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An optical microplate biosensor for the detection of methyl parathion pesticide using a biohybrid of *Sphingomonas* sp. cells-silica nanoparticles

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ABSTRACT

The previously developed *Sphingomonas sp.* based optical microplate biosensor for methyl parathion (MP) was good as it detected multiple samples but had poor stability and low sensitivity. The present study aims to overcome these limitations. Silica nanoparticles (Si NP) were thus functionalized with polyethyleneimine (PEI) and the functionalized silica nanoparticles (^{f}Si NP) were then integrated with *Sphingomonas* sp. cells. The process was optimized for hydrolysis of MP into p-nitrophenol (PNP). Integration of ^{f}Si NP with cells was confirmed by FT-IR analysis. Biohybrid of *Sphingomonas sp.*-^{f}Si NP was immobilized on the wells of microplate and associated directly with the optical transducer of microplate reader. Immobilized biohybrid of *Sphingomonas sp.*-^{f}Si NP was characterized using SEM. A detection range of 0.1–1 ppm MP was achieved from the linear range of calibration plot. After integration with ^{f}Si NP the storage stability of biohybrid was enhanced ten times from 18 to 180 days. This study proves that after interaction of cells with ^{f}Si NP, improved the sensitivity and stability of the biosensor. Spiked samples were also analyzed and correlated using this biohybrid based biosensor.

1. Introduction

Methyl parathion (MP) is an organophosphate (OP) compound that is used as a non-systemic insecticide in agriculture to protect the crops from insect pests. However, MP can cause problems to human health related to acetylcholinesterase inhibition. Thus, it has been classified by the World Health Organization (WHO) under 'Category Ia' (extremely toxic) and by the United States Environmental Protection Agency (US EPA) under 'Toxicity Category I' (most toxic) insecticide (FAO/WHO, 1986; Kumar and Melo, 2015). Although it is banned in developed countries, it is still being used in developing countries like India as a restricted insecticide. As per the statistical report from the Directorate of PPQS, India and the Centre of Science and Environment (CSE), consumption of MP in India was 2739 MT during 2009–10 alone (CSE, 2011; Kumar and Melo, 2015; PPQS 2016). Presence of this insecticide is thus expected in the soil samples, water resources and even in food materials. The FAO/WHO Codex Alimentarius Commission has recommended the Maximum Residue Limits (MRLs) in several food commodities in the range between 0.1 and 1 ppm (FAO/WHO, 1986). Food Safety and Standards Authority of India (FSSAI) has set MRLs of MP in food commodities in the range of 0.2–1 ppm (FSSAI, 2011, 2016). Therefore, there is an urgent need to develop a method that is rapid, sensitive, selective, reliable, economically feasible and able to monitor a large number of MP samples simultaneously.

Most of the studies till date for the detection of MP/OP are based on the use of acetylcholinesterase (AChE) and organophosphorus hydrolase (OPH) (Mulchandani et al., 2001; Lan et al., 2012; Chen et al., 2011; Kumar and Melo, 2015). Biosensors based on the AChE are related to its irreversible inhibition and although they are very sensitive face limitations with respect to single use, poor specificity, longer incubation time, interference from other substances like heavy metals and are unable to handle many samples in short period of time (Mulchandani et al., 1998; Pundir and Chauhan, 2012; Yang et al., 2013; Liang et al., 2014). To overcome the drawbacks of inhibition-based system, focus has been shifted towards systems based on hydrolysis of the substrate using OPH enzyme which hydrolyzes MP into an optically detectable product p-nitrophenol (PNP) as shown below in structural representation. PNP can be detected by electrochemical or

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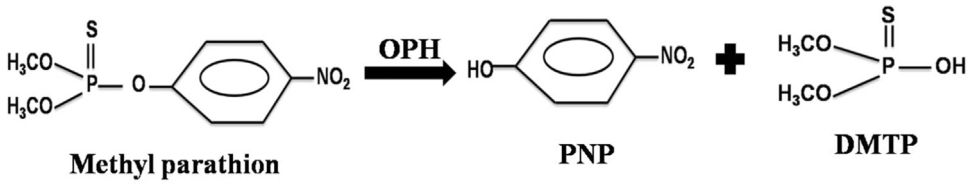
colorimetric methods and in turn can be exploited to develop a biosensor for detection of MP (Kumar and D'Souza, 2010, 2011a, 2011b).



