Biotechnol Lett (2021) 43:933–944
https://doi.org/10.1007/s10529-021-03078-1

ORIGINAL RESEARCH PAPER

MPH-GST sensing microplate for easy detection of organophosphate insecticides

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Received: 15 October 2020 / Accepted: 6 January 2021 / Published online: 29 January 2021

Abstract
Objective To develop a convenient and efficient means for organophosphate (OP) insecticide detection, a simple, cost-effective, and easy-to-use absorbance-based sensing device was generated using methyl parathion hydrolase fused with glutathione-*S*-transferase (MPH-GST) covalently immobilized onto a chitosan film-coated microplate.
Results With methyl parathion (MP) as a representative substrate, this MPH-GST sensing microplate had the detection limit of 0.1 μM and the linear range of 0.1–50 μM. Despite its highest stability at 4 °C, it was considerably stable at 25 °C with high activity for 30 days. It was also most stable at pH 8.0 and could be efficiently reused up to 100 rounds. The device revealed a high percentage of recovery for tap water spiked with a known concentration of MP, which was also comparable to the result obtained from gas chromatography-mass spectrometry. It also showed a high recovery of 82–100% with MP spiked agricultural products and satisfactory results with non-spiked samples. This immobilized enzyme sensing system was more sensitive and efficient than the whole cell system from our previous work.
Conclusions All of the advantages of the MPH-GST sensing microplate developed have rendered it suitable for rapid and convenient OP screening, and for being a bio-element for fabricating a potential optical biosensor in the future.

Keywords Organophosphate · Methyl parathion hydrolase · MPH-GST · Covalent immobilization · Sensing microplate

Abbreviations

OP	Organophosphate insecticide
MP	Methyl parathion
AChE	Acetylcholinesterase
MPH	Methyl parathion hydroloase
MPH-GST	Methyl parathion hydrolase fused with glutathione-*S*-transferase
GC–MS	Gas chromatography–mass spectrometry
LOD	Limit of detection
NTA	Nitrilotriacetic acid

Supplementary Information The online version of this article (https://doi.org/10.1007/s10529-021-03078-1) contains supplementary material, which is available to authorized users.

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Introduction

Neurotoxic organophosphate (OP) insecticides, such as methyl parathion (MP) and chlorpyrifos, are extensively used in agricultural areas in many countries, causing contamination in the environment and agricultural products, which leads to nerve function loss and death in humans (Simon 2015; Tchounwou et al. 2015; Kumar et al. 2018; Jain et al. 2019). FAO/WHO have set maximum residue limits (MRLs) for pesticides in Codex Alimentarius International Food Standard (FAO/WHO 2020). In order to ensure safety for consumers and agricultural products, an effective OP sensing device is urgently needed for efficient OP detection (Zhang et al. 2012; Mishra et al. 2017). Biosensor/sensing devices have advantages over conventional methods such as gas chromatography (GC) (Kumar et al. 2018; Kaur and Singh 2020) in their simplicity, cost-effectiveness, rapidity, and suitability for onsite detection.

Methyl parathion hydrolase (MPH), an OP-degrading enzyme capable of hydrolyzing MP to dimethylthiophosphoric acid and a yellow product, *p*-nitrophenol (PNP), has been isolated (Cui et al. 2001; Liu et al. 2005a; Schenk et al. 2016). In Thailand, the isolated MPH was encoded from methyl parathion degrading gene (*mpdB*) of MP-degrading *Burkholderia cepacia* (Keprasertsup et al. 2001). The *mpdB* gene was cloned and transformed into *Escherichia coli* BL21, yielding the recombinant clone BpGP, capable of expressing MPH fused with glutathione-*S*-transferase (GST) at the N-terminus (MPH-GST). The purified recombinant MPH had the molecular mass of approximately 35 kDa and the recombinant MPH-GST was shown to have cross-reactivity with other OP insecticides (Ekkhunnatham et al. 2012). The chromophoric and electroactive products can be detected by optical and electrochemical sensing methods, respectively (Kumar et al. 2018; Jain et al. 2019; Kaur and Singh 2020). With the chromophoric nature of PNP, a colorimetric dipstick assay based on MP hydrolysis using this recombinant MPH-GST was developed, which could detect MP as low as 1 mg/L (3.8 μM) (Anh et al. 2011). MPH-GST was also used in our previous work as an electrochemical sensing component of an amperometric OP biosensor (Wichitwechkarn et al. 2018). Although it could detect MP efficiently, there were certain disadvantages such as interference by electrolytes, complicated assembly steps, and high development cost. Therefore, a simple optical OP biosensing device, which has no electrolyte interference, is the main focus of our work presented here.

