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Immobilization of microbial cells on inner epidermis of onion bulb scale for biosensor application

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ABSTRACT

Inner epidermis of onion bulb scales was used as a natural support for immobilization of microbial cells for biosensor application. A bacterium *Sphingomonas* sp. that hydrolyzes methyl parathion into a chromophoric product, *p*-nitrophenol (PNP), has been isolated and identified in our laboratory. PNP can be detected by electrochemical and colorimetric methods. Whole cells of *Sphingomonas* sp. were immobilized on inner epidermis of onion bulb scale by adsorption followed by cross-linking methods. Cells immobilized onion membrane was directly placed in the wells of microplate and associated with the optical transducer. Methyl parathion is an organophosphorus pesticide that has been widely used in the field of agriculture for insect pest control. This pesticide causes environmental pollution and ecological problem. A detection range 4–80 μM of methyl parathion was estimated from the linear range of calibration plot of enzymatic assay. A single membrane was reused for 52 reactions and was found to be stable for 32 days with 90% of its initial hydrolytic activity. The applicability of the cells immobilized onion membrane was also demonstrated with spiked samples.

1. Introduction

Biosensor is an analytical device in which biological systems are integrated with the transducer for detection and quantification of the specific analyte. Microbial biosensor is generally an immobilized cell that is combined with a transducer to monitor a specific change in the microenvironment (D'Souza, 2001b; Lei et al., 2006; Su et al., 2011). Biocomponent can be integrated with transducer directly or in combination with immobilizing support. Fundamental requirement of biosensor is that the biological component should bring the physico-chemical changes in close vicinity of a transducer and in this direction immobilization technology has played a key role (Turner et al., 1987; D'Souza, 1999, 2001a, 2001b; Tembe et al., 2006, 2008; Kumar and D'Souza, 2008, 2009, 2010). The choice of support and techniques should be such that it maintains the microbial enzyme activity and has reusability as well as storage stability. A variety of synthetic as well as natural polymers have been exploited for immobilization of cells and enzymes for biosensor preparation. Natural polymers in living organisms are composed of biomolecules like carbohydrates, lipids and proteins and therefore it can provide a biocompatible microenvironment for optimum functioning of enzyme and microbial cells. Natural polymers like eggshell membrane and bamboo inner shell membrane have been proved to be useful support for enzyme immobilization for biosensor application (Wu et al., 2004; Yang et al., 2006; Tembe et al., 2008). Recently our group has reported a new natural polymer, inner epidermis of the onion bulb scales as a support for immobilization of glucose oxidase enzyme for biosensor application (Kumar and D'Souza, 2009). Subsequently based on our work, Wang et al. (2010) immobilized glucose oxidase/*O*-(2-hydroxyl) propyl-3-trimethylammonium chitosan chloride nanoparticles on onion inner epidermis for development of glucose biosensor. Inner epidermis of the onion bulb scales consists of elongated tubular cells, blunt or tapering ends along with numerous guard cells (Scott et al., 1958; Bruce and Hepworth, 2004; Kumar and D'Souza, 2009). Structural features of the cell wall of inner epidermis cells are an elaborate extracellular matrix consisting of a microfibrillar cellulose phase and a matrix phase that contains a variety of polymers such as polygalacturonic acid (PGA), hemicelluloses, proteins, and phenolics, including lignin (Carpita and Gibeaut, 1993; Brett and Waldron, 1996; Kumar and D'Souza, 2009) and therefore it is mechanically stronger than the other reported natural polymer and can provide a biocompatible microenvironment and stable support for immobilization of biocomponent like enzymes and microbial cells.

In the case when enzymes are expressed in periplasm or in cytoplasmic membrane of cells, whole cells directly can be immobilized even without permeabilisation and they can be used for simple biosensor applications (Svitel et al., 1998; D'Souza, 2001a,

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2001b). Passive trapping of cells into the pores or adhesion on the surfaces of glass fibre or other synthetic membrane has been well documented (D'Souza, 1999, 2001a, 2001b; Jha et al., 2009; Kumar et al., 2006; Kumar and D'Souza, 2010). The major advantage of the cells immobilized through adhesion or adsorption is that they are in direct contact with the liquid phase containing the substrate thus eliminating the mass transfer problem which is commonly associated with entrapment and other methods of immobilization (D'Souza, 2001a, 2001b). A basic limitation of adsorption method or adhesion of cells on immobilizing support is the possibility of cell wash out from the immobilized support during continuous use and hence the biocomponent can be used only as disposable but not as reusable (Kumar et al., 2006). Adsorption followed by cross-linking eliminates cell wash out from the immobilized biocomponent and therefore it increases the reusability and stability of the immobilized biocomponent.

