

Review Article

Microbial Biosensors as Pesticide Detector: An Overview

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Farmers are highly dependent upon agrochemicals to boost crop production through soil fertilization and insect pests, pathogens, parasites, and weeds management. However, contentious application of agrochemicals on the farm has aggravated residual accumulation and has become problematic for environmental safety besides causing disease to humans and other animals. Thus, the analysis of chemical residues from the environment is vital for policymakers and communities. Mostly, chemists were devoted to analyzing the existing contaminants from different sources by using highly sophisticated chromatographic equipment, although it is time taking, laborious, costly, and that required well-trained professionals. However, biosensors are more important to analyze chemical contaminants from different samples using various bioreporters integrated with electrochemical and optical transducers. Microbes are metabolically diverse, amenable for genetic engineering, cost effective in culturing, and tolerant to diverse conditions. Thus, microbial biosensor is capturing attention and becoming more effective for environmental monitoring. Therefore, this review assessed the implication of microbial biosensors for pesticide detection and the role of genetic engineering for strain improvement.

1. Introduction

Farmers are applying substantial amounts of different agrochemicals to increase crop production [1, 2]. These agrochemicals are deliberately applied for soil fertilization for managing insect pests, bacterial and fungal disease, weeds, nematodes, and rodent management. Residues of these agrochemicals then directly or indirectly flow into the ecosystem and food chain. This continued entrance of agrochemicals into the ecosystem increases residual accumulation and induces an effect on living creatures including human beings [3, 4].

Mainly, organochlorine, organophosphate, organonitrate, and their derivatives are the most important classes of pesticides and toxic to several living organisms in the environment [5]. This could require clear understanding of the existing levels of residual accumulation of pesticides, and the events endured by the pesticides, interaction mechanisms with the soil, and the biota found in a specific location [6]. Therefore, scientists have developed the most sophisticated,

sensitive, reliable, and efficient chromatographic methods to detect chemical residues from environmental samples [7]. However, these methods are time taking, laborious, and need expensive equipment and highly trained professionals [8]. To solve these concerns, for almost a decade, considerable attention was given to biosensors, which are the easiest method and best alternative for chemical analysis [9]. Hence, bioreporters (whole cells, enzymes, antibodies, DNA, and RNA) have been used for biosensor construction and have become promising tools. These components can easily be refined through evolution to perform specific tasks [10].

Microbial biosensors, therefore, consist of whole cells as bioreporters through coupling with physiochemical transducers to produce signals for specific analyte(s) [10]. Signal production could be through proton concentration change, gas liberation or uptake, light emission, etc., depending on the nature of the microbial metabolic processes of certain compound(s) [11]. The intensity of signals generated during the process directly or indirectly indicates the concentration

of target analyte(s) in a given amount of sample. A signal-sensing transducer converts this phenomenon into a measurable response such as a current, potential, or absorption of light using electrochemical or optical energy convertors [12]. Thus, several types of biosensors are therefore designed and used for different types of pollutant analysis [11, 13]. Researches of whole-cell, enzymatic, immunochemical, and DNA-based biosensors have been designed and used for pesticide detection [11, 14–16].

A microbial biosensor is one of the promising devices for analyzing targeted contaminants through coupling microbes with a transducer to enable rapid, accurate, and sensitive detection of analytes from the different sources [11, 14, 17]. The earlier microbial biosensors were only dependent upon viable cell respiration and their metabolic functions to detect a substance that either was a substrate or an inhibitor of their metabolic processes [11, 15]. However, the current microbial biosensors consist of transducers which work in conjunction with immobilized viable or nonviable microbial cells including genetically modified ones [10, 14, 18]. Application of nonviable microbes targeting periplasmic enzymes found in permeable cells or whole cells was cost effective than using cellular enzymes [10]. Portable cell arrays of biosensors also have been designed from freeze-dried biosensing strains of microbes for high-throughput pollutant analysis [19].