There were several reports related to OP optical sensing device using whole cells of *Flavobacterium* sp. (Kumar et al. 2006) and *Sphingomonas* sp. (Kumar and D'Souza 2010, 2011), with the linear range of 4–80 μM. A biohybrid of *Sphingomonas* sp. cells with functionalized silica nanoparticles was developed yielding MP detection range of 0.1–1 μg/mL (Mishra et al. 2017). Another study was done on whole cells of recombinant *E. coli* expressing organophosphate hydrolase (OPH) to create an OP electrochemical device with the MP detection range of 2–80 μM (Kumar and D'Souza 2011). In our recent work, an absorbance-based OP sensing device using whole cells of clone BpGP immobilized onto the microplate surface was generated, demonstrating the linear range of 7.6–760 μM and the detection limit of 7.6 μM for MP (Pootawee et al. 2018). Here, the development of a simple, easy-to-use, and cost-effective MPH-GST-based sensing device is described. The MPH-GST was covalently immobilized on the chitosan-coated microplate well surface and the detection of MP, as an OP representative, was performed through an optical microplate reader. Its performance characteristics and tests on real samples were also determined.

Materials and methods

Reagents and materials

MP (purity 98.3 %, analytical grade), medium molecular weight chitosan, glutaraldehyde (50 % in H_2O), bovine serum albumin (BSA), sodium borohydride ($NaBH_4$), isopropyl β-D-1-thiogalactopyranoside (IPTG), and dithiothreitol (DTT) were purchased from Sigma-Aldrich (Singapore). A standard *p*-nitrophenol (PNP) was purchased from Merck (Germany). BLUeye Prestained Protein Ladder was obtained from GeneDireX, Inc. (Taiwan). Polystyrene microplates were ordered from SPL Life Sciences (Korea).

Production of recombinant MPH-GST fusion protein

The methyl parathion degrading (*mpdB*) gene encoding for methyl parathion hydrolase (MPH), isolated from MP-degrading *Burkholderia cepacia* indigenous to Thailand (Keprasertsup et al. 2001), was digested by BamHI and XhoI. The BamHI-XhoI DNA fragment was cloned into the pGEX-6P-1 vector (GE Healthcare Life Sciences, USA) for expression as an enzyme fused with glutathione-*S*-transferase (GST) at the N-terminus. The recombinant plasmid was transformed into *E. coli* BL21, resulting in clone BpGP capable of expressing recombinant MPH-GST fusion protein (Ekkhunnatham et al. 2012; Pootawee et al. 2018).

The recombinant clone BpGP was grown on Luria-Bertani (LB) agar supplemented with 100 µg/mL ampicillin at 37 °C overnight. Each single colony was picked using a sterilized toothpick and determined for MPH activity by microtiter plate activity assay (Fu et al. 2004). Only the colonies expressing the yellow PNP, an MP-degrading product, were then grown in LB broth containing 100 µg/mL ampicillin for 16–18 h with shaking at 37 °C. A preculture was transferred into fresh LB broth with the same antibiotic and incubation condition for 3–4 h. When the OD_{600} of 0.5 was reached, the induction was done by adding 1 mM IPTG in the presence of 0.5 mM $CoCl_2$. The induced cells were grown further at 18 °C for 6 h and harvested by centrifugation at 7000×*g*, 4 °C for 10 min. Precipitated cells were washed twice and resuspended with 50 mM phosphate buffer saline (PBS), pH 8.0. Resuspended cells were disrupted on ice with a 3 mm probe at 20 % amplitude for about 1 min using an ultrasonicator (Sonics Vibra cell™, USA). The lysate was then centrifuged at 17,000×*g*, 4 °C for 20 min to remove cell debris and the supernatant having MPH activity was collected. This partially purified MPH-GST was further used in all subsequent experiments. Protein concentration was measured at OD_{595} according to the Bradford method using BSA as a standard solution.

MPH activity assay

MPH activity was determined according to the previously reported method (Ekkhunnatham et al. 2012). Briefly, it was measured spectrophotometrically at 410 nm for the yellow PNP arising from MP-degrading reaction using a spectrophotometer Genesys 8 (Spectronic Instruments, UK) for cell extracts or free MPH-GST, and a microplate reader (Tecan, Switzerland) for immobilized MPH-GST sensing device. The reaction mixtures consisted of assay buffer (pH 8.5) and 100 µg/mL MP. The glutaraldehyde-activated, chitosan-coated microplate well containing assay mixture without immobilized MPH-GST was used as a blank. One unit (U) of activity was defined and expressed as the quantity of the enzyme that was necessary for the formation of 1 µmol of PNP per min.

Fabrication of MPH-GST sensing microplate

The conditions for coating chitosan film onto polystyrene microplate surface were optimized. With 50 µL chitosan solution, its varying concentrations in the range of 1–3% (w/v) in 1% (v/v) acetic acid were added to individual wells. They were dried at room temperature for about 6 h for chitosan film formation. With the optimum concentration obtained, chitosan volume was then varied within the range of 35–80 µL for optimum volume determination. The chitosan film-coated microplate prepared with optimized conditions was further used for MPH-GST immobilization.

