



# A cell-based potentiometric biosensor using the fungus *Lentinus sajor-caju* for permethrin determination in treated wood

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## ABSTRACT

The characteristics of a potentiometric biosensor for the determination of permethrin in treated wood based on immobilised cells of the fungus *Lentinus sajor-caju* on a potentiometric transducer are reported in this paper. The potentiometric biosensor was prepared by immobilisation of the fungus in alginate gel deposited on a pH-sensitive transducer employing a photocurable acrylic matrix. The biosensor gave a good response in detecting permethrin over the range of 1.0–100.0  $\mu\text{M}$ . The slope of the calibration curve was 56.10 mV/decade with detection limit of 1.00  $\mu\text{M}$ . The relative standard deviation for the sensor reproducibility was 4.86%. The response time of the sensor was 5 min at optimum pH 8.0 with 1.00 mg/electrode of fungus *L. sajor-caju*. The permethrin biosensor performance was compared with the conventional method for permethrin analysis using high performance liquid chromatography (HPLC), and the analytical results agreed well with the HPLC method (at 95% confidence limit). There was no interference from commonly used organophosphorus pesticides such as diazinon, parathion, paraoxon, and methyl parathion.

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## 1. Introduction

Chemicals are used as a protector due to their impregnation ability into the wood cells to improve their physical properties [1]. A range of different chemical treatments are able to extend the durability of wood cells as well as to improve the resistance from decay, insects, weather, and fire [2]. Chromated Copper Arsenate (CCA) is an approach that is widely used in wood treatment. CCA is the best option for wood treatment because of its strong chemical bonding to the wood hence no leaching problems. However, there are limitations to this approach especially in solvent (water) and in its aesthetic value. The water in CCA will wet the wood and consequently will subject the wood to dimensional movement upon drying [3]. CCA also imparts a green colour to the treated wood hence lowering the aesthetic value. In many countries, CCA is considered unsafe and unfriendly chemical to the environment [3]. Therefore, to overcome this problem, Light Organic Solvent Preservative (LOSP) is the most popular alternative treatment used in industrial process in preparation of softwood and hardwood products [4].

In order to achieve quality assurance of the treatment process, the quantity of the LOSP used must be sufficient as stated in the standard requirements [5]. Permethrin, as the main ingredient of LOSP formulations, is therefore used as an indicator to determine the usage of LOSP in treated wood [6]. Up to now, the techniques employed for permethrin detection mainly involve chromatography such as High Performance Liquid Chromatography (HPLC) [7] and Gas Chromatography (GC) [8–11]. Other techniques are UV–vis Spectroscopy [12], Fourier Transform Infrared Spectroscopy [13], Flow Injection Analysis [14], and Surface Plasma Resonance [15]. Although these techniques offer reliable results, they are non-portable devices. Therefore, simple and portable devices are being explored, as have recently been reviewed. For examples, an electronic nose device that has been developed to detect the amount of permethrin in water [16], a DNA-based biosensor for permethrin using single strand *calf thymus* (ssCT-DNA) immobilised onto chitosan-iron oxide nanobiocomposite film deposited on indium tin oxide (ITO) coated glass [17], and an immunoassay method utilising enzyme-linked immunosorbent assay (ELISA) with different antibodies to determine permethrin in river [18–19].

Therefore, more alternative methods that are simple, easy in sensor design, and of wide range applications should be explored [20–24]. Baronian [25] has suggested simpler approach by using cell-based biosensors, which demonstrate similar practice and principles of nucleic acid biosensors. For permethrin, toxicity

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towards fungus has been studied and fungal bioassay using novel bioluminescence has been established for toxicity testing [26,27]. The use of the fungus *L. sajor-caju* for biosorption of cadmium (II) has been reported; the fungus is entrapped in alginate gel via a liquid curing method in the presence of Cd(II) ions [28].

Until now, the fungus, *L. sajor-caju* has not been employed as a cell-based biosensor for specific determination of permethrin although this fungus is known to be capable of degrading permethrin. Therefore, the objective of this study was to design a new cell-based biosensor from the fungus *L. sajor-caju*, which was immobilised in an alginate matrix to construct a potentiometric device that consisted of three layers of membranes. The biosensor can be used as a simple device to detect pyrethroid compounds, especially permethrin in treated wood.

