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Polyaniline-Based Highly Sensitive Microbial Biosensor for Selective Detection of Lindane

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Supporting Information

ABSTRACT: A highly sensitive, selective, and rapid, whole-cell-based electrochemical biosensor was developed for detection of the persistent organochlorine pesticide γ-hexachlorocyclohexane (γ-HCH), commonly known as lindane. The gene *linA2* encoding the enzyme γ-hexachlorocyclohexane (HCH) dehydrochlorinase (LinA2), involved in the initial steps of lindane (γ-HCH) biotransformation, was cloned and overexpressed in *Escherichia coli*. The lindane-biodegrading *E. coli* cells were immobilized on polyaniline film. The rapid and selective degradation of lindane and concomitant generation of hydrochloric acid by the recombinant *E. coli* cells in the microenvironment of polyaniline led to a change in its conductivity, which was monitored by pulsed amperometry. The biosensor could detect lindane in the part-per-trillion concentration range with a linear response from 2 to 45 ppt. The sensor was found to be selective to all the isomers of hexachlorocyclohexane (HCH) and to pentachlorocyclohexane (PCCH) but did not respond to other aliphatic and aromatic chlorides or to the end product of lindane degradation, i.e., trichlorobenzene (TCB). The sensor also did not respond to other commonly used organochlorine pesticides like DDT and DDE. On the basis of experimental results, a rationale has been proposed for the excellent sensitivity of polyaniline as a pH sensor for detection of H^+ ions released in its microenvironment.

Lindane (γ-hexachlorocyclohexane, HCH), an organochlorine pesticide, is a widely distributed contaminant.[1] Among all the HCH stereoisomers, the β-HCH[2,3] and γ-HCH (lindane)[4] are extremely persistent and cannot be easily degraded aerobically. The persistence and organic phase solubility of HCH isomers cause significant biological accumulation and biomagnification in cells and tissues. Its well-established neurotoxicity, carcinogenicity, and consequent health risks led to a worldwide ban on use of lindane.[5] However, lindane is still found in ecological niches such as water bodies and in crops resulting in major environmental problems.[6] In addition, huge amounts of unused obsolete stockpiles of both technical grade HCH (2785 tons), lindane (304 tons), and 45 tons of unspecified HCH exist in dump sites in Africa and the Near East.[7]

Common methods of detection and estimation of lindane are (a) colorimetric measurement of chloride ions,[8,9] (b) phenol red based colorimetric method,[6] (c) colorimetric measurement using zinc in acetic acid;[10] (d) determination of complete mineralization to $^{14}CO_2$,[11] (e) thin-layer chromatography,[8,9] and (f) gas chromatography.[12,13] The detection limits of these methods are tabulated in the Supporting Information (Table S1). The relative merits/demerits of different methods have been discussed earlier.[6] Though the methods are reasonably sensitive, they involve solvent extraction, are time-consuming and expensive, and hence not considered suitable for on-site monitoring.[14] A rapid method for sensitive (part-per-trillion level) and selective detection of HCH contamination is therefore highly desirable.

Biosensors present a promising alternative to conventional techniques as they offer a rapid, specific, and sensitive way of detection of environmental pollutants, including pesticides, without the need of sophisticated instruments.[15] The electrochemical biosensors can be miniaturized to develop a hand-held device and offer the advantage of low detection limit, specificity, and ease of operation for on-site monitoring. However, several challenges need to be addressed in the case of on-site monitoring, like proper storage of the biosensor (as whole cells are used). Indirect acetyl cholinesterase (AChE)-based biosensors have been widely explored for detection of several classes of pesticides, wherein the inhibition of the enzyme activity after exposure to pesticide has been measured using an amperometric,[16] potentiometric,[17] or conductometric transducer.[18] Though the AChE-based biosensors are sensitive, their application is limited due to the irreversible loss of enzyme activity upon inhibition by organophosphorous pesticides.[19] Moreover, as the activity of AChE is inhibited by several organophosphorus and carbamate pesticides, the AChE biosensors are not selective.[20,21]

A major limitation in the development of a biosensor is the availability of a probe or sensor biomolecule which can specifically detect the target analyte.[22] Genetic engineering of microbes can be employed to provide a plethora of

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biomolecules which can be used as the active components for specific biorecognition. A genetically engineered whole-cell-based potentiometric biosensor for organophosphate pesticides has been reported by Mulchandani et al.[21] They modified a pH electrode with an immobilized layer of recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. Schulze et al.[23] have carried out protein engineering of acetylcholinesterase of *Nippostrongylus brasiliensis* by introducing mutation into the AChE peptide chain. The combination of three mutants with the wild-type enzyme in a multienzyme biosensor array enabled the detection of 11 out of the 14 most important organophosphate and carbamate pesticides. Su et al.[24] have recently reviewed the progress in the field of microbial biosensors.

