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Biosensor Technology for Pesticides—A review

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Abstract Pesticides, due to their lucrative outcomes, are majorly implicated in agricultural fields for crop production enhancement. Due to their pest removal properties, pesticides of various classes have been designed to persist in the environment over a longer duration after their application to achieve maximum effectiveness. Apart from their recalcitrant structure and agricultural benefits, pesticides also impose acute toxicological effects onto the other various life forms. Their accumulation in the living system may prove to be detrimental if established in higher concentrations. Thus, their prompt and accurate analysis is a crucial matter of concern. Conventional techniques like chromatographic techniques (HPLC, GC, etc.) used for pesticides detection are associated with various limitations like stumpy sensitivity and efficiency, time consumption, laboriousity, requirement of expensive equipments and highly trained technicians, and many more. So there is a need to recruit the methods which can detect these neurotoxic compounds sensitively, selectively, rapidly, and easily in the field. Present work is a brief review of the pesticide effects, their current usage scenario, permissible limits in various food stuffs and 21st century advancements of biosensor technology for pesticide detection. Due to their exceptional performance capabilities, easiness in operation and on-site working, numerous biosensors have been developed for bio-monitoring of various environmental samples for pesticide evaluation immensely throughout the globe. Till date, based on sensing element (enzyme based, antibody based, etc.) and type of detection method used (Electrochemical, optical, and piezoelectric, etc.), a number of biosensors have been developed for pesticide detection. In present communication, authors have summarized 21st century's approaches of biosensor technology for pesticide detection such as enzyme-based biosensors, immunosensors, aptamers, molecularly imprinted polymers, and biochips technology. Also, the major technological advancements of nanotechnology in the field of biosensor technology are discussed. Various biosensors mentioned in manuscript are found to exhibit storage stability of biocomponent ranging from 30–60 days, detection limit of $10^{-6}-10^{-16}$ M, response time of 1–20 min and applications of developed biosensors in environmental samples (water, food, vegetables, milk, and juice samples, etc.) are also discussed. Researchers all over the globe are working towards the development of different biosensing techniques based on contrast approaches for the detection of pesticides in various environmental samples.

Keywords Pesticides · Biosensors · Acetylcholinesterase (AChE) · Oragnophosphate (OP) · Aptamers · Transducers

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Introduction

Detection and quantification of the pesticides has become critically important in the recent years due to their exhaustive use in the agriculture. They act as a chemical agent to prevent the proliferation of harmful pests (weeds, insects, mould or fungi) but in parallel, also interfere with the normal biological processes of other living organisms [1–3]. Pesticides are indispensable to the modern agriculture and used extensively in agricultural fields to control attack of insects, fungus, and rodents on crops; reduce the growth of weeds; boost agricultural productivity, thus increases crop yields; and reduce post-harvest losses [4–7]. Large amount of pesticides reaches the soil, air, water, food, and other various systems through drift that takes the pesticides to places downwind the point of application. This may prove to be detrimental to the various life forms if it enters into the food chains [8, 9]. Some of these pesticides are very much toxic [10] mutagenic, carcinogenic, and tumorogenic [11, 12].

Majorly of the pesticides are neurotoxic compounds (Organophosphorus pesticides (OP)) and irreversibly inhibit the enzyme acetylcholinesterase, an essential enzyme for the functioning of the central nervous system in humans and insects. This inhibition leads to the accumulation of the neurotransmitter acetylcholine in nerves which interferes with muscular activities and the functioning of vital organs, produces serious symptoms, and may even lead to death [13, 14]. Target site of other various pesticides are—human erythrocytes [15], reproduction and development system [16], endocrine system [17], liver, central nervous system, and immune system [18, 19], etc. Thus, the analysis of pesticide residues is an important concern due to their bioaccumulation effect, high toxicity and their long-term damage risk to both the environment and lives even though their concentration is very low.

Pesticide analysis is carried out usually by employing conventional techniques like gas chromatography (GC) or high-performance liquid chromatography (HPLC) and by other spectroscopic methods [20]. These means follow laborious procedures, performed by highly trained technicians, undergo cleanup steps that increase analysis time and the risk of errors, and are not convenient for on-site or on-field detection [21, 22]. Biosensor technology (Fig. 1) resolves these snarls by using a biological sensing element connected to a transducer to convert an observed response into a measurable electrical signal. Magnitude of signal is proportional to the concentration of a specific chemical under investigation to which biosensing element binds [23, 24]. Biosensors exhibit exceptional performance capabilities, which include simplicity, high specificity and sensitivity, rapid response, low cost, portability, relatively compact size, user-friendly operation, and continuous real time analysis. Thus, it has become an important mean for the detection of chemical and biological components for clinical food and environmental monitoring [25].

Biosensors broadly classified on the basis of (1) the type of bio-recognition elements (i.e., enzyme, antibody, nucleic acid, whole cell, etc.) and (2) the signal transduction method (optical, electrochemical, peizoelectric, etc.) used for detection [26, 27]. Biosensor as an alternative to the conventional analytical techniques has been directed mainly towards health care, environmental applications, food industry, and agricultural practices [21]. Development of biosensors is a growing area, in response to the demand for rapid, simple, selective, and low-cost techniques for pesticide analysis. Biosensor technology used for the direct, fast, and easy determination of various pesticides (organophosphates, organochlorides, carbamates, etc.) and has been achieved by integrating various biocomponents with different transducers [28]. Majorly used OP's detection was initially done by enzyme-based OP biosensors which monitored the inhibition of acetylcholinesterase by various neurotoxins [29–32]. These inhibition-based sensors are very sensitive (able to detect 10^{-10} M), but they offer poor specificity, as there are many other substances (carbamates, heavy metals, etc.) that also inhibit these enzymes [33, 34]. Biosensors have also been developed on the principle of the working of organophosphorus hydrolase (OPH)

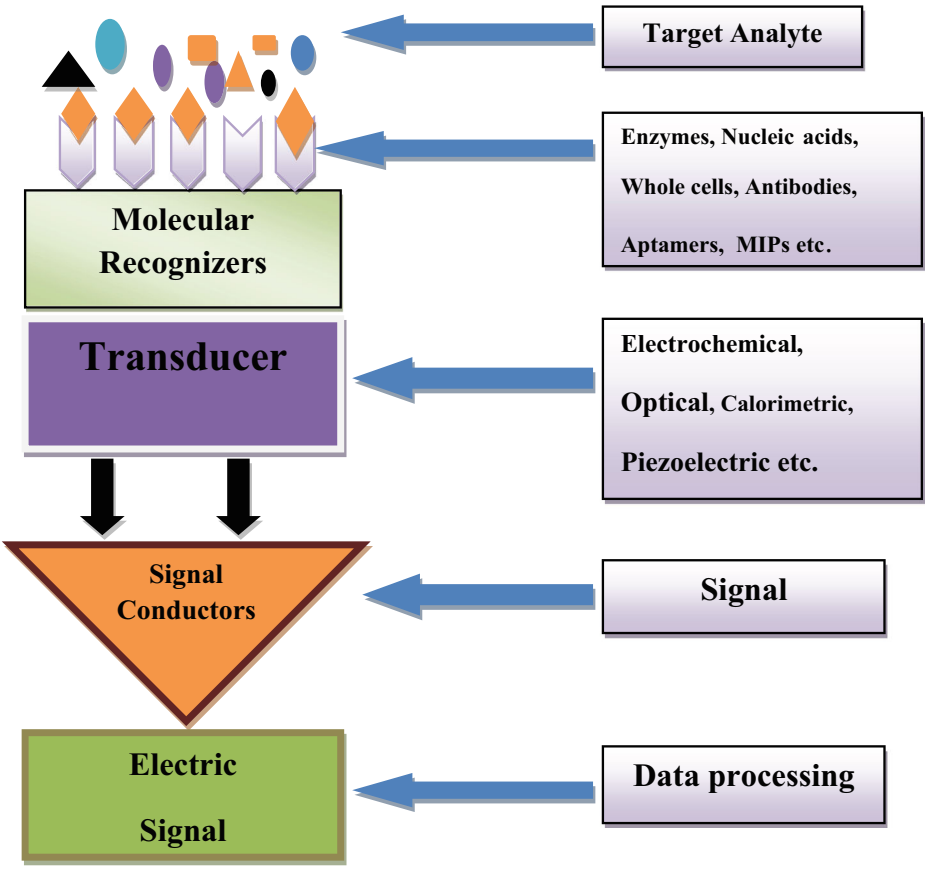

Fig. 1 Basic layout of elements of biosensor for its construction

[35] and organophosphorus acid anhydrolase enzymes [36]. They are not susceptible to non-specific inhibition, and they offer much greater specificity than the cholinesterases enzymes. Immuno-sensing technologies also have great potential for rapid detection of pesticide residues in food and environment [22]. From the literature, many examples of biosensors successfully developed for the detection of variety of pesticides has been found. Present review focused the 21st century's basic biosensor research applied and developed for the detection of pesticides in various samples. Over the last few years, the promising synergy among biosensor and nano-technology has also been exploited and discussed here. Some recent trends like aptamers, biochips, and molecular imprinting also specially mentioned. Although all of these devices have not been marketed commercially, yet they were successfully tested in the laboratories.

