Plant Archives Vol. 20, Supplement 2, 2020 pp. 2509-2516 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MICROORGANISM BASED BIOSENSORS TO DETECT SOIL POLLUTANTS

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Abstract

Biosensors play a vital role in the field of environmental biotechnology. Biosensors are devices that can be used for analysis which include a biological sensing element integrated with a physical transducer to generate a signal. The signal produced is proportional to the concentration of analyte. Microorganisms can also be used as response element to obtain biosensor. Biosensor has many applications that include environmental monitoring of toxic chemicals or compounds that may affect the ecosystems as well as the life and development of humans. In this review, use of various types of microorganisms to obtain electrochemical, optical and other biosensors for pollutants detections have been discussed. Applications of biosensors for monitoring environmental pollutants such as polychlorinated biphenyls, nitrogenous compounds, pesticides as well as aromatic hydrocarbons are also addressed. Overall, the use of biosensor could serve as an alternative and a promising technology that can be used effectively for the detection and hence remediation of toxic pollutants in the environment as well as the industrial waste contaminated soil.
Keywords: Sensor, Biosensor, Environmental pollution, Soil.

Introduction

Pollution causes a very serious change in the environment and the ecology. However, alternative arrangements of power generation energy resources and reserve in India are discussed (Kumar *et al.,* 2019). It also has an effect on the economic growth of the area (Segura *et al.,* 2017). Anthropogenic activities are one of the causative agents of environmental hazard faced globally. Both developing and developed countries are suffering from industry-based pollutant (Jarque *et al.,* 2016). Human activities related to industrial production of day-to-day use goods, medicines, agricultural products are major source of pollutants into the environment. These pollutants such as trace element ions, toxic gas causes pollutants in air, water, soils that are harmful to the life and human development (Renella and Giagnoni, 2016; Kumar and Dwivedi, 2018a; Kumar and Kumar *et al*. 2018b; Kumar *et al*., 2018c; Kumar and Dwivedi, 2018d; Kumar *et al*., 2018e). Because of the increasing public awareness and strict regulation of environmental issues, monitoring of environmental pollutants related to soil, water as well as air has become necessary. Pollutants from petroleum sources due to leaking on the ground, underground storage, and spillage while transportation of petroleum products and such waste from related industries are serious soil pollutants. These kinds of pollutants are very costly to be decontaminated. Just like petroleum-based pollutants, overall methods for remediating the polluted sites by mixture of pollutant like lake water attracts more attention (Alamri, 2009). To overcome these challenges, remedial actions need to be done with a high frequency and accuracy (Geetha *et al.,* 2013, Kumar and Pathak, 2019f; Kumar *et al*., 2019g; Siddique and Kumar, 2018h; Siddique *et al*., 2018i; Pathak *et al.,* 2017j; Prakash and Kumar, 2017k; Kumar and Mandal, 2014L). To detect initial pollutant level and whether a remedial method is removing pollutants from contaminated water and soil we require accurate sensing technique, procedures and sensors. Many sensing techniques have been used for the detection of environmental monitoring; however, the techniques that have been used have some drawbacks. In a recent development, the use of biosensor serves as a promising technology that can be used for continuous detection of pollutants in the environment. Biosensors in combinations with some new technologies related to nanomaterial, molecular biology as well as microfluidic has many applications for environmental monitoring. They include real time detection of pesticides, and other environmental toxic chemicals (Dorst *et al.,* 2010; Pakshirajan *et al.,* 2015; Timur *et al.,* 2015). Use of microorganisms for the detection of soil pollutant is low cost effective approach and hence has been discussed in detail.