Some bacterial species of *Sphingomonas* are able to metabolize lindane as a sole carbon source for growth.[25] *Sphingomonas paucimobilis* strain B90 can degrade lindane aerobically. However, the rate of degradation is quite slow because of low-level expression of the proteins involved in biodegradation. The HCH dehydrochlorinase (LinA2) gene is involved in the initial steps of lindane biotransformation through a three-step dehydrochlorination to 1,3,5- or 1,2,4-trichlorobenzene and concomitant release of three hydrochloric acid molecules as shown in Figure 1.[4,25]

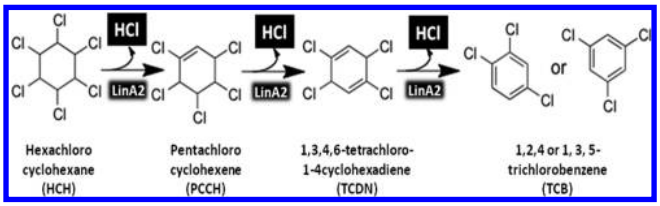

Figure 1. Proposed mechanism for biotransformation of hexachlorocyclohexane (HCH) by stepwise dehydrochlorination using the LinA2 HCH dehydrochlorinase.[4,25]

Since HCl is produced as the byproduct of lindane metabolism, use of a pH transducer was considered appropriate for quantitative estimation of lindane. Polyaniline has been found to be highly suitable for pH sensing in aqueous media as it offers a variety of oxidation states and colors with change in pH and can be used for both electronic[26] and optical pH sensing.[27] The protonation of polyaniline is accompanied by an increase (9−10 orders of magnitude) in its conductivity, thus enhancing its utility as a pH sensor.[28] Due to its conductivity and entrapment capacity, polyaniline has been extensively utilized for development of biosensors for detection of metabolites[29] (glucose, urea, cholesterol), environmental pollutants, gases,[30] etc. However, polyaniline has seldom been used for detection of pesticides. Ivanov et al.[31] have reported polyaniline-based cholinesterase potentiometric biosensor for detection of pesticides. Manisankar et al.[32] have made an attempt to analyze several pesticides by cyclic voltammetry using polyaniline and a multiwall carbon nanotube (MWCNT) modified electrode. However, these sensors responded to a variety of pesticides and were not specific.

In the present study, we developed a polyaniline-based electrochemical sensor for detection of lindane. The HCH dehydrochlorinase encoding gene *linA2* (Figure 1) from *S. paucimobilis* B90 was cloned and overexpressed from a strong inducible T7 promoter in *E. coli* strain BL21 (DE3). Genetically modified *E. coli* cells expressing HCH dehydrochlorinase encoding *linA2* gene were immobilized in polyaniline matrix. Lindane biodegradation and concomitant generation of HCl was detected from the change in the conductivity of polyaniline. A schematic for the sensing mechanism is depicted in Supporting Information Figure S1. Such direct monitoring of catalytic reaction products scores over prevalent enzyme inhibition methods which are indirect. Moreover, the *E. coli* cells are robust and can tolerate high temperatures (up to 42 °C), salinity in the agricultural land, or other toxicity. The purified HCH dehydrochlorinase has optimal activity at 25 °C, and it is likely to get inhibited by several physicochemical factors in case of on-site monitoring. The biosensor is sensitive in the part-per-trillion concentration range, is selective, and has an active shelf life of 15 days when stored at 4 °C. On the basis of our experimental results, an attempt has been made to provide a rationale for the high sensitivity based on detection of acid liberated in polyaniline matrix.

EXPERIMENTAL SECTION

Lindane Sensor Design and Measurement. Polyaniline films were deposited by electropolymerization method.[33] The recombinant *E. coli* BL21 (DE3, pET16blinA2, pLysS) strain overexpressing LinA2 protein (hereafter referred to as *Ec*Lb strain) was immobilized on polyaniline film by physical adsorption method. The cell suspension was diluted to attain OD_{600nm} values of 0.5, 1, and 1.5 (1 OD = 1×10^8 cells/mL), and 10 μL was loaded on sensor area of 0.25 cm^2. Details of the polymer deposition, cell culture, cloning, overexpression, cell immobilization, and scanning electron microscopy (SEM) sample preparation are described in the Supporting Information. All electrochemical experiments were conducted using a three-electrode electrochemical cell with Ag/AgCl, KCl (3 M) reference electrode and platinum wire auxiliary electrode (Supporting Information Figure S2). Response of the sensor under optimized conditions was measured using pulsed amperometry. Specificity of the sensor was studied by continuous amperometry. For details of sensor response measurement and specificity studies see the Supporting Information.