Methyl parathion is a nitro-aromatic organophosphate compound, which has been widely used as an insecticide although it is toxic to mammals (Melnikov, 1995; Stolyarov, 1998). Organophosphorus (OP) pesticides have been widely used in the field of agriculture for insect pest control. These pesticides and their degradation products cause environmental pollution and ecological problem. Recently, there has been an intense research effort to develop biosensor devices for the determination of organophosphorus pesticides (A. Mulchandani et al., 2001; P. Mulchandani et al., 2001; Kumar et al., 2006; Kumar and D'Souza, 2010). Methyl parathion can be hydrolyzed by organophosphorus hydrolase (OPH) enzyme, which was first discovered in soil microorganisms *Pseudomonas diminuta* MG and *Flavobacterium* sp. (Dumas et al., 1989; Munnecke and Hsieh, 1974). In each of these organisms enzyme was coded by *opd* gene (Mulbry et al., 1986; Harper et al., 1988; Somara et al., 2002) and hydrolyzes the methyl parathion into detectable product *p*-nitrophenol (PNP) (Kumar et al., 2006). PNP can be detected by electrochemical or colorimetric methods which can be exploited to develop a biosensor for detection of organophosphate pesticide. Our laboratory has been working in this field and reported a few microbial biosensors for detection of methyl parathion pesticide (Kumar et al., 2006; Kumar and D'Souza, 2010).

In the present study, we describe inner epidermis of onion bulb scale as natural support for immobilization of microbial cells of *Sphingomonas* sp. and its association with optical transducer of microplate reader for detection of methyl parathion pesticide. The analysis was based on the relationship between the amount of methyl parathion hydrolyzed and the amount of chromophoric product, PNP formed, which was quantified by measuring the absorbance at the λ_{max} 410 nm (Kumar et al., 2006; Kumar and D'Souza, 2010). Cells immobilized onion membranes were placed on bottom surface of the wells of microplate, which was associated with the optical transducer. Association of cells immobilized onion membrane with microplate having 96 reaction vessels provides a convenient system for detecting multiple numbers of samples in a single platform. Here biocomponent, cells immobilized onion membrane was reusable in nature.

2. Materials and methods

2.1. Materials

Methyl parathion (*O*,*O*-dimethyl *O*-4-nitrophenyl phosphorothionate) purity, 98.5% analytical grade was purchased from Dr. Ehrenstorfer Schorfers Augsburg, Germany, *p*-nitrophenol from Central Drug House, New Delhi, India. Glutaraldehyde (25%, w/w) solution was purchased from Sisco Research Laboratories, Mumbai. A bacterium *Sphingomonas* sp. JK1 was isolated from field soil for hydrolyzing methyl parathion pesticide up to a chromophoric product PNP, using oligonucleotide primers from *opd* gene (Accession No. EU709764) and organophosphorus hydrolase enzyme activity. Isolate was identified on the basis of morphological, biochemical and 16S rRNA gene (Accession No. EU616621) (Kumar and D'Souza, 2010). All other analytical grade chemicals were purchased from Sisco Research Laboratory, Mumbai, India.

2.2. Microorganism and culture condition

Isolate *Sphingomonas* sp. JK1, having methyl parathion hydrolyzing activity, was grown in 100 mL modified Wakimoto broth media (consist of 15 g sucrose; 5 g peptone; 2 g $Na_2HPO_4{\cdot}12H_2O$; 0.5 g $Ca(NO_3)_2{\cdot}4H_2O$; and 0.5 g $FeSO_4{\cdot}7H_2O$ in 1 L milliQ water) for 24 h at 30 °C. Growing time of cell biomass was studied by measuring the absorbance λ_{max} 600 nm for optimum enzyme activity. Inoculation of Luria broth (200 mL) was carried out with the 1/100th volume from the overnight grown culture (from modified Wakimoto broth) and incubated for 36 h at 30 °C on a rotary shaker at 140 rpm. Cells were harvested by centrifugation at 5000 × *g* for 10 min and washed twice with buffer. Finally, the pellet was resuspended in 1/10th volume in phosphate buffers (pH 8.0) and stored at 4 °C (Kumar and D'Souza, 2010).