Pesticides—Introduction and Threats

The basic need behind the usage and evolvement of huge number of pesticides is to obtain higher yields from agricultural fields. It was estimated that about 45 % of annual food production was lost due to the pest attack [37]. Most superlative way to increase crop productivity is the successful pest control system.

During the past five decades, the use of agrochemicals (pesticides) has contributed to significant increase in the crop production by checking the growth of pests. Many developing countries like India are agriculturally dominant countries and use pesticides in ample amount. India is the largest producer of pesticides in Asia and ranks 12th in the world in the use of pesticides [37]. The worldwide consumption of pesticides is about 2 million tonnes annually: Out of which 45 % is utilized by Europe alone, 25 % is used in the USA, and 25 % in the rest of the world. India's consumption is just 3.75 %, and usage of pesticides is only 0.5 kg/ha. Insecticide consumption in India was reported highest (76 %) among other pesticides followed by fungicides (13 %), herbicides (10 %), and others (1 %) [38]. This high consumption of insecticides in India may be due to the fact that Indian climate is warm (tropical) and provides favorable breeding environment and enhances the survival and reproduction rates of insects. Comparison of pesticides usage in India and worldwide is given in Fig. 2.

Based on the chemical constituents, pesticides are classified as organochlorine, organophosphate, carbamate, synthetic pyrethroides, and inorganic pesticides, etc. [2, 40, 41]. Pesticides can also be classified on the basis of the target against which it is applied like insecticides—used against insect pests, nematocides—used against nematodes, fungicides—used against fungi, weedicides used against weed pests, in addition to miscellaneous other substances [6, 41, 42]. There is another class of pesticides which is produced from natural materials called as biopesticides.

Impregnable usage of pesticides is a great matter of concern as they impart many toxicological impacts on living creatures by direct and indirect exposure to pesticides and their residues. Humans come in contact with pesticides found in environment by different routes of exposure like skin, respiratory tract, ingestion, etc. There are many evidences that the pesticide imposes undesirable side effects to environment and thus generates risk to all kinds of life forms [43–46]. In nature, the pesticide residues usually undergo various degradation processes, but because of their high stability and low water solubility, pesticide residues can persist in ecosystem for a long time. For instance, organophosphates (Parathion) and organochlorides (DDT) can be detected for 15–20 years after their use [47, 48]. Mortality and chronic illness caused by pesticide and their residues poisoning numbers about 1 million per year [49]. Among the various pesticides used, organochlorine pesticides are of serious environmental concern and create serious health issues. In most of the developed countries, application of organochlorine pesticides like dichlorodiphenyltrichloroethane (DDT), Lindane, Aldrin, Atrazine, etc. has been reduced significantly due to their ability of long persistence, propensity

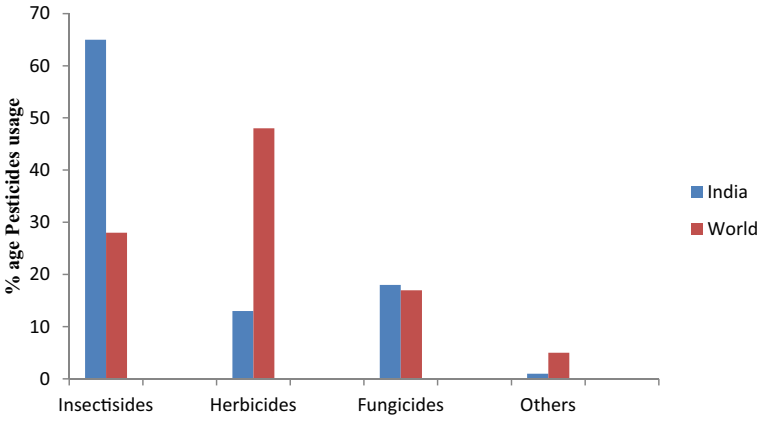

Fig. 2 Comparison of pesticide usage in India and worldwide [39]

of bioaccumulation, and the toxicity which they impose on other nontarget organisms [50]. These compounds may also get transferred from nursing mother to the young ones via breast milk [51]. Moreover, pesticide residues in food samples have also become a topic of concern. They got entered in food because of direct spraying and by transfer through food chain.

Initially (before the 1970s), organochlorine pesticides were widely concerned around the world as a pest control agent but as they found to impose toxicological effects, their use have been substituted by the less toxic and more effective organophosphorus pesticides [1, 52]. Development of organophosphorus compounds as insecticides took place firstly in late 1930s and early 1940s [53, 54]. Initially, use of OPs was also considered as a safe alternative to organochlorines but over the years their indiscriminate use, accumulation capability, and exposure imparts acute toxicological effects on nontarget organisms. Organophosphorus compound poisoning is also a worldwide health problem with around 3 million poisonings and 200,000 deaths annually [55, 56]. Basic classification of pesticides with their chemical terminology and the respective effects on humans is given in Table 1.

Steep increase in the incidences of cancer, chronic kidney diseases, suppression of the immune system, sterility among males and females, endocrine disorders, neurological and

Table 1 Classification of pesticides with their biological effects

Class	Group	Chemical Name	Target sites/effects
Insecticides	Organochlorines	DDT	Liver and lungs [57] Reproductive system [58] Immune system [59]
		Hexachlorohexane	Immune system [60] Liver [57] Blood dyscrasias anemia [61] Reproductive system [62]
	Organophosphates	Chlorpyrifos	Immune system [63] AChE activity in developing fetus [64] Mammalian cell cultures [65] Neurodevelopmental disorders [66]
		Methyl parathion	Neurotoxic effects (CNS) [21, 67, 68]
		Malathion	Reproductive system [69] AChE activity [70] Lipid peroxidation [71] Genotoxic effects [72]
	Pyrethroids	Allethrin, Permethrin	Neurotoxic effects and NA^+-K^+ ions channels [73, 74]
	Carbamates	Aldicarb, Carbaryl Propoxur	Nervous system [75, 76]
Fungicides	Dicarboximide	Mancozeb	Endocrine disruptor [77]
	Dithiocarbamates	Vinclozolin, Asomate, Amobam,	Antiandrogenic effects [78]
	Organomercuricals	Methyl mercury, Phenyl mercuric acetate	Central nervous system [79]
Herbicides	Sulfonylureas	Chlorosulfuran	Embryo development [80]
	Chlorophenoxy compounds	MCPA, MCPP	Human carcinogens [81]
		2,4-D	Gastrointestinal and peripheral neuromuscular systems [82]

DDT dichlorodiphenyltrichloroethane, *AChE* acetylcholinesterase, *CNS* central nervous system, *MCPA* 4-chloro-2-methyl phenoxyacetic acid, *MCPP* 2-(4-chloro-2 methylphenoxy) propionic acid, *2,4-D* 2,4-dichlorophenoxy acetic acid

behavioral disorders, especially among children, have been attributed to chronic organophosphate pesticide poisoning [83]. As mentioned earlier, organophosphorus compounds halt the activity of enzyme acetylcholinesterase by changing its structure and function. It results in the accumulation of acetylcholine at synapses and leads to extra stimulated and jammed nerves [84]. Carbamate pesticides resemble to organophosphates according to the action, but they are less detrimental. Concentration required for producing minimum poisoning and mortality is more for carbamate pesticides than organophosphorus compounds [85, 86]. Due to the lack of timely degradation, these persist in the soil and not efficiently escaped out from the ecosystem [6]. Lack of degradation may be due to different reasons. Since these compounds have great potential to pose adverse health threats on human lives, so the accurate monitoring of these compounds in different environments is very much required. Permissible limits of some pesticide residues in various food samples are mentioned in Table 2.