RESULTS AND DISCUSSION

Cloning and Overexpression of LinA2. Restriction endonuclease digestion of the plasmid pET16b with *Nde*I having a unique site in the vector yielded an expected product of 5.7 kb (Figure 2A; lane 2). The restriction endonuclease digestion of pET16blinA2 with *Nde*I and *Bam*H1 yielded 471 bp *linA2* insert and 5.7 kb pET16b vector (Figure 2A; lane 3) confirming proper cloning of the *linA2* gene. The isopropylthiogalactopyranoside (IPTG) induction of LinA2 was maximal in the *Ec*Lb strain after 3 h. The identity of the recombinant LinA2 (rLinA2) protein and the incremental increase in its expression with time was assessed by Western blotting and immunodetection (Figure 2B). The rLinA2 protein matched the calculated molecular mass (~17 kDa) of LinA2 protein of *S. paucimobilis* strain B90 (Figure 2B). The lindane biodegradation ability of recombinant *Ec*Lb cells was found to be several hundred fold higher than the wild-type *S. paucimobilis* strain B90 because of overexpression of LinA2 protein.

Lindane Biodegradation by *Ec*Lb Strain. The *Ec*LB cells displayed no lag in biodegradation of lindane in spite of poor miscibility and uptake of lindane. The *Ec*LB cells could degrade 50 ppm of lindane within 30 min as estimated by phenol red method (experimental details are in the Supporting Informa-

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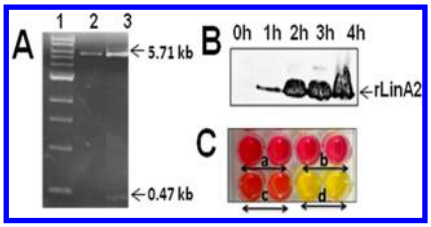

Figure 2. Cloning, overexpression, and activity of LinA2 in recombinant *E. coli* cells. (A) Cloning of *linA2* gene into pET16B vector. Various lanes show the following: 1 kb DNA ladder, lane 1; pET16b restriction digested with *Nde*I−*Bam*HI, lane 2; pET16blinA2 restriction digested with *Nde*I−*Bam*HI depicting 5.7 kb pET16b and 0.471 kb *linA2* gene insert, lane 3. (B) Overexpression of recombinant (rLinA2) protein in *Ec*LB strain. Western blot of protein extract from *Ec*Lb cells induced with IPTG for 1−4 h. The LinA2 protein was immunodetected using anti-His antibody. (C) Detection of lindane biodegradation using pH indicator based phenol red method. Microtiter plate showing the lindane biodegradation. All the wells contained dehydrochlorinase buffer (pH 8) and phenol red. Other additions were the following: (a) nil; (b) lindane, 50 ppm; (c) lindane plus *Ec*pET16b (empty vector control cells); (d) lindane plus *Ec*Lb.

tion) resulting in dramatic visual color change (Figure 2C), which could also be estimated spectrophotometrically (data not shown). Strain *Ec*LB could also degrade various other isomers of hexachlorocyclohexane (HCH) as verified by gas chromatography (data not included).

Polyaniline as a H^+ Sensor. Figure 3 shows the amperometric response of polyaniline-modified electrode to

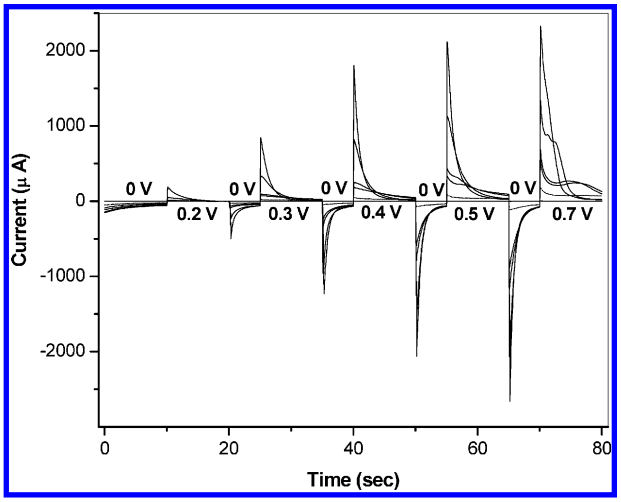

Figure 3. Pulsed amperometric response of polyaniline electrode to different concentrations of HCl ranging from 10^{-5} to 10^{-1} M at the specified voltage values.

