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# Review: Biosensor for detection of pesticide residue

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**Abstract:** Pesticide residue is a common contamination in the environment and food. Various analytical methods have been developed to detect and analyze the residues. Biosensor is one of the fast detection technologies. In this paper, we reviewed those biosensors according to their unique detection mechanism, fabrication and incorporation with nanomaterials.

**Keywords:** Biosensor, Pesticide Detection, Enzyme Immobilization, Nanomaterials

## 1. Introduction

Pesticide residue is a consequence of utilizing the pesticide in the agriculture. In recent years, organophosphorus, carbamates, and pyrethroid pesticide have been widely used because of their low toxicity and rapid degradation. However, there is still amount of pesticide residue remaining in the soil and water, being absorbed by plants, vegetables or fruits, and resulting in poisoning and neural system damage. Advanced analytical method, such as gas chromatography–mass spectrometry, is of limited use on site because it's time consuming and costly. Therefore fast, selective and reliable detection methods are highly demanded. Due to high specificity of the enzyme based biological reaction with pesticides, biosensors are good candidates for this detection application.

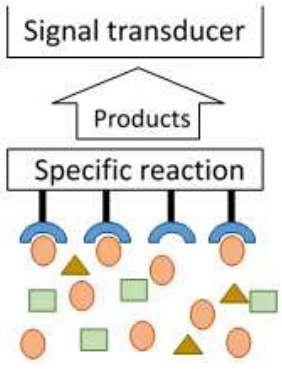


***Figure 1.*** *Schematic of the detection mechanism of biosensor*

The detection mechanism of the biosensor is shown in Figure 1. Most biosensors are designed based on the catalyticreaction (mostly enzymatic reaction). The catalytic substance is immobilized on the sensor as receptor. In normal conditions, the substrates (reactants) produce the products which can be detected by the detection part in the sensor. When pesticide presents, it can specifically bond with the biosensor receptor, and inhibit the catalytic activity, therefore, the resulted products would make a chemical or physical change in the system compared with normal condition. Such change usually indicates the pesticide concentration and new product formation. The attached signal transducer can collect, amplify and visualize these changes, and calculate the pesticide concentration.

## 2. Biosensor Classification and Application

In recent studies, researchers are focusing on increasing the sensitivity and selectivity of biosensors. Modification work has been done to improve the enzyme based receptor layer. A variety of Nano-scaled materials and composites have been combined with the biological molecule to form the novel receptor to enhance the detection sensitivity. Other advanced detection technology was also applied into the signal transducer part. In this paper, we reviewed various novel biosensors regarding the different incorporated

materials and detection technology at nano-micron scale.

### *2.1. Enzyme Immobilized Biosensor*

The pesticides can damage the neural system by inhibiting the activity of acetylcholinesterase (AChE). The most current biosensors take advantage of that inhibition effect. The activity level of the AChE on the biosensor is related to the amount of pesticide in the testing sample. [1, 2]

Bachmann et al. developed an AChE based biosensor for the detection of insecticide residues in infant food. [3] The electrode was fabricated by screen printing technology using commercial screen printer. [4] The working electrode and reference electrode were firstly printed on the conducting tracks containing polyvinylchloride sheets, and then an insulation layer was printed on the track to separate the testing electrodes and signal collection ends. After curing, the enzyme ink containing bovine serum albumine (BSA) and various types of AChE were printed on the top of working electrode. AchE was then immobilized by cross-linking in glutaraldehyde vapor. In a typical test, the biosensor was firstly measured the AChE enzyme activity, and then incubated in the food samples for 30 mins. During the incubation, the pesticide residues in the food sample would inhibit the AChE. The activity of inhibited AChE in biosensor was then measured again. The amount of pesticide residue can be calculated by the activity difference before and after incubating in the food sample. That biosensor was able to detect the neurotoxic pesticide levels of organophosphates and carbamates lower than 5 μg/kg, which is below the maximum residue limit of pesticide (10 μg/kg) in infant food in European Union.

