Biosensors

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Biosensors for Pesticides: From Concept to Technology

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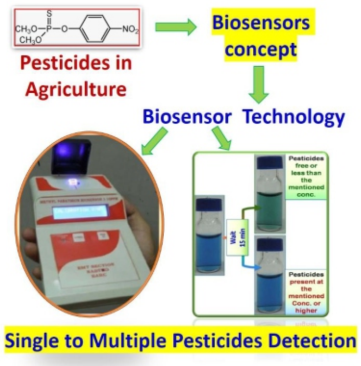

BARC technology for detection of single to multiple pesticides

ABSTRACT

Pesticides, especially insecticides, are chemicals used in many different sectors, such as agriculture, forestry, and the and the food industry, to kill or control insects. The accumulation of pesticides in the ecosystem, which are harmful to human and animal health, is detrimental to the environment. Thus, there is a need to monitor these pesticides with prompt and accurate analysis. The present article is a brief review of the work carried out at BARC for biosensor-based detection of pesticides using different biocomponents. We have developed the concept of microbial biosensors by immobilizing microbial cells on different matrices and associated them with different transducers for the detection of single to multiple samples of methyl parathion in the laboratory or field. Later on, this concept was translated into a handheld optical biosensor device for the detection of methyl parathion directly in the field. Further, a concept of enzyme-based biosensors was also developed for the detection of various pesticides belonging to the same group, and the same was translated into the technology of Biokit for the qualitative detection of multiple pesticides belonging to the organophosphate and organocarbamate groups.

KEYWORDS: *Biosensors, Pesticides, Insecticides, Microbial biosensors, Transducers, Methyl parathion*

Introduction

Due to the extensive use of pesticides in agriculture and other allied sectors, the presence of pesticides and their residues in food chains or food commodities has become a major cause of concern all over the world. Food safety has become crucial for all, and consumers have to be assured that they are not exposed to an unacceptable level of pesticide residues. Many newspapers, including The Hindu (10 June 2015 title: Chemical contaminants in household spices), The Deccan Chronicle (8 June 2015 title: Washing vegetables does not reduce pesticides), have reported the presence of pesticides in vegetables and spices [1-2]. On May 7, 2017, The Hindu and The Deccan Chronicle, reported the presence of pesticides in dried ginger powder [3–4]. In 2018, the Government of India banned 18 pesticides, which cover many pesticides belonging to the organophosphate (OP) and organocarbamate (OC) groups [5–6]. Keeping in view the dietary exposure and risk assessment, the Food Safety and Standard Authority of India (FSSAI) under the Ministry of Health and Family Welfare uses the Good Agricultural Practice (GAP) data for the fixation of the Maximal Residual Level (MRL) of each pesticide. A central scheme, "Monitoring of Pesticide Residues at National Level," was set up, and NABL-accredited laboratories located in different parts of India under the Department of Agriculture, Cooperation, and Farmers Welfare, Ministry of Agriculture and Farmers Welfare, participated in collecting and analysing the food samples for the possible presence of pesticide residues [7].

Many traditional analytical methods like HPLC and GC/LC MS-MS have been widely used for pesticide analysis, but they require not only expensive equipment but also highly-trained technicians and are time consuming and laborious. Over a course of time, researchers have put efforts to develop biosensors, which can be used for easy, online and prompt detection of pesticides with accuracy and sensitivity comparable to that of other techniques. A biosensor is an analytical device that integrates an immobilized biological element with a transducer to recognize the analyte. The signal generated by the interaction of analyte and the biological element is proportional to the concentration of analyte. Contrast to the traditional analytical methods, biosensor facilitates onsite detection of a large number of samples with almost no or very little preparation, in less time and without the requirement of expensive apparatus or trained [7-8]. In this direction, NABTD, BARC, has also developed the concept of microbial and enzyme-based biosensor technology for the detection of pesticides.

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Concept of Microbial Biosensors for Detection of Methyl Parathion Pesticide in Laboratory

Methyl parathion (MP), an extremely toxic organophosphate pesticide that was used as a non-systemic insecticide in agriculture to protect crops, is currently banned

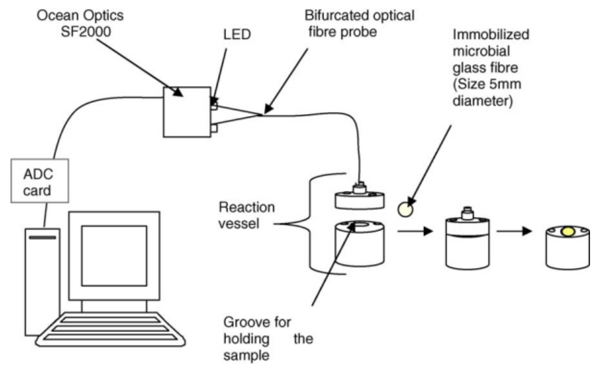


Fig.1: Schematic diagram of optical biosensor using disposable microbial biocomponent .