## 2. Experiment

### 2.1. Materials

Chemicals used in this study were as follows: 2-hydroxyethyl methacrylate (HEMA), 2,2-dimethoxy-phenyl-acetophenone (DMPP), n-butyl acrylate (nBA), sodium tetrakis [3,5-bis (trifluoromethyl) phenyl] borate (NaTFPB), poly(2-hydroxyethyl methacrylate) (pHEMA), 1,6-hexane diolacrylate (HDDA), tris(hydroxymethyl)aminomethane (Tris-HCl), sodium alginate, hydrogen ion ionophore (tridodecylamine), citrate buffer, permethrin, and dioxin. All the chemicals were from Merck.

### 2.2. Fungus culture

Regular forays were conducted by Wood Mycology Unit of Forest Research Institute Malaysia (FRIM) in 2006–2012 with the aim to study the occurrence of *Lentinus* species and population in the Malaysian forests concentrating only in Peninsular Malaysia. A number of *Lentinus* species were found at various locations in the forests, with *L. sajor-caju* showing the widest distribution in the forays. 15 specimens, which were identified only up to genus level, were collected in Endau-Rompin National Forest. *L. sajor-caju* was found as the most frequent species collected in this study.

*L. sajor-caju* was maintained by subculturing the species on malt extract agar slants. The growth medium consisted of the following (in gL<sup>-1</sup> of distilled water): D-glucose, 10.0; KH<sub>2</sub>PO<sub>4</sub>, 20.0; MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.5; NH<sub>4</sub>Cl, 0.1; CaCl<sub>2</sub> · H<sub>2</sub>O, 0.1; and thiamine, 0.001. The growth medium was also supplemented with 1.0 mL of trace element solutions as follows (in gL<sup>-1</sup>): nitrilotriacetate, 1.5; NaCl, 1.0; MnSO<sub>4</sub> · H<sub>2</sub>O, 0.5; FeSO<sub>4</sub> · 7H<sub>2</sub>O, 0.1; ZnSO<sub>4</sub>, 0.1; CaSO<sub>4</sub>, 0.01; CuSO<sub>4</sub> · 5H<sub>2</sub>O, 0.01; H<sub>3</sub>BO<sub>3</sub>, 0.01; and NaMoO<sub>4</sub> · 2H<sub>2</sub>O, 0.01. The pH of the medium was adjusted to 4.5, which was the optimum growth pH of *L. sajor-caju*.

### 2.3. Electrode preparation method

An Ag/AgCl screen-printed electrode (SPE) of size 25 mm × 12.5 mm with an electrode diameter 2 mm was used for the biosensor construction (Fig. 1). Monomer of 2-hydroxyethyl methacrylate was

mixed with 1.6 wt% 2,2-dimethoxy-2-phenylacetophenone photoinitiator and the mixture was deposited on the surface of a Ag/AgCl screen-printed electrode (SPE) and photopolymerisation was conducted using ultraviolet radiation (~200 nm) under nitrogen gas purging for 3 min. The polymer film formed was then hydrated for 10 min using Tris-HCl 0.1 mM buffer solution at pH 7. Next, a mixture composed of 99.5 wt% n-butyl acrylate monomer, 0.1 wt% 2-hexanedioldiacrylate crosslinking agent, 2 wt% photoinitiator, 2,2-dimethoxy-2-phenylacetophenone, sodium tetrakis[bis-3,5(trifluoromethyl)phenyl]borate and 5 wt% of hydrogen ion ionophore was deposited on the surface of the poly(hydroxyethyl methacrylate) film for the design of a pH sensor. The photopolymerisation was conducted by exposure to ultraviolet radiation (~200 nm) under nitrogen atmosphere for 3 min. The design of the hydrogen ion sensor is shown in Fig. 1. The response of the sensor to hydrogen ion was tested with a double junction Ag/AgCl electrode as the reference electrode. The electrode and the sensor were connected to an Orion ion metre where the difference in the potential of the cell (electromotive force, EMF (mV)) was recorded when a stable value was reached. The sensor was examined with 0.1 mM Tris-HCl buffer at pH 4.0–9.0. The pH of each buffer solution was measured with a pH electrode before use. The EMF response of the test cell was plotted against the logarithmic concentrations of the test solutions according to the Nernst equation.

