissolved oxygen diffuses from the aerated phosphate buffer through the dialysis membrane, where a part of the oxygen is consumed by the immobilized bacteria. The remaining oxygen then continuously diffuses through the gas-permeable Teflon membrane and can be measured by the oxygen electrode. When 2,4-dichlorophenol is injected into the sensor system, it diffuses through the dialysis membrane where it is biodegraded by the immobilized bacteria, resulting in an increase of bacteria respiration rate and oxygen consumption. Therefore, less oxygen diffuses through the Teflon membrane, resulting in a decrease in the output sensor signal. Because the process is controlled by substrate diffusion, the sensor signal should be proportional to the concentration of the pollutant in the sample.

The sensitivity and the response time of the biosensor were optimized by injecting DCP at concentrations ranging from 0.03 to 0.50 mM under various conditions of flow rates, sample volumes, buffer pH values, and buffer concentrations (Table 1). For each set of parameters tested, the sensitivity of the biosensor was calculated as the regression coefficient of the sensor response vs. DCP concentration. Thereafter, all experiments were conducted under optimum conditions.

To determine the linear pollutant concentration range for analysis and the limit of detection, DCP was analyzed at concentrations ranging from 0.01 to 0.75 mM. The influence of phenol, 2-chlorophenol, 4-chlorophenol, trichlorophenol, glucose, sucrose, sodium acetate, and citric acid was then tested by injecting mixtures of either of these compounds at 0.10 mM together with 0.10 mM DCP. Finally, the stability of the biosensor was tested every day by assaying injections of DCP in the concentration range 0.02–0.30 mM. The calibration graphs were plotted, and the sensitivity at each day was determined. When the sensor was not used, the flow injection system was slowly perfused with 100 mM phosphate buffer.

Table 1. Biosensor optimization

Parameter[a]	Optimum values	Sensitivity	Response time
Flow rate ($mL\,min^{-1}$)	0.10	19.2	12
Sample volume (μL)	100	69.4	16
Buffer pH	7.50	25.0	12
Buffer concentration (mM)	100	25.0	12

[a]The default parameter values are (only one parameter was changed at the time): flow rate, 0.10 $mL\,min^{-1}$; sample vol., 100 μL; buffer pH, 7.50 and buffer concentration, 100 mM.

Analysis

Gas samples were analyzed by GC-TCD according to Borde et al. (2003). Liquid samples were analyzed for chlorophenol content by HPLC-UV (Elution with methanol:H_2O:acetic acid 60:39:1 v:v at 0.8 mL·min^{-1} and separation with Supelco LC-8 column, detection at 280 nm).

Results were analyzed with one-way and two-way ANOVA ($p \leq 0.05$), and outliers were eliminated by using the Grubb's test.

RESULTS AND DISCUSSION

Preliminary Tests

After 13 days of incubation, the oxygen levels in the gas phases of the flasks supplied with DCP at concentration of 20, 50, and 80 mg L^{-1} had decreased from 20.7 ± 0.1% to 18.3 ± 0.1, 14.1 ± 0.0, and 15.9 ± 0.0%, respectively. No significant decrease in the oxygen concentration was recorded in the flasks supplied with 110 mg DCP L^{-1}. No CO_2 production and no O_2 consumption were observed in the control flasks not supplied with DCP, and chemical analysis confirmed that DCP was removed after incubation in the flasks where microbial activity was recorded (data not shown). Hence, although the consortium tested was inhibited at high concentration of DCP, microbial activity could be followed by monitoring the oxygen consumption, showing that the system was suitable for use as whole cell biosensor combined with O_2 measurement for DCP quantification. On the basis of these results, log phase bacterial cells grown in the presence of 50 mg L^{-1} of DCP culture were used in the following experiments.

Optimization of the Sensor Response

Flow Rate

In a flow system, the flow rate of the solution passing by the detector is the main factor affecting the dispersion of the analyte molecules, the yield of the reaction, and the response of the detector. Increasing the flow rate can reduce dispersion effects and the response time but also decrease the yield of the reaction and thereby the sensor response. It is, therefore, important to test and optimize this parameter.