In this regard, microbial cells with OPH enzyme which are able to hydrolyze MP into an optically detectable product PNP, were immobilized onto various supports and associated with optical transducer for MP detection (Mulchandani et al., 1999; Roger et al., 1999; Kumar et al., 2006; Kumar and D'Souza, 2010, 2011b). Studies from our laboratory have shown that *Flavobacterium* sp. expressing OPH enzyme when immobilized on glass fiber filters could detect MP in the range of 4–80 μM (Kumar et al., 2006) which is equivalent to 1–20 ppm. In another study, when *Sphingomonas* sp. cells were immobilized on surface of microplate wells directly or with the help of inner epidermis of onion bulb scales, it showed the same detection range of 4–80 μM of MP (1–20 ppm) and further displayed a storage stability of only 18 and 32 days, respectively (Kumar and D'Souza, 2010, 2011b). Microplate based optical microbial biosensor has its own advantages in terms of being a very good system for detection of multiple samples of MP simultaneously on a single platform in a very short time. However, the low sensitivity and storage stability with respect to what has been reported till date is a matter of concern. Thus, the challenge is to improve the sensitivity (up to 0.1 ppm) which is in the range of MRL value for MP in food commodities as well as to enhance the storage stability. Longer storage stability of biosensors is crucial for many practical/routine applications in diverse fields, including decentralized field testing, screening and rapid detection of multiple samples and avoiding the time consuming preparation of a fresh biosensor.

For improving the sensitivity and stability of the biosensor, immobilization of biological component on a suitable matrix can play a crucial role. Now a day, researchers are trying to design materials which are inspired by nature, so that they can offer a favorable environment to the immobilized biomolecule (Ponamoreva et al., 2015). It has been reported that silica which forms a major component of cell wall of diatoms, acts as a protective shell of cells (Hamm et al., 2003). Due to the protective nature, it has been exploited as an immobilizing matrix for living cells (Nassif et al., 2002; Blondeau and Coradin, 2012; Ponamoreva et al., 2015). Silica based materials have several desirable properties like chemical inertness, high mechanical strength, bio-compatibility, good thermal stability, controlled porosity, optical transparency, resistance to microbial attack and the ability to maintain cell viability for an extended period of time (Bhatia et al., 2000; Depagne et al., 2011; Ye et al., 2016). Thus, immobilization of microbial cells with Si NP could be a way to exploit the beneficial characteristics of silica as well as microbe, for synthesizing a stable functional biohybrid material. In this context yeast cells have been integrated with silica through the sol-gel technique for biosensor application (Ponamoreva et al., 2015). Although, sol-gel technique is a commonly used method for immobilization of biomolecules in silica matrix (Livage et al., 2001; Yu et al., 2005), its major disadvantage lies in the release of alcohol which could be detrimental for the biomolecule (Avnir et al., 2006; Trogl et al., 2010). While people have tried different ways to overcome this limitation, still there is a need to develop the process for the synthesis of functional biohybrid using silica, which is economical and safe for the environment (Depagne et al., 2011; Ponamoreva et al., 2015). Therefore, colloidal silica has emerged as material of choice which is widely used for a range of commercial applications and available at relatively low cost. Our group has earlier reported a facile route for synthesizing microstructures of Si NP and microbial cells using spray dryer (Sen et al., 2011; Melo et al., 2013; Mishra et al., 2014).

In the present work, we have modified our approach to integrate Si NP with *Sphingomonas* sp. cells to design a more sensitive and stable optical microbial biosensor for the detection of MP. Microbial cells integrated with fSi NP were immobilized on the microplate well surface and further used for detecting multiple samples of MP on a single platform.

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. Silica colloidal dispersion (~15 nm) was obtained from VISA Chemicals, Mumbai, India. Polyethyleneimine (PEI), average $M_w \sim 7.5 \times 10^5$) was purchased from Sigma Chemical Co., MO, USA. Microplates (96 wells) used for immobilization were procured from BD Flacon (USA). All other analytical grade chemicals were purchased from Sisco Research Laboratory, Mumbai, India.

2.2. Micro-organism, culture medium, harvesting of cells and hydrolytic activity assay

Sphingomonas sp. JK1 culture was available in our laboratory. *Sphingomonas* sp. JK1 was first cultivated in modified Wakimoto 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 milli Q water) for overnight at 30 °C. After this, 1/100th volume from the overnight grown culture was sub-cultured in Luria broth (200 mL) and grown for 48 h at 30 °C on a rotary shaker at 150 rpm till absorbance of 2.2 at λ_{max} 600 nm for optimum MP hydrolytic activity (Kumar and D'Souza, 2010). Cells were harvested by centrifugation at 5000 × *g* for 10 min and washed three times with 50 mM phosphate buffer (pH 8.0). Finally, the pellet was resuspended in 1/10th volume in phosphate buffer (pH 8.0) and stored at 4 °C. Microbial OPH activity of the cells was measured by adding 50 μL of whole cell suspension in 1 mL of MP and production of PNP was determined by measuring absorbance at λ_{max} 410 nm (ε_{410}=16,500 M^{-1} cm^{-1} for PNP) (Kumar and D'Souza, 2010).