Sensors to Detect Environmental Pollutants

Sensors, term has been derived from Latin word "Sentire" which means to observe or perceive. Biotechnology play an important role regarding the monitoring as well as treatment of environmental pollutants prevailing in contaminated air, water, and soil. Among the different biosensing approaches available currently some are well established and few are new (Kumar *et al*., 2014m; Kumar *et al*., 2014n; Kumar, 2013o; Kumar and Dwivedi, 2015p; Gogia *et al*., 2014q; Kumar, 2014r; Kumar *et al*., 2012s). Overall the reliability regarding the performances of most of these sensors is under scrutiny. This is difficult due to challenges observed regarding sample isolation and processing in a complex environment. Continuous efforts are being made to design wearable accurate personalized sensor for robust human use Different sensor types for monitoring of environmental pollutants are categorized. The employment of these sensors depends upon the type of analyte to be detected as well as the transducer to be used (Dorst *et al.,* 2010; Pakshirajan *et al.,* 2015; Timur *et al.,* 2015; Çevik *et al.,* 2016; Lei *et al.,* 2006). Biosensor integrates biological sensing elements namely, enzymes, nucleic acids, proteins, and microorganisms with transducer to generate a signal, which is proportional to the analyte concentration under investigation. In biosensor, transducer has the responsibility of producing a response that is measurable which includes potential, current as well as absorption via electrochemical or optical process by the action of the transducer that alter the biological signal. This can further be amplified, processed that can be used for future analysis (Pakshirajan *et al.,* 2015; Lei *et al.,* 2006; Mishra *et al*., 2012t; Kumar *et al*., 2011u; Kumar *et al*., 2011v; Kumar and Pathak, 2016w; Pathak *et al*., 2016x; Kumar *et al*., 2018y; Kumar *et al*., 2018z). So, Microbial sensor is an analytical device that integrates microorganisms such as bacteria, fungi, with a physical transducer thereby generating a measurable signal (Su *et al.,* 2011). The signal produced is due to the action of the microorganism or biological recognition element produced by microorganism over analyte leads to difference in signal produced. On the other hand, such biosensor can simply be said as a device with two intimately or closely related elements (Sassolas *et al.,* 2012). In biosensor, enzymes, oligonucleotides and antibodies are commonly utilized as biosensing elements than microbes. Use of microorganisms as response element has advantages in terms of more robust and economic as compared to other biological elements (Kumar *et al*., 2018aa; Kumar *et al*., 2018bb; Kumar *et al*., 2018cc). The first biosensor for the direct quantification of glucose concentration in the sample was designed in 1962 by Clark and Lyons. In this study, glucose oxidase enzyme was immobilized on the surface of an amperometric oxygen electrode through a semi permeable dialysis membrane. This approach was further used to design various types of sensor for the detection of different substances such as cholesterol, glucose or lactic acid in biological fluid, blood, serum or urine for biomedical and environmental applications. For the past decades, growing interest has put in place biosensor for direct toxicity assessment also (Sassolas *et al.,* 2012; Chaubey and Malhotra, 2012). The use of biosensors for detecting pollutant plays a vital role in the field of biotechnology. Microbial biosensor has many applications that include environmental monitoring of toxic chemicals or compounds that may affect the ecosystems as well as the life and development of humans. In microbes ,based biosensor the amplitude of signal generated is corresponding to the growth or other metabolic activity of the microbes. Various types of microbial sensors have been designed for the detection of soil pollutants as discussed in following sections.

Types of Microbial Sensors for the Detection of Environmental Pollutants

Microbial biosensors are commonly categorized on the basis of transducers to electrochemical, optical and others (Chaubey and Malhotra, 2012; Monosik *et al.,* 2012).

Electrochemical Microbial Sensor

Electrochemical biosensor makes use of analytical power of the electrochemical techniques with specificity of biological recognition processes. The method can employ various electrodes or a three-electrode system that include reference, counter and working electrode. The bio receptor will be immobilized on a working electrode. The main target of this is to biologically generate an electrical signal that associate with the analyte concentration. The most widely employed technology is sophisticated and state-of-art to produce easy-to-use and very affordable devices (Chaubey and Malhotra, 2012). In this system, the chemical reaction that takes place between the immobilized biological sensing elements and the targeting analyte produce or make use of ions or electrons thereby affecting the properties of electric which are measurable in the solutions such as an electrical current or potential (Monosik *et al.,* 2012).

Enzyme, antibody, nucleic acid are commonly used as biological recognition element due its their specificity from analyte. However these elements are costly and require time-consuming purification steps, activities in complex test sample are some of the challenges. Use of microbes as recognition elements is cost effective and comparatively easy. The basic principle behind microbial biosensor is the use of microorganisms to produce signal or change in signal in response to the presence of analyte (Fig. 2). Although bacterial biosensors have disadvantages in terms of being less sensitive and long response time (Dai and Choi, 2012). According to the detection principle, electrochemical approaches that are commonly used for the designing of microbial sensor are divided into amperometry, potentiometry, conductometry, voltammetry and microbial fuel cell (MFC) based sensors (Su *et al.,* 2011).