To fabricate an enzyme-based biosensing device, MPH-GST was covalently immobilized onto the chitosan-coated polystyrene microplate using glutaraldehyde as a cross-linker, in accordance with Zhang method (Zhang et al. 2011). The chitosan-coated microplate was treated with 2% (w/v) BSA at 4 °C for 2 h, washed with 50 mM PBS (pH 8.0), cross-linked with 1% glutaraldehyde at 25 °C for 2 h, and covalently immobilized with MPH-GST solution at 4 °C overnight. Subsequently, the MPH-GST microplate wells were incubated with freshly prepared 0.1 M $NaBH_4$ in 50 mM PBS (pH 8.0) at 25 °C for 1 h, gently washed with 50 mM PBS (pH 8.0) containing 0.1 M NaCl and 0.5% (v/v) Tween 20, and washed with 50 mM PBS (pH 8.0). The MPH-GST sensing microplate fabricated was then subjected to MPH activity determination by detecting yellow PNP liberated at 410 nm using an optical microplate reader.

Optimization of MPH-GST biosensing device

To determine the optimum temperature of the MPH-GST biosensing device, its MPH activity was

investigated at different temperatures (4, 25, 37, 60, 70, and 80 °C). MP substrate (100 µg/mL) in the assay buffer (pH 8.5) was added to each well of the sensing device. After 10 min incubation, the OD_{410} was measured and the MPH activity calculated. Each assay was performed in triplicate. For optimum pH determination, MP substrate (100 µg/mL) with different pH values (5.0–12.0) was added into individual wells. The MPH activity was determined from the OD_{410} value measured after 10 min incubation at 37 °C. All experiments were conducted in triplicate.

Performance characteristics of MPH-GST biosensing device

Detection limit and linear range

The MPH-GST sensing wells were incubated with different MP concentrations (0.1–100 µM) at optimum conditions for 10 min. The yellow PNP development was detected at OD_{410} using an optical microplate reader as described above. The detection limit and linear range were determined from the MP calibration curve plotted. All reactions were performed in triplicate.

Stability and reusability of MPH-GST biosensing device

To determine the temperature stability of the MPH-GST biosensing device, the sensing microplates were stored in 50 mM PBS (pH 8.0) at different temperatures (4, 25, 37, and 60 °C). MPH activity was measured every day for 30 days by an optical microplate reader (Tecan, Switzerland) using the assay procedure described above. For pH stability, the sensing microplates were stored at 4 °C in buffers with different pH (5.0, 6.0, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0). MPH activity was measured. To investigate its reusability, the wells containing fresh 100 µg/mL MP were measured for the MPH activity and washed with 50 mM PBS (pH 8.0) in each round of detection. This was repeatedly done for 100 cycles. All experiments were conducted in triplicate.

MP detection of spiked water samples by gas chromatography–mass spectrometry (GC–MS) and MPH-GST sensing microplate

Tap water from Silpakorn University was collected using standard method (Water Quality Management Division 2010). Different MP concentrations (7.6, 15.2, 22.8, 30.4, and 38.02 µM) were spiked into the water samples. Spiked MP was extracted from each water sample (Ekkhunnatham 2009) and analyzed by a 7890A Agilent GC equipped with a flame photometric detector (FPD) and a 5975C mass spectrophotometer (MS) (Agilent Technologies, USA) using a DB-5MS capillary column (Agilent Technologies, USA) (Sapahin et al. 2014).

The spiked MP in water samples was also detected by MPH-GST sensing microplate. Tap water samples were spiked with MP at different concentrations of 7.6-38.02 µM. They were then tested for MP using MPH-GST sensing microplate. The experiments were done repeatedly for 5 times.

MP Detection of spiked and real (non-spiked) samples by MPH-GST sensing microplate

Agricultural products such as Chinese kale, holy basil, coriander, morning glory, asparagus, and gotu kola (Asiatic pennywort) were collected from agricultural areas in Nakhon Pathom and Phetchaburi provinces. Fruit samples such as durian, guava juice, and mangosteen juice were taken from fresh markets. Each sample (5 g) was washed with distilled water, chopped into small pieces, mixed with 50 mM PBS (pH 8.0) at the ratio of 10 mL per 5 g samples, and stirred for 1 h at room temperature. The sample was then filtered, centrifuged at 10,000×*g*, and the precipitate discarded (Pootawee et al. 2018). The clear fluid of each sample was then spiked with 190 µM MP. The spiked MP was detected by the MPH-GST sensing microplate and the percentage of recovery was calculated from the MP calibration curve. For real (non-spiked) samples, MP residues were measured following the same protocol. All experiments were conducted in triplicate.

Determination of OP cross-reactivity by MPH-GST sensing microplate

To determine OP cross-reactivity of the MPH-GST biosensing device, the sensing microplate was tested with various phenyl-substituted OP insecticides: methyl parathion, paraoxon, parathion (each at 100 μM), and fenitrothion (50 μM). The OD_{410} values detected by an optical microplate reader were then calculated for the percentages of recovery. The experiments were done in triplicate.