2.3. Integration of functionalized silica nanoparticles (fSi NP) with

Sphingomonas sp.

2.3.1. Optimization of polyethyleneimine (PEI) concentrations

Si NP (0.1%) was treated with varying concentrations (0.001–0.1%) of PEI solution for 30 min with continuous stirring at 150 rpm. After this, suspension was centrifuged and washed properly to remove unbound PEI. ^{f}Si NP were suspended in distilled water.

2.3.2. Optimization of ratio of Sphingomonas cells and ^{f}Si NP

Different ratio of *Sphingomonas* cells to ^{f}Si NP (1:3, 1:2, 3:4, 1:1, 4:3, and 2:1) was mixed together and incubated for overnight with stirring at 30 °C. After this, cells bound with ^{f}Si NP were separated by centrifugation, washed thoroughly with phosphate buffer (pH 8.0) and used further. A 100 μL of harvested cell suspension contains $\sim 2 \times 10^{9}$ CFU.

2.4. FTIR study

Fourier transform infrared spectroscopy (FTIR) study of Si NP, ^{f}Si NP, *Sphingomonas* sp. cells and biohybrid of *Sphingomonas* sp.-^{f}Si NP were carried out and spectra were recorded using Jasco FT-IR 660 plus spectrometer. Dried samples were mixed with KBr in the ratio of 1:20 and used for FTIR study. All spectra were recorded over wavenumber 3700–400 cm^{-1} region with a resolution of 4 cm^{-1}.

2.5. Immobilization of biohybrid (Sphingomonas sp.-^{f}Si NP) onto microplate

Different concentrations of biohybrid (10–60 μL) were immobilized onto the interior surface of the wells of microplate (96 well) and was air dried for 1 h at room temperature. After washing the wells, immobilized biohybrid was cross-linked with different concentrations (0.5–5%) of glutaraldehyde. Immobilized wells were washed with buffer and stored at 4 °C until use. The production of PNP was determined by measuring absorbance at λ_{max} 410 nm.

2.6. SEM study of the biohybrid immobilized onto microplate

A scanning electron microscope (PS-230, Pemtron) was employed to observe the surface morphology of the wells of microplate after immobilization of *Sphingomonas* sp. cells and biohybrid. For SEM study, well of the microplate was mounted on stubs and coated with gold (Au) using a sputter coater. The SEM micrographs of the immobilized well were acquired at magnification 6000X.

2.7. Transducer and operating system for optical detection of MP using immobilized biohybrid

For optical detection of MP, multi detection microplate reader (MDMR) from Bioteck (Synergy™ HT) was used. A xenon flash light source in conjunction with the photomultiplier tube (PMT) detector helped to make time-resolved measurements. Wavelength range in this reader can be selected from 200 to 999 nm with an increment of 1 nm. In this, the transducer was optical and microplate reader was completely controlled via KC4™ PC software for all operations (Kumar and D'Souza, 2010; Saini et al., 2014). MP was added into the biohybrid immobilized wells and formation of PNP was measured at 410 nm. The difference in absorbance between the initial and final reading corresponds to the amount of PNP formed.

2.8. Calibration studies using biohybrid immobilized onto microplate

Biohybrid immobilized microplate was integrated with the optical microplate reader. It was calibrated using MP concentration ranging from 0.1 to 5.0 ppm in phosphate buffers (pH 8.0) at room temperature for 5 min and the formation of PNP was measured on optical microplate reader at 410 nm. All experiments were carried out with phosphate buffer (pH 8.0) at room temperature in triplicates.

2.9. Storage stability and reusability study of biohybrid immobilized onto microplate

Biohybrid was stored at 4 °C and the storage stability of immobilized biohybrid was determined for a period of 200 days using 1 ppm of MP and PNP was detected at regular interval. Reusability of the immobilized biohybrid (stored at 4 °C) was also studied using 1 ppm of MP. After each reaction, the hydrolyzed MP was removed; immobilized biohybrid was washed with buffer and fresh samples of MP were analyzed up to 10 cycles.

2.10. Analysis of spiked sample with developed biosensor

For real sample analysis, water sample was spiked with different concentrations of MP (0.2, 0.4, 0.5, 0.6, 0.8 and 1 ppm) and then subjected to quantification using biohybrid immobilized optical microplate biosensor.

In another study, 50 g of grapes were spiked with different concentrations of MP, adsorbed MP was extracted in phosphate buffer and the same was used for detection of MP on the developed microplate based biosensor.