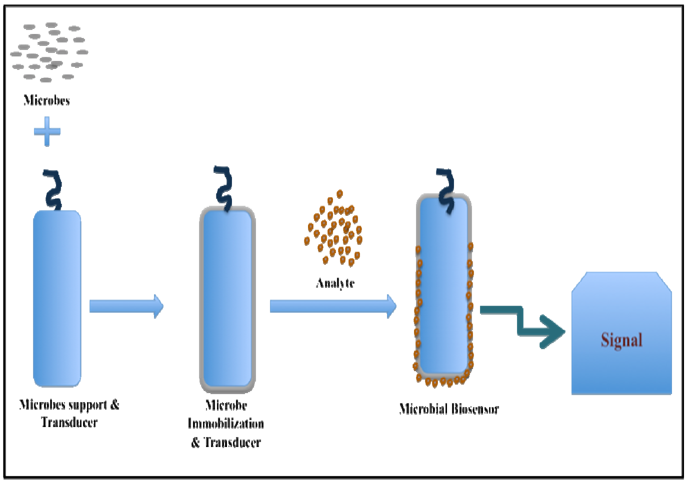

Fig. 2: Schematic diagram showing the basic mechanisms of microorganism based electrochemical biosensors.

Amperometric microbial sensor

In this type of sensor, a fixed applied potential is operated in relation to the electrode thereby leading to current detection that produced due to oxidation-reduction species over electrode surface. Amperometric sensors are widely developed for biochemical oxygen demand (BOD) detection for bioremediation of organic pollutants in water-based samples (Lei *et al.,* 2006; Su *et al.,* 2011; Chong *et al.,* 2008). In this method, a three conventional electrode system dipped into the reaction cell or container can be used under constant magnetic stirring. The analyte can be injected into the reaction cell and the change of the current as well as the responses are observed and recorded. Along with natural, genetically modified microorganism has also been used in constructing amperometric sensor. The engineered microorganism surface contains organophosphorus hydrolase enzyme that hydrolyses organophosphorus pesticides to produce oxidation current. The oxidation current is proportional to concentration of pesticide (Su *et al.,* 2011; Mulchandani *et al.,* 1998). Similarly, for zinc detection microalgae *Chlorella vulgaris* was immobilized over the surface of the diamond electrode. The presence of zinc produced change in current that was detected using cyclic voltammeter. *C. vulgaris* was entrapped in bovine serum albumin membrane to avoid fouling before immobilization over electrode surface (Verma and Singh, 2006).

Potentiometric microbial sensors

An ions-selective electrode and or gas-sensing electrode are used in this type of sensor. In this system, the electrodes mentioned above are coated by immobilization of microbial layer, thereby causing microbes to consume the analyte leading to generation of change in potential. Therefore, the transducer used here measure the variations that exists between reference electrode and working electrode. Hence, the correlation can be obtained between the signal generated and concentration of the analyte (Su *et al.,* 2011). Genetically engineered *E. coli* cells producing organophosphorus hydrolase at intracellular space have been explored for potentiometric detection of organophosphorus pesticides (Mulchandani *et al.,* 1998). Excess nickel in soil causes stress to growing plants. It is also toxic to humans at higher concentration. Various industries discard nickel and hence its detection as pollutant is much needed. Urease producing engineered *Bacillus sphaericus* strains has been designed to detect nickel (Verma and Singh, 2006). pH electrode modified by permeabilized P. aeruginosa modified pH electrodes has been used for the detection of cephalosporin antibiotics (Kumar *et al.,* 2008). The method is based on measurements of CO_2 produced by living bacteria and is related to the growth of microbial cell growth is inhibited by antibiotics. As the antibiotic are well known to inhibit microbial growth. So, the rate of CO_2 produced is directly proportional to the amount of antibiotic pollutant in a test sample. *Bacillus stearothermophilus* based potentiometric biosensor has been designed on this basis to detect Beta-lactam class of antibiotic in milk samples. This approach may be implemented for processed beta-lactam antibiotic contaminated soil samples also (Ferrini *et al.,* 2008).