Therefore, microbial biosensors are more helpful for pesticide analysis, since microbes are highly capable of using a wide range of chemical substrates because of their metabolic diversity [20], amenability for genetic modification [14], and a broad spectrum of environmental factor tolerance [13]. Thus, microbial biosensor devices were constructed for use as amperometers [21, 22], potentiometers [23], calorimeters [24], conductometers [25], colorimeters [26], transducers, and luminescent [27, 28] and fluorescent biosensors [26, 29]. Conductometric, amperometric, and potentiometric biosensors can detect the electroactive types of pesticides, whereas luminescent and fluorescent biosensors detect light-emitting ones during microbial metabolic processes [14]. This is then a promising technique for the analysis of versatile types of pesticides from the environment. Therefore, the main objective of this review was to assess the current progress of microbial biosensors and their role for the detection of pesticides for environmental monitoring.

2. Whole-Cell Microbial Biosensor

Microorganisms are highly versatile in nature and can endeavor to survive in various adverse conditions such as extreme temperatures and different salinity levels, pH, and environments with several toxic chemicals [30]. Microbial biosensors were then designed since 1990s [31] and were used as bioreporters of environmental pollution. Microbial biosensor construction was continuously upgraded through time using different types of transducers and microbial strains [32]. Its construction was mostly dependent upon the close contact of sensing microbial cells and the sense-reporting transducers [11]. Thus, the microbial cell immobilization on the transducer is vitally required, and this

necessitates a critical choice of microbial cell immobilization techniques by considering currently developed technologies.

The chemical and physical immobilization techniques were commonly applied to immobilize bioreporter elements on the transducer during biosensor construction. Similarly, microbial cells were immobilized on the transducer or support matrices by using these methods [33, 34]. From these, covalent bonding and cross-linking were chemical immobilization techniques. Covalent bonding forms the stable covalent bond between functional groups of the biological components (elements mostly found on the microbial cell wall) such as amine, carboxylic, or sulphhydryl groups and the transducer such as amine, carboxylic, epoxy, or tosyl components [11]. Covalent bonding was applied to develop disposable biosensors used to detect different analytes [35] and avidin-biotin interactions to attach biotinylated biocomponents to the electrode surface [36].

Cross-linking is another chemical immobilization technique used to bridge molecules between functional groups found on the outer membrane of microbial cells. Cross-linking uses multifunctional reagents like glutaraldehyde and cyanuric chloride to form the networked molecular interactions. The process was fast, simple, and widely accepted for immobilization of microorganisms. Cells can be fixed directly onto the surface of electrodes or on a removable support membrane, which can be placed on the transducer surface [37]. Thus, cross-linking is suitable to construct microbial biosensors where cell viability is not important and only intracellular enzymes are required for analyte detection [38].

Physical immobilization on the other hand, includes both adsorption and cell encapsulation techniques in microbial biosensor construction. Adsorption is the simplest method for biological element immobilization. Disposable microbial biosensors were constructed through growing a microbial suspension on the electrode or an immobilization matrix, such as alumina and glass beads. It requires subsequent rinsing with a buffer to remove nonadsorbed microbial cells from the surface/matrix. Microbes were immobilized due to ionic, polar, or hydrogen bonding and hydrophobic adsorptive interactions [37]. Furthermore, cell encapsulation is another physical immobilization technique. It requires bounding enzymes found in semipermeable membranes, which allow the substrate and products to pass through, but block the biocomponent [39]. Encapsulation techniques commonly used agar/agarose, carrageenan, alginate, polyurethane-polycarbonyl sulfonate (PCS), and polyacrylamide as reagents to encapsulate the cells [29]. Encapsulated microbial biosensors are protected from temperature, pH, and ionic force changes and other adverse conditions. However, the rate of the biochemical reaction is low since analytes have to pass through the membrane to reach the biocomponent, which implies a less comprehensive analysis.

The agar method of the strain immobilization technique was expected to improve the sensing efficiency and viability of microbes due to the presence of nutrients within the matrix [40]. Thus, there is a report on the agar immobilization of 20 sensor bacterial cell arrays with different promoters and constructed with the transducer as biosensor [41]. This

technique makes the living microorganisms to serve as biocatalytic agents [32] for several types of pollutants, simultaneously. The main advantage of a microbial biosensor is that it is easy to develop and there is no need for isolating subcellular components like enzymes, antibodies, and antigens [42]. In other reports, gram-positive actinomycetes are indicated as a broad-spectrum sensor by degrading halogenated hydrocarbons. This showed the existence of a potentially inciting broad-spectrum microbial biosensor fabrication for halogenated hydrocarbons [43].