Under the range of DCP concentrations analyzed, the biosensor exhibited linear responses (with correlation factor >0.98) at the three flow rates tested (Figure 1). The highest sensitivity was achieved at a flow rate of 0.10 mL min^{-1} and decreased by 49% and 68% when the system was operated at flow rates of 0.20 and 0.30 mL min^{-1}, respectively (Figure 1).

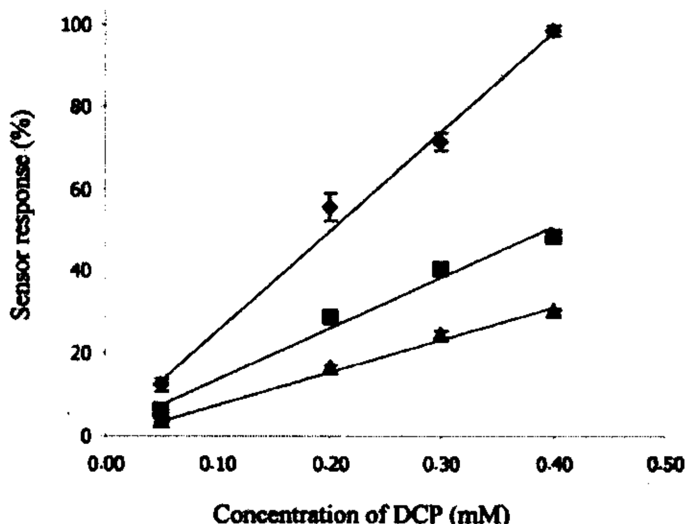

Figure 1. Effect of DCP concentration on the sensor response (%) when the sample were injected at a flow rate of 0.30 (▲), 0.20 (■), and 0.10 (◆) $mL\,min^{-1}$. The results are calculated as the percentage of the analyte peak area compared with the maximum area recorded during this experiment. Vertical bars represent the standard deviation on triplicates.

Experiments at flow rates lower than 0.10 $mL\,min^{-1}$ were not performed because of the restriction of the measuring time and the pump linearity at low flows. Although the analysis took longer time at a flow rate of 0.1 $mL\,min^{-1}$ (~14.6 min at this flow rate compared with 9.1 and 7.6 min at flow rates of 0.20 and 0.30 $mL\,min^{-1}$, respectively, at a DCP concentration of 0.20 mM), emphasis was given to sensitivity at low-analyte concentration, and the results showed that the sensor response at a DCP concentration of 0.05 mM was significantly higher at a flow rate of 0.10 $mL\,min^{-1}$. Hence, all subsequent experiments were performed at a flow rate of 0.10 $mL\,min^{-1}$.

Sample Volume

The response of the biosensor is directed by the amount of oxygen consumed by the bacteria, which is itself correlated to the amount of DCP injected. The amount of DCP then depends on the injection volume and the concentration in the sample. Larger injection volumes should then normally produce higher response signals and thereby improve the sensor sensitivity and detection limit. However, in a cell-based biosensor system, the response also depends on the amount of immobilized cell and the sensor can be saturated at too high concentrations of analyte due to oxygen limitation.

There was no significant difference between the sensor responses when 3 nM of DCP were injected from a 100-μL sample containing DCP at 0.03 mM or from a 10-μL sample containing DCP at 0.30 mM (Figure 2). Clearly, the best sample volume depends on the range of concentration and the limit of detection required. Sample volumes >100 μL were not studied because of saturation effects. The duration of an analysis was proportional to the injection time because it took about 5 min to complete the analysis of 0.1 mM of DCP injected at 10 μL compared with 7, 9, and 16 min when injected at 25, 50, and 100 μL, respectively. Once again, because this sensor was designed to operate at low DCP concentrations, an injection volume of 100 μL was preferred in the following tests.

Buffer pH

Because the catalytic activities of the enzymes involved in DCP catabolism are a function of the pH, this parameter can potentially affect the sensor response. The effect of the pH was studied by varying the pH of the 100 mM of potassium phosphate buffer used as carrier stream (Figure 3).