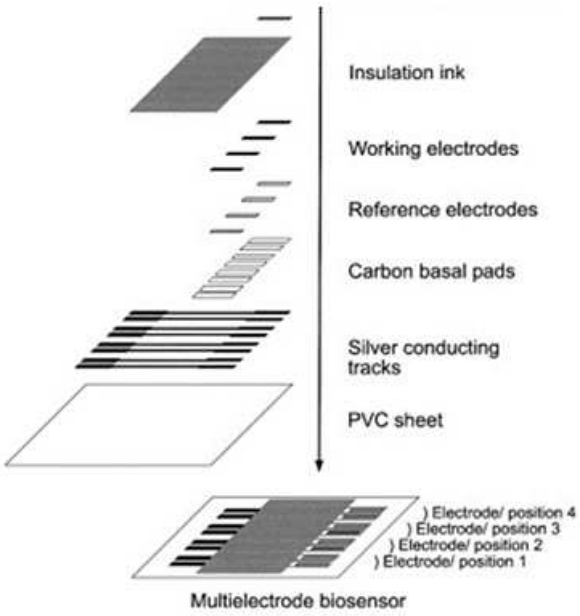


***Figure 2.*** *Multielectrode biosensor assembly by screen printing with enzymes with different specific targets [4]. Reprinted from Analytica ChimicaActa, Volume 401, Issues 1–2, Till T. Bachmann, Rolf D. Schmid, A disposable multielectrode biosensor for rapid simultaneous detection of the insecticides paraoxon and carbofuran at high resolution, 29 November 1999, Pages 95-103, Copyright (1999), with permission from Elsevier*

The biosensors with AChE-only enzyme are usually lack of repeatability. Sarkar et al. modified the enzyme electrode by co-polymerizing the AChE with acrylamide and N,Ndimethylene-bis-acrylamide (BIS) to immobilize the enzyme directly on the working electrode surface. [5] Thismodified biosensor showed enhanced stability and repeatability in detecting organophosphorous and carbamate pesticides.

Besides the inhibition principle, people become interested in hydrolase, which can directly hydrolyze the pesticide, such as organophosphorus hydrolase (OPH). [1]

### *2.2. Whole Cell Immobilized Biosensor*

The real food or water contains various toxic pesticide residues and heavy metals. Sensors have been developed to detect the total toxic compounds at one measurement. Because the detection is based on the enzyme inhibition, certain compounds in the sample require specific enzyme for each single compound. However, those enzymes are very difficult to be immobilized on one electrode to achieve the same optimized operation condition. Therefore, the living cells containing a large number of enzymes were used in the biosensor instead of pure enzymes. The measurement can be implemented by measuring the change of ions surrounding the cells or the potential between cell membranes. Furthermore, that cell based biosensor can be developed into bioassay for multiple detection. Because the various enzyme has unique inhibitor and substrate, enzymes in the biosensor can selectively detect different toxic pollutants, distinguish their types and determine their concentration respectively.

Among those live cells, algae have been widely used in biological reactors. Jean-Marc Chovelon et al. utilized the Chlorella vulgaris microalgae to make a bi-enzymatic biosensor. [6] The algae contain both alkaline phosphatase for heavy metal ion detection and acetylcholinesterase for organophosphorous pesticide detection. The local conductivity variations detected by the sensor would indicate the concentration of the above two pollutants respectively, and integrate the true toxicity for an organism in water samples. Kintzios et al. entrapped two cell types of neuroblastoma and fibroblast in sodium alginate beads with bead diameter of ~2mm and cell number of ~50k/bead. [7] Silver electrode coated with Ag/AgCl was inserted into the cell entrapped bead to become working electrode. That electrode was directly immersed into the standard pesticide solutions to establish standard curve and then into blended tomato samples to measure the pesticide concentration, while the bare electrode without connecting to the bead was used as reference electrode. When pesticide presented, Calcium ion signals increased because of the overstimulation by the pesticide. That Calcium ion overstimulation resulted in the potential change of the cell membrane. By measuring the potential change, the concentration of the pesticide can be determined. Using the two types of cell also increased the selectivity of the biosensor.

### *2.3. Simulating Neural Network*

The real food and water usually contains multiple types of pesticide residues, so sensitive, selective and cost-efficient detection methods are demanded to discriminate and quantify the various pesticides in one sample for the fast detection purpose. As discussed earlier, different pesticides have specific inhibiting enzyme target at certain optimized condition. People utilized that unique character to apply the various enzymes or cells into one detecting system, and analyze the data by artificial neural networks (ANNs) to achieve multi-target detection of various pesticide residues.

Schmid et al. combined four types of AChE (from electric eel, bovine erythrocytes, rat brain, Drosophila melanogaster) into one working electrode using the screen printing to simultaneously detect the paraoxon and carbofuran (Figure 2). [4] For the ANN training of the multi biosensor system, each enzyme electrode inhibiting profile to each individual pesticide was obtained at different pesticide concentration, and the inhibition data processing and network training were performed using feed forward neural networks generated with software of NEMO and resilient backpropagation algorithm. That multibiosensor system could simultaneously detect and quantify 0 – 20 μg/L of paraoxon and carbofuran mixture in 60 mins.

Instead of using the enzyme based multibiosensor system, Kintzios et al. applied mouse neuroblastoma, human neuroblastoma and African green monkey kidney cell to the customized device with eight detection channels to obtain an artificial neural network based bioelectric cellular biosensor. [8] The cultured cell suspension was directly deposited onto the detecting channels containing electrode strips, and then the pesticide sample and ACh substrate were added into the channels. That ANN biosensor was operated as the bioelectric recognition assay (BERA), and was able to simultaneously detect eight samples in three minutes. This is because the cell-pesticide interaction canbe immediately recorded by the device without diffusion limitation from the immobilization.