2.1. Reporter Genes Used in Microbial Biosensors. The principle of a reporter gene is that it encodes easily detectable signals showing the presence of, or its exposure to, certain analytes [44]. Therefore, microbial cells are frequently equipped with purposely designed reporter genes to show the production of certain biomolecules that can influence gene expression [14, 45]. Reporter gene expression under the control of a specific regulatory network gives increased sensitivity with a simple and easy sensor platform [44].

Enzymes are frequently used as outcome indicators of reporter genes to detect contaminant residues with activities of colorimetric, fluorescent, or luminescent readouts [46–48]. A commonly used enzyme for this purpose is β -galactosidase (β -gal) encoded by the *lacZ* gene to detect pollutants based on either colorimetric or fluorescent characteristics in a simple and rapid manner [49]. The availability of chemiluminescent and electrochemical substrates for β -gal gives increased sensitivity with a wide and dynamic range of detection [20, 50].

The bioluminescence gene *lux* cloned in *Vibrio fischeri* and *Photobacterium luminescens* [51] codes a popular enzyme luciferase that catalyzes the light-emitting reaction and serves as the light reporter gene. This gene was regularly used for the construction of whole-cell microbial biosensors to monitor environmental pollution [49]. Bacterial luciferase catalyzes the oxidation of a long-chain fatty aldehyde in the presence of molecular oxygen, resulting in a blue-green light emission [29] and was advantageous due to its broad dynamic ranges, sensitivity, and simplicity. Bioluminescence was successful in detecting pollutants using sensitive instrumentations including fiber optic probes and integrated circuit chips to detect produced light [34, 52].

Fluorescent proteins were also widely utilized in microbial biosensors as reporters without adding any additional substrates due to their autofluorescence ability. Green fluorescent protein (GFP), for instance, encoded by the *Gfp* gene was used to detect pollutants by emitting light that was easily detectable using a modern potentiometer with little or no damage to the host system [53]. In addition to its role as a reporter gene in the biosensing process, GFP served as a fusion tag and pH indicator in environmental pollution analysis [11]. Clearly, harmful chemicals in the environment can develop stress in living cells by changing the metabolic equilibrium and other physiological processes [54]. To detect the existing situations, the process-sensitive green fluorescent protein, which is called the roGFP variation, was developed to monitor the redox status of cells [18]. Hence, the reporter gene (*roGFP2*) was expressed in *E. coli* in large quantities and immobilized on the κ -carrageenan matrix and became a

more stable and sensitive biosensor to detect oxidative stress-inducing chemicals in a short time [18].

The other reporter gene (*crtA*) obtained from purple photosynthetic bacteria, *Rhodovulum sulfidophilum*, was used to synthesize carotenoids through the spheroidene pathway [53] and used to detect environmental pollutants. When the bacterium containing *crtA* gene is grown in media with the presence of a pollutant, it results in a colour change in the solution because of the accumulation of spheroidene. The advantage of using such a gene is that it does not need the addition of a special reagent or substrate for colour production. The fluorescent detection method of this gene also does not require sophisticated equipment or chemiluminescence to determine the existing pollutant. The resulting colour was monitored in the samples simply by naked eye observation under sunlight, even in areas where electricity is not available. The *crtA* in these bacteria is responsible for changing the colour of the cultures from yellow to red due to the presence of spheroidene [53].

2.2. Gene Promoter and Regulator Elements in Microbial Biosensor. The promoter is the segment of a gene used to initiate the reporter gene to reflect the ongoing metabolic character of the host. Selection of the appropriate promoter portions is crucial for biosensor construction based on the target molecules being monitored [55]. A selected promoter sequence is normally placed at the 5'-segment of the reporter scheme where it can be switched on in the presence of the target pollutant and initiates the turning on of the expression reporter. During promoter selection, their sensitivity and specificity should be considered [14]. Most promoters respond to groups of compounds than a specific one. Sometimes it may also behave differently in different microorganisms. Some other promoters are substrate dependent and host specific to monitor a given process [56].