A rapid, sensitive, selective, and reliable determination of these pesticides and their residues is very crucial. Current techniques used for the analysis of pesticides in environmental samples include HPLC, GC, MS, ELISA, and various other spectrophotometric methods [87]. All these conventional techniques have some specific drawbacks due to which they are not suitable under field condition investigations [88]. Chemicals of the highest purity and equipment of

Table 2 Permissible limits or tolerances for pesticide chemical residues in food (United States of America Environmental Protection Agency 2014)

S. no.	Pesticide name	Various food samples	Tolerance level /permissible limit (in moles)
1	Methyl parathion	Corn grain	3.4×10^{-6}
		Rice grain	3.5×10^{-6}
		Wheat grains	3.4×10^{-6}
2	Diazinon	Carrot	2.3×10^{-6}
		Onion and tomato	2.5×10^{-6}
3	Carbyl	Wheat grain	5.0×10^{-6}
		Milk	4.8×10^{-6}
		Corn grain	5.0×10^{-7}
4	Endosulfan	Vegetables	5.0×10^{-6}
		Corn seed	2.5×10^{-6}
		Milk	5.0×10^{-6}
5	Atrazine	Corn grain	9.2×10^{-7}
		Milk	9.8×10^{-8}
		Wheat grain	4.6×10^{-7}
6	Dichlorovos	Milk	9.0×10^{-8}
		Egg	2.3×10^{-7}
		Agricultural	9.0×10^{-6}
7	Carbofuran	Milk	4.5×10^{-7}
		Wheat and corn grain	9.0×10^{-6}
8	Chlorpyrifos	Wheat grain	1.4×10^{-6}
		Cotton	5.7×10^{-7}
9	Malathion	Potato	3.0×10^{-6}
		Wheat and corn grain	2.4×10^{-5}
10	Trichlorforton	Milk	2.0×10^{-6}

grade 1 are required in these techniques which make the overall process less cost effective. Sample preparation like for GC is an elaborative task, often needing derivation, thus, limiting the number of samples in a specific interval of time. Several pesticides under chromatographic investigation get decomposed at high column and injection head temperatures. Matrix interference and longer duration of test are common problems with spectrophotometric methods which use complex of color reactions [28].

Biosensor Technology and Its Remunerative Aspects in Pesticides Detection

Biosensors emerge as a highly efficient analytical tool for the resolution of above mentioned snarls linked with conventional techniques of pesticide detection. Biosensors pave way for easier and efficient pesticide detection with immense accuracy [89]. The ability to detect very minute amounts of the target substance (pesticides), continuous monitoring, low cost, and decentralized infield analysis are the key features of biosensor technology. The close association of the biological events with the generation of a signal creates a path for developing compact and easy-to-use analytical tools of high sensitivity and specificity. Their biological base makes them ideal for toxicological measurement of pesticides (qualitative), while conventional techniques can only measure pesticides quantitatively. Rapid advancement in development of biosensors was noticed during the 21st century. Fabrication of biosensors for determination of pesticides by applying different biosensing elements and numerous different methods of immobilization has been done. Construction of immune-sensors also came in to play in a rapid pace. Various profitable aspects of nanotechnology also have been collaborated with biosensor technology. Several review articles are already published explaining all these new advancements in the field of biosensors technology [28, 90, 91]. Development of biosensor requires the immobilization of biosensing element with the interface of transducer. The immobilization secures both the stabilization of the biorecognition material and the proximity between the biorecognition material and the transducer [92]. The immobilization method which are generally used are: physical adsorption at a solid plane, crosss-linkage between molecules, covalent attachment to a surface, entrapment within a membrane system or polymer [93] and are summarized in Table 3.

Progress in the field of biosensors technology is mainly made by the upgrading of the biological components (use in the construction of biosensor) and the implementation of

Table 3 Methods of immobilization of the biological component in biosensors

S. no	Method	Advantages	Limitations
1	Physical adsorption	No modification of biological component is required Matrix can be regenerated Maximal retention of activity Prepared simply and low cost	Binding is Susceptible to changes in pH and temperature Binding is not strong Loss of biological activity
2	Cross liking	Less chances of reduction of biological activity of biocomponent Strong binding force	High preparation cost High running problem Treatment with toxic chemicals
3	Covalent binding	Strong binding force No loss of biological activity Less affected by adverse conditions	High preparation cost Difficult to prepare Matrix not regenerable
4	Entrapment	No direct chemical modification Preparation cost moderate Specificity retained	Weak binding force Loss of biological activity Applicable for small analyte detection

nanotechnology (microsystems technologies). Regarding biosensing elements, enzymes are one of the most appropriate choices as biosensing part in pesticide biosensors construction, because they possess high chemical specificity and have natural bio-catalytic signal amplification property [94]. In enzyme-based biosensors, the biological element is the enzyme which is combined with a transducer. Enzymes react selectively with its analyte and produce a signal proportional to the target analyte concentration [95]. This signal can result from a change in proton concentration, release or uptake of gases, light emission, absorption or reflectance, heat emission, and so forth, brought about by the reaction catalyzed by the enzyme. The transducer converts this signal into a measurable response, such as current, potential, temperature change, or absorption of light through electrochemical, thermal, or optical means. This signal can be further amplified, processed, or stored for later analysis [96]. An overview showing the types of transducers used for biosensor construction is given in Fig. 3. Immunosensors based on the principle of immunoassays have grown steadily in the recent years for the environmental monitoring. Immunosensors collaborate the characteristic of antibodies as a biosensing element and a suitable transduction method to change the recognition events in readable form [97]. A huge number of research papers have been materialized over the last years describing the development of novel immunosensors for detecting trace amounts of chemical residues in environmental and food samples [98]. The integration of advanced materials (nanomaterials) promoted the sensitivity, stability, and detection limit of enzyme-based biosensors.

Enzyme-Based Biosensors

Enzymatic biosensors for the detection of pesticides are fabricated on the basis of two principles: Based on measurements of enzyme inhibition (indirect approach) or on direct measurement of compounds involved in the enzymatic reaction (direct approach).

Inhibition Biosensor

Pesticide as Inhibitor

Most simple and innovative biosensor approach for the development of enzyme biosensors for pesticide detection is based upon the inhibition of enzyme ChE (cholinesterase). Two types of

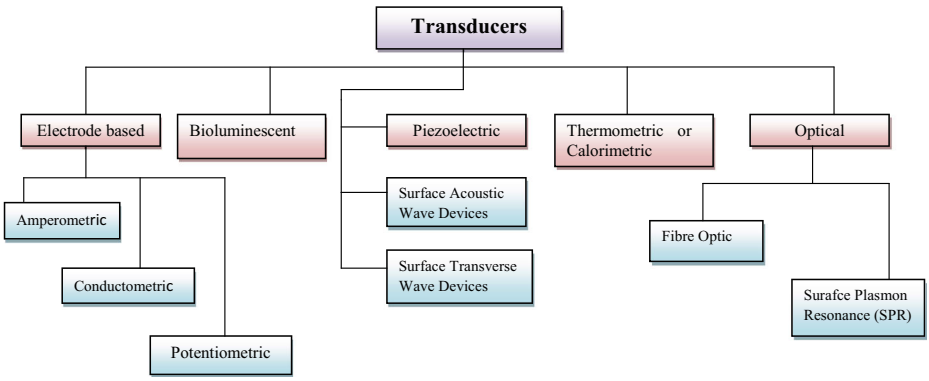

Fig. 3 Classification of transducers employed in biosensor construction

enzymes are known: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Mostly, the acetylcholinesterase (AChE) activity-based biosensors for pesticide (mostly organophosphorus pesticides) detection have been developed [99]. AChE belongs to the family of hydrolases and at its active site three amino acids: histidine, serine, and aspartic acid are present. When the binding site attracts the positively charged quaternary ammonium group of ACh, serine hydroxyl group attacks and hydrolyzes the compound by deprotonation. In the presence of pesticide like OP, the nucleophilic serine present at the active site is covalently bound to the phosphorus atom of the organophosphate, and this blocking of the serine inactivates the enzyme [100]. The detection methods of organophosphate and carbamate pesticides are mostly based on the principle of inhibition of cholinesterases. Acetylcholinesterase enzyme, isolated from various organisms, was initially used in the early 1950s for determination of pesticides with potentiometric transducers. Biosensors based on the acetylcholinesterase (AChE) inhibition test, using AChE-modified amperometric transducers (measuring hydrogen peroxide generated as a result of the oxidation of choline produced from acetylcholine hydrolysis in the presence of choline oxidase) have been reported [101–104]. Compounds other than pesticides such as heavy metals, fluoride, nerve gas, or nicotine, etc. can also hold back ChE enzyme and thus may interfere the pesticide detection process specificity. Following reaction shows the degradation of acetylcholine.