### 2.4. Fungus immobilisation on electrode

Alginate was used to immobilise the fungus *L. sajor-caju* and it was rinsed several times with Tris-HCl buffer before being mixed with alginate solution, which contained CaCl<sub>2</sub>. Finally the alginate solution was spread onto the pH-sensitive membrane of the potentiometric transducer. This solution was then dried at 4 °C overnight before measurements with permethrin were carried out.

### 2.5. Evaluation of biosensor response

Permethrin solutions in the range of 0.1 to 0.1 mM were prepared in Tris-HCl buffer (0.1 mM pH 7). Measurements were carried out as described above. Before use, the electrode was equilibrated in 0.1 mM Tris-HCl buffer (pH 7) for at least 30 min. Measurements were conducted at room temperature (25 °C). The EMF readings in mV were recorded after 10 min and were plotted against the logarithmic of permethrin concentration to establish the calibration curve. The optimum pH, effect of buffer concentrations, enzyme optimisation, effect of temperature, dynamic response range, response time of the sensors, repeatability, reproducibility, lifetime and interference characteristics, and the sensor regeneration capability were evaluated. Possible interference of the sensor from cypermethrin, deltamethrin, diazinon, parathion, paraoxon, and methyl parathion was investigated.

### 2.6. Extraction of permethrin from wood samples

In this study, 1.00 g of wood powder was weighed and transferred into a 50 mL Erlenmeyer flask. Then, 15–20 mL of n-hexane was added into the flask, covered with aluminium foil and placed on an electric sieve. Wood powder sample immersed in the n-hexane was filtered for 30 min at a speed of 100 revolutions per minute. After 30 min, the sample was separated from the extraction solvent by using a size 4 filter paper. Further addition of n-hexane to resulting extract was carried out to make up to a final volume of 25 mL. The permethrin in the extract was then analysed.

### 2.7. Validation of sensor response

Wood sample used in this study was rubberwood (*Hevea brasiliensis*). The wood was treated with permethrin from 0 to 100 μM

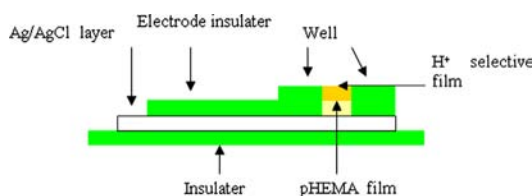


Fig. 1. The design of screen-printed electrode (SPE) ion sensor.

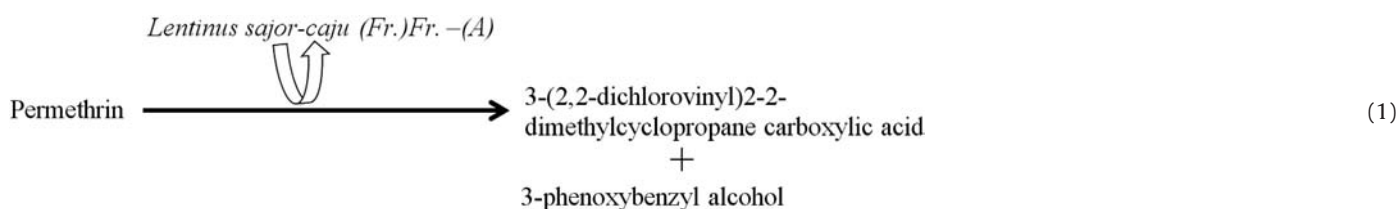
using vacuum impregnation vessel. Untreated wood samples were used as a control for the measurements. The performance of the biosensor was compared with established method using permethrin-treated wood samples. In this study, Australian/New Zealand Standard [29] for the HPLC method was employed. The organic solvent (n-hexane) and mobile phase (99.5% n-hexane: 0.5% THF) were used for separation and determination of permethrin in treated wood samples. The HPLC conditions were as follows: photodiode detector at 260 nm for detection; solvent flow rate of 1.5 mL/min; loop injector of 20  $\mu$ L; and column of 5  $\mu$ m Luna silica (brand Phenomenex) size 150  $\times$  4.6 mm<sup>2</sup> to separate the *cis* and *trans* isomers.