Conductometry microbial sensor

In this type of sensor, microorganisms produce changes in ionic species by the catalytic actions. There is a net change in the conductivity of the sensor due to the bio catalytic reactions. In this system the measurements are very sensitive even though the detection conductance in solution is non-specific. In a recent development, to study the effect of species and concentration of the *E. coli* metabolic activity, a microbial sensor was developed (Lei *et al.,* 2006; Su *et al.,* 2011). The immobilization of *Chlorella vulgaris* microalgae over conductometric electrodes was used to design a conductometric biosensor for heavy metals, and pesticides. The biosensor could selectively detect cadmium and zinc by inhibiting alkaline phosphatase activity of microorganism. Similarly, carbamates and organophosphorus pesticides selectively inhibited acetylcholinesterase activity. Inhibiting enzyme activity led to difference in electrode conductivity in a pesticide and heavy metal concentration dependent manner (Chouteau *et al.,* 2005).

Voltammetry Microbial Sensor

This is widely adaptable method in electrochemical analysis. Here in, measurements and recording of the current and potential observe. The peak position of the current that is related to its current density and concentration of the analyte species. One major significant of this type of sensor is that, there is low noise with this system that can give rise biosensor with high sensitivity. Moreover, multiplex detection of compounds can also be possible by this sensor in samples having peak potential differences (Su *et al.,* 2011). Voltammetric detection using microorganism can also be used for the detection of life in extraterrestrial space. In this study *Pseudomonas putida* was used to reduce dye molecules to produce signal (Crawford *et al.,* 2002). Recently, a voltammetric Rhodotorula mucilaginosa based biosensor has been designed for the detection of copper ions (Yuce *et al.,* 2010). Recombinant *E. coli* and *Pseudomonas putida* expressing organophosphorus hydrolase enzyme has been used for voltammetric detection of organophosphorus pesticide, methyl parathion. Hydrolysis of methyl parathion produces oxidation current that is proportional to pesticide concentration The biosensor could selectively detect Methyl parathion among complex soil and water samples containing similar interfering pesticides like atrazine, coumaphos, sutan, sevin, and diazinon (Kumar and D'Souza, 2011a; Kumar and D'Souza, 2011b). Immobilization of Sphingomonas cell over inner epidermis of onion bulb scale was used to produce colorimetric and electrochemical detection of organophosphorus pesticide, methyl parathion. Sphingomonas contain hydrolytic activity that converts methyl parathion to produce chromophoric and electrochemically active p-nitrophenol (Kumar and D'Souza, 2011b). Enzymes based methods of detection are discussed in detail elsewhere (Nigam and Shukla, 2015).

Microbial fuel cell based sensor

Microbial fuel cells (MFC) sensor makes use of conversion of chemical energy into electrical energy due to metabolic activity of the microbes. This type of sensor can be suitable for in situ analysis as well as target chemical monitoring (Fig. 3). The target compounds are utilized by microorganisms and/or pathways metabolic inhibition by toxic compounds alter the electricity production (Su *et al.,* 2011).

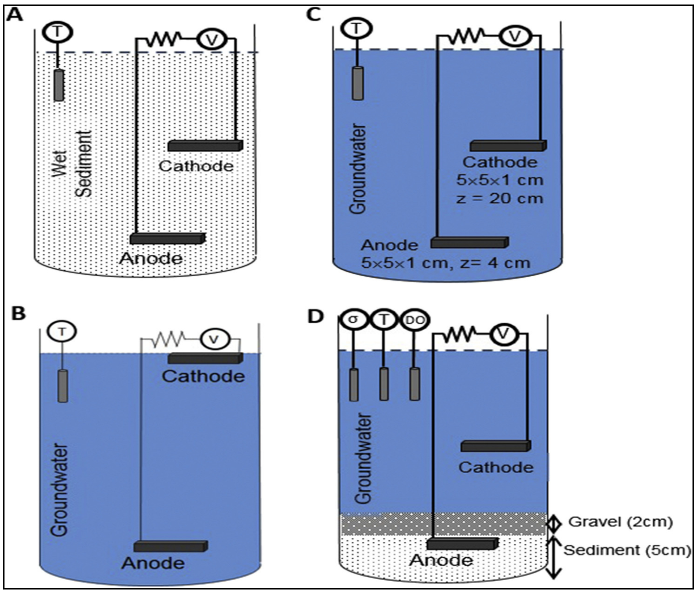

Fig. 3: Schematic diagram depicting basic components of MFC reactor. A) SED/SED MFC (sediment/sediment MFC); B) BL/Air MFC (bulk liquid/air MFC); C) BL/BL MFC (bulk liquid/bulk liquid MFC); D) SED/BL MFC, SMFC (sediment/bulk liquid MFC). Sensors used to record data were T: temperature sensor; DO: dissolved oxygen sensor; s: conductivity sensor; V: voltage sensor. Reprinted with permission, (Copyright Elsevier: Velasquez-Orta *et al.,* 2017).