different concentrations of HCl ranging from 10^{-5} to 10^{-1} M at various applied potentials. For all the potentials studied, the amperometric current was found to increases with increase in HCl concentration. The operating potential of the sensor was optimized at 0.4 V, since above this potential polyaniline is known to undergo slow degradation due to oxidation.[34] Moreover, at a potential of 0.4 V, polyaniline is in the emeraldine form (ES/EB), which is its most sensitive form to H^+ and leads to a large change in conductivity with small changes in H^+ concentration.[28,35]

For the applied potential of 0.4 V, the current was found to increase with decrease in pH (Supporting Information Figure S3) as the degree of protonation of polyaniline, and hence its conductivity, are known to increase with decrease in pH. The increase in current was more prominent in the pH range of 1−3. These observations are in line with the conductance surface state diagram reported by Gholamian et al.[36] for thin film of polyaniline grown between two parallel platinum wires. Similarly, MacDiarmid et al.[37] have reported a sigmoidal variation of the logarithm of conductivity as a function of pH. But over a narrow range of pH, it can be approximated to be linear.[38] These results indicate that polyaniline can be used as a good transducer for detection of lindane by monitoring lindane degradation by *Ec*Lb cells to produce HCl.

Lindane Sensor. *Morphological Studies.* An SEM image of the film with polymer−cell−polymer sandwich structure as mentioned in the Experimental Section is shown in Figure 4a.

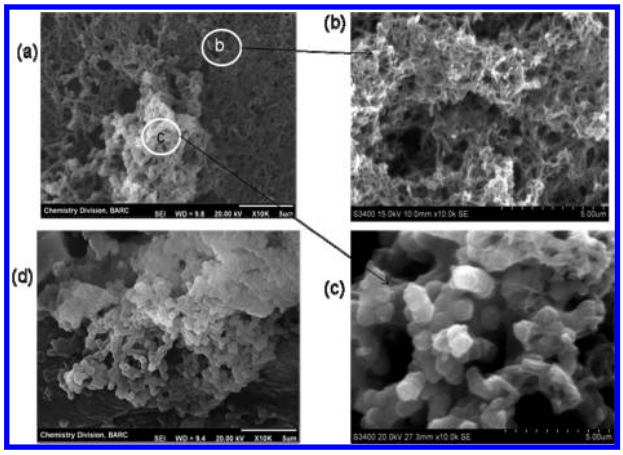

Figure 4. SEM images of (a) *Ec*Lb cells entrapped in polyaniline film, (b) magnified image of the polyaniline region, and (c) magnified image of the cell-entrapped region. (d) SEM image of *Ec*Lb cells drop cast on polyaniline film.

The film showed two distinct regions, i.e., the polyaniline region and the cell-entrapped region, which are further magnified as Figure 4, parts b and c, respectively. The polyaniline region is composed of entangled microfibers or fibrils of polyaniline with diameter in the range of 20−100 nm and length of a few micrometers as seen in the magnified image of this region (Figure 4b). The SEM image of pure polyaniline is also shown for reference (Supporting Information Figure S4). The cell-immobilized region showed the presence of *Ec*Lb cells with mean diameter of ∼1 μm and length of 2 μm, as seen in Figure 4c. The *Ec*Lb cells were found to be sandwiched in polyaniline layers and entrapped in polyaniline microfibrils. This would be useful in order to avoid leaching of the cells during repeated successive usage. The *Ec*Lb cells were also separately drop cast on polyaniline film for comparison. In this case the cells formed clusters over the polymer surface (Figure 4d).

Sensor Response. The pulsed amperometric response of the sensor to varying concentration of lindane at different *Ec*Lb cell loadings is illustrated in Figure 5. The average response of the control films with different loadings of *Ec*pET16b (empty vector control cells) and polyaniline film (without immobilized cell) are also shown for comparison. With increase in loading of the cells, the response current was found to increase due to enhanced catalysis and degradation of lindane.

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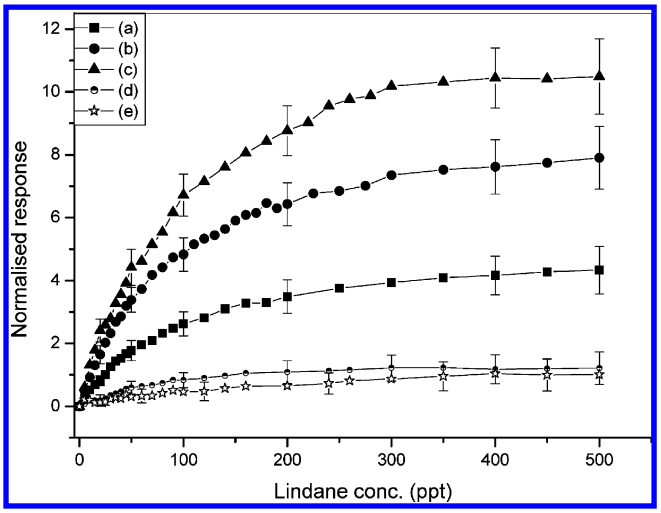

Figure 5. Normalized response of the *Ec*Lb-immobilized polyaniline film to lindane at cell densities (OD_{600nm}) of (a) 0.5, (b) 1, and (c) 1.5. The response of (d) corresponding control films (with *Ec*pET16b; empty vector control cells) and (e) blank polyaniline film (without any cells) is also shown.