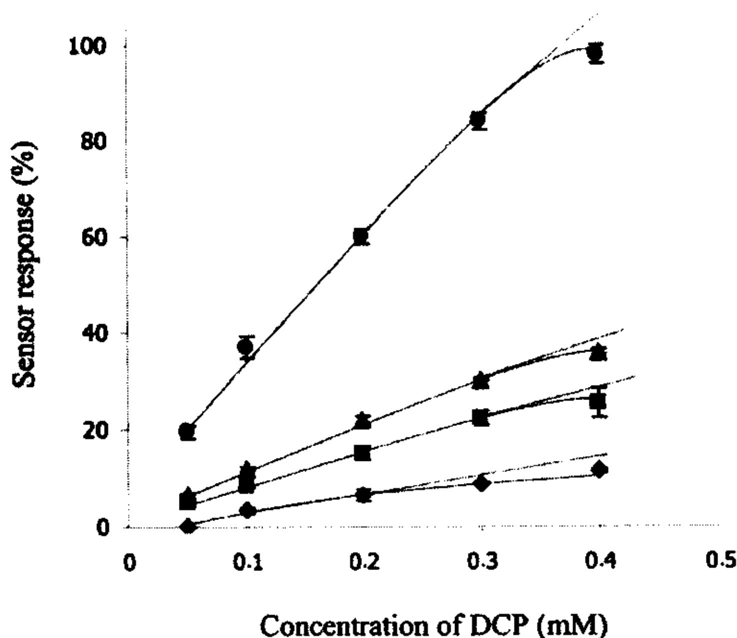

Figure 2. Effect of DCP concentration on the sensor response (%) when the injection volume was set to 100 (●), 50 (▲), 25 (■), and 10 (◆) μL. The results are calculated as the percentage of the analyte peak area compared with the maximum area recorded during this experiment. Vertical bars represent the standard deviation on triplicates.

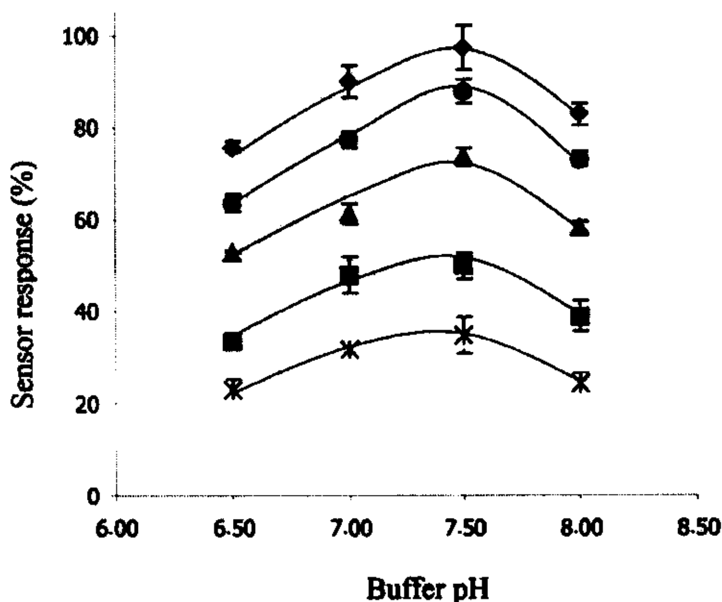

Figure 3. Effect of buffer pH on the sensor response (%) when the DCP concentration was set to 0.05 (×), 0.10 (■), 0.20 (▲), 0.30 (●), and 0.40 (◆) mM. The results are calculated as the percentage of the analyte peak area compared with the maximum area recorded during this experiment. Vertical bars represent the standard deviation on triplicates.