3. Results and discussion

3.1. Optimization of amount of Sphingomonas cells, ^{f}Si NP and PEI for synthesis of biohybrid

Various permutations were carried out for optimizing the integration of *Sphingomonas* sp. cells with ^{f}Si NP and quantified in terms of maximum MP hydrolysed or PNP formed. Different concentrations of PEI (0.001–0.1%) for functionalization of Si NP were optimized for maximum formation of PNP (Fig. S1). It was observed that 0.01% PEI is sufficient for functionalization of 0.1% Si NP.

Results of optimization of ratio of cells to ^{f}Si NP are shown in Fig. 1a. It was observed that 1:2 ratio of cells to ^{f}Si NP showed maximum formation of PNP and on further increase in the ratio there is no increase in PNP formation. An optimum concentration of ^{f}Si NP is required for binding on the cell surface and to cover the whole surface of cell. At lower ratio of 1:3, the amount of ^{f}Si NP may not be sufficient to cover the surface of cells. Therefore, for further study, 1:2 ratio of cells to ^{f}Si NP was used.

3.2. FTIR study of biohybrid of Sphingomonas sp.-^{f}Si NP

FTIR spectra of Si NP, ^{f}Si NP, *Sphingomonas* sp. cells and biohybrid of *Sphingomonas sp.*-^{f}Si NP was recorded and shown in Fig. S2. FTIR spectra of the Si NP showed peaks at 1109 cm^{-1}, 956 cm^{-1}, 800 cm^{-1} corresponding to the asymmetric vibration of Si-O-Si, asymmetric vibration of Si-OH and symmetric vibration of (Si-O-Si) groups, respectively (Mishra et al., 2014). After functionalization of Si NP with PEI new peaks appears at 3500–3200 cm^{-1} which are assigned to the NH stretch and OH stretch and peak at 1464 cm^{-1} assigned to stretching vibration of -CH_2 and -CH_3 functional groups which confirms the functionalization of Si NP.

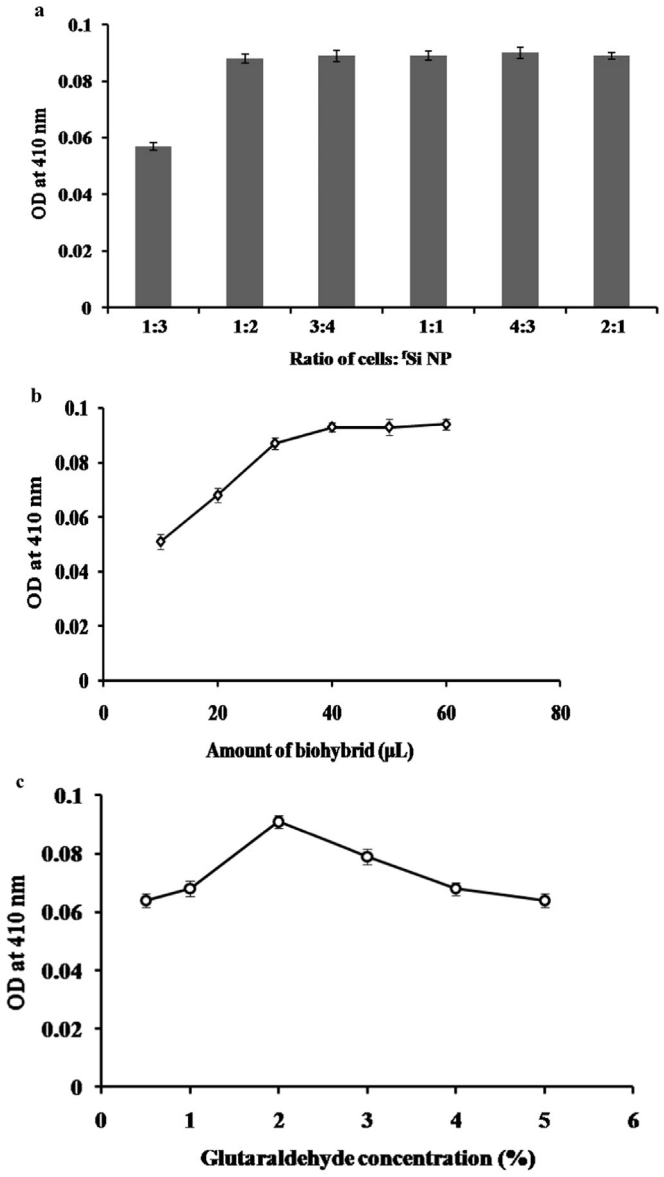


Fig. 1. (a) Optimization of ratio of cells:^{f}Si NP. (b) Optimization of amount of biohybrid for immobilization onto microplate. (c) Optimization of glutaraldehyde concentration for immobilization.