$$\text{Acetyl choline} \xrightarrow{\text{Acetylcholine esterase (AChE)}} \text{Choline} \xrightarrow{\text{Choline oxidase}} \text{Betain} + H_2O_2$$

Although inhibition-based system lacks the property of high selectivity, instead, those ChE-based biosensors are established as a powerful tool for the rapid screening of pesticides. Exhaustive research from various authors has been applied to design and development of AChE-based biosensors for pesticide analysis applying different configurations and detection methods. Genetically modified AChEs have been broadly used in the construction of enzyme inhibition-based biosensors as they exhibited the enhanced performance than indigenous/native enzymes. Various immobilization approaches [105] have been examined with various AChE mutants [106, 107] and immobilization surfaces, in order to ensure high enzymatic activity, stability, and sensitivity of the biosensing materials to OPs. Different types of transducers have been applied by various researchers worldwide [108]. An amperometric transducer developed to analyze the oxidation or reduction current was generated as a result of hydrolysis products of the enzymatic reaction of AChE [109, 110]. A potentiometric transducer like pH electrode [111, 112] or a field effect transistor [113] can be used to measure the pH changes produced as a result of enzymatic reaction and can be then correlated to the concentration of the pesticides. A highly sensitive biosensor for determination of organophosphorus pesticides based on mechanism of acetylcholinesterase inhibition was developed [7]. For the construction of system, authors used the phenomenon fluorescence quenching of CdTe QDs (quantum dots) in the presence of H_2O_2. Two enzymes (AChE and ChOx) were used in the construction of the biosensor along with the usage of QDs and acetylcholine (ACh). In the presence of OP pesticide (Dichlorvos), AChE was inhibited which consecutively inhibit the enzyme ChOx. This leads to the diminished fluorescence of quantum dots due less H_2O_2 production. As the concentration of OP increases, quenching of the quantum dots also decreases. QDs used here as a fluorescent probe because of its photophysical properties. Developed biosensor has shown the lower detection limit of 4.4×10^{-9} M. Using this approach/principle (AChE inhibition), other numerous biosensors have been developed till date. A basic plan of working of AChE-based biosensor for OPs detection is given in Fig. 4. An AChE inhibition-based disposable biosensor for monitoring of malathion (OP) residues

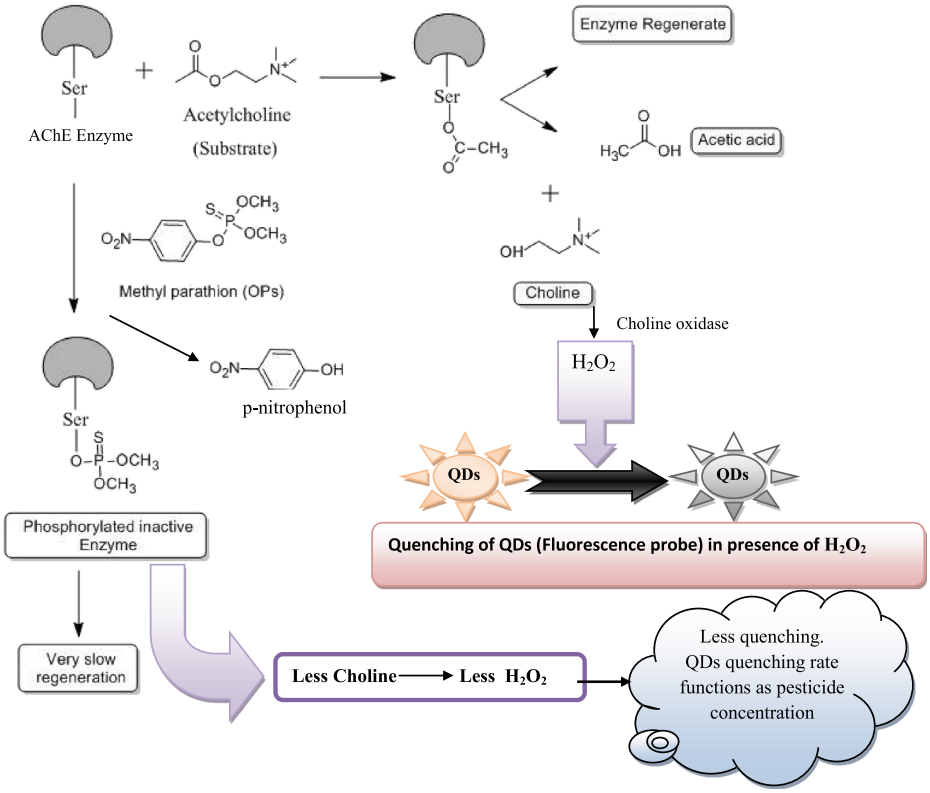

Fig. 4 Principle of working of acetylcholinesterase (AChE) based biosensor for OP detection. *OP* organophosphorus pesticides, *AChE* acetylcholinesterase, *QDs* quantum dots, H_2O_2 hydrogen peroxide

was developed [114]. Source of enzyme used in investigation was blood, and linear range obtained for malathion concentrations was 0.5–1.0 μg. Instead of using AChE, lipase enzyme was used as a biocomponent for the first time to develop an electrochemical biosensor for chlorpyrifos (OP) detection [115]. Authors have used the silver nanoparticles in biosensor's development which enhanced the overall performance of biosensors. Their investigation was novel in terms of application of lipase as biocomponent and also the reaction volume has been miniaturized to 2 ml.

Peroxidase-based biosensors are the other options which can be exploited for the construction of enzymatic-based biosensor [116]. In some cases, bi-enzymatic and tri-enzymatic approaches are also used for the biosensor development. Alkaline phosphatase (ALP)-based biosensors, tyrosinase-based biosensors, and other than enzymes, a number of microbial/whole cell-based biosensors also have been developed for pesticides like paraxon, carbamates, malathion, thiodicarb, etc. Microorganisms offer a number of advantages as sensing elements for the development of biosensors if compared to enzyme-based systems. Microorganisms have the ability to metabolize broad range of chemical which is not possible with enzymes. In case of whole cell system, expensive procedures of enzyme purification are not required, and they themselves behave as a source of intracellular enzymes. Another important merit of a whole-cell biosensor is the ability to analyze the sample through processes in which many enzymes are involved [117]. The enzymes are retained in its natural environment improving

their stability and activity and overall performance. The main limitation linked with use of whole cells is the substrate and products diffusion through the cell wall which alternatively lowers the response as compared to enzyme-based biosensors.

Catalytic Biosensors

Pesticides as Substrate

Another approach for the development of enzyme biosensors for pesticides is based on the activity of enzyme organophosphorus hydrolase (OPH). OPH is one of the typical bacterial enzymes known as phosphotriesterase, and instead of enzyme inhibitor, pesticides act as a substrate for the enzyme. OPH causes the hydrolysis of organophosphorus pesticides, such as parathion, methyl parathion, or paraoxon. Inhibition-based biosensors are very sensitive (able to detect up to 1×10^{-10} M, but they are slow, indirect, and have poor specificity, because number of other substances (carbamate, heavy metals, etc.) have the ability to inhibit these enzymes [33, 34]. OPH-based biosensors are found to be a much more preferred approach for direct biosensing of OP pesticides and involve the biocatalytic activity of organophosphorus hydrolase [118]. Biosensors based on a catalytic reaction are superior to the inhibition mode since they can be potentially reused and are also suitable for continuous monitoring [119]. Till date, several OPH-based amperometric, potentiometric, and optical devices have been described and introduced [120–122]. Amperometric OPH transducers monitor the oxidation of the *p*-nitrophenol, product generated in the reaction. OPH catalyzes the hydrolysis of organophosphorus pesticides with p-nitrophenyl substituent such as paraoxon, parathion, and methyl parathion to *p*-nitrophenol. OPH has a broad substrate specificity and is able to hydrolyze a number of OP pesticides such as paraoxon, parathion, coumaphos, diazinon, dursban, methyl parathion, and chemical warfare agents like sarin and soman [123].