2.3. UV–visible spectral study of methyl parathion hydrolysis by *Sphingomonas sp.*

UV–visible spectral scanning from 240 to 440 nm wavelengths was carried out for observation of hydrolysis of methyl parathion on UV/Visible spectrophotometer, from Jasco, Model V-530. The maximum absorption peaks of methyl parathion and *p*-nitrophenol are 273 nm and 410 nm, respectively. A 20 μL of harvested microbial cells were incubated with 1 mL of 200 μM methyl parathion and were scanned before and after hydrolysis of methyl parathion. A blank carrying only of 1 mL of 200 μM methyl parathion was used as control.

2.4. Optimization of cell loading and glutaraldehyde concentration for immobilization

Amount of cell suspension to be immobilized and concentration of glutaraldehyde for cross-linking were optimized. Different amounts of cell suspensions (0, 5, 10, 15, 20, 25 μL) were immobilized on membrane of inner epidermis of onion bulb scale and hydrolytic activities were observed. Immobilized cells were also cross-linked with different concentrations (0.2, 0.5, 1, 2, 5%) of glutaraldehyde. The immobilized microbial hydrolytic activity was observed in response to methyl parathion.

2.5. Immobilization of microbial cells on inner epidermis of onion

A circular membrane (diameter = 5 mm; area = 19.64 sq mm) of inner epidermis of onion bulb scale was cut and used for immobilization of bacterial cells. A 20 μL cell suspension was adsorbed onto the inner epidermis of onion bulb scale and was dried in air for 1 h at room temperature. Cross-linking was then activated by addition of 3 μL of 2% glutaraldehyde followed by incubation at room temperature and binding the cells to the surface and to each other. After immobilization, microbial onion membrane was washed with buffer and stored at 4 °C until use.

2.6. Transducer and operating system

Optical transducer integrated in multidetection microplate reader (MDMR) from Biotek (model Synergy™ HT) was used for the detection of absorbance at 410 nm (Kumar and D'Souza, 2010). The

Synergy Time Resolved (TM) option allows time-resolved measurements by using the xenon flash light source in conjunction with the photomultiplier tubes (PMT) detector. The monochromator provides wavelength selection from 200 to 999 nm in 1 nm increments. The reader is completely controlled via KC4^{TM} PC software for all operations including data reduction and analysis. Online monitoring of each well of 96 wells microplate can be carried out using this software. Cells immobilized onion membranes were placed into the wells and 200 μL volume of methyl parathion sample was added into the wells and readings were acquired at 410 nm wavelength that corresponds to PNP. The absorbance differences between the initial and final readings were proportional to the concentration of PNP. Cells modified onion biocomponent was washed with buffer after every analysis of sample and reused. All the experiments were carried out at room temperature.

2.7. SEM study of the microbial cells immobilized onion membrane

A scanning electron microscope (Model XL30 Philips, Netherlands) was employed to observe the surface structure of the cells immobilized onion membrane. For SEM study, cells immobilized onion membrane was mounted on stubs and coated with Au/Pd using a sputter coater. The SEM micrographs of the whole cells immobilized and unimmobilized (blank) onion membranes were taken at magnification 10,000×.

2.8. Enzymatic assay using cells immobilized onion membrane

Optical microplate transducer was calibrated using standard concentration (4–400 μM) of PNP by determining the absorbance at λ_{max} 410 nm (ε_{410} = 16,500 M^{-1} cm^{-1} for PNP). Enzymatic assays were carried out with cells immobilized onion biocomponent using methyl parathion concentration ranging from 4 to 400 μM in phosphate buffer (pH 8.0) at room temperature for 5 min and appearance of PNP was measured on optical microplate reader at λ_{max} 410 nm (Kumar and D'Souza, 2010).