Results and discussion

Production of recombinant MPH-GST fusion protein

The active BpGP colonies, possessing MPH activity and yielding yellow PNP upon MP hydrolysis, were subjected to recombinant MPH-GST production. In this work, the recombinant MPH was produced in the form of GST-tagged MPH, MPH-GST. From SDS-PAGE analysis, a protein band in the supernatant with the molecular mass of about 61 kDa was clearly shown, which corresponded to that of MPH-GST [MPH ~ 35 kDa and GST ~ 26 kDa] (Fig. 1). As summarized in Table 1, its specific activity (72 U/mg) was 5.54 times higher than that of cell debris (13 U/mg). Even though the purified enzyme had high specific activity, its practical application as a bio-component has certain limitations in expensive and time-consuming enzyme purification and the need for essential factors to produce the detectable products (Kumar et al. 2018). The use of crude enzyme was reported to be advantageous due to the presence of cofactors and coenzymes essential for the enzymatic activity (Colmati et al. 2019). Hence, this MPH-GST supernatant is most suitable for use as a biomolecule for the fabrication of the OP sensing device.

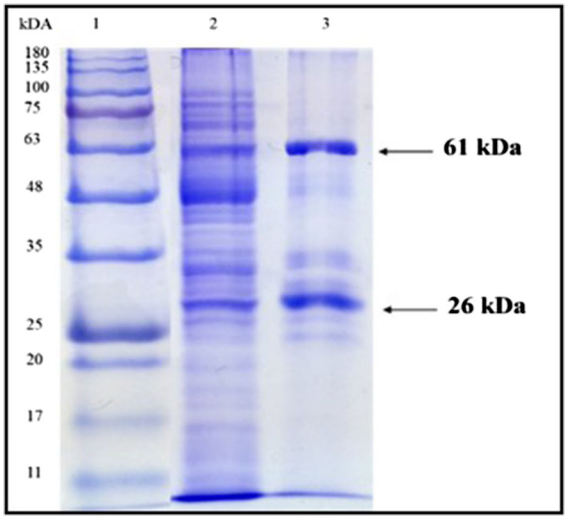

Fig. 1 SDS-PAGE of MPH-GST supernatant from recombinant clone BpGP, lane 1: standard protein ladder; lane 2: cell debris; lane 3: MPH-GST supernatant

Optimization of chitosan film formation for immobilizing MPH-GST

Our proposed OP detection system was successfully developed based on the chromogenic detection using chitosan-coated polystyrene microplate as the matrix for immobilizing biomolecules. After coating the chitosan to the microplate surface, the glutaraldehyde was then added for crosslinking using its carbonyl group to form a covalent bond with the amine group of chitosan (Islam et al. 2019). With the other free carbonyl group, the glutaraldehyde that was attached to the chitosan film was then able to form a covalent bond with MPH-GST through the amino group at the N-terminus of the GST molecule (Ekkhunnatham et al. 2012). The crosslinking schematic diagram of the immobilized MPH-GST sensing microplate was shown in Fig. 2. This simple immobilization approach provided an advantage in that it scarcely interfered with the optical transparency of the microplate, as reported earlier (Kildeeva et al. 2009; Zhang et al. 2011).

The optimum conditions for the formation of the chitosan film-coated 96-well plate prepared for MPH-GST immobilization were determined and the activity expressed in terms of maximum OD_{410}. For the effect of chitosan concentration, the relative MPH activity of the proposed biosensing device was highest at 2% (w/v) chitosan and dramatically decreased with increasing concentration (Fig. S1). Hence, 2% (w/v) chitosan was chosen for further covalent coupling of MPH-GST with different volumes of chitosan. The optimum chitosan volume for MPH-GST immobilization was determined to be 35 μL (Fig. S2). At higher chitosan volume, the relative MPH activity decreased slightly, this would probably arise from the fragility of the dried, thick films (Yi et al. 2003). These optimum

Table 1 Extraction parameters of MPH-GST supernatant from clone BpGP

Extracted fraction	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
Cell debris	79	6	13
MPH-GST supernatant	652	9	72

conditions for chitosan film formation were consequently utilized for immobilizing MPH-GST in all experiments.