### 3. Results and discussions

#### 3.1. Detecting principle

The biosensor consisted of four layers of membranes deposited on top of an Ag/AgCl screen-printed electrode (SPE). The uppermost layer was the alginate gel membrane immobilised with the fungus *L. sajor-caju* (Fig. 2). The use of alginate gel to entrap fungus *L. sajor-caju* has also been reported by Bayramoglu (2002) where the fungus was immobilised in Ca-alginate beads for the removal of Cd(II) ions from aqueous solution [28]. In a hydrophilic environment, alginate provided a stable two-phase piqued system and this caused the enhancement of the operational stability of the fungi entrapped within the alginate layer. Besides alginate, other organic and inorganic materials such as polypyrrole films, biocompatible synthetic latex, and laptonite have also been reported as suitable matrix for fungi entrapment [30]. The function of the second layer that contained hydrogen ionophore was to create a pH transducer that detected the pH changes occurred in fungus immobilised layer (Fig. 1). The sample that contained permethrin first diffused through the alginate gel membrane and reaction between permethrin and enzymes in the living fungal cells led to the formation of a weak acid. As a result, the changes in pH due to the hydrolysis of permethrin were detected by the plasticizer-free H<sup>+</sup> selective membrane. However, there was no response from the sensor towards permethrin if the fungus was not immobilised in the sensor membrane.

There is little information in permethrin pesticide biotransformation by fungi [31,32]. *L. sajor-caju* is a white-rot fungus that has several extracellular enzymes for bioremediation of various xenobiotics. The reaction of *L. sajor-caju* fungus with permethrin has not been reported before but the possible mechanism of the reaction between permethrin and other similar fungi might be inferred from the work reported by Yurekli et al. [32]. Saprophytic white-rot fungi have the ability to degrade permethrin to yield the degradation product of 3-(2,2-dichlorovinyl)2-2-dimethylcyclopropane carboxylic acid [32]. As the *L. sajor-caju* (Fr.)Fr.-(A) fungus is from the saprophytic white-rot fungi group, it is assumed that the fungus reacts with permethrin to produce a carboxylic acid. The proposed reaction mechanism is as Eq. (1).



Some preliminary investigations of the reaction kinetics of the *L. sajor-caju* (Fr.)Fr.-(A) fungus with permethrin under solution conditions at 25 °C indicated a behaviour of first-order kinetics and

the rate constant was 0.081 s<sup>-1</sup> over the concentration range of permethrin from 0.0008 to 0.01 mM permethrin. In this concentration range, the reaction half-life was 8 s and the reaction is fast in solution.

#### 3.2. Effect of pH

The pH of the buffer could affect the potentiometric biosensor response. It was found that the pH for maximum response of the biosensor was at pH 8.0 (Fig. 3). This observation was different from that reported by Gülay et al. [28] for biosorption of Cd(II), Cr (VI), and uranium by *L. sajor-caju* encapsulated in alginate beads. The optimum pH in their study was at pH 5 because these metal ions are more stable under acidic conditions. In this study, slightly basic condition was required for measuring the pH change that occurred from the reaction between permethrin and *L. sajor-caju* since a weak acid was produced from the reaction.

#### 3.3. Effect of buffer concentration

The best buffer concentration that yielded the highest potentiometric sensitivity slope was found at 1.0 mM (Table 1). This is because at lower concentration of buffer, the biosensor detected more protons and this generated optimum signal. With the increase of buffer capacity, more protons will be neutralised by the buffer and the transducer will detect a smaller pH variation. Similar results were obtained by other potentiometric biosensors developed by Aurelia-Magdalena et al. [33].

#### 3.4. Effect of fungus loading on biosensor response

The amount of fungus that can be loaded onto the electrode surface was limited by the size of the electrode area. As shown in Fig. 4, the maximum weight of fungus loading was 1.00 mg/electrode. The increase of mycelia weight was proportional to the increase in the biosensor response. The response became constant after a loading of 1.00 mg/electrode of fungus cells because all the fungal mycelia had already reacted with permethrin, and the permethrin was in excess. Mulchandani [34,35] also reports similar finding in the construction of potentiometric microbial biosensor for the direct measurement of organophosphate nerve agents.