2.9. Analysis of methyl parathion spiked samples

Synthetic methyl parathion spiked samples were prepared by incubating methyl parathion in the tap water at room temperature with the final concentrations of 5, 10, 20, 30, 40, 50 and 60 μM. The pre-incubated tap water samples were mixed with phosphate buffer (pH 8.0) in ratio 3:1 and analyzed by microbial biosensor at room temperature.

3. Results and discussions

3.1. UV–visible spectral study of methyl parathion hydrolysis

Hydrolysis of methyl parathion using *Sphingomonas* sp. JK1, which has *opd* gene for organophosphorus hydrolase enzyme, was studied by UV–visible spectral scanning. The scanning results are shown in Fig. 1; the maximum absorption peak at 273 nm was recorded when only methyl parathion sample was scanned. Just after addition of microbial cells in methyl parathion sample, a new absorption peak appeared at 410 nm and after complete hydrolysis of methyl parathion, peak at 410 nm increased and peak at 273 nm decreased.

3.2. Optimization of cell loading and glutaraldehyde for immobilization

Because of the limited space of circular membrane of inner epidermis of onion bulb scale (diameter = 5 mm; area = 19.64 sq mm),

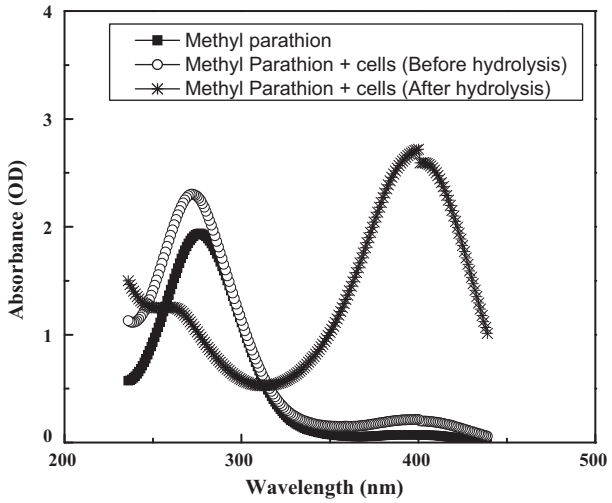

Fig. 1. UV–visible spectral study for the hydrolysis of methyl parathion using isolate *Sphingomonas* sp.

there is need to optimize the effect of biomass (cells) loading for immobilization of cells to achieve the optimum hydrolytic activity. In order to investigate the effect of biomass loading, different amounts of cell suspension were used for immobilization and hydrolytic activities were observed. As shown in Fig. 2a, 20 μL cell suspension used for immobilization was having optimum hydrolytic activity. Glutaraldehyde concentration was also optimized for cross-linking. It was also observed in Fig. 2b that 2% glutaraldehyde concentration was optimum for cross-linking and binding the cells to the surface and to each other to prevent the leaching effects and thereby increases the stability and longevity of the biocomponent. Optimized glutaraldehyde concentration was similar to that of the previous report (Kumar and D'Souza, 2009, 2010).

3.3. SEM study of the cells immobilized inner epidermis of onion membrane

SEM study was carried out to investigate the surface characteristics of unimmobilized (blank) and whole cells immobilized onion membrane and described the occurrence of microbial cells on the natural support. In this part, surface morphologies of the immobilized area of both blank and cells immobilized inner epidermis of onion bulb scale were imaged by SEM (Fig. 3). SEM micrographs of inner epidermis of onion bulb scale showed that it consists of cellulosic fibrous structures. A bunch of bacterial cells (sizes 0.4–0.8 μm) were observed in the micrograph of cells immobilized inner epidermis of onion bulb scale (Fig. 3b) and was absent in the micrograph of unimmobilized surface (Fig. 3a). Presence of bacterial cells confirms the immobilization of *Sphingomonas* sp. onto the inner epidermis of onion bulb scale.