For DCP concentrations >0.05 mM, the sensor response was statistically higher at pH 7.50 than at other pH values. There was no statistical difference between the duration of analysis at analyte concentration of 0.05 and lower but at higher DCP concentration, the duration of analysis increased with the pH. However, these differences were not very relevant in regards to the total analysis time (the larger difference recorded was 3 min between the analysis of 0.40 mM of DCP at pH 6.00 and pH 8.00). It is, however, not directly obvious how the pH dependence of the electrode response is, because there is a surplus of cells (the reaction should be diffusion limited), and several different enzymes with potentially different pH profiles are involved in the processes eventually leading to the sensor response.

Buffer Concentration

The buffer concentration on the sensor should be optimized because it must be sufficiently high to compensate variations of pH in different samples permeating through the dialysis membrane. The highest sensor response was obtained

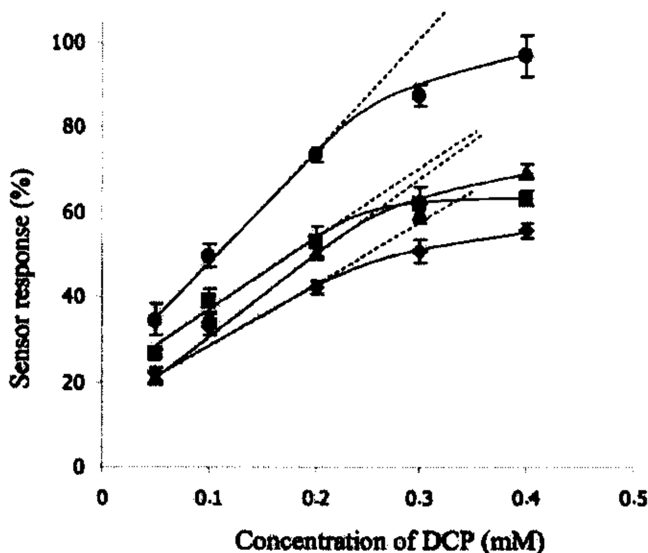

Figure 4. Effect of DCP concentration on the sensor response (%) when the buffer concentration was set to 10 (◆), 30 (▲), 50 (■), and 100 (●) mM. The results are calculated as the percentage of the analyte peak area compared with the maximum area recorded during this experiment. Vertical bars represent the standard deviation on triplicates.

at a buffer concentration of 100 mM (Figure 4) and, therefore, this concentration was chosen in further experiment.

Analytical Characteristic of the DCP Microbial Sensor

When the flow rate, sample volume, buffer pH, and buffer concentration were set to 0.10 mL min^{-1}, 100 μL, 7.50 and 100 mM, respectively, a linear correlation was observed between the sensor response and the DCP concentration ($r^2 = 0.9937$) for DCP concentrations ranging from 0.01 to 0.30 mM (Figure 5). The calibration equation was $y = 36.537x - 0.0436$ where y is response indicated as oxygen consumption measured by oxygen electrode and x is the concentration of DCP in mM. At optimum conditions, the lower detection was down to 0.02 mM of DCP by using statistical method (Miller and Miller 2000), a value rather typical for catalytic sensors based on polarographic oxygen sensors.

Sensor Stability

The stability of the sensor was investigated by evaluating the response of the same electrode to DCP in 100 mM phosphate buffer at pH 7.50 and

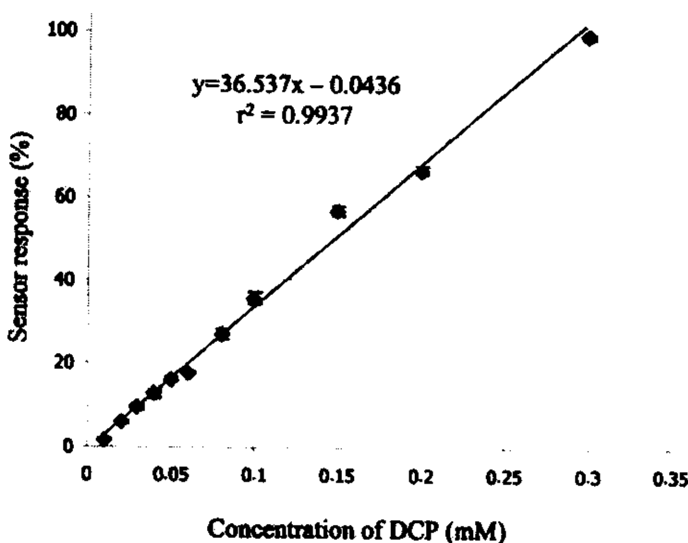

Figure 5. Effect of DCP concentration on the sensor response (%) when DCP concentration was set to 0.01–0.30 mM at optimum conditions. The results are calculated as the percentage of the analyte peak area compared with the maximum area recorded during this experiment. Vertical bars represent the standard deviation on triplicates.