Though the response was found to increases with cell concentration, the differential increase in response was less at higher loadings. This could be due to the transport resistance experienced by lindane to reach the cells embedded deeper in the film at higher loadings. The trend observed was similar to that reported for several microbial biosensors earlier.[21,39] Further increase of cell loading was, however, restricted by the small size of the polyaniline film (10 mm × 5 mm) which could hold only up to 20 μL of the cell suspension.

For a cell loading of 10 μL of 0.5 OD (1 OD = 1×10^8 cells/1 mL) cell suspension, the amperometric response was found to be linear as a function of lindane concentration up to 45 ppt (R^2 = 0.995) after which it saturated. The curve followed the Michaelis–Menten profile, indicating an enzyme-catalyzed process. Similar behavior was observed for higher cell loading. The apparent Michaelis–Menten parameters for the degradation of lindane were derived using a Lineweaver–Burk plot (Table 1).

Table 1. Apparent Michaelis–Menten Parameters and Experimentally Determined Linear Region Slope for the Biosensor with Different Cell Loadings

amount of cells loaded (OD)	$K_{M,app}$ (ppt)	$V_{max,app}$	sensitivity (ppt^{-1})[a]	linear range (ppt)	R^2 [b]
0.5	85.86	4.8	0.0368 ± 0.0014	5–45	0.995
1	87.62	9.21	0.0696 ± 0.0024	5–45	0.995
1.5	101.23	13.24	0.1077 ± 0.0075	5–25	0.993

[a]Slope of linear region of the calibration curve. [b]Correlation coefficient of the linear range.

It is to be noted that, due to the entrapment of cells inside polymer matrix and the restriction on diffusion of substrate, the Michaelis–Menten model might not be strictly obeyed.[40] The apparent K_m and V_{max} values would indicate whether the response of the biosensor is limited by the diffusional limitation or controlled by the enzyme reaction.[41] The observed response was compared with ideal Michaelis–Menten plots calculated by using the experimental K_m and V_{max} values for the lowest cell loading (0.5 OD) and increasing the enzyme amount by 2 (for 1 OD) and 3 (for 1.5 OD) times, respectively (Supporting Information Figure S5). The observed response was lower than the calculated curve, suggesting restriction for diffusion of substrate to the cell embedded deep in the polymer matrix with increase in cell loading. The linear range and sensitivity (slope of the linear region) of the sensor for different cell loadings are given in Table 1. It can be observed that, with the increase in cell loading, the sensitivity increased, whereas the linear range decreased. As far as the response time is concerned, the biosensor showed a fast response to lindane. For each aliquot of lindane added, the biosensor reached the steady-state current in ~60–100 s. The biosensor demonstrated good reproducibility as reflected from the low relative standard deviation of ~1–2% (n = 5) for detection of 25 ppt lindane. The detection limit (estimated at 3σ, σ = standard deviation of background signal) was found to be ~2 ppt. The present method has lower detection limit as compared to most of the known methods for estimation of lindane (Supporting Information Table S1). The gas chromatography-electron capture device (GC-ECD) method is reported to have a detection limit in the part-per-trillion range.[42] However, the current method has several advantages like direct detection without the need of solvent extraction and potential for miniaturization to develop a low-cost, hand-held device for on-site monitoring.

Selectivity and Specificity of the Sensor. Experiments were performed to investigate selectivity of the microbial lindane sensor by directly injecting several analogous compounds in the electrochemical cell and monitoring the chronoamperometric response. The biosensor was equally sensitive to α, β, and γ isomers of HCH (Figure 6a) and would therefore be useful for detection of levels of all HCH isomers and some of their degradation products. The response to pentachlorocyclohexane (PCCH), the product of first dehydrochlorination step, was about 3 times higher as compared to lindane for the same concentration (Figure 6b). In contrast, the sensor did not respond to trichlorobenzene (TCB) (Supporting Information Figure S6), the final product of the dehydrochlorination steps. This indicates that the *Ec*Lb strain immobilized on biosensor film expressing HCH dehydrochlorinase used PCCH preferentially with a high substrate specificity, which was also verified by phenol red based microtiter plate assay. This could be because the rate of catalytic dehydrohalogenation of PCCH by *Ec*Lb cells is higher as compared to that of lindane.