Optimization of MPH-GST sensing microplate

Since the changes of the conditions around the enzyme-based biomolecules might influence the

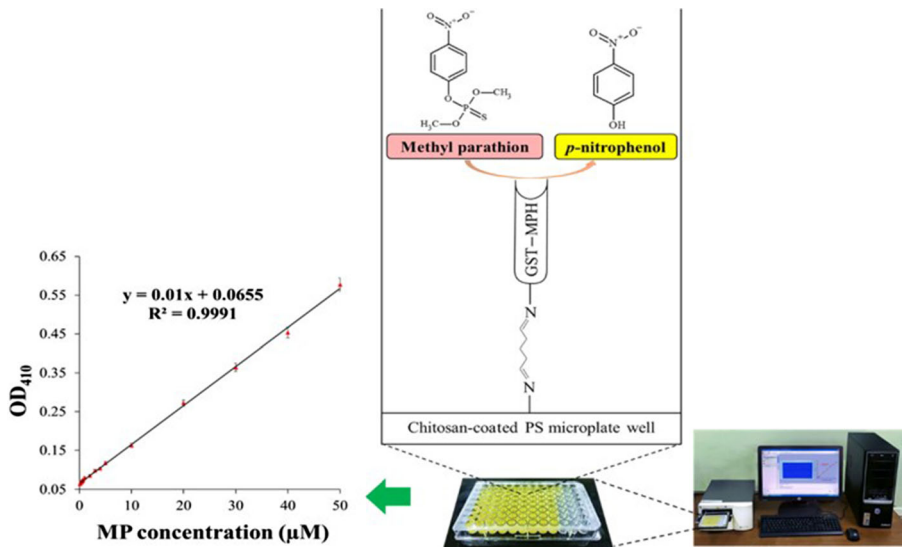

Fig. 2 Schematic diagram of immobilized MPH-GST sensing device for OP detection

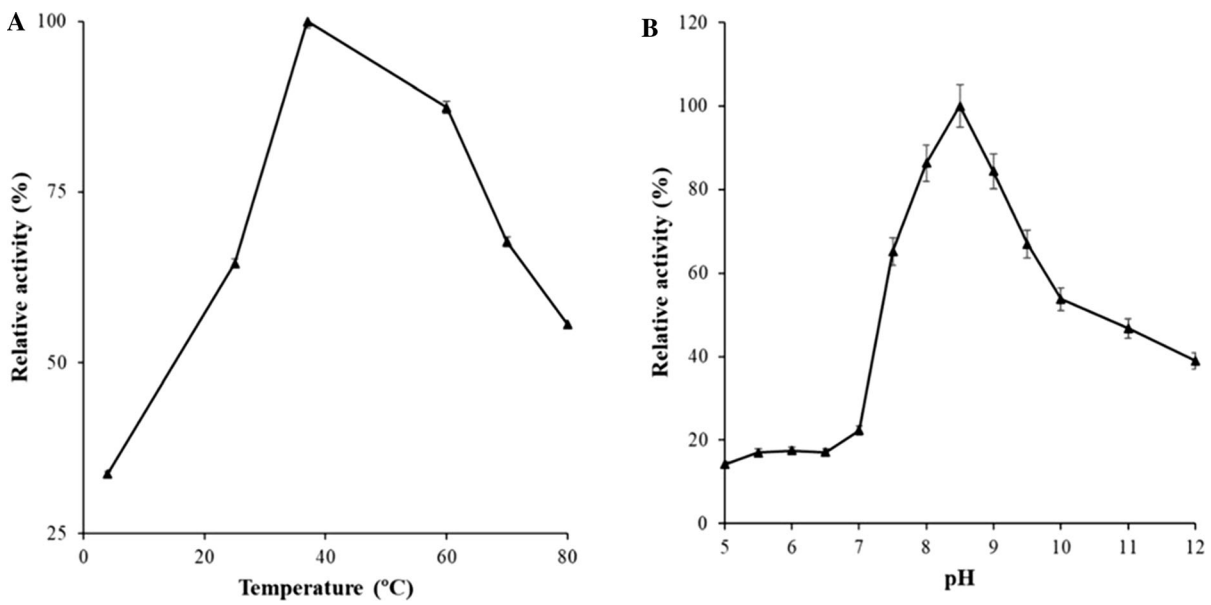

Fig. 3 Optimum temperature (**a**) and optimum pH (**b**) of MPH-GST biosensing device

detectability of the biosensing instruments and biosensors (Tran 1993), it was essential to optimize the conditions for their maximum activity (Bisswanger 2012; Brena et al. 2013). With the use of an optical microplate reader, the effects of temperature and pH for the MPH-GST biosensing device were determined. As presented in Fig. 3a, the optimum temperature of the biosensing device was 37 °C and the activity decreased thereafter at higher temperatures. This was probably caused either by the fact that the enzyme was denatured (Kaushal et al. 2018) or that the chitosan film matrix was unable to protect the immobilized enzyme from high temperatures (Kumari and Kayastha 2011). At 37 °C, the effect of pH on the biosensing device activity was determined with different pH ranging from 5.0 to 12.0 in various buffer solutions. Figure 3b indicates that the MPH-GST sensing device was capable of detecting MP with high activity at the pH range of 8.0–9.0, with the optimum pH being 8.5. This could be due to the fact that the ion charges of the buffer system could possibly change the pH, which affected the activity and the folding of the enzyme (Kumar and Upadhyay 2020).