#### 3.5. Effect of temperature on biosensor response

As shown in Fig. 5, it is clear that temperature affected the performance of the biosensor. Maximum biosensor response was observed at lower temperatures. At the temperature of 4 °C, it was found that the *L. sajor-caju* fungus showed a response of 57.20 mV/decade while at the temperature of 25 °C, a similar response of 56.80 mV/decade was obtained. Although a temperature of 4 °C

yielded among the highest response, statistical tests using a *t*-test showed that there was no significant difference in the results when compared with measurements at temperature of 25 °C.

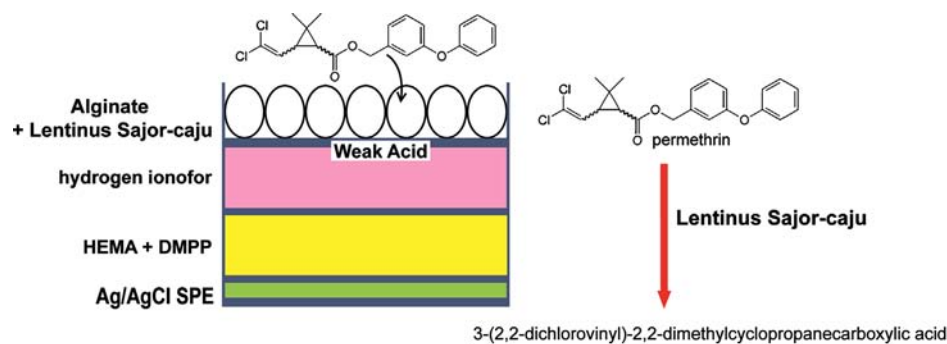


Fig. 2. The design of a potentiometric permethrin biosensor.

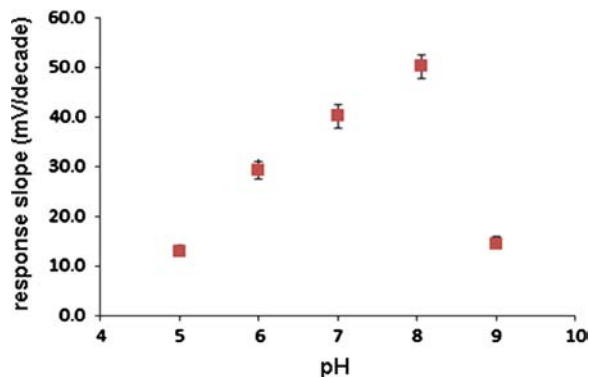


Fig. 3. Effect of pH on permethrin biosensor response. The permethrin concentrations range from  $2.00 \times 10^{-8}$  M to 2.51 mM.

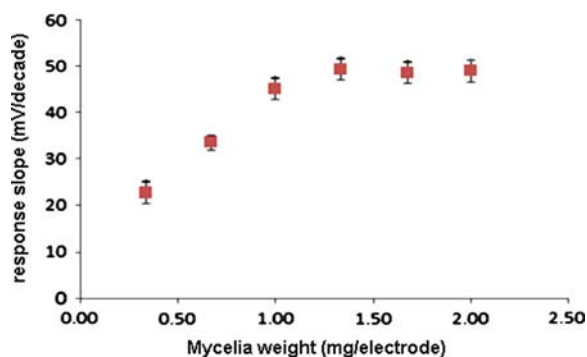


Fig. 4. Effect of fungus loading on the biosensor response. Permethrin concentrations  $2.00 \times 10^{-8}$  M to 2.51 mM.

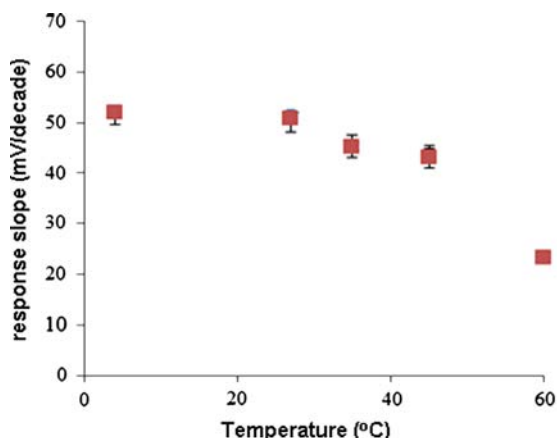


Fig. 5. The effect of temperature on the biosensor response. Permethrin concentration range tested was from  $2.00 \times 10^{-8}$  M to 2.51 mM.