Trantírek et al.[43] have carried out detailed stereochemical analysis of the reaction products formed during conversion of γ-HCH by LinA using GC/MS, NMR, and circular dichroism. It is reported that the enzymatic transformation of PCCH results in the formation of 1,2,4-TCB and the reaction proceeds through 1,3(*R*),4,6(*R*)-tetrachlorocyclohexa-1,4-diene (TCDN) as an intermediate. 1,3(*R*),4,6(*R*)-TCDN was never directly detected in the reaction mixture, suggesting that 1,4 elimination of HCl from TCDN proceeds at the same or higher rate than enzymatic 1,2 elimination of HCl from γ-PCCH. Thus, our observation of higher rate for PCCH degradation based on amperometric studies is in line with the above literature reports. Detailed electrochemical studies of such multistep reactions not only gave the solution for the pertinent problem of toxicity detection but also provide an insight for the reaction mechanism.

Immediately upon injection of lindane into the electrochemical cell, a characteristic step response was observed with a rapid rise in current followed by a steady-state plateau

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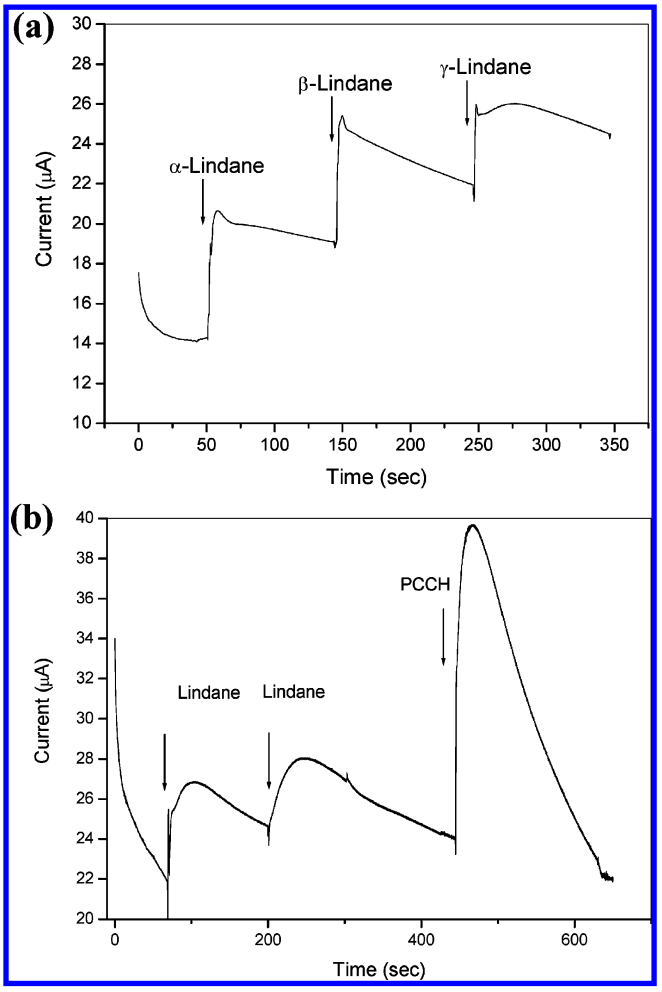

Figure 6. Continuous amperometric response of the sensor to 50 ppt concentrations of (a) α, β, and γ isomers of HCH and (b) lindane and PCCH.

(Supporting Information Figure S6). In contrast, no detectable amperometric response was observed on injection of 1 mM each of aliphatic and aromatic halides like chlorobenzene, 1,2,3-trichlorobenzene, chlorobutane, and trichloroethane, or to cyclohexane (Supporting Information Figure S6a). No significant interference from other commonly used organochlorine pesticides like DDT and DDE was observed. Even at a very high concentration of 10 ppm, the chronoamperometric response was very small as compared to the response for 50 ppt lindane (Supporting Information Figure S6b). The observed small response could be because of the presence of traces of free acid contamination in the pesticide sample. These results indicated that the sensor is specific to hexachlorocyclohexane (HCH) isomers and PCCH but does not respond to other pesticides or other organic halogen compounds. The specificity of the sensor to lindane over other commonly used pesticides is distinct advantage of the present system. As discussed earlier, most of the electrochemical sensors for pesticides reported earlier were based on measuring the inhibition of acetylcholinase activity and were not specific.[19]