Direct, selective, rapid, and simple determination of organophosphate pesticides has been achieved by integrating organophosphorus hydrolase with electrochemical and optical transducers [118]. The sensor developed was based on a carbon paste electrode containing genetically engineered cells expressing organophosphorus hydrolase (OPH) on the cell surface. A microbial biosensor with high specificity and sensitivity for rapid detection of p-nitrophenyl-substituted OPs by inclusion of OMCs (ordered mesopore carbon) with OPH was developed [124]. Under the optimized experimental conditions, authors found the lower detection limits of 9.0×10^{-9} M for paraoxon, 10×10^{-9} M for parathion and 15×10^{-9} for methyl parathion. Electrochemical microbial biosensor developed by them exhibited good operating stability and long-term stability and is well suited for meeting the challenges of on-site determination. The proposed system was also applicable for the detection in real samples.

Immunosensors for Pesticides

Immunosensors showed a great potential and has become a small and cost-effective device for on-site environmental sample's monitoring. Unlike enzyme-based biosensors, they are able to evaluate total toxicity as immunosensors are specific for a particular chemical moiety. Different pesticides have a similar mode of action altering the activity of the same enzyme. Also, most of enzyme-based biosensors are used for screening purposes and are not capable of differentiating a particular pesticide. Enzyme-based biosensors can only detect total pesticide content and do not provide specific information about a particular pesticide. Overcoming all these limitations, immunosensor is an alternative approach for pesticides detection and uses

antibodies or antigens as the specific biosensing element. In recent years, the use of antibodies against pesticides has gained tremendous importance which has led to the introduction of several immunoassays for pesticides detection [125]. Antibodies generation against pesticide residues has seen significant progress in 21st century [22, 126]. Immunoassay based on the antibody–hapten reaction has become a complementary method for the analysis of agrochemicals and pollutants because of their specificity, cost-effectiveness, and high sample throughput [127, 128]. Technology of immunosensing is a rapid, versatile, and attractive mean for the on-site detection of small molecules (pesticides) in food and other environmental samples [129]. Various transduction systems, such as electrochemical, optical, piezoelectric, and nanomechanics methods have been reported in the literature for the fabrication of immunosensors for pesticide detection. Immunosensors are based on the principles of solid-phase immunoassays. Immunosensors imply the feature of antibodies as biosensing elements and a suitable transduction mechanism to convert biosensing event in to a readable form [127]. Numerous biosensors have been developed for various pesticides detection using different technologies in recent years. Some of the recently (21st century) developed biosensors for pesticides detection using various approaches showing transducer used, their limit of detection, response time, storage capacity, and applications are given in Table 4.

A large number of research papers have been appeared over the last many years describing the development of immunosensors for detecting trace amounts of pesticide chemical residues in environmental and other food samples [98]. Basic scheme of working of a immunosensor is given in Fig. 5. Monoclonal antibody specific for a particular pesticide is linked to the linking surface which is further connected to a transducing system. In competitive assays, the analyte (antigen or pesticide of interest) competes with labeled analyte for a limited number of antibody binding sites. As the pesticides concentration increases, more labeled analyte is displaced, giving a decrease in signal if antibody-bound labeled analyte is detected [166].

It is a well-established fact that the competitive assay is more complex and to obtain simpler and faster immune-sensing procedure, direct detection of pesticide residues in food products without the use of labels (fluorescent compounds, enzymes, etc.) has increasingly come in to power. Compared with label-dependent immunoassays, label-free direct detection represents a crucial advantage of the simplicity, low cost, and real-time response. Based on signal transduction mechanisms, existing label-free immunosensors chiefly involves surface plasmon resonance (SPRs), modified electrochemical sensors, microcantilever and quartz crystal microbalances, (QCMs) etc. [167–169]. Formation of Ab-Ag complexes is measured by changes in the optical behavior. This complex formation also underlay the basis of the construction of optical immunosensors. Development of the mechanical immunosensors is based on a response generated due to either changes in surface characters or mass loading. The interaction between a surface-adhered ligand (e.g., antibody) and an analyte (e.g., an antigen) causes a surface stress change of cantilever and can be detected as alterations in the cantilever deflection. Various advantages of using microcanlitever sensors include label-free detection, high accuracy, consistency, and reduced size.

A label-free immunosensor based on film bulk acoustic resonator have been developed for the detection of paraxon pesticide residues [116]. Artificial antigens immobilized on the sensing surface of bulk acoustic resonators (FBARs) and obtained ultra low limit of detection for parathion as 2×10^{-10} M. Immunosensors for the detection of the pesticides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been reported [170]. An immunosensor was fabricated (with Potentiometric transducer) for the detection of pesticide Simazine using HRP (Horse Radish Peroxidase) as label [171]. Limit of detection was recorded up to 1.4×10^{-14} M. Also, immunosensors

Table 4 Biosensors developed for pesticides detection in the 21st century using various approaches

Year	Biosensing element	Pesticide detected	Transducer	Limit of detection (moles)	Sample detected	Response time	Storage stability	References
2001	OPH	Methyl parathion	Amperometric	2.0×10^{-8}	Water	2 min	30 days	[130]
	OPH	Paraoxon	Amperometric	2.0×10^{-7}	Water	2 min	45 days	[120]
		Methyl Parathion		1.0×10^{-6}				
2002	AChE	Carbaryl Dichlorvos	Fiber-optic	5.4×10^{-7}	Water	12 min	21 days	[131]
	AChE	Diazinon	Potentiometric	2.3×10^{-10}	Purchased	Not given	Not given	[132]
2003	AChE	Carbofuran	Photothermal	Not given	Vegetable	2 min	Not given	[133]
	AChE and CHOx	Methyl parathion	Amperometric	1.0×10^{-10}	Purchased	Not given	180 days	[134]
2004	AChE	Methyl parathion	Amperometric	5.0×10^{-8}	Purchased	Not given	Not given	[135]
2005	OPH	Paraoxon	Amperometric	1.5×10^{-7}	Purchased	Not given	Not given	[136]
		Methyl parathion		8.0×10^{-8}				
	AChE	Carbofuran,	Potentiometric	9.5×10^{-8}	Water	4–6 min	Not given	[112]
		Carbaryl	Conductimetric	1.3×10^{-7}				
2006	OPH	Paraxon	Fibre-optic	5.0×10^{-8}	Purchased	Not given	Months	[121]
	AChE	Paraxon	Piezoelectric	8.5×10^{-10}	Purchased	Not given	60 days	[137]
2007	AChE	Dichlorvos	Optical	1×10^{-10}	Water	Not given	50 days	[138]
	AChE	Paraoxon	Surface	1×10^{-7}	Purchased	10 min	30 days	[139]
		Parathion	Acoustic wave	8.5×10^{-7}				
2008	Anti-Atrazine Ab	Atrazine	Conductimetric	4.6×10^{-10}	Red wine	Very Fast	Not given	[140]
	AChE	Methyl parathion	Opto-fluidic	1×10^{-1}	Purchased	0.5 min	Months	[141]
2009	AChE	Methyl Parathion Chlorpyrifos	Electrochemical	1×10^{-12}.	Water	15 min	15–20 days	[142]
	AChE	Methyl paraxon	Amperometric	2.5×10^{-7}	Purchased	Not given	14 days	[143]
2010	AChE	Dichlorvos	Amperometric	1.1×10^{-11}	Purchased	10 min	Not given	[144]

Table 4 (continued)