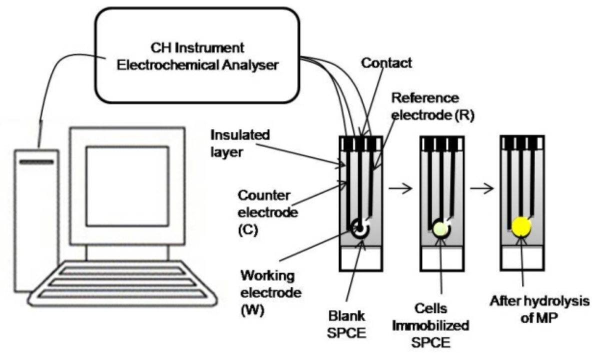


Fig.2: Schematic diagram of electrochemical biosensor using cells immobilized SPCE.

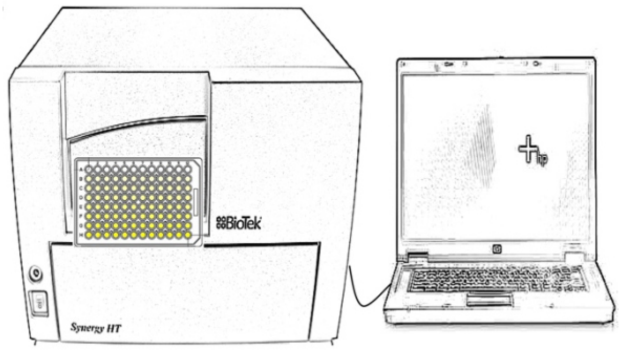


Fig.3: Schematic diagram of microplate based optical biosensor for multiple samples.

[9–11]. Our laboratory has established the concept of microbial biosensors for the detection of this pesticide. The characteristic feature of the developed microbial biosensors was their ability to detect MP in the laboratory from single or multiple samples. The first optical microbial biosensor was based on the immobilisation of whole cells of *Flavobacterium sp.* (containing organophosphorus hydrolase enzyme) on glass fibre filters. This biosensor dealt with a disposable biocomponent [12] and consequently, these biocomponents could be used for single-sample analysis only. In the second study, an electrochemical microbial biosensor was developed by immobilising recombinant *E. coli* on a screen-printed carbon electrode (SPCE) and integrating this system with an electrochemical analyzer [13]. Here, the biocomponent was reusable and required a low amount of sample. In the third study, a microplate-based optical biosensor was developed by immobilising *Sphingomonas sp.* (a) directly onto the surface of the 96-well microplate or (b) indirectly on an onion membrane fixed inside the wells of the microplate. These two types were linked with the optical transducer of a multi-detection microplate reader (MDMR). The microplate technique enables the acquisition of the whole array of data simultaneously and provides an innovative concept where multiple samples can be detected in a very short period of time [14–15]. Further integrating *Sphingomonas sp.* cells with fSi nano-particles increased the sensitivity and stability of the biocomponent [16].

All these microbial biosensor techniques require high-end, costly detection transducer systems and can only be used in laboratories due to their large size and high cost. Therefore, later on, the concept of microbial biosensors was exploited and translated into a technology for the simplified detection of MP.

Microbial Biosensors Based Technology for Detection of MP

BARC has exploited the concept of microbial optical biosensors for detection of MP and translated the research work into technology for developing a prototype handheld colorimetric biosensor, which can be used in the field for monitoring of MP pesticides (Fig.4). It has two components: The first component is the biocomponent, consisting of immobilised microbial cells of *Sphingomonas sp.* with the organophosphorus hydrolase (OPH) enzyme. The second component is the handheld optical colorimeter with an ultraviolet 3W LED light source, a small cuvette, and a microcontroller circuit. This handheld biosensor is small in size, battery-operated, and directly displays the concentration of MP in ppm (detection range 1–10 ppm) and can be utilised for the detection of MP directly in the field [17]. A microplate-based optical biosensor was able to detect MP pesticides in the range of 0.1–1.0 ppm (the FSSAI set MRL) and can detect many or multiple samples (No. 96) in a very short period of time (10 min). This was subsequently translated into technology (AB35NABTD, 18).

Biosensor Kit (Biokit) For Detection of Organophosphate and Organocarbamate Pesticides

Using the above technology, one could detect only one pesticide, methyl parathion. However, under the real field conditions, multiple pesticides are used in large amounts on crops, resulting in the accumulation of many pesticides in fruits and vegetables as well as in water bodies. Organophosphate (OP) and organocarbamate (OC) pesticides are the most commonly used pesticides for agriculture and domestic use to control insects. BARC has developed a colorimetric visual biosensor kit (BioKit: AB37NABTD) for the detection of safe levels of OP (Methyl Parathion, Parathion, Monocrotophos, Chlorpyrifos, Phorate, Profenfos, Quinalphos, and Dichlorvos) and OC (Aldicarb, Carbaryl, Carbofuran, and Carbosulfan) pesticides (Fig.5). This qualitative type of detection indicates the presence of either a single or all the 12 pesticides (as mentioned in Table included in Fig.5) that belong to the OP and OC groups. In this Biokit, a colour code of blue and green was optimised and calibrated with their respective concentrations of pesticide (Fig.5). If pesticides are either absent or present at a concentration lower than the mentioned concentration (Fig.5), then the colour will change from blue to green, and if pesticides are present at concentrations higher than the mentioned concentration, then there will be no change in colour and the blue colour will persist. This biokit is able to detect six pesticides among the 18 banned pesticides. This technology was recognised as a rapid food testing kit by