## 3. Biosensor Detection Improvement

In recent years, more and more restrict policies for environmental and food safety have been established in a wide range of countries. The biosensors based on simplex enzyme or cell can't satisfy the current detection requirement. People have been focusing on incorporating new material or technology to improve the biosensor selectivity and sensitivity. The detection principle can be generally described as immobilizing enzyme/cell, inhibiting/hydrolyzing enzyme/cell, sending electron signal to detector and amplifying the signal, so the new material and technology has been applied to those steps to enhance the entire biosensor performance.

### *3.1. Enhance Enzyme/Cell Immobilization*

In order to maintain bioactivity, the enzyme/cell was entrapped into network substrate to achieve the immobilization. However, such entrapment is not strong binding and usually causes elution. Novel nanomaterials with porous nanostructure and biocompatible binding sites or spaces have been developed to improve the immobilization and preserve the bioactivity simultaneously.

Lia Stanciu et al. developed a zinc oxide sol-gel biosensor to immobilize the AChE. [9] The ZnO sol was prepared and mixed with poly ethylene glycol (PEG) 6000 and AChE. The mixture was then spread on the screen printed electrodes (SPE) and dried in air. The gelled ZnO matrix immobilized the AChE and preserved the enzymatic activity up to three months. Jingming Gong et al. used layered double hydroxides (LDHs) to immobilize the AChE. [10] The LDHs are nano-structured anionic clays. The layered structure provides enough space for the interaction of substrates and enzyme, and the hydroxyl groups provide biocompatible microenvironment to preserve enzyme activity. Other commonly used immobilization matrixes include chitosan [11], mesocellular silica foam (MSF) [12] and multiple types of sol-gel films.

### *3.2. Enhance Electron Transition from Sensing Part to Detector*

The electron transition formed during the enzyme inhibition reaction is a key for the biodetection, therefore, nanoparticles and carbon nanotubes (CNTs) are embedded into the immobilization matrix to enhance the transition and consequently increase sensitivity. Furthermore, those modified composite are usually multifunctional. They can also protect the enzyme from degradation.

Aidong Zhang et al. embedded gold nanoparticles (AuNPs) into the silica sol-gel film to form the AChE immobilization matrix. [13] AuNPs was able to catalyze the electro-oxidation of thiocholine, which was the product of AChE catalyzed hydrolysis of acetylthiocholine chloride, to increase detection sensitivity. AleksandrL.Simonian et al. developed a bi-enzyme biosensor by immobilized AChE and OPH onto CNTs to discriminatively detect the organophosphorus and non-organophosphorus pesticides. [14] The CNTs not only enhanced the direct electron transfer, but also preserved the enzyme activity because of the physical adsorption between the enzyme and nanotube surface.

Researchers are also interested in modifying the CNTs to increase the sensor conductivity. As shown in Figure 3, after immobilizing the ssDNA wrapped CNTs on the gold electrode surface, Jerzy Radecki et al. coated a layer of conducting polymer polyaniline (PANI) on the CNTs surface followed by AChE immobilization to reach high sensitivity. [15]