Year	Biosensing element	Pesticide detected	Transducer	Limit of detection (moles)	Sample detected	Response time	Storage stability	References
		Trichlorfon		1.9×10^{-11}				
	OPH	Methyl parathion	Amperometric	3.4×10^{-9}	Purchased	Not given	30 days	[145]
	OPH	Paraoxon	Electrochemical	1.2×10^{-7}	Purchased	<10 s	Not given	[146]
	Anti-Atrazine Ab	Atrazine	Electrochemical	4.6×10^{-12}	Purchased	Not given	Not given	[147]
	AChE	Malathion	Amperometric	3.0×10^{-9}	Purchased	Not given	2 weeks	[148]
2011	Methyl parathion hydrolase	Methyl parathion	Electrochemical	1.0×10^{-9}	Garlic	Not given	30 days	[149]
	AChE	Paraxon	Optical	1.0×10^{-11}	Fruit	Not given	35 days	[150]
		Parathion		4.4×10^{-12}				
	AChE	Monocrotophos	Amperometric	1.0×10^{-9}	Garlic	10 min	30 days	[151]
	Anti-atrazine Ab	Atrazine	Amperometric	5.0×10^{-11}	Milk	15 min	Not given	[152]
	Anticarbofur an Ab	Carbofuran (Carbamate)	Electrochemical	1.3×10^{-8}	Vegetable	20 min	10 days	[153]
2012	AChE	Carbaryl	Electrochemical	1.4×10^{-6}	Food	Not given	60 days	[154]
		Methomyl		9.5×10^{-6}				
	AChE	Methyl parathion	Amperometric	2.7×10^{-9}	Purchased	1 min	30 days	[155]
		Acephate		4.4×10^{-7}				
	Anticarbofuran MAbs	Carbofuran	Amperometric	4.5×10^{-11}	Purchased	Not given	10 days	[156]
	AChE	Chlorpyriphos	Amperometric	5×10^{-12}	Milk	2 min	Not given	[157]
	AChE	Malaoxon		5×10^{-10}				
		Monocrotophos	Potentiometric	4.4×10^{-10}	Fruit	Not given	Not given	[158]
2013	AChE and ChOx	Dichlorvos	Optical	4.49×10^{-9}	Purchased	Not given	Not given	[7]
	AChE	Methyl parathion	Amperometric	2.5×10^{-6}	Purchased	Not given	Not given	[159]
	Anti paraxon Ab	Paraxon	Electrochemical	7.2×10^{-9}	Purchased	Not given	Not given	[160]

Table 4 (continued)

Year	Biosensing element	Pesticide detected	Transducer	Limit of detection (moles)	Sample detected	Response time	Storage stability	References
	AChE	Methyl parathion Chlorpyrifos	Optic	5×10^{-14}	Purchased	10 min	30 days	[161]
	AChE	Paraxon	Amperometric	7.2×10^{-9}	Water	Not given	Not given	[162]
2014	Antigens	Parathion	Electrochemical	2.7×10^{-9}	Leaves	Not given	30 days	[163]
	OPH	Paraoxon	Electrochemical	9×10^{-9}	Water	Not given	35 days	[121]
		Parathion		10×10^{-9}				
		Methyl parathion		15×10^{-9}				
	Heptan	Endosulfan	Electrochemical	1.2×10^{9}	Purchased	Not given	30 days	[164]
		Paraoxon		7.2×10^{-9}				
	AChE	Chlorpyrifos	Electrochemical	1.3×10^{-13}	Cucumber juice	Not given	30 days	[22]
	AChE	Methyl parathion		4.9×10^{-13}				
	AChE	Carbofuran	Electrochemical	3.6×10^{-9}	Cabbage sample	Not given	Not given	[165]

AChE acetylcholinesterase, *OPH* organophosphorus hydrolase, *CHOx* choline oxidase, *Ab* antibody, *Mab* monoclonal antibody

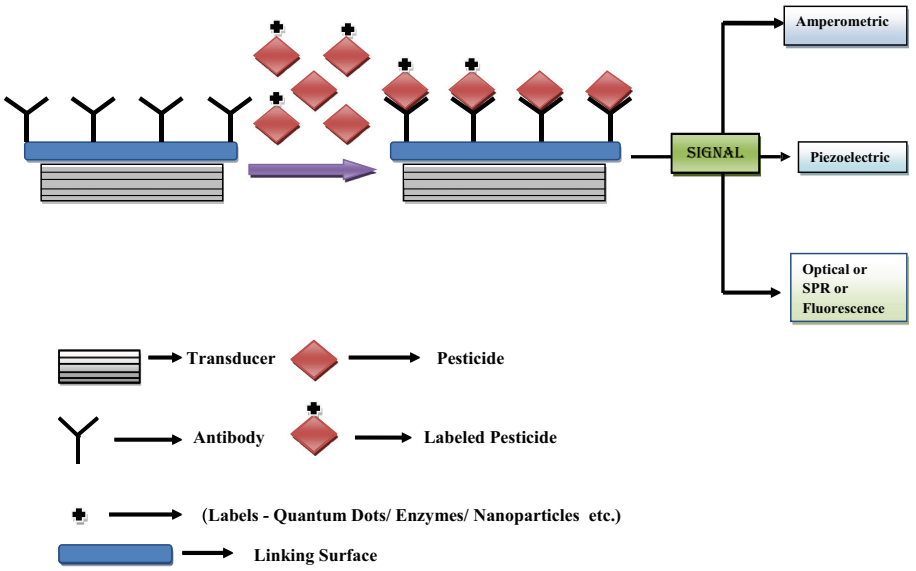

Fig. 5 Basic scheme of the development of immunosensor for pesticide detection

[172, 173] for the Isoproturon pesticide detection have been developed and detect pesticide with the lower limit of 4.8×10^{-16} and 4.4×10^{-13} M, respectively. Fluorescein was used as a label molecule and employed with fluorescent detector. An electrochemical immunosensor for detection of atrazine (OC) by immobilizing gold nanoparticles on gold electrode surface [174]. Increase in surface area by the use of nanoparticles result in capture of more anti atrazine monoclonal antibodies which makes the overall process highly sensitive. Interaction between atrazine and anti-atrazine antibody triggers the signal via differential pulse voltametry (DPV).

Recent Advancements in Pesticide Analysis

Aptamers

Aptamers are single-stranded specific DNA or RNA sequences prepared by technique called SELEX selection evolution of ligands by exponential enrichment (SELEX) in vitro from libraries of synthetic oligonucleotides that bind non-nucleic acid sequences with elevated affinity and specificity [175]. Immunosensors are associated with difficulties like antibodies generation for very small molecules that cannot educe an immune response and the unstability of antibodies under unfavorable environment conditions, thus made immunosensor less preferable for wide applications in detection [176]. The most significant factors support the use of an aptamer over antibodies—they can be generated against a number of targets including small molecules such as heavy metals, antibiotics, pesticides, amino acids, nucleotides, and biomacromolecules like carbohydrates, proteins, and nucleic acids. They also possess prolonged shelf life, better detection range, attainable cross reactivity and efficient and limited production. Merits of aptamers over antibodies as biosensing element in biosensor technology are given in Table 5. As aptamers exhibit numerous profitable aspects, till date numerous aptamer-based biosensor systems have been developed for analysis of small molecules of

Table 5 Differences between use of antibodies and aptamers in biosensor technology

S. No.	Antibodies	Aptamers
1	Stable under narrow range of conditions	Stable under wider physiological physiological conditions
2	Production process costly and time consuming	Production process is cost effective and efficient
3	Shelf life is short and limited	Shelf life is prolonged
4	Detection range is moderate	Detection range is vast
5	Less physically stable	More physically stable
6	Narrow chemical modification	Wide chemical modifications
7	Toxins difficult to detect	Can detect all targets
8	Cross reactivity not attainable	Cross activity is attainable

various classes [177]. The potential of aptamer-based biosensor for the detection of pesticides is not fully exploited, yet only few aptamers have been selected for pesticide detection.

For fast, highly selective and sensitive detection of acetamiprid (neonicotinoid insecticide), a novel aptamer-nanozyme-based reversible biosensing strategy was developed [178]. Authors were able to detect of 4.5×10^{-7} M acetamiprid within an assay time of 10 min. An engineered aptamer-based approach for the detection of organophosphorus pesticides was successfully developed [179]. Recombinant broad-specificity DNA aptamer bound with a molecular beacon (MB). This binding of the MB to the aptamer results in the generation of the fluorescent signal, amount of which can be measured for pesticide quantification. [180] exploited the specificity of DNA aptamer recognition and demonstrated a method for direct detection of malathion using DNA apta-SERS substrates. Developed apta-sensing microspheres were well suited for industrial and agricultural applications. A highly simple, sensitive, and selective colorimetric method has been developed for detection of acetamiprid pesticide [181]. By employing acetamiprid-binding aptamer (ABA) as recognition element, excellent level of selectivity for acetamiprid determination was achieved. Specific interaction between the pesticide and biosensing element found to be responsible for this high level of selectivity. Target induced conformation changes of ABA upon binding of acetamiprid were responsible for the abovementioned fact. Developed assay was successfully employed in determining acetamiprid in real samples and exhibits superiority in the simple sample treatment, stability, reliability, and repeatability. At present stage, noteworthy interest is concentrated onto the development of small molecule aptamers for their applications in biosensing and need to be explored to the full extent. Upcoming research in future should be focused on to the screening of novel potential aptamers and developing relevant assay kits for detection of various classes of pesticides with highest specificity and greatest affinity.