3.4. Association of microbial onion membrane with microplate

Onion membrane is a natural polymer, comprises biomolecules like microfibrillar cellulose, PGA, hemicelluloses, proteins and phenolics including lignin (Carpita and Gibeaut, 1993; Brett and Waldron, 1996; Kumar and D'Souza, 2009) and therefore it provided a biocompatible microenvironment and stable support for optimum functioning of microbial enzyme. Cells immobilized onion membranes were placed into the wells of microplate. Microplate technology was utilized to develop an effective biosensor tool for detection of multiple numbers of samples (Kumar and D'Souza, 2010). There are two characteristics of microplate which

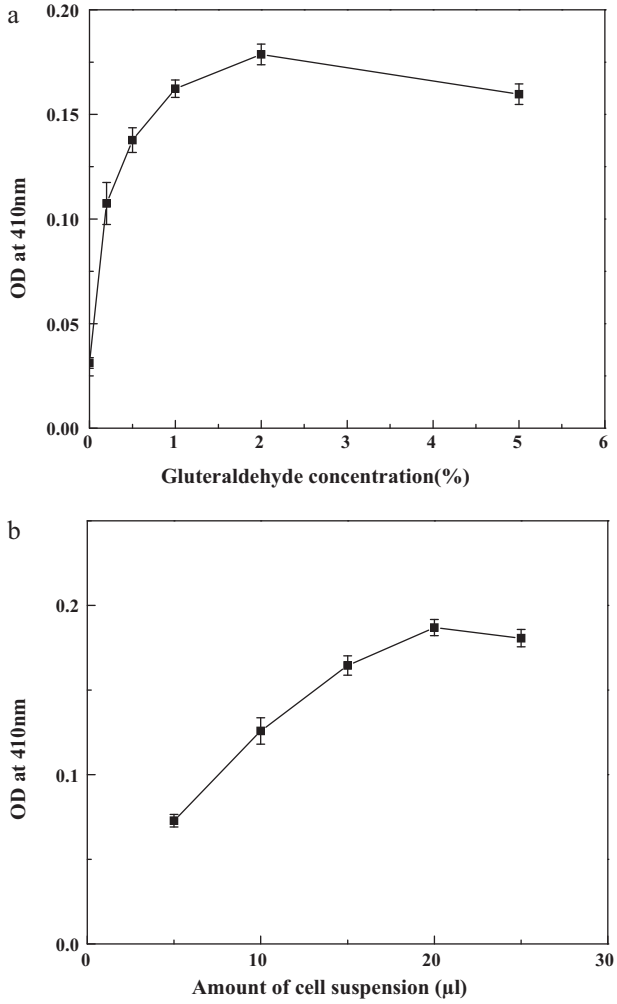

Fig. 2. Optimization of cell biomass loading (a) and glutaraldehyde concentration (b) for immobilization on onion membrane (size 5 mm diameter).

were utilized for developing an improved biosensor, first characteristic is that the microplate is having 96 wells (reaction vessels) and therefore multiple number of samples can be handled simultaneously on a single platform and second characteristic is that it provides a simpler approach of two-dimensional micropositioning, which enables the acquisition of time independent measurement of

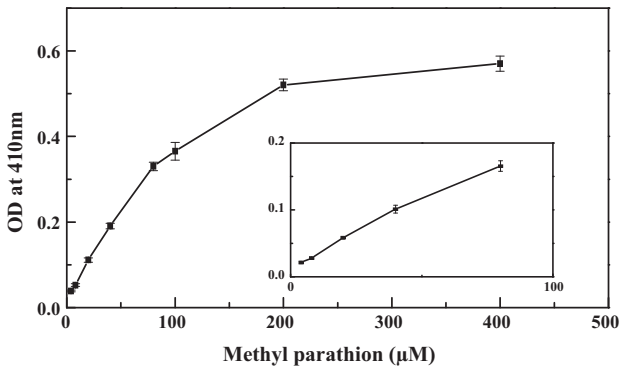

Fig. 4. Calibration of the optical biosensor using cells immobilized onion membrane with methyl parathion (4–400 µM) (inset: linearity between 4 and 80 µM with linear regression equation, $y = 0.01296 + 0.00212x$, $r^2 = 0.9819$ where 'y' represents the absorbance at 410 nm and 'x' the substrate concentration).

the whole plate irrespective of the number of samples present on the plate (Filippini et al., 2003). Cells modified onion membrane on the bottom surface of microplate in association with optical transducer (MDMR) and KC4 software was capable for rapid data acquisition and efficient data handling and therefore this system became capable for online monitoring of multiple samples, even with different concentrations, on a single platform simultaneously. This is the first report where microbial cells have been immobilized on the onion membrane and integrated with optical transducer of microplate reader for biosensor application.