Recently, promoters have been improved and specified by using different modifications. For instance, there are reports on metal-induced promoter regions identified and arranged in cassettes that can be easily used to activate reporter systems such as the *lux* or *GFP* reporter genes or the expression of outer membrane epitopes that can be easily detected [18]. The high specificity of such induced gene expression has been used to report the existence of lead and cadmium ions.

Furthermore, there are a number of well-characterized promoters used for the construction of pesticide biosensors [57, 58]. Promoters are also available for the evaluation of general toxicity [59–61]. The main drawback of microbial biosensor development is the decreased availability of strong promoters that respond only to relevant products generated from pollutants. To overcome this problem, more knowledge on gene regulatory networks in microbes is needed [29]. Linking metagenome information with the metatranscriptome analysis of microbial communities using microarray technology could provide an enormous source of new regulatory elements in the future [62]. Another option is to synthesize “super promoters” based on consensus sequences obtained from comparative studies of different promoters in known regulatory networks [62].

2.3. Genetic Modification and Whole-Cell Microbial Biosensor. A microbial biosensor mostly utilizes nucleic acid oxidation properties based on the interaction of DNA molecules or its product with pesticides [63] can be monitored by detecting the change in reduction oxidation (redox) potential. Thus, scientists are devoting their time to produce genetically engineered microbes which are responsible to recognizing certain existing chemical or physiological stresses through reporter protein synthesis [19]. For instance, Tibazarwa et al. [64] constructed *Ralstonia eutropha* AE2515 through transcriptional fusing of *cnrYXH* regulatory genes to the bioluminescent *luxCDABE* as reporter to fabricate a whole-cell biosensor for Ni^{2+} and Co^{2+} detection in the soil. The optical biosensors are also constructed from bacteria by fusing genes between the regulatory region of the *mer* operon (*merR*) and the reporter *luxCDABE* to detect the quantitative response of Hg^{2+} accumulation. The *mer* promoter is activated when Hg^{2+} binds to *merR*, which then results in the transcription of the *lux* reporter gene and causes subsequent light emission [65, 66]. The existence of copper in the soil is also monitored by using engineered *P. fluorescens* through mutagenesis of *P. fluorescens* containing copper-induced genes and the *Tn5::luxAB* promoter probe transposition [66].

Recently, highly sensitive, selective, and rapid whole-cell electrochemical biosensors were also developed to detect the persistent organochlorine (γ -hexachlorocyclohexane) pesticide, commonly known as lindane, using microbial gene modification [67]. The gene *linA2*-encoded enzyme (γ -hexachlorocyclohexane dehydrochlorinase) was involved in the initial steps of lindane biotransformation. Then, this gene (*linA2*) was cloned and overexpressed in *E. coli*. The lindane-biodegrading *E. coli* cells were immobilized on a polyaniline film. The rapid and selective degradation of lindane and concomitant generation of hydrochloric acid by recombinant *E. coli* cells in the microenvironment of polyaniline led to a change in its conductivity and monitored by pulsed amperometry. The sensor was found to be selective to all isomers of hexachlorocyclohexane and pentachlorocyclohexane but not to other aliphatic and aromatic chlorides or end products of lindane (trichlorobenzene) isomers [67].

Mostly, online pollutants and toxicity-detecting bioluminescence biosensors are considered as very effective, sensitive, and more reliable. Then, *Lux*-marked rhizobacterium *P. fluorescens* was developed through gene transfer to evaluate the induced stress of certain pollutants, which influences carbon flow in the bacterium and results in bioluminescence output. This is directly correlated with metabolic activity and a report on carbon flow in root exudates [68]. Therefore, the *Lux*-marked whole-cell biosensor is developed for the evaluation of the interactive toxicity of chlorophenol [69] and the toxicity level of a wastewater treatment plant treating phenolic-containing waste in a fast and rapid manner [70].