Molecularly Imprinted Polymers

Molecular imprinting allows the fabrication of molecularly imprinted polymers (MIPs) that has been created to mimic biological receptors. By generating specific recognition sites on to the polymers, MIP-based biosensors have been developed for the analysis of the chemical residues in various samples [182]. Molecular polymer complexes are formed by interacting the template and functional monomers. These monomers then polymerize with the help of a cross linker in the presence of a specific solvent. Finally, template removal yields the expected polymer exhibiting specific sites complementary to the template in size, shape, and location of

functional groups [183]. MIPs have extensively been used as sensitive sensing element in sensors due to their associated advantages over enzyme-based biosensor and immunosensors such as simplicity, reliability, superior stability, low fabricating cost, easy operation, and a wide choice of templates and functional polymers [184].

Numerous reports are available in the literature that reveals the use of MIPs as artificial recognition elements in the biosensor development for pesticide detection. A novel, highly sensitive and selective electrochemical sensor by electro polymerization of (MIP) synthesized onto Au electrode in phosphate buffered saline (PBS) [185]. Methyl parathion (MP) served as template molecules and phenol as functional monomer. Developed system was ABLE to detect hexazinone (herbicide) up to concentrations of 10^{-10} M. High selectivity for this target analyte was the main attribute of developed sensor, unlike other enzyme-based biosensors where the response of system relies onto the nonselective enzyme inhibition. Authors also revealed that developed sensor could be used for the detection of pesticide in different types of environmental samples. A very recent potentiometric sensor for lindane (γ-hexachlorocyclohexane), an organochlorine pesticide detection was designed and fabricated by molecularly imprinted polymer on to the surface modified multiwalled carbon nanotube [186]. The sensor response to pesticide was in the range of 1×10^{-10}–1×10^{-3} M, and the detection limit was found to be 1.0–10^{-10} M. Based on molecular imprinted polymers (MIPs), another highly selective and sensitive sensor was developed for the determination of hexazinone pesticide in environmental samples [187]. MIPs were synthesized using a non-covalent approach, and molecular modeling technique was applied for the selection of the monomers employed in the polymerization reaction. Workers concluded that for the identification of functional monomers, molecular modeling can be employed. System constructed by them was highly sensitive and enable to detect pesticide at concentrations in the region of 10^{-10} M. Literature survey supported the fact of utilization of MIPs for molecular recognition in analytical chemistry and environmental analysis. Still, there are many imprinted polymeric systems which remain less explored, particularly for the direct recognition and sensing of pesticides [188].

Biochips

A biochip may be explained as assortment of miniaturized test sites (microarrays) arranged on to a solid surface (glass or silicon) having the size of a finger nail that permits many tests to be performed at the same time in order to achieve higher throughput and speed [189]. Biochips work on to basis of principle of biosensor technology and involve the specific recognition and binding of target analyte to biosensing elements present on the solid surface in a well-ordered manner and allow detection of the specific analyte in sample mixture [190]. Similar to biosensors, various classes of biosensing materials like nucleic acids, antibodies, enzymes, and cellular components can be employed for the biochip fabrication. Other artificially constructed sensing elements like aptamers, molecular imprinted polymers, synthetic peptides, etc. can also be employed [191]. All these sensing materials arrayed on to the biochips are very much specific, sensitive, and can detect the target analyte up to very low limits. Due to its numerous promising characters and recent advancements, biochip technology has emerged as the striking tool in multiplex chemical analysis. But there are few reports in literature revealing that still biochip technology is not fully explored for the detection of pesticide. A rapid, highly sensitive, chemiluminescence enzymatic assay for organophosphate (OP) pesticide residues detection in milk was developed [192]. The assay was based on the inhibition of enzyme butyrylcholinesterase (BChE). It was tested for different OPs such as methyl paraoxon, methyl parathion, and malathion in milk and also evaluate their synergistic effects. Enzyme was stabilized and preloaded in 384-well plates. The assay permits rapid determination of OPs in

milk within 12 min at 30 °C. Developed system was able to detect methyl paraoxon, methyl parathion, and malathion milk with a detection range of range 0.005–50, 0.5–1000, and 0.5–1000 μg/L, respectively, for individually or in mixtures. Detection of pesticide coumaphos employing the biochip method was proposed and was based on surface plasmon resonance (SPR), and immune reaction belongs to the suppression method [193]. This method was a label-free type, used an unpurified single antibody, and continuously tested at least 80 groups of samples continuously. Advantages of the developed system were cost effectiveness of equipment, label-free detection, high accuracy, specificity, portability, environmentally friendly, and easy control. Developed system detected pesticide residues in fruits and vegetables and generates quick quantitative results. Real-time detection and rapid screening of large amount of samples were the key features of the system. From literature survey, we found that multiplex pesticide analysis is not fully explored and need much more attention.

Nanotechnology in Biosensors/Nanobiosensors

High degree of sophistication has been achieved by the science in these days. The promising association between nanotechnology and sensors technology has come into play over the past few years. Origination of nanoscience technology, using the materials of nanosize (nanomaterials), has shown tremendous profitable aspects to the biosensors technology. Importance of these nanomaterials to the basic development of biosensors has been recognized. Nanomaterials show immense capabilities to the further elaborative development to biosensors technology. Whenever materials scaled down to nanosize, surface area increases, and most of materials display some novel attributes that are not present in their large-size counterparts. Nanomaterials-based biosensor developed from the association of material science, molecular engineering, and biotechnology, improve overall sensitivity and specificity of biomolecule recognition in various environmental samples. It also possesses the characteristic of manipulating atoms and molecules and has properties of recognition of biomolecules, pathogenic diagnosis, and environment monitoring. The immobilization of nanomaterials with sensing part of biosensor generates novel interface thus enhances the sensitivity of the detection of analytes. Because of their unmatchable physical, chemical, mechanical, magnetic, and optical properties, various classes of nanomaterials, like gold nanoparticles [194], carbon nanotubes (CNTs) [195] magnetic nanoparticles, and quantum dots [196], have been applied in association with biosensors and distinctly enhance the sensitivity and specificity of detection. Nanomaterials improve (mechanical, electrochemical, optical, and magnetic properties) overall performances of working of the biosensors and in future will come forward as a single molecule biosensor [197]. The over potential related to electroactive compounds can be lowered down through electrocatalytic action of nanomaterials, thus finally reduces the interventions present in the sample under investigation. At some instances, nanomaterials also have been applied as label to amplify the signal measured.

Gold nanoparticles (GNPs) provide a biocompatible microenvironment for biosensing material through which quantity of immobilized biomolecules on to the electrode surface increases which improve the sensitivity and overall show of the biosensor [198, 199]. A nanoparticle-based (gold nanoparticles) optical biosensors for the direct detection of organophosphate chemical warfare agents and pesticides was developed [200]. Authors obtained greatest sensitivity to paraoxon pesticide detection (minimum detection limit 20×10^{-6} M) when gold nanoparticle conjugates of enzymes were present. Another gold nanoparticle-based electrochemical biosensor for pesticide detection was also developed [201]. The proposed electrochemical biosensor exhibited high sensitivity, advantageous accuracy, low cost, and low detection limit of 1.2×10^{-9} M. AuNPs nanoparticles assembled on a sol–gel matrix and

derived silicate network for immobilization of AChE, leading to a stable AChE biosensor for investigation of pesticide. The network figured out after assembling sol–gel-derived silicate network and gold nanoparticles (AuNPs-SiSG) offered a biocompatible microenvironment around the enzyme which provided the stability to the system and halted the leaking of the enzyme from working interface.

CNTs have attracted enormous authors due to their different novel properties such as unique mechanical, physical, and chemical properties. In the field of nanoelectronics, biomedical engineering, biosensing and bioanalysis, CNTs play different profitable roles [195]. For example, polymer-CNTs composites have the capability to attain good electrical conductivity and good mechanical strength, thus provides the possibility of developing ultrasensitive, electrochemical biosensors. An extremely sensitive amperometric biosensor for organophosphate pesticides based on self-assembled acetylcholinesterase (AChE) on a carbon nanotube (CNT)-modified glassy carbon (GC) electrode was fabricated [202]. Nanostructures on the CNT surface provide a constructive microenvironment to preserve the bioactivity of AChE and to prevent enzyme molecule leaking. The electrocatalytic activity of CNT leads to a highly improved electrochemical detection limit of the biosensor system with higher sensitivity, stability, reproducibility, and good precision. The developed biosensor detected paraoxon having lower limit of detection of 4×10^{-13} M with a 6-min inhibition time. Enzymes used in biosensors development acetycholinesterase (AChE)/choline oxidase (CHO) provided a high sensitivity, large linear range, and low detection limits for the analysis of OP compounds, and all these features attributed to the catalytic activity of carbon nanotubes.