3.5. Calibration of the biosensor and detection range

Biosensor was calibrated in association with microbial onion membrane using different (4–400 µM) concentrations of methyl parathion and absorbance was observed. As shown in Fig. 4, a linear range between 4 and 80 µM of methyl parathion was estimated using cells immobilized onion membrane and the detection range of biosensor was calibrated between 4 and 80 µM methyl parathion. Detection range of biosensor using cells immobilized onion membrane was equally comparable to that of the earlier report (Kumar et al., 2006; Kumar and D'Souza, 2010).

3.6. Reusability, reproducibility and stability of the whole cells immobilized onion membrane

Reusability, reproducibility and stability are desirable characteristics of a good biosensor. Reusability is one of the desirable

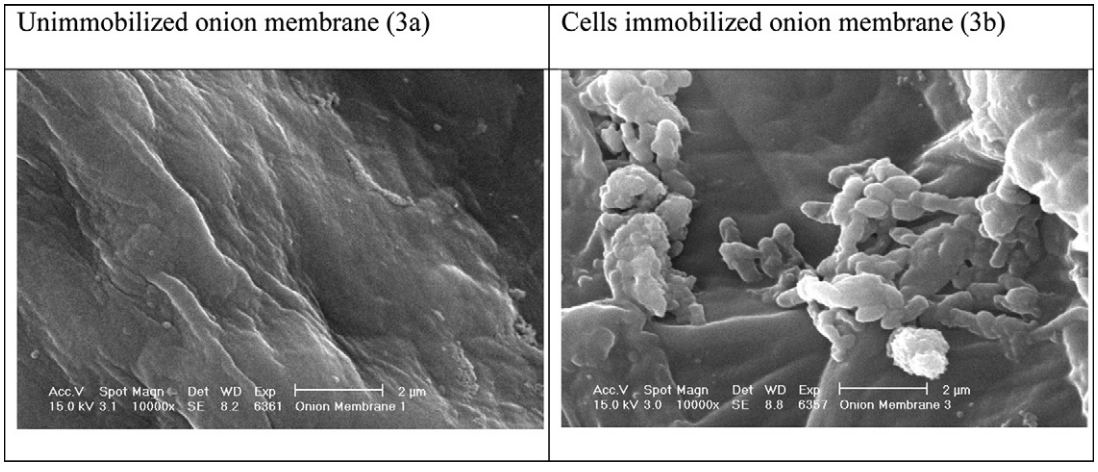

Fig. 3. SEM study of the cells immobilized onion membrane at 10,000×. Micrograph of unimmobilized (a) and cells immobilized (b) onion membrane.

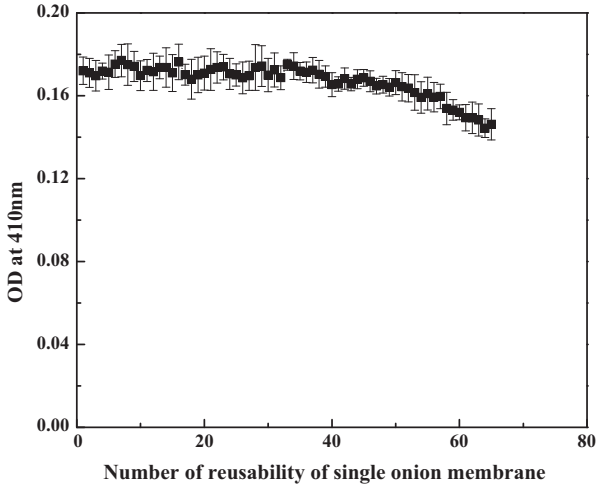

Fig. 5. Reusability of the cells immobilized onion membrane. 80 μM methyl parathion was used for the study of reusability.