Spectra of *Sphingomonas* sp. cells showed peak at 3500–3200 cm^{-1} which are assigned to the NH stretch and OH stretch. The peak at 1395 cm^{-1} was characteristic of C=O symmetric stretching of the COO^{-} group of biomolecules. A peak at ~1654 cm^{-1} and ~1530 cm^{-1} corresponds to amide I and amide II, respectively that characterizes the presence of protein (Mishra et al., 2014). Stretching vibration of -CH_2 and -CH_3 groups were detected at 1400–1460 cm^{-1}. Peak at ~1240 cm^{-1} is due to the -C-O stretching and C-O-H asymmetric stretching of -COOH group. After integration of cells with ^{f}Si NP the biohybrid showed the peaks at 3500–3200 cm^{-1}, 2929 cm^{-1}, 2357 cm^{-1}, 1652 cm^{-1}, 1534 cm^{-1}, 1454 cm^{-1}, 1390 cm^{-1}, 1105 cm^{-1} and 798 cm^{-1}. Peaks of cells and ^{f}Si NP were present in the spectra of biohybrid of *Sphingomonas sp.*-^{f}Si NP. The disappearance of peak at 1240 cm^{-1} and changes in the region 1380–1400 cm^{-1} suggests the role of carboxylic group for integration with ^{f}Si NP. Peak at ~ 950 cm^{-1} present in ^{f}Si NP was missing in spectra of biohybrid which shows involvement of silanol (Si-OH) groups in the integration of silica with PEI. The presence of peaks due to ^{f}Si NP and cells in the biohybrid supports the fact of integration of cells with Si NP.

3.3. Immobilization of biohybrid onto microplate

Immobilization of microbes either on a suitable matrix or the transducer plays a very important role in development of a microbial biosensor. Various techniques such as layer-by-layer (LbL) approach, adsorption, adsorption followed by cross-linking, covalent attachment etc. have been extensively exploited for the immobilization of microbes/enzymes (Turner et al., 1987; Melo and D'Souza, 1992; Melo and D'Souza, 1999; Kumar and D'Souza, 2009, 2010, 2011a, 2011b; Manjunatha et al., 2011; Karimpil et al., 2011, 2012; Lan et al., 2012). In our previous work, microbial cells and enzyme were immobilized onto the well surface of microplate by adsorption followed by glutaraldehyde cross-linking and used for developing biosensors (Kumar and D'Souza, 2010, 2011b; Saini et al., 2014). In the present work, microplate made up of polystyrene was used for the immobilization of biohybrid component. The microplate are usually vacuum gas plasma treated, and in the process, highly energetic oxygen ions are generated, which then oxidize and get grafted onto the surface of polystyrene chains (Ryan, 2008; Saini et al., 2014). Due to this the interior surface of wells are negatively charged and are suitable for immobilization of positively charged biomolecules on interior surface of the microplate. Cells integrated with ^{f}Si NP are positively charged due to PEI and easily get adsorbed on negatively charged interior surface of wells of microplate. The main advantages of microplate based assays are the use of small sample volumes and simultaneous analysis of multiple samples on a single platform. The response time which is an important factor was only 5 min for the measurement of multiple samples on single platform. This is the first report where *Sphingomonas* sp. cells were integrated with ^{f}Si NP and further immobilized onto polystyrene microplate and used for optical biosensor application. Supplementary schematic 1 shows the functionalization of Si NP, integration of cells with ^{f}Si NP, immobilization of biohybrid onto microplate and its application as a biosensor for detection of methyl parathion. For immobilization of biohybrid onto wells of microplate, different parameters were optimized.

3.3.1. Optimization of amount of biohybrid for immobilization onto microplate

Optimization of amount of biohybrid immobilized onto surface of wells is important due to availability of limited space on the bottom surface of the wells (diameter = 5.7 mm; area = 25.74 mm^2) of microplate. Therefore, in order to understand the effect of biohybrid loading, different amounts of biohybrid were used for immobilization and PNP formation was measured. As shown in Fig. 1b, by varying the amount of biohybrid loaded from 10 to 60 µL, 40 µL of biohybrid used for immobilization showed highest formation of PNP. With further increase beyond 40 µL, there was no increase in PNP formation which may be due to leaching of excess cells during washing. This suggests that on the limited space at the bottom of the well, 40 µL of biohybrid can be immobilized to get best signal for detection. Therefore, 40 µL of biohybrid was used for the subsequent experiments.