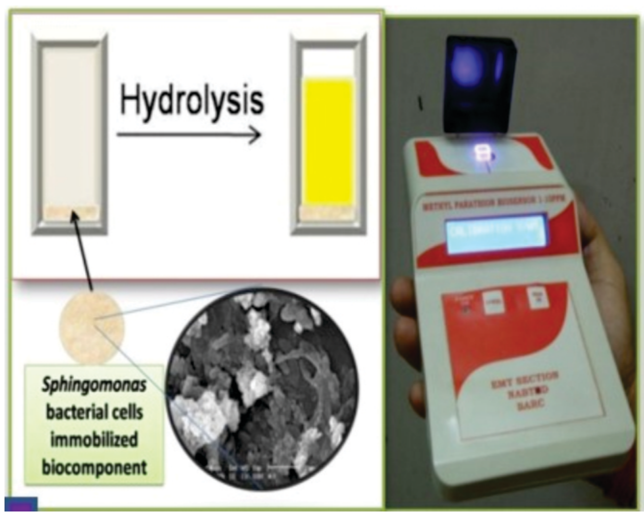
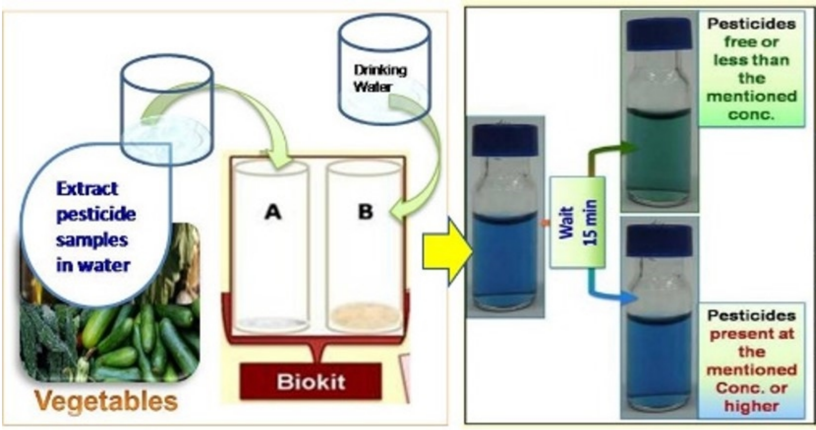


Fig.4: Handheld colorimetric microbial biosensor for detecting methyl parathion (AB28NABTD: http://barc.gov.in/technologies/biosensor/index.html)



BioKit testing 12 pesticides (OP and OC groups):

Organophosphate (OP)	Conc. (ppm) Where no colour changes	Organocarbamate (OC)	Conc. (ppm) Where no colour changes
Dichlorvos	0.2	Carbaryl	0.05
Methyl Parathion	1.0	Carbofuran	0.01
Monocrotophos	1.0	Carbosulfan	0.01
Chlorpyrifos	2.0	Aldicarb	0.05
Phorate	2.0		
Profenofos	1.0		
Parathion	0.005		
Quinalphos	0.01		

Fig.5: Biokit for detection of OP and OC pesticides (AB37NABTD:https://www.barc.gov.in/technologies/biokit/index.html)

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"TO WHOM IT MAY CONCERN"

No. FSC 20/2021/609

This is to certify that the "Blokit for detection of Pesticides" developed and demonstrated by Dr. Jitendra Kumar, Scientist-G, Bhabha Atomic Research Centre (BARC), Mumbai on 1st May, 2023 at Conference Hall of Directorate of Horticulture & FP, Khanapara, Guwahati-22 was found to be functioning satisfactorily and qualitatively response to 12(twelve) numbers of Organophosphate (8 nos.) and Organocarbonate (4 nos.) pesticides as per its detection protocol. The kit was found to be easy to handle and use.

Dated Guwahati
the 3rd May, 2023

05-05-23
Food Analyst to the Govt. of Assam, Guwahati-21
(ANUPAM GOGOI)
Food Analyst to the Govt. of Assam,
State Public Health Laboratory, Assam,
Bamunimaidam, Guwahati-21

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Fig.6: Validation certificate from Food Analyst, Governemnt of Assam, for satisfactory performance of Biokit detecting pesticides.

FSSAI in their press release (December 31, 2019) (20). Recently, the technology was validated by the State Food Analyst, Govt. of Assam (Fig.6) and transferred to many entrepreneurs, including Patanjali.

Conclusion

In this article, we have summarised the research work carried out at BARC, which has led to the development of biosensors for the detection of pesticides. Initial research work was carried out to develop the concept of biosensors. After a thorough validation, these were later translated into technologies such as handheld biosensors for the detection of methyl parathion pesticides for field application and biokits for the detection of multiple pesticides belonging to organophosphate and organocarbamate groups.

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