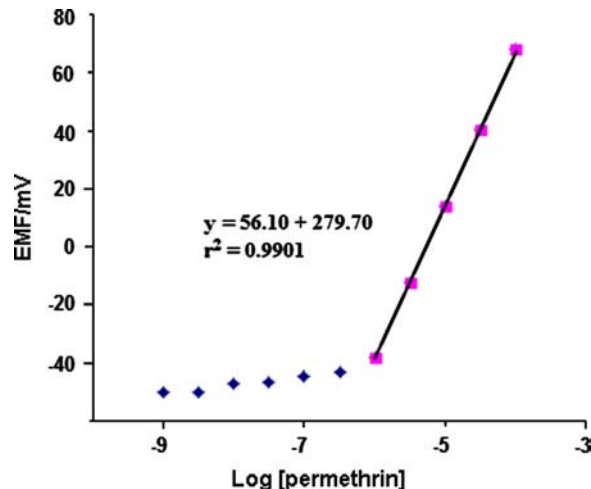


Fig. 6. Study of the dynamic response range of the permethrin biosensor in permethrin concentrations from 1.0 to 100.0  $\mu$ M under optimised conditions.

This shows that the enzyme in the mycelial cell of *L. sajor-caju* (Fr.) Fr.-(A) fungus behaves similarly within this range of temperatures. Above 25 °C, the biosensor response was significantly reduced and this was followed by a sharp decline after 45 °C. This could be due to the effect of heat that disrupted the properties of fungus cells or the enzyme involved in the process of hydrolysis, which eventually reduced the biosensor response. This observation is similar to that obtained by Karube et al. [36] for a biosensor used in the detection of BOD with fungus *Pseudomonas fluorescens*.

### 3.6. The dynamic response range of the biosensor

At the optimised condition of pH, buffer concentration and fungus loading, the biosensor demonstrated a near-Nernstian response to permethrin concentrations from 1 to 100  $\mu$ M with a sensitivity slope of 56.10 mV/decade (Fig. 6), which was near to the theoretical value of 59.6 mV/decade [37]. However, the response slope was lower, i.e. 50.10 mV/decade before the optimisation of sensor working conditions such as pH and the amount of fungus immobilised. The limit of detection, as determined from the intersection of the two extrapolated segments of the calibration curve, was 1.0  $\mu$ M. For the repeatability evaluation, the same biosensor was exposed to the range of 1.0–100.0  $\mu$ M of permethrin for 10 times and the RSD of the response slopes obtained was 3.10% while for the reproducibility evaluation, 10 different biosensors were exposed to 1.0–100.0  $\mu$ M of permethrin and the RSD determined was 4.86%. The low RSD values showed that the method was analytically acceptable [38]. The permethrin biosensor had a response time of 6 min.



**Table 1**

Study on the effect of buffer concentrations on permethrin biosensor response. The permethrin concentrations range from 1.0 to 80.0  $\mu\text{M}$ .

Buffer concentration (mM)	Sensitivity slope (mv/decade)	$R^2$
100.00	17.10	0.9714
10.00	25.30	0.9823
1.00	50.10	0.9993
0.10	45.60	0.9997
0.01	40.30	0.9985

**Table 2**

Interference study of a biosensor towards interference chemicals concentrations from 1.0 to 30.0 mg/L.

Analyte	Concentration for linear response ( $\mu\text{M}$ )	Sensitivity slope (mv/decade)	$R^2$
Rubber wood extract	No response	No response	–
Cypermethrin	2.50–100.00	50.10	0.9991
Deltamethrin	3.40–100.00	49.20	0.9990
Diazinon	6.30–12.10	10.10	0.9566
Methyl parathion	6.10 to 11.90	12.30	0.9512
Paraoxon	5.00–10.00	11.10	0.9451
Parathion	5.20 to 11.10	13.20	0.9490

**Table 3**

Recovery study of the permethrin biosensor.