Performance characteristics of MPH-GST sensing microplate

For the determination of detection limit and linear range for MP detection, MPH-GST sensing microplate was calibrated with various MP concentrations (0.1–100 μM) and OD_{410} was detected. As seen in Fig. 4, the OD_{410} increased linearly with MP concentration. The linear regression equation was $y = 0.01x + 0.0655$, and the determination coefficient (R^2) was 0.9991, which demonstrated the reliability of MP detection by this sensing microplate. The detection limit was 0.1 μM and the linear range was 0.1–50 μM. Its detection limit was approximately 76 folds lower than that of the absorbance-based sensing microplate using the immobilized whole cells of the same clone from our previous work (Pootawee et al. 2018) (Table 2). Although whole cell sensing system was considered most suitable for maintaining high stability of the enzyme (Kumar et al. 2006; Kumar and D'Souza 2010; Tang et al. 2014; Mishra et al. 2017), it was reported to have several limitations due to slow substrate diffusion and slow product passage through the cell wall, resulting in long time response and low specificity (D'Souza 2001). The

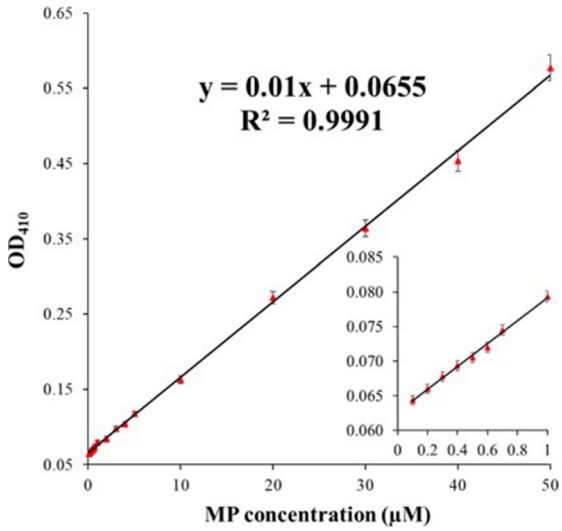

Fig. 4 Calibration curve of MPH-GST sensing device with various MP concentrations, showing the linear equation $y = 0.01x + 0.0655$ ($R^2 = 0.9991$), where Y-axis was expressed as OD_{410} and X-axis as MP concentration

detection limit of our immobilized MPH-GST sensing microplate was also about 40 folds lower than that of absorbance-based, immobilized MPH-His system from another report (Lan et al. 2012). The results indicated high sensitivity of MPH-GST sensing microplate for MP detection via the absorbance-based method.

In determining the temperature stability of the MPH-GST sensing device for MP detection at different temperatures for 30 days, the system was most stable at 4 °C (Fig. 5a). The relative activity remained stable during the first half of the month, and then gradually decreased to the final activity of about 80% at day 30. At 25 °C, the relative activity appeared constant for about 7 days, after which it gradually dropped to about 70% at day 30. This is an advantage of the sensing device that allows its practical use at around room temperature. These results indicated that the proposed system could effectively maintain MPH activity for a long time and hence support the tolerance of immobilized MPH-GST sensing device. At 37 °C and 60 °C, the relative activities rapidly dropped to about 37% and 32%, respectively, at day 30. For pH stability of the MPH-GST sensing system for MP detection, the sensing microplate was tested with various buffer solutions with different pH values (pH 5.0–10.0) at 25 °C for 30 days. It can be seen in

Table 2 Comparison of the OP absorbance-based sensing system using microbial cells/enzyme

OP sensing system	Insecticide	LOD (μM)	Linear range	Reference
Immobilized whole cells expressing MPH-GST on microplate	MP	7.6	7.6–760 μM	(Pootawee et al. 2018)
Immobilized MPH-$(His)_6$ with Ni^{2+}/NTA agarose	MP	4	0–1 × 10^{-4} M	(Lan et al. 2012)
Immobilized MPH-GST on chitosan-coated microplate	MP	0.1	0.1–50 μM	This study