3.3.2. Optimization of glutaraldehyde (GA) concentration for immobilization

Different concentrations of glutaraldehyde (0.5–5% (v/v)) were used for the cross-linking of biohybrid onto the microplate wells as shown in Fig. 1c. A 2% glutaraldehyde concentration was found optimum for cross-linking of biohybrid on the microplate. Further increase in glutaraldehyde concentration caused decrease in formation of PNP as shown in Fig. 1c. This observation is in the agreement of previous results (Kumar and D'souza., 2010).

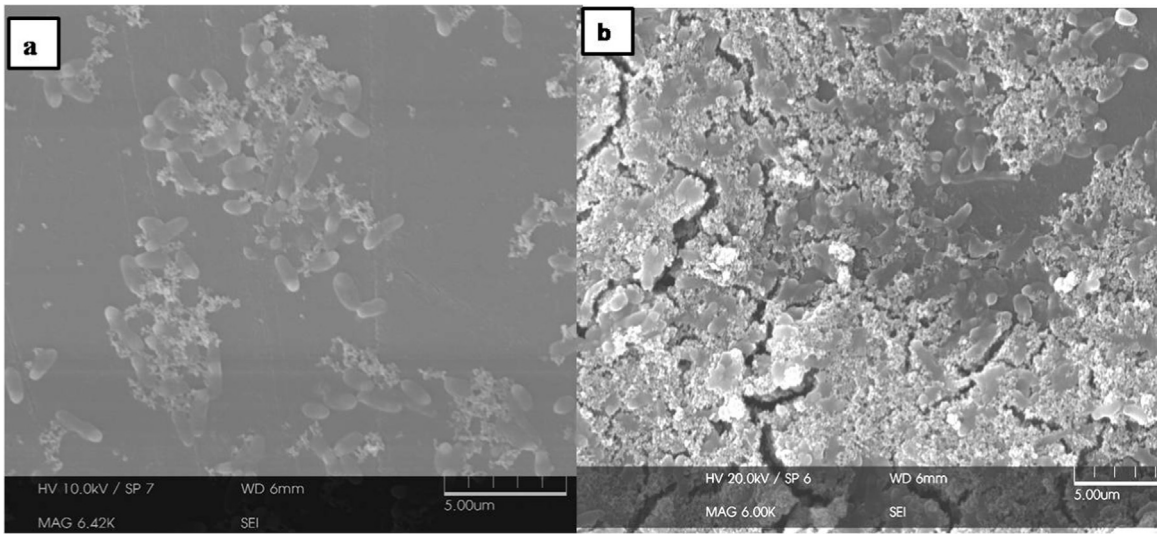


Fig. 2. SEM micrographs of (a) *Sphingomonas* sp. cells and (b) biohybrid of *Sphingomonas sp.*-^{f}Si NP immobilized on microplate.

Glutaraldehyde is a cross-linking agent as well as a protein denaturing agent; excess glutaraldehyde may have an adverse effect on microbial enzyme which further leads to the decrease in PNP formation.

3.4. SEM study of the biohybrid immobilized onto microplate

SEM studies were carried out to observe the changes in the surface characteristics of wells of microplate when *Sphingomonas* sp. and biohybrid were immobilized. SEM micrographs were acquired of interior surface of the wells of microplate. Fig. 2. shows the SEM image of wells immobilized with cells and biohybrid. In the micrograph of cells immobilized onto surface of well, one observes only bacterial cells (Fig. 2a) while in case of biohybrid immobilized well, cells were embedded in silica (Fig. 2b). As seen in Fig. 2a., there are less number of cells when plain cells were immobilized while the number of cells were much higher when biohybrid was immobilized (Fig. 2b.). Better binding seen in case of biohybrid immobilized onto wells is probably because the biohybrid is positively charged and surface of well is negatively charged but in case of plain cells immobilized on wells, the cells and surface of wells both are negatively charged and limited binding seen may be due to the cross-linking. Presence of bacterial cells and biohybrid confirms the immobilization onto the wells of microplate.

3.5. Calibration studies using biohybrid immobilized onto microplate

Developed biosensor was calibrated using different (0.1–5 ppm) concentration of MP as shown in Fig. 3. A linear range between 0.1 and 1 ppm (with linear regression equation, $y = 0.0443x + 0.0387$, $r^2 = 0.997$) was achieved from calibration plot which indicates the detection range of the biosensor. This biosensor is thus able to detect 0.1–1 ppm of MP and is better than our earlier reported microplate based optical microbial biosensor (1–20 ppm) (Kumar et al., 2006; Kumar and D'Souza, 2010, 2011b). This multiple sample analyzing biosensor will be able to detect MP in several food commodities in the range between 0.1 and 1 ppm which is the MRL value as per FAO/WHO Codex Alimentarius Commission and FSSAI recommendation.