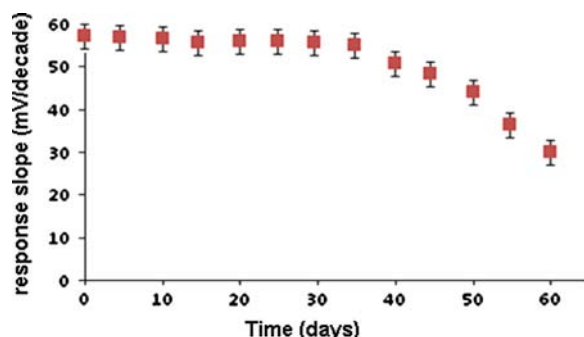
Spike permethrin concentration (mg/kg) <sup>a</sup>	Determined concentration (mg/kg) ( $n=7$ )	Recovery (%) ( $n=7$ )
10.0	$9.0 \pm 2.3$	90.0
20.0	$22.0 \pm 3.0$	110.0
30.0	$28.5 \pm 2.1$	95.0
40.0	$42.1 \pm 3.1$	105.0
50.0	$52.1 \pm 3.5$	104.0
60.0	$57.7 \pm 4.5$	96.0
80.0	$82.1 \pm 2.5$	102.0
100.0	$97.3 \pm 3.1$	97.3

<sup>a</sup> Using rubberwood sawdust.

**Table 4**

Validation study of the biosensor (determination of permethrin in rubberwood).

Standard method using HPLC (mg/kg) ( $n=7$ )	Biosensor method (mg/kg) ( $n=7$ )
$10.1 \pm 1.1$	$9.1 \pm 2.5$
$30.5 \pm 1.2$	$29.3 \pm 2.9$
$50.4 \pm 1.5$	$49.3 \pm 2.3$
$70.6 \pm 1.9$	$68.9 \pm 2.6$
$100.5 \pm 1.3$	$102.5 \pm 2.9$



**Fig. 7.** Lifetime of a biosensor towards permethrin concentrations from 1.0 to 100.0  $\mu\text{M}$  measured under optimised conditions.

### 3.7. Interference study

There was only a slight response to organophosphorus group of insecticides that are likely to be possible interference substances. But, the biosensor also responded to other pyrethroids that were similar in structure to permethrin because of the presence of the carboxyester linkage that could be hydrolysed by carboxylesterase from *L. sajor-caju* (Table 2). Thus, the biosensor was selective to pyrethroid group of pesticides and not others such as organophosphorus pesticides.

### 3.8. Lifetime of the biosensor

The stability and durability of the biosensor based on fungi for the detection of permethrin were evaluated for its response to permethrin over a period of 10–20 days. As demonstrated in Fig. 7, the permethrin biosensor response remained constant over a period of more than 35 days. The reduction of the response beyond that was attributed to the deterioration of the immobilised fungal cells and thus indirectly impacted on the production of  $\text{H}^+$  ions [39].

### 3.9. Recovery study and validation of sensor with standard method

The results from the determination of known concentrations of permethrin spiked into treated wood samples are shown in Table 3. The recovery of spike permethrin was from 90 to 110%. Thus, the biosensor was considered an acceptable method of permethrin determination in treated wood [40]. The analytical performance of the biosensor was further ascertained by comparing it with analysis of permethrin using the Australian/New Zealand Standard [29].

As shown in Table 4, the concentrations of permethrin determined using the sensor did not differ statistically ( $\alpha=95\%$ ) from that determined using the standard HPLC method. This demonstrated that the permethrin biosensor can be used for permethrin determination [40]. In comparison with other electrochemical sensors or biosensors for permethrin, which are mainly based on amperometry, the potentiometric biosensor for permethrin reported in this paper demonstrated a much larger linear response range of up to 100  $\mu\text{M}$  [17,18,41].

## 4. Conclusion

The potentiometric biosensor for permethrin based on the concept of reaction between permethrin and the fungus *L. sajor-caju* cells has been successfully developed. It appeared that the action of the fungus on the permethrin produced acid that led to the response of the potentiometric pH transducer. This is probably the first potentiometric biosensor based on such a fungus developed for the determination of permethrin in treated wood. The biosensor demonstrated a large response range to permethrin when compared to other non-potentiometric devices such as amperometry based sensors. The biosensor is not only useful for the determination of permethrin but also other pyrethroid insecticides. There is almost no interference from other insecticides such as the organophosphorus group since the biosensor is only selective to the pyrethroid group.

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