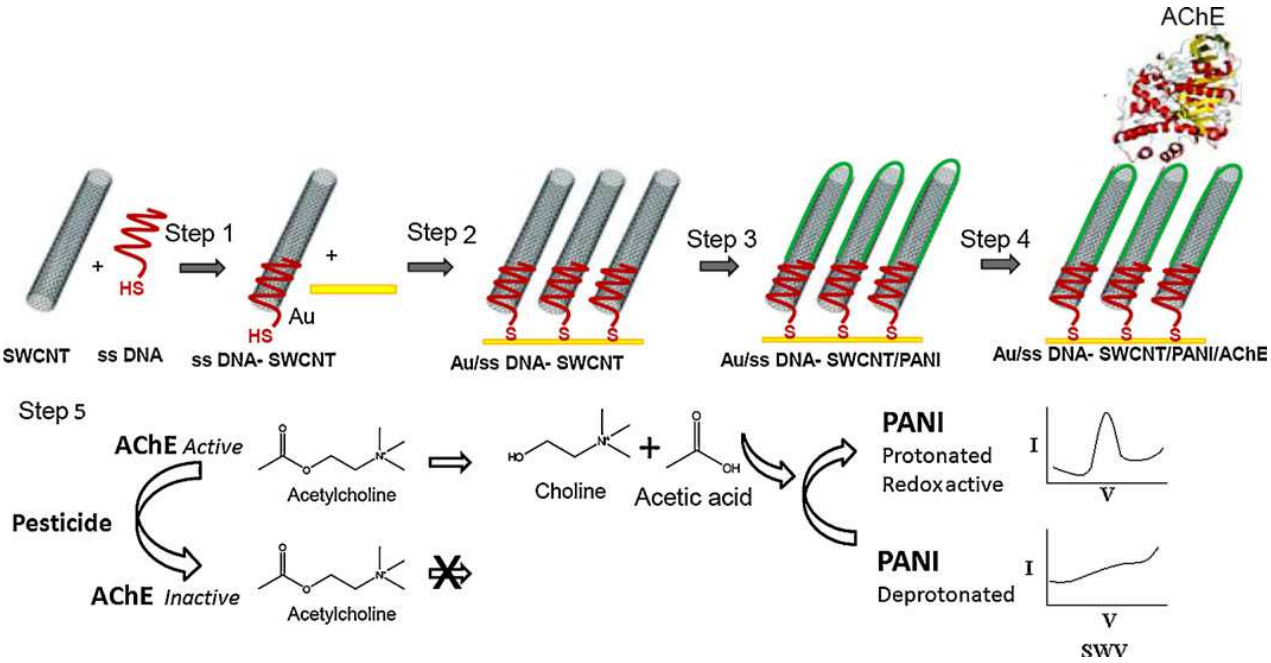


***Figure 3.*** *Modify the ssDNA wrapped CNTs with conducting PANI on gold electrode surface [15], Reprinted from Biosensors and Bioelectronics, Volume 24, Issue 9, Subramanian Viswanathan, Hanna Radecka, Jerzy Radecki, Electrochemical biosensor for pesticides based on acetylcholinesterase immobilized on polyaniline deposited on vertically assembled carbon nanotubes wrapped with ssDNA, 15 May 2009, Pages 2772-2777, Copyright (2009), with permission from Elsevier*

### *3.3. Amplify the Signal for Detection*

Amplification of the signal is crucial to detecting sensitivity. Because the very small amount of the reactants are on the biosensor, the resulting product and signals are also very low. The amplification can be achieved by either incorporating with other more sensitive detection technology or using large amount of reactants amount in short term.

Tsao-Jen Lin et al. applied the localized surface plasmon resonance (LSPR) to the AChE based biosensors (Figure 4). [16, 17] The detection principle is based on the change of light attenuation caused by inhibition of pesticide to AChE. The AChE were immobilized on the gold nanoparticles, which already self-assembled on the optical fiber core surface. In the LSPR sensor, the absorbance band of gold nanoparticle is sensitive to the refractive index (RI) of the surrounding local micro environment change. When the pesticide inhibited the AChEbonded on the gold nanoparticles, it would decrease the light intensity. By measuring the degree of intensity change, the concentration of the pesticide can be obtained.S.F. D'Souza et al. immobilized the Flavobacterium sp. whole cells on glass fiber filters, and detected the methyl parathion pesticide by measuring the p-nitrophenol (PNP) hydrolyzed from parathion by electrochemical and colorimetric methods. [18]

In order to increase the reactants amount in short term, various microfluidic devices have been developed. [19, 20] Those flow-based device can dynamically detect the large amount of the samples, and they can also be incorporated with automated system to obtain online high throughput detection.

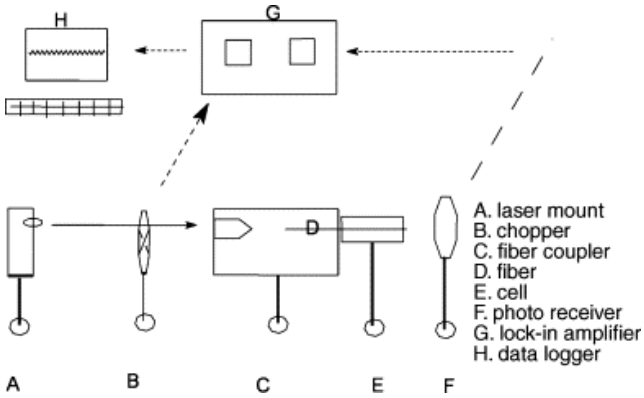


***Figure 4.*** *The schematic of LSPR based biosensor [16], Reprinted from Biosensors and Bioelectronics, Volume 22, Issue 4,Tsao-Jen Lin, Kuang-Tse Huang, Chia-Yu Liu, Determination of organophosphorous pesticides by a novel biosensor based on localized surface plasmon resonance, 15 October 2006, Pages 513-518, ISSN 0956-5663, Copyright (2006), with permission from Elsevier*

## 4. Conclusion

In this paper, we reviewed multiple types of current biosensors for pesticide residue detection. The enzyme or whole cells immobilized on the biosensor can selectively bind with the detection target and respond at very low concentration. The detection selectivity and sensitivity have been enhanced by various novel nanomaterials, nanotechnology and detection technology. Developing rapid reliable and sensitive biosensors is a promising area for the environment and food safety.

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