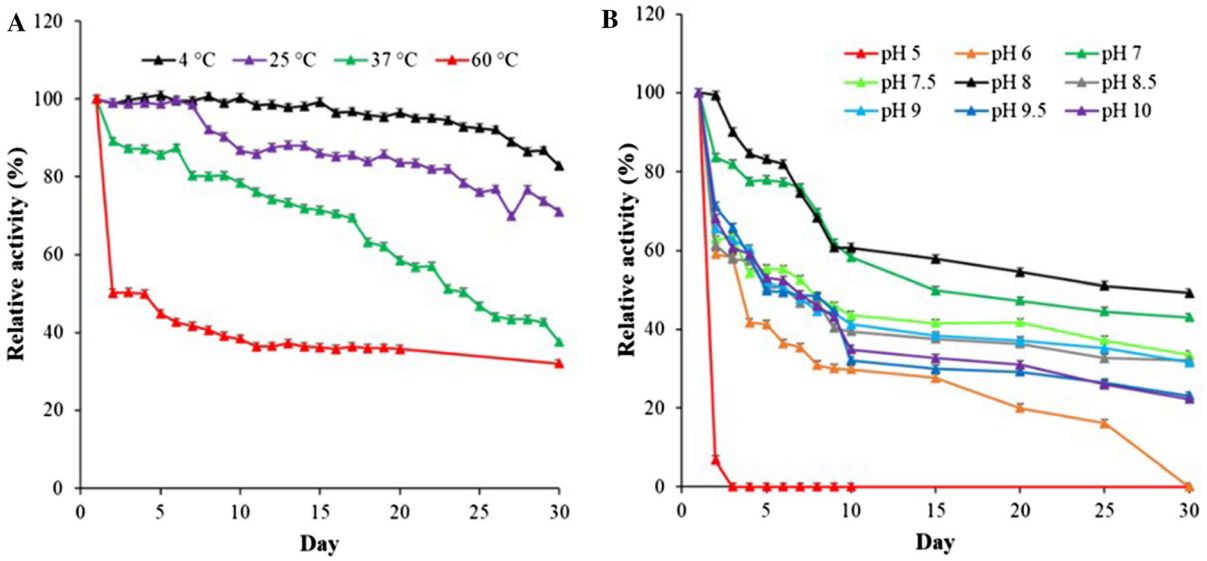

Fig. 5 Temperature stability (**a**) and pH stability (**b**) of MPH-GST sensing device

Fig. 5b that the sensing system was most stable at pH 8.0. The relative activity gradually dropped to about 61% at day 9 and decreased to the final activity of about 49% at day 30. At pH 7.0, the relative activity decreased faster and to lower percentages than at pH 8.0 most of the time until the final activity reached about 43% at day 30.

Reusability is another important property for biosensing devices and biosensors (Tran 1993; Kumar and D'Souza 2010). Here, the MPH-GST sensing microplate was tested for 100 rounds of MP detection under the optimum conditions. As shown in Fig. 6, the result appeared that this system could be reused up to 92 rounds with high activity of more than 80%. After that, it still held the activity of more than 60% until round 100. The possible reason why the sensing device could be reusable for 100 rounds with high activity was that, with the immobilization method used here, the amino acid residues that function as MPH active site did not involve in the matrix binding (Mohamad

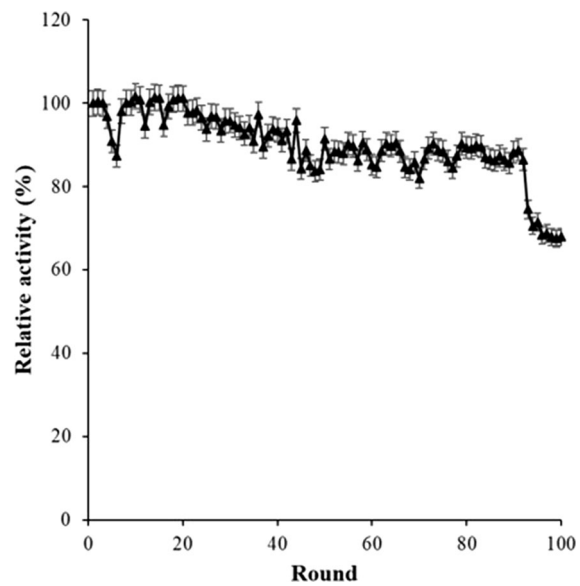

Fig. 6 Reusability of MPH-GST sensing device for MP detection

et al. 2015). Consequently, the catalytically active tertiary structure of MPH-GST was still preserved, resulting in the maintenance of its high activity and reactivity (Norouzian 2003; Mohamad et al. 2015). It was clearly evident that the covalent bonding method provided a strong bond that allowed a longer time of reusability than that of other immobilization methods (Sheldon 2007; Mohamad et al. 2015).

Detection of MP spiked in water samples

In order to assess the accuracy of MP detection, MPH-GST sensing microplate was determined for the percentage of recovery of MP spiked in water samples. When spiked MP concentration detected by MPH-GST sensing microplate was compared with that detected by GC-MS method, the percentage of MP recovery was only 3% different (Table 3), indicating high accuracy of the device. When the sensing microplate was further tested with water samples spiked with varying MP concentrations, the concentrations of the MP detected were in agreement with the known spiked MP concentrations (Fig. S3). The linear regression equation $y = 1.0787x - 0.0849$ from the graph arising from the experimental results, together with the determination coefficient (R^2) of 0.9991, also revealed the device potential for MP detection with high accuracy in field use.

Detection of spiked and real (non-spiked) samples

The OP contamination in agricultural products and the environment has been of great concern and remarked in many articles (Liu et al. 2005b; Hassan et al. 2010; Sapahin et al. 2014; Srivastava et al. 2014). In order to evaluate the feasibility of the MPH-GST sensing device developed here to detect MP in agricultural products, the sensing microplate was further tested on spiked and real vegetable and fruit samples. Table 4 displays the percentages of MP recovery upon the detection of MP in the spiked samples using the sensing microplate, which appeared to be in the range of 82–100. These results of high recovery were comparable to those of MP-spiked water sample test, indicating high reliability of detection by the sensing device. The MPH-GST sensing microplate was also demonstrated for its ability to detect MP in real, non-spiked agricultural samples. The results were shown in Table 5, proving that the device has high reliability and efficiency for field work application.