3.6. Storage stability and reusability of biohybrid immobilized onto microplate

The storage stability of the immobilized biohybrid sensor was

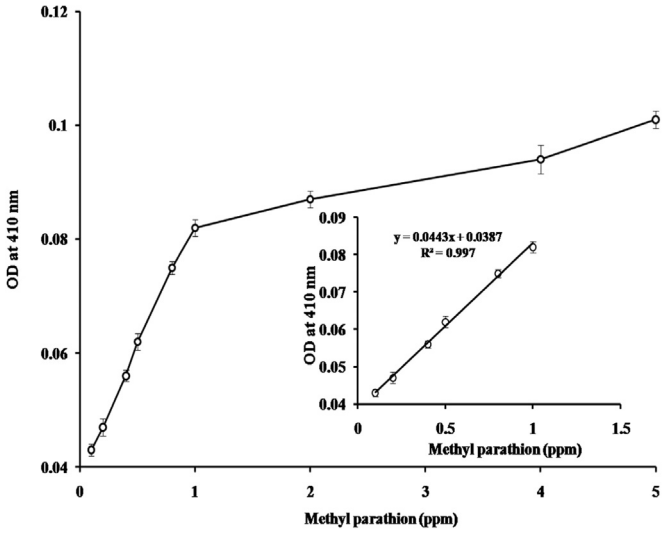


Fig. 3. Calibration of the biosensor using biohybrid immobilized onto microplate with MP (0.1–5 ppm). (inset: linearity between 0.1 and 1 ppm with linear regression equation, $y = 0.0443x + 0.0387$, $R^2 = 0.997$ where 'y' represents the absorbance at 410 nm and 'x' the substrate concentration).

also studied (Fig. 4a). The stability of the immobilized biohybrid was observed at regular intervals by measuring its MP hydrolyzing ability. Biohybrid immobilized microplate biosensor showed storage stability upto 180 days with a retention of 85% activity, when stored at 4 °C. In our previous study, biocomponent was stable only for 18 days and current study has increased the storage stability by ten times from 18 days to 180 days (Kumar and D'Souza, 2010). The increase in storage stability may be due to the binding of ^{f}Si NP on microbial cells surface, which provides a protective covering around the cell and also reduces the leaching of hydrolytic enzyme (Hamm et al., 2003). This observation is in the accordance with the work of Nassif et al. where it was reported that silica imparts positive impact on survival of microbes and helps to maintain enzyme activity [Nassif et al., 2002]. Increase in storage stability of biohybrid sensor will help to increase the application of biosensor for a longer period of time. The extended storage stability of 180 days enables many practical/routine applications in diverse fields, including decentralized field testing, screening and rapid detection of multiple samples and avoiding the time

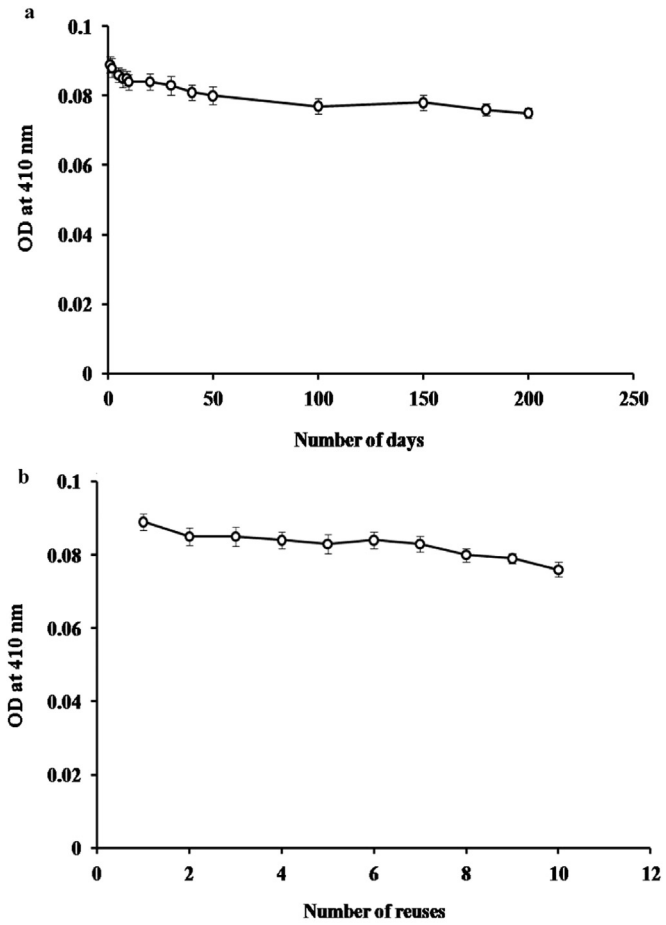


Fig. 4. a. Storage stability of the biohybrid-immobilized onto microplate. b. Reusability of the biohybrid-immobilized onto microplate.