factors which is important for the applicability of the immobilized biocomponent in biosensor application. The reusability of microbial cells immobilized onion membrane, prepared in the presence and absence of glutaraldehyde was studied. Whole cells immobilized onion membrane, prepared in the presence of glutaraldehyde showed a high number of reusability. Glutaraldehyde treatment bound the adsorbed microbial cells onto the onion membrane and to each other by cross-linking and therefore reduced the leaching of whole cells, thus increasing the reusability of the immobilized microbial cells. It was observed that 90% activity of immobilized whole cells enzyme was retained up to 52 repeated reactions with single cells immobilized onion membrane (Fig. 5). Although cells immobilized onion biocomponent was less reusable as compared to our earlier report (Kumar and D'Souza, 2010) but was better than the other report on disposable biocomponent (Kumar et al., 2006). The reproducibility of the measurement in different wells using different cells immobilized onion membrane across the microplate was quite good. The low relative standard deviations (RSD), 0.156 (mean = 8.48×10^{-6} A, when $n = 6$) in response of cells immobilized onion membrane against 80 μM methyl parathion also demonstrated high reproducibility. Microbial cells immobilized onion membrane was stable for 32 days of investigation (Supplementary Fig. 1) with retention of 90% activity, when stored at 4 °C which is better than that of the earlier report which was stable for only 18 days (Kumar and D'Souza, 2010).

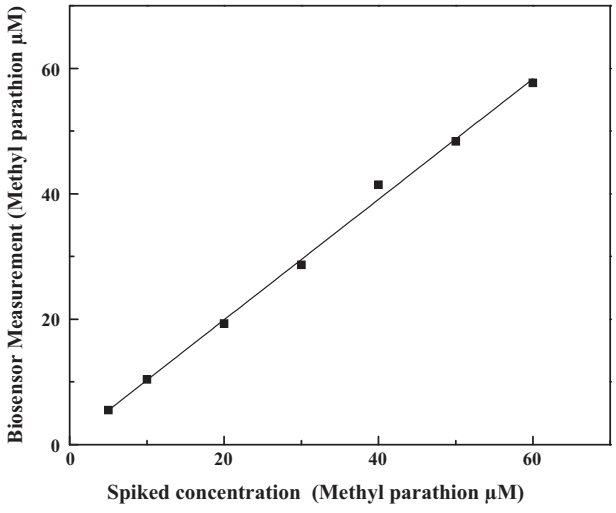

Fig. 6. Correlation of the biosensor measurement with actual methyl parathion spiked concentration. Spiked methyl parathion concentrations (5, 10, 20, 30, 40, 50 and 60 μM).

3.7. Applicability of biosensor for methyl parathion spiked samples

The pre-incubated tap water samples were mixed with phosphate buffers (pH 8.0) in ratio 3:1 for maintaining the optimum pH for optimum functioning of microbial enzyme and spiked samples were analyzed by *Sphingomonas* sp. immobilized onion based optical biosensor. As shown in Fig. 6 the straight line fit plot between the spiked concentration of methyl parathion and the biosensor results yielded a slope of 0.961 with a positive correlation ($r^2 = 0.9963$) which demonstrates the feasibility of the cells immobilized onion membrane for biosensor application.

4. Conclusion

We described immobilization of microbial cells on inner epidermis of onion bulb scale and its application in development of microbial biosensor. A bacterium *Sphingomonas* sp. which hydrolyzes the methyl parathion up to a chromophoric product, *p*-nitrophenol was immobilized directly onto the surface of inner epidermis of onion bulb scale by adsorption followed by cross-linking. Immobilization of microbial cells was confirmed by SEM study. Cells immobilized onion biocomponent was placed on the bottom surface of microplate and associated directly with the optical transducer, microplate reader. Multiple cells immobilized biocomponents were placed on reaction vessels of microplate for analysis of multiple samples on a single platform. Detection range of the biosensor was calibrated between 4 and 80 μM methyl parathion and cells modified onion biocomponent had shown a reusability up to 52 reactions. This is the first report on employing inner epidermis of onion bulb scales as natural support for microbial cells immobilization and its application as reusable microbial biocomponent for biosensor application.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2011.04.049.