Rationale for Sensitivity. Polyaniline sensors based on H^+ ion concentration change have seldom been reported to have high sensitivity in the part-per-trillion concentration range. Singh and Contractor[44] have reported a polyaniline-based sensor for detection of Hg^{2+} ions at low concentration levels. In this case, a ligand, dimercaprol,2,3-dimercapto-1-propanol (BAL), was entrapped in polyaniline matrix. On complexation of Hg^{2+} ions with BAL, H^+ ions were released, which led to change in conductivity of polyaniline. On the basis of the conductivity change, very low concentrations of Hg^{2+} ions, of the order of 10^{-12} M, could be detected. Yan et al.[45] have developed a gold nanoparticle−AgCl@PANI core−shell nanocomposite-based sensor for detection of glucose. The amperometric sensor was found to be highly sensitive and had a detection limit of 4 pM with a linear response in a narrow range of 4−34 pM. It was therefore interesting to elucidate the reason for high sensitivity of the sensor at very low concentration of lindane.

Earlier Fraoua et al.[46] made an attempt to obtain the local pH in case of polyaniline film dipped in HCl solution. By using electrochemistry and X-ray photoelectron spectroscopy (XPS), they studied the relaxation phenomena of the first redox system of polyaniline after polarization of the film in its reduced state and showed that the concentration of protons inside the polyaniline film increased during negative polarization leading to shift in peak position. An empirical relation was derived to obtain the local pH based on the peak shift, pH of bulk solution, and the polarization time. For polyaniline film dipped in HCl solution of pH 2, they have reported a local pH of 1.[47] Thus, in their case, the local H^+ concentration was found to be 1 order of magnitude higher than the bulk electrolyte on application of negative polarization.

In the present study, we have made an attempt to estimate the change in pH produced due to degradation of lindane. For this purpose, polyaniline was deposited on gold interdigitated electrodes in 15 scans by potentiodynamic method. The amperometric response of the electrode, on application of potential pulse of 0.4 V, to HCl solutions of varying concentrations (1 to 10^{-7} M) showed an increase in the response current with increase in H^+ ion concentration as depicted in Figure 7a. The electrode was rinsed several times with distilled water in order to remove excess of H^+ ions, and 0.5 OD *Ec*Lb cells were drop cast on the same electrode. The pulsed amperometric response of the *Ec*Lb cell-immobilized electrode to lindane in the concentration range of 5−500 ppt is

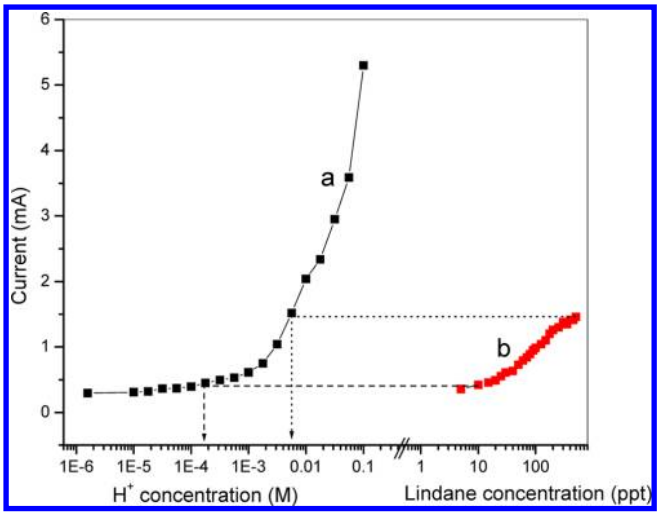

Figure 7. Comparison of the current obtained at (a) polyaniline electrode at varying acid concentrations and (b) *Ec*Lb immobilized (on the same) polyaniline electrode at varying lindane concentrations (0−500 ppt).

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depicted in Figure 7b. Since the area of the polyaniline electrode remained same, the response current of the *Ec*Lb cell-immobilized electrode was compared with the current obtained for a plain polyaniline electrode. On the basis of this, the change in pH due to the HCl generated on lindane degradation was estimated and the results were compiled for two different concentrations of lindane (in Table 2).

Table 2. Estimation of the Equivalent pH in the Microenvironment of the *Ec*Lb Cell-Immobilized Polyaniline Electrode at Different Concentrations of Lindane (Based on Figure 7)

lindane concn (ppt)	amperometric current (A)	equiv H^+ concn in microenvironment of polyaniline electrode (M)	equiv pH in the microenvironment of the polyaniline electrode
0	2.96×10^{-4}	1.58×10^{-6}	5.8
10	4.2×10^{-4}	1.778×10^{-4}	3.75
500	1.4×10^{-3}	5.62×10^{-3}	2.25

For a concentration of 10 ppt lindane, the amperometric current produced at the modified electrode was 4.2×10^{-4} A, which is same as the current value produced at the plain polyaniline electrode when 1.778×10^{-4} M HCl (pH = 3.75) is used as the bulk electrolyte. Similarly, addition of 500 ppt lindane leads to a conductivity change equivalent to that produced by an electrolyte containing 5.62×10^{-3} M HCl (pH = 2.25).