As reports indicated, promoter-reporter biosensor modifications are related to the cloning of a promoter upstream of a reporter gene cassette through subsequent transfer of the plasmid constructs into specific strains [71, 72]. However, the loss of these plasmids due to starvation [73] and expression reduction of the reporter genes due to multiple copies

of the promoter binding region on the plasmid [74] were the resulting problem of the applicability of biosensors under *in vivo* conditions. On the other hand, biosensors constructed through the chromosomal insertion of the promoter reporter gene were very limited, but their product is more stable and efficient for pollution analysis [75].

Concerning this, there are some reports which described three constructed chromosome-based biosensors from *P. fluorescens* F113rifgfp, *P. fluorescens* F113rifPCBgfp, and *P. fluorescens* F113L::1180gfp [76]. The integration of the *gfp* reporter gene into the chromosome affects the growth ability of *Ralstonia eutropha* on 2,4-dichlorophenoxyacetic acid [77] and on biphenyl [78]. However, the chromosomal insertion of the *Gfp* construct did not affect any phenotypic character, rhizosphere colonization ability, and major metabolic pathways of these biosensor strains [29]. It also has no effect on the *bph* operon during modification by insertion of the *Gfp* construct [79]. Although random chromosomal insertion offers many advantages over plasmid-based construction of biosensors, an ideal approach for commercial applications of targeted insertion to known chromosomal sites is not clear [29].

2.4. Microbial Cell Array Biosensor. Microbial cell array construction is less costly and easier to obtain large and homogeneous populations, which can survive in a variety of physical and chemical environments. It is evidently possible to obtain microbial cells that can be genetically modified to respond in a dose dependent and give readily quantifiable optical or electrochemical signals to predetermined analytes for environmental monitoring [80]. Advances in genetic engineering also give the chance to construct two independent reporter genes in a single microbial strain that can serve to express for multiplex analysis and particular logic operations of microbial cell arrays [81, 82]. Thus, genetically modified cells are therefore responsible to sense multiple types of analytes simultaneously from a given polluted environmental sample [83].

A genetically engineered *E. coli* strain was constructed using the *lacZ* reporter gene that encodes β -galactosidase, by fusing to the promoter of a heavy metal-responsive gene. Simultaneously, an enhanced cyan fluorescent protein (CFP) coding plasmid gene was also subsequently introduced into this sensing strain to produce associated optical signals in proportion to the amount of the target heavy metal (Hg^{2+}) detection at low (100 nM) concentration [84]. Moreover, arsenic and cadmium were also simultaneously quantified by using a multichannel bioluminescent *E. coli* array system [85]. There are reports that also indicate the existence of an *E. coli* array, which consists of optically coded functional microbeads with both a bioluminescent reporter bacterial gene and fluorescent microspheres used for broad-range toxicity analysis [86]. The most hazardous chemicals such as paraquat, mitomycin-C, and salicylic acid are successfully detected within 2 h, using a bacterial cell array of bioluminescent *E. coli* [41].

2.5. Nanotechnology Used in Microbial Biosensor Improvement. Mostly, the performance of a microbial

biosensor was challenged by some obstacles such as problems of the whole-cell transducer immobilization technique, size inappropriateness, sensitivity, and selectivity. Nanotechnologies are becoming attractive and efficient technologies with their small size and large surface area ensuring improved surface activity and electrical conductivity [87]. From nanotechnology, nanotubes, nanoparticles, and fiber optics are widely applicable in microbial biosensor construction [88]. Because of their electronic properties and large surface area, carbon nanotubes are ideal for the immobilization of microbial biosensors and exhibit excellent electrochemical performance with better stability [89]. In addition to those characteristics, carbon nanotubes increase the cell loading processes [90] hestin, ecatalytic activity [91], and improve the electrical conductivity [92].

A microbial biosensor was constructed through capturing bacterial cells on a carbon nanotube-modified chitosan membrane, with better repeatability and high operational stability [93]. Hnaïen et al. [90] also developed a bacterial impedimetric biosensor for trichloroethylene detection by immobilizing *Pseudomonas putida* F1 strain on gold microelectrodes which is working with single-wall carbon nanotubes. This biosensor achieved a good linearity with the concentration of contaminants.