0.10 mL min^{-1}. DCP was injected to the sensor system every day at 0.02, 0.05, 0.10, 0.15, 0.20, and 0.30 mM. Although the sensor sensitivity decreased with time, the microbial electrode was reasonably stable, and the response remained linear after 1 week of operation during which it was used a total of 90 times.

Interferences

Despite the fact that a microbial consortium was used, the sensor was quite specific to phenol and chlorophenols, it exhibited no or very little response to generic substrates such as glucose, sucrose, sodium acetate, and citric acid (Table 2). The specificity of the sensor could be improved by using pure isolate of DCP-degrading microorganisms from the consortium. However, bacteria that can degrade DCP are also often capable of using phenol and other chlorophenols as carbon sources. This remains an intrinsic limitation to the use of cell-based biosensors for analysis of environmental samples because chlorophenols are often found as mixtures. However, the lack of specificity for chlorophenols could even be exploited because the sensor may react to many different toxic compounds, yielding an integrating signal for the present of a group of contaminants (e.g., chlorophenols or just phenols) for the detection of other compounds.

Table 2. Interfering effect calculated as the increase (%) of sensor response when the target substance was injected with DCP in the sample at 0.1 mM in comparison with the response of 0.1 mM DCP

Substance	Interference (%)[a]
Phenol	103 ± 5
2CP	82 ± 8
4CP	135 ± 9
TCP	77 ± 9
Glucose	13 ± 46
Sucrose	0
Sodium acetate	13 ± 7
Citric acid	0

[a]These results were not statistically different at 5%.

CONCLUSION

The microbial biosensor for direct measurement of 2,4-dichlorophenol was developed. The developed biosensor was constructed with microbe immobilized electrode and flow system. The sensor gave response to other phenolic compounds and very little response to generic substrate. The sensor had an excellent lower detection limit and stability.

The conditions obtained for optimal performance of the electrode may not be ideal if the electrode is going to be used for process control, because then quicker analyses are needed. One way to achieve that would be to use a faster flow, but that is not compatible with the bioelectrode design used. If, however, a small precolumn with immobilized cells is used, and then a plain pO_2-electrode at the effluent, then a very high cell density can be used in the precolumn. Under such conditions, a higher flow rate can be used, and still good signals with regard to oxygen consumption can be registered.

REFERENCES

Abd-El-Haleem, D., Beshay, U., Abdelhamid, A.O., Moawad, H., and Zaki, S. 2003. Effects of Mixed Nitrogen Sources on Biodegradation of Phenol by Immobilized *Acinetobacter sp.* Strain W-17. *Afr. J. Ecol.*, 2: 8–12.

Anh, T.M., Dzyadevych, S.V., Soldatkin, A.P., Chien, N.C., Renault, N.J., and Chovelon, J.M. 2002. Development of Tyrosinase Biosensor based on pH-sensitive Field-effect Transistors for Phenol Determination in Water Solutions. *Talanta*, 56: 627–634.

Annachhatre, A.P. and Gheewala, S.H. 1996. Biodegradation of Chlorinated Phenolic Compounds. *Biotechnol. Adv.*, 14: 35–56.

Antonopoulos, V.T., Rob, A., Ball, A.S., and Wilson, M.T. 2001. Dechlorinations of Chlorophenols using Extracellular Peroxidases Produced by *Streptomyces albus* ATCC 3005. *Enzyme Microb. Technol.*, 29: 62–69.