This is a comparison of the "pH change of the microenvironment of polyaniline on degradation of lindane" with the "pH change of the bulk electrolyte solution required to produce the same current". Though the actual amount of H^+ ions released on degradation of lindane at part-per-trillion concentration level was very low, the response produced is comparable to that by increasing the pH of HCl in bulk electrolyte by 2−3 units (or change in H^+ concentration by 2−3 orders of magnitude). Our data thus demonstrate that polyaniline is very sensitive to change in pH of the microenvironment and is able to detect very low concentration of protons liberated in its matrix.

Changes in the microenvironment have led to several interesting observations in the course of development of polyaniline-based sensors. During an attempt to develop amperometric ascorbate sensor, Jureviciute et al.[48] observed an autocatalytic oxidation of ascorbic acid in solution of pH of 7.2. As the oxidation of ascorbic acid is accompanied by liberation of proton, they observed a local acidification of polyaniline layer leading to a local decrease in pH leading to proton doping of polyaniline. The protonated conducting form of polyaniline enabled efficient electrooxidation of ascorbate.

The pH in the microenvironment of a polymer also has significant impact on its physical properties. Polyaniline is known to lose its electrochemical activity in solutions of pH greater than 4. But its electroactivity and conductivity are retained in neutral or slightly alkaline solutions when doped with polymeric anions such as polystyrene sulfonate (PSS).[49] This is because the anionic polyelectrolytes attract hydrogen ions, and hence, the pH in polyelectrolyte-doped polymer microenvironment is lower than that of bulk electrolyte.

The other factor contributing to the higher sensitivity is the use of genetically engineered *E. coli* cells, which possess several hundred fold higher enzyme activity than that of native lindane biodegrader *S. paucimobilis* strain B90. The genetic engineering approach in whole-cell-based biosensor development provides means to generate high-efficiency clones of viral enzymes to eukaryotic cellular enzymes which broaden the scope for sensitive detection of various analytes.

Stability of the Sensor. The long-term storage and operational stability of the sensor is another important issue for its practical application in lindane detection. The storage stability of the polyaniline sensor was investigated over a period of 1 month. In between measurements, the sensor electrode was kept in a dry state in the refrigerator (4 °C). Since live bacterial cells were used and stored in encapsulated form without nutrition in our experiments, they will senesce with time. Nutrients were not provided during storage in order to prevent cells from outgrowing outside the polymer matrix. This would also lead to change in the response of the sensor as observed in studies with different cell loadings. It must be noted, however, that the LinA2 dehydrochlorinase does not require any inputs of electrons/energy from cells and its efficiency is not regulated by metabolic performance of cells.

The normalized response for 50 ppt lindane recorded at different time points during storage is shown in Supporting Information Figure S7. The activity was measured three times on the same day. The error bars represent standard deviation for $n = 3$. A small loss (∼5%) in catalytic activity was noticed after 15 days of storage, while after 30 days the activity was significantly reduced by ∼35%. These results indicate a good stability of the sensor and its utility for repeated measurements over a period of 2 weeks.

Conceptual Design for Environmental Monitoring. In employment of the lindane biosensor for detection of lindane in environmental samples, the initial pH of the different solutions to be analyzed may adversely influence the sensor response which could lead to error. For practical application, the response can be measured as the difference in current of two similar polyaniline electrodes, one of which contains the genetically engineered *E. coli* cells and the other one which does not. Such a differential measurement/circuit would practically eliminate the influence of interferences due to presence of electroactive species and pH of the sample.

CONCLUSION

In the present study, the sensitive nature of the microenvironment of polyaniline has been judiciously utilized for development of a biosensor with very high sensitivity and specificity for HCH isomers. For this purpose, genetically modified *E. coli* cells expressing the LinA2 dehydrochlorinase were immobilized and sandwiched in polyaniline matrix. The LinA2 protein expressed by the cells could degrade lindane to produce HCl leading to protonation of polyaniline microenvironment and an increase in its conductivity. The sensor showed an excellent response to part-per-trillion levels of lindane up to 15 days of storage at 4 °C. The sensor was found to be specific to hexachlorcyclohexane and did not respond to several other organic halogen compounds studied. Commonly used chloropesticides like DDT and DDE did not interfere with the sensor response. A rationale has been provided for the high sensitivity of polyaniline as a transducer for H^+ ions liberated in its microenvironment. This work opens up a platform for highly sensitive detection of analytes based on liberation of protons during the course of chemical/biochemical process using polyaniline.

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■ ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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