Table 4 Percentages of MP recovery by MPH-GST sensing microplate on 190 μM MP-spiked samples

MP spiked samples (190 μM)	Recovery (%)
Chinese kale	95
Holy basil	97
Coriander	93
Morning glory	98
Asparagus	87
Gotu kola (Asiatic pennywort)	100
Durian	100
Guava juice	82

Table 5 MP concentrations detected in real (non-spiked) samples by MPH-GST sensing microplate

Real (non-spiked) samples	MP concentration (μM)
Chinese kale	10.7
Holy basil	24.4
Coriander	14.12
Morning glory	15.83
Asparagus	0
Gotu kola (Asiatic pennywort)	0
Durian	3.65
Guava juice	0
Mangosteen juice	0

Table 3 Percentage of MP recovery determined by MPH-GST sensing device and GC-MS method

Detection method	MP spiked (μM)	MP detected (μM)	Recovery (%)
MPH-GST sensing device	38.02	31.18	82.00
GC-MS	38.02	32.32	85.00

Table 6 Cross-reactivity with phenyl-substituted OP insecticides using MPH-GST sensing microplate

OP insecticides	Spiked concentration (μM)	Detected concentration (μM)	Recovery (%)
Methyl parathion	100	99	99
Paraoxon	100	98	98
Parathion	100	96	96
Fenitrothion	50	49	99

Even though this work was carried out using MP as an OP representative, the MPH-GST sensing device can be used to detect other types of phenyl-substituted OP, since the recombinant MPH was shown to have cross reactivity with other OP substances, e.g., paraoxon, parathion, fenitrothion, coumaphos, chlorpyrifos, and malathion (Ekkhunnatham et al. 2012). The cross reactivity among phenyl-substituted OP insecticides using the sensing device was shown in Table 6, where the percentages of recovery determined were considerably high. While many researches have focused on fabricating expensive sophisticated devices for insecticide detection, a simple, inexpensive, and convenient OP sensing device with reliability and good detection limit like this MPH-GST sensing microplate developed here is still in demand in certain circumstances. It could be beneficial for both household use and large-scale screening as an economical and rapid way to monitor and control OP contamination. With all the good performance characteristics, this highly sensitive and efficient MPH-GST sensing microplate was proved to be feasible for field use and further fabrication of an optical biosensor for OP detection.

Conclusions

In this study, we have developed a simple, cost-effective, and efficient absorbance-based sensing device for OP detection. The MPH-GST was immobilized onto the chitosan-coated polystyrene 96-well plate using covalent immobilization and the detection of MP, as a representative of OP, was performed spectrophotometrically through the production of the yellow hydrolytic product, PNP. The MPH-GST sensing microplate was demonstrated to be highly advantageous due to its simplicity, cost-effectiveness, stability, and reliability. The possibility of the device to detect OP in multiple samples simultaneously, due to the use of a 96-well plate as a matrix, also added up to its advantages. Its linear detection range was 0.1–50 μM. With the detection limit as low as 0.1 μM, together with its good reusability, the device proved suitable for practical application in field work. This sensing device was able to detect MP reliably in both spiked and non-spiked agricultural samples. Although simply designed on a small budget, the device has rendered itself to be an efficient tool in real world. Hence, the immobilized MPH-GST microplate fabricated here as an OP sensing device has a great potential to be a favorable alternative method for fast and easy screening of OP contamination in agricultural products and the environment. It also offers the promise for further development into an efficient OP optical biosensor in the future.

Acknowledgements We thank the Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University and the Division of Science and Technology, Faculty of Science and Technology, Phetchaburi Rajabhat University for providing facilities and equipment necessary for this research. We also thank Miss Tipaporn Subsomboon for her helpful technical support and Ms. Jongkonrat Wichitwechkarn for proofreading and editing the manuscript.

Author contributions Conceptualization: WR and JW; Methodology: WR, SC, JM, and JW; Formal analysis and investigation: WR; Writing—original draft preparation: WR; Writing—review and editing: WR, SC, and JW; Funding acquisition: JW; Project administration: JW; Supervision: JW and JM.

Funding This research was funded by Research and Researcher for Industry (RRI) grant, Thailand Research Fund, Contract No. PHD59I0024. Mixberry Co., Ltd. joined in part in the RRI funding program. The research work was also financially supported by the Research Network for Higher Education in Lower Central Region of Thailand through Silpakorn University Research, Innovation and Creativity Administration Office, a part of which was jointly funded by A4 Fruit Trading Co., Ltd.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research using human and animal consents This article does not contain any studies with human participants or animals performed by any of the authors.

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