Bagheri, H., Mohammadi, A., and Selemi, A. 2004. On-line Trace Enrichment of Phenolic Compounds from Water using a Pyrrole-based Polymer as the Solid-phase Extraction Sorbent Coupled with High-performance Liquid Chromatography. *Anal. Chim. Acta*, 513: 445–449.

Borde, X., Guieysse, B., Delgado, O., Muñoz, R., Hatti-Kaul, R., Nugier-Chauvin, C., Patin, H., and Mattiasson, B. 2003. Synergistic Relationship in Algal-bacteria Microcosms for the Treatment of Aromatic Pollutant. *Bioresour. Technol.*, 86: 293–300.

Czaplicka, M. 2004. Sources and Transformations of Chlorophenols in the Natural Environment. *Sci. Total Environ.*, 322: 21–39.

Dzyadevych, S.V., Anh, T.M., Soldatkin, A.P., Chien, N.C., Renault, N.J., and Chovelon, J.M. 2002. Development of Enzyme Biosensor based on pH-sensitive Field-effect Transistors for Detection of Phenolic Compounds. *Biochemistry*, 55: 79–81.

Håkansson, K. and Mattiasson, B. 2004. A Biosensor for the Analysis of Acetronitrile. *Biosens. Bioelectron.*, 19: 721–726.

Kang, C., Wang, Y., Li, R., Du, Y., Li, J., Zhang, B., Zhou, L., and Du, Y. 2000. A Modified Spectrophotometric Method for the Determination of Trace Amounts of Phenol in Water. *Microchem J.*, 64: 161–171.

Kojima, M., Tsunoi, S., and Tanaka, M. 2004. High Performance Solid-phase Analytical Derivertization of Phenol for Gas Chromatography-Mass Spectrometry. *J. Chromatogr. A*, 1042: 1–7.

Lee, M.R., Yeh, Y.C., Hsiang, W.S., and Hwang, B.H. 1998. Solid-phase Microextraction and Gas Chromatography-Mass Spectrometry for Determining Chlorophenols from Landfill Leaches and Soil. *J. Chromatogr. A*, 806: 317–324.

Liu, J., Björnsson, L., and Mattiasson, B. 2000. Immobilised Activated Sludge based Biosensor for Biochemical Oxygen Demand Measurement. *Biosens. Bioelectron.*, 14: 883–893.

Mattiasson, B., ed.: 1983. Analytical applications of immobilized cells. In *Immobilized Cells and Organelles*; CRC-Press: Boca Raton, FL; Vol. 2, 95–123.

Miller, J.N. and Miller, J.C. 2000. Calibration Methods in Instrumentatal Analysis: Regression and Correlation. In *Statistics and Chemometrics for Analytical Chemistry*, 4th Ed.; Pearson Education Limited: England, 120–123.

Nandakumar, R. and Mattiasson, B. 1999. A Tyrosinase based Enzyme Sensor for the Determination of Phenolic Compounds in an Expanded Bed System. *Resour. Environ. Biotechnol.*, 2: 327–338.

Puig, D. and Barceló, D. 1996. Determination of Phenolic Compounds in Water and Waste Water. *Trends Anal. Chem.*, 15: 362–375.

Rajesh, Takashima W. and Kaneto, K. 2004. Amperometric Tyrosinase based Biosensor using an Electropolymerized PTS-doped Polypyrole Film as an Entrapment Support. *React. Funct. Polym.*, 59: 163–169.

Škládal, P., Morozova, N.O., and Reshetilov, A.N. 2002. Amperometric Biosensor for Detection of Phenol using Chemically Modified Electrodes Containing Immobilized Bacteria. *Biosens. Bioelectron.*, 17: 867–873.

Timur, S., Pazarlioğlu, N., Pilloton, R., and Telefoncu, A. 2003. Detection of Phenolic Compounds by Thick Film Sensors based on *Pseudomonas putida*. *Talanta*, 61: 87–93.

Yee, D.C. and Wood, T.K. 1997. 2,4-Dichlorophenol Degradation using *Streptomyces viridosporus* T7A Lignin Peroxidase. *Biotechnol. Prog.*, 13: 53–59.