Nanoparticles (NP), especially gold (Au) NPs, due to their high conductivity, biocompatibility, and catalytic activity, and serve for modification on the surface properties of electrodes to achieve good immobilization performance [94]. They promote electron transfer between the microbial cell wall and the electrode surface without any denaturalization of redox active proteins [95]. Furthermore, an Au NP-modified conducting polymer, which was used as a platform for immobilization for glucose analysis, showed significant advantages in biocompatibility, stability, sensitivity, and possibility of electrocatalysis [96].

For the rapid detection of analytes, fiber optic-based platforms have been constructed for the immobilization of microorganisms [97]. The fiber optic-based biosensors have advantages over other biosensors because of their fast response and stable immobilization capabilities. A flow through a fiber optic-based real-time biosensor for the detection of toxicity in water was fabricated [98]. By immobilizing the bacteria strains on the fibre optic biosensor, the biosensor has advantages with respect to regulation, as the bacteria do not leave the device in the water.

2.6. Success of Pesticide Detection Scenario Using Microbial Biosensor. A number of highly selective, sensitive, rapid, and cost-effective amperometric, potentiometric, colorimetric, and other microbial biosensors were constructed and used for direct determination of several pesticides from different samples [99–102]. Biosensors constructed from Cyanobacteria. *Anabaena variabilis* was an alginate entrapped on a carbon-felt electrode and used for online herbicide detection through inhibition of a generated photocurrent [100]. This biosensor showed sensitivity to two herbicides (atrazine and diuron) by current generation. The cell entrapment prevented the release of p-benzoquinone in the solu-

tion and improved the operating range of the sensor, with a limited decrease of sensitivity that does not hinder its application [100].

Organophosphorous insecticides, particularly parathion and chlorpyrifos pesticides, were detected using the *Flavobacterium* spp. bacterium immobilized on the surfaces of pH electrodes, and the bacterium degrades compounds up to 100 and 33% within 48 h of incubation, respectively [101]. *Flavobacterium* produces an enzyme, organophosphorous hydrolase, and has been used to degrade these compounds and generate two protons from each substrate. This stoichiometry forms the electrochemical basis for a potentiometric biosensor to detect product amounts [101, 102]. The hydrolyzed products are p-nitrophenol and 3,5,6-trichloro-2-pyridinol that accumulated in the medium and did not serve as substrate for the bacterium [101]. Furthermore, a microbial biosensor consisting of a dissolved oxygen electrode modified with the genetically engineered PNP-degrader *Moraxella* sp. was used for direct determination of p-nitrophenyl- (PNP-) substituted organophosphates. At the optimum condition, the biosensor detects the smallest (27.5 ppb) of paraoxon with best selectivity [103]. Moreover, a genetically engineered PNP-degrading *Pseudomonas putida* JS444 biosensor was constructed to determine fenitrothion and ethyl p-nitrophenyl phenylphosphorothioate (EPN) pesticides and release PNP and 3-methyl-4-nitrophenol, respectively. In this case, the bacterial biosensor released organophosphorous hydrolase on its cell surface as a biological-sensing element and used a carbon paste electrode as the amperometric transducer to detect a readout of the products [103]. Thus, the electrooxidation current of the intermediates was measured and was supposed to correlate with concentrations of fenitrothion and EPN up to 1.4 and 1.6 ppb without any interference, respectively [99]. In general, microbial biosensor development for pesticide detection was increasing and becoming more specific to target analytes.

3. Conclusion

A microbial biosensor is an analytical device which is constructed from the whole cell through integrating with sense-transmitting transducers to convert the sensed information from analyte(s) into understandable signals. It is a promising alternative to solve the problems which were faced in conventional contaminant analysis from various sources using chromatographic techniques. Although microbial biosensor construction is easy, introducing an appropriate gene through genetic engineering is highly challenging. This approach was currently given high attention, particularly in the model with bacterial (*E. coli*) modifications. Recent reports clearly showed success to this approach include the multiplex-sensing ability of a given strain for a number of analytes in a sample by developing the cell array. Furthermore, knowledge on nanotechnological science has its own contribution on microbial biosensor qualification by improving cell immobilization, specificity, portability, and durability patterns.

Data Availability

The data of this review is found in the sources cordially cited in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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