References

Brett, C.T., Waldron, K.W., 1996. Physiology and Biochemistry of Plant Cell Walls. Chapman & Hall, London, pp. 42–43.
Bruce, D.M., Hepworth, D.G., 2004. J. Texture Stud. 35, 586–602.
Carpita, N.C., Gibeaut, D.M., 1993. Plant J. 3, 1–30.
D'Souza, S.F., 1999. Curr. Sci. 77, 69–79.
D'Souza, S.F., 2001a. Appl. Biochem. Biotechnol. 96, 225–238.
D'Souza, S.F., 2001b. Biosens. Bioelectron. 16, 337–353.
Dumas, D.P., Caldwell, S.R., Wild, J.R., Raushel, F.M., 1989. J. Biol. Chem. 264, 19659–19665.
Filippini, D., Andersson, T.P.M., Svensson, S.P.S., Lundstrom, I., 2003. Biosens. Bioelectron. 19, 35–41.
Harper, L.L., McDaniel, C.S., Miller, C.E., Wild, J.R., 1988. Appl. Environ. Microbiol. 54, 2586–2589.
Jha, S.K., Kanungo, M., Nath, A., D'Souza, S.F., 2009. Biosens. Bioelectron. 24, 2637–2642.
Kumar, J., D'Souza, S.F., 2008. Talanta 75, 183–188.
Kumar, J., D'Souza, S.F., 2009. Biosens. Bioelectron. 24, 1792–1795.
Kumar, J., D'Souza, S.F., 2010. Biosens. Bioelectron. 26, 1292–1296.
Kumar, J., Jha, S.K., D'Souza, S.F., 2006. Biosens. Bioelectron. 21, 2100–2105.
Lei, Y., Chen, W., Mulchandani, A., 2006. Anal. Chim. Acta 568, 200–210.

Melnikov, N.N., 1995. Pesticides and Plant-growth Regulators: A Handbook. Khimiya Publishers, Moscow (in Russian).
Mulbry, W.W., Karns, J.S., Kearney, P.C., Nelson, J.O., McDaniel, C.S., Wild, J.R., 1986. Appl. Environ. Microbiol. 51, 926–930.
Mulchandani, A., Chen, W., Mulchandani, P., Wang, J., Rogers, K.R., 2001. Biosens. Bioelectron. 16, 225–230.
Mulchandani, P., Chen, W., Mulchandani, A., 2001. Environ. Sci. Technol. 35, 2562–2565.
Munnecke, D.M., Hsieh, D.P.H., 1974. Appl. Microbiol. 28, 212–217.
Scott, F.M., Hammer, K.C., Baker, E., Bowler, E., 1958. Am. J. Bot. 45, 449–461.
Somara, S., Manavathi, B., Tebbe, C., Siddavattam, D., 2002. Indian J. Biochem. Biophys. 39, 82–86.
Stolyarov, M.V., 1998. Zaschita I Karanthin Rastenii 3, 16–17.
Su, L., Jia, W., Hou, C., Lei, Y., 2011. Biosens. Bioelectron. 26, 1788–1799.
Svitel, J., Curilla, O., Tkac, J., 1998. Biotechnol. Appl. Biochem. 27, 153–158.
Tembe, S., Karve, M., Inamdar, S., Haram, S., Melo, J., D'Souza, S.F., 2006. Anal. Biochem. 349, 72–77.
Tembe, S., Kubal, B.S., Karve, M., D'Souza, S.F., 2008. Anal. Chim. Acta 612, 212–217.
Turner, A.P.F., Karube, I., Wilson, G.S. (Eds.), 1987. Biosensors Fundamentals and Applications. Oxford University Press, Oxford, UK.
Wang, F., Yao, J., Russel, M., Chen, H., Chen, K., Zhou, Y., Ceccanti, B., Zaray, G., Choi, M.M.F., 2010. Biosens. Bioelectron. 25, 2238–2243.
Wu, B., Zhang, G., Shuang, S., Choi, M.M.F., 2004. Talanta 64, 546–553.
Yang, X., Zhou, Z., Xiao, D., Choi, M.M.F., 2006. Biosens. Bioelectron. 21, 1613–1620.