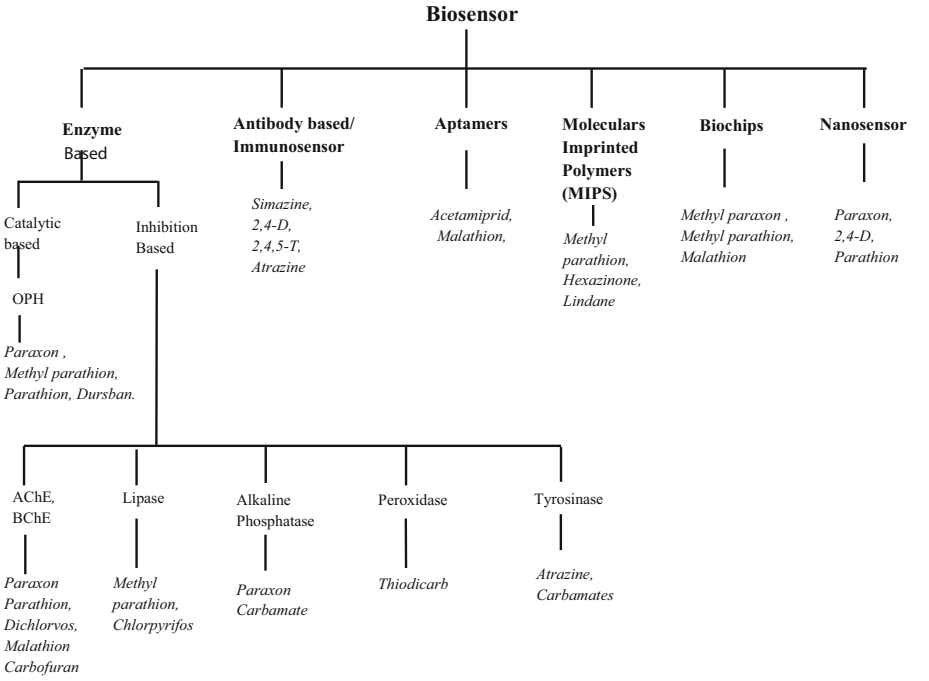

Fig. 6 Biosensor's classification used for the pesticide analysis. The specific pesticide examples cited here are described in the text. *2,4-D* dichlorophenoxyacetic acid, *(2,4,5-T)* trichlorophenoxyacetic acid, *AChE* acetylcholinesterase, *BChE* butyrylcholinesterase, *OPH* organophosphorus hydrolase

Quantum dots are fluorescent nanoparticles which can be used to examine pesticides with high sensitivity and specificity. Due of their unique photoluminescent properties and other potential applications, quantum dots have also came into play in various exhaustive investigations and biologically relevant studies [203]. These quantum dots offers significant advantages, including better stability, stronger fluorescent intensity, and different colors adjusted by controlling the size of the dots, made quantum dots a new functional platform for bioanalytical sciences and biomedical engineering. Quantum dots (CdTe) were used to detect pesticide 2,4-dichlorophenoxyacetic acid in food [204]. A highly sensitive biosensor, comprised of an optical transducer of CdTe semiconductor quantum dots (QDs) integrated with actylcholinesterase enzyme (AChE) for detection of OPs in vegetables and fruits, was developed [144]. The detection limits of the biosensors were described as low as 1.05×10^{-11} M for paraoxon and 4.47×10^{-12} M for parathion. A biosensor for determination of organophosphorus pesticides in real samples based on inhibition mechanism of acetylcholinesterase using QDs was fabricated [153]. For the development of biosensor, they used the phenomenon of fluorescence quenching of CdTe QDs in the presence of H_2O_2. The proposed biosensor exhibited many advantages, such as easy sample treatment, simple process of detection, high sensitivity, and low cost. Thus, it is also clear from the discussion that nanotechnology also offers many profitable effects on to the biosensor technology use for the detection of pesticides. Biological molecules being occupied with special structures and functions, the complete use of the structure and function of both nanomaterials and biomolecules to develop single molecule multifunctional nanocomposites is still a big challenge.

Conclusions

Biosensors (enzyme based, antibody based [immunosensor], aptamers based, MIPs based, nanosensors, and biochip based) have been developed for the analysis of various pesticides and summarized in Fig. 6. Detection limit for different pesticides ranges from 10^{-6}–10^{-14} M having storage stability of 30–60 days. Response time of various biosensors was found in the range of 2–20 min, and in some cases less than 1 min was also achieved. Developed biosensors have been applied for the monitoring of pesticides in water, milk, vegetables, and fruit juice samples. AChE-based biosensors are found to exhibit detection limit of 10^{-10}–10^{-12} M and nanoparticle-based biosensor shows it in the range of 10^{-7}–10^{-13} M. Antibody-based exhibited the lowest detection limit of 10^{-16} M. Recent progresses in the biosensor technology like aptamers and MIPs showed the lower detection limit of 10^{-8} M and 10^{-10} M, respectively. Biochips are also able to detect pesticides up to ppt (parts per trillion) range, but their utilization for multiplex pesticide analysis needs to be explored fully. Immunosensors were found to exhibit the most promising one, depending upon the lowest detection limit. Storage stability of biosensors needs to be improved.

Future Perspectives

Biosensors proved to be a good nominee in the environmental sample's monitoring for the detection of pesticides. Till date, a variety of biosensor have been developed for pesticides detection using diverse approaches. A range of enzymes based biosensors and immunosensors have been developed throughout the globe by various authors. The detection limit of the almost all biosensors developed is very high and capable of detecting the pesticides more far than their permissible limits in food and water (Table 3). Enzyme inhibition-based biosensors are found to be most promising, and their performance level can be further enhanced using

various approaches like enzyme engineering. This will increase the sensitivity of the enzyme and can lower the detection limit of the pesticides. Besides the advantages, the enzyme-based system has broad specificity and only used for the screening of pesticides. Immunosensors as a substitute approach for pesticides detection uses antibodies or antigens as the specific biosensing element to detect pesticides. This system is quite specific than enzymatic system. The applicability and advantages of immunosensors have been particularly established in the field of pesticide analysis. The concept of biosensors development could be extended to assemble other biological molecules, such as DNA, tissue as biosensing materials for wide bioassay applications in pesticides detection; as it has been reported that plant tissue-based bioelectrodes exhibited longer shelf life than pure enzyme-based biosensors. Biosensors other than enzyme based should be developed in the future so that sensitivity and overall performance level of system can be accentuated. Newly emerging technologies like aptamers molecular imprinting polymers biochips for multiple pesticide detection are also gaining importance day by day due to the benefits they impose. Aptamers can be generated against a range of target compounds such as heavy metals, antibiotics, pesticides, amino acids, nucleotides, and biomacromolecules such as carbohydrates, proteins, and nucleic acids, possess prolonged shelf life and better detection range. So further research in the field of aptamers should be focus on to the screening of more potential aptamers for various classes of pesticides with high specificity and with greatest affinity. Also, MIPs which mimic biological receptors and detect specifically a single analyte in mixture sample with exact quantification is still not fully explored particularly for the direct recognition and sensing of various kinds of pesticides and to be work on.

Also application of nanotechnology in biosensors offering novel prospects for developing a new era of biosensor technology. Nanomaterial-based biosensors impose immense developments and usually applied in clinical diagnosis, food sample analysis and environmental monitoring in the near future in an exhaustive manner. Newer kinds of the developments should be made in the field of nanotechnology so that the collaborative approach of biosensor and nanotechnology proves to be more productive. The application of biosensor technology in environmental field is until not fully explored in comparison to medical applications. Most of commercialized biosensors are related to the medical field applications, whereas only few are tailored for the monitoring of environmental samples. So, immense magnitude of effort is still required to develop more improved and more reliable devices allowing the detection of pesticides up to the lowest limits. Improvement of storage stability of biocomponent needs attention.

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