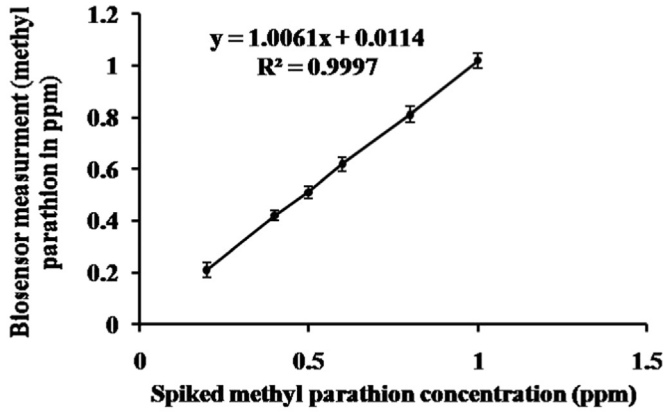


Fig. 5. Correlation between the spiked concentrations of MP in water and corresponding biosensor reading.

consuming preparation of a fresh biosensor.

The reusability of biohybrid immobilized on microplate (stored at 4 °C) was also studied (Fig. 4b). It was observed that 81% of enzyme activity was retained even after 10 repeated reactions.

3.7. Interference study in presence of different organic compounds

Interference study was carried out using different organic compounds like sucrose, glucose, carbaryl, malathion and monocrotophos to study the specificity of developed biosensor for MP detection. It was observed (Supplementary table 1) that glucose and sucrose were not interfering in hydrolysis of MP. Carbaryl is one of the non-specific pesticide which did not interfere during detection of MP. Although malathion and monocrotophos are the substrates of OPH enzyme, these organophosphate pesticides did not interfere during detection because their hydrolysis products are not chromophoric like p-nitrophenol (PNP). Basic principle of the biosensor is to detect the production of PNP by *Sphingomonas* cells which is determined by measuring absorbance at λ_{max} 410 nm (ε_{410}=16,500 M^{-1} cm^{-1} for PNP). Another reason may be that biosensor is working at low concentrations (detection range 0.1–1 ppm).

Table 1
Biosensor measurement of MP in grape samples soaked with different concentrations of MP.

MP concentration used for soaking grapes (ppm)	MP concentration adsorbed by grapes after soaking (ppm)*	Biosensor measurement of adsorbed MP (ppm)
10	0.2	0.186 ± 0.013
20	0.8	0.776 ± 0.042
30	1	0.963 ± 0.068

* Grape samples were soaked in different concentrations of MP (10, 20, 30 ppm) for 30 min, air dried and extracted as mentioned in Section 2.10. Remaining concentration of MP was measured in the spent and washing to know how much MP was adsorbed by grapes.

3.8. Analysis of spiked sample with developed biosensor

The practical applicability of the proposed biosensor was studied using spiked sample. Water samples were spiked with different concentrations of MP (0.2, 0.4, 0.5, 0.6, 0.8 and 1 ppm) and then subjected to quantification using biohybrid immobilized microplate biosensor. In all, there were 5 replicates for each of the six different concentrations studied. All spiked samples were analyzed at the same time using microplate biosensor. As shown in Fig. 5, the values of MP concentrations obtained by using the biosensor showed good correlation with that of known spiked concentration values of MP. The straight line fit gave a linear regression equation of y = 1.0061x + 0.0114 (R^2=0.9997), which demonstrated the feasibility of the developed biosensor for stable and sensitive detection of multiple MP samples.

In another study, grape samples were soaked with different concentration of MP, adsorbed MP was extracted in phosphate buffer and the same was used for detection of MP on the developed microplate based biosensor. Results of MP concentration on grapes and the biosensor reading value is mentioned in the Table 1. Results show a good correlation between known spiked concentrations of MP.

4. Conclusions

In the present study, we describe the synthesis of a functional biohybrid component by integrating *Sphingomonas* sp. cells with ^{f}Si NP. Biohybrid was further immobilized onto 96 well microplate and immobilization was confirmed by SEM. The detection range of this optical biosensor for the detection of MP is 0.1–1 ppm which is in the range of MRL value of MP and the storage stability is 180 days. Spiked samples of MP were also analyzed using the developed optical biosensor and showed a good correlation with the known spiked concentrations. Present study thus opens a window of interest where *Sphingomonas* cells can be associated with silica to overcome the poor storage stability of enzymes present in cells.

This innovative concept leads to the development of a stable and sensitive optical microbial biosensor for detection of MP.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.048.

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