Silica based MFCs have been used for detection of formaldehyde. The MFCs contain *Geobacter sulfurreducens* in the form of film over electrode. *G. sulfurreducens* is used as catalyst to produce current. Microfabricated silicon plates that acts as a current collector. So, the thickness of film over electrode is proportional to the amount of current produced. Toxic compound or chemical try to reduce growth of biofilm that is proportional to reduction in current recorded (Davila *et al.,* 2011). Likewise, the growth inhibition property of chromium (VI) has been explored for designing MFCs. Facultative anaerobe, Ochrobactrum anthropi has been used as recognition element to detect chromium (VI). The voltage output of MFCs decreased directly with level of O. anthropi growth inhibition that is directly related to chromium (VI) concentration (Wang *et al.,* 2016). Ferric phosphate NPs based MFCs has been used for the detection of soil contaminating antibiotic, levofloxacin (Zeng *et al.,* 2017).

Microbial Cell Based Optical Sensors

The use of optics for sensor development has been widely used. Measurements by optical methods are normally based on fluorescent, bioluminescent, calorimetry and other optics signal that is generated association of microbes with analytes. The observation of the optical signal generated with target compounds concentrations is correlated. The use of genetically modified microbes has been largely developed for whole-cell optical biosensors (Lei *et al.,* 2006).

Fluorescence Based Microbial Sensor

Here, the fluorescent biosensor can be categorized into in vivo and in vitro methods. In vivo biosensors use a mutant or genetically modified microbes with a reporter gene that encode fluorescent protein and combine with promoter inducible. The commonly well-known tool is the green fluorescent protein that can be encoded by gfp gene for the efficiency of detection (Su *et al.,* 2011). Recombinant Escherichia coli strain having the ability to grow in the presence of heavy metal cadmium has been used to develop colorimetric sensor. The heavy metal ion resistant bacterial cells also contain green fluorescent protein producing genes. So, the amount of green fluorescent protein expression and hence its intensity under ultraviolet illumination is proportional to concentration of cadmium heavy metals in soil and wastewater samples (Raja *et al.,* 2011). Likewise lead, Arsenite and Arsenate detection is achieved using green fluorescent protein expressing recombinant E. coli strain (Stocker *et al.,* 2003; Chakraborty *et al.,* 2008).

Bioluminescence based microbial sensor

In this type of sensor, microbes emits light, this process play a significant role in real time process detection. The gene lux of bioluminescent bacteria has largely employed as a reporter gene. This reporter gene is combining with a promoter that is regulated by the target compound concentration. For this reason, the target compounds can be analyzed by the intensity of bioluminescent quantitatively (Su *et al.,* 2011). Recombinant *Staphylococcus aureus* containing firefly luciferase has been used to develop a selective lumisensor for arsenite, antimonite, and cadmium. The expression of luciferase was proportional to the amount of arsenite, antimonite, and cadmium in test sample (Tauriainen *et al.,* 1997). Similarly, recombinant strains of *Staphylococcus aureus* and *Bacillus subtilis* have been documented for the detection of toxic heavy metals, cadmium and lead (Tauriainen *et al.,* 1998). Recombinant *E. coli* strain containing luciferase-expressing plasmid that express Lucifer enzyme in response to the presence of cadmium in a concentration dependent manner has also used to design a luminescence (Hou *et al.,* 2014). Engineered *E. coli* has also been used for the detection of nickel metal ions (Cayron *et al.,* 2017). Similar strategy is applied using *E. coli* to construct luminescence biosensor for cadmium, zinc, mercury, chromium and various other metal ions in the soil samples (Ivask *et al.,* 2002; Ivask *et al.,* 2009; Hou *et al.,* 2015). Acinetobacter and *Pseudomonas fluorescens* based bioluminescence based biosensor has been for the detection of naphthalene and phenol in contaminated soil samples (Abd-El-Haleem *et al.,* 2002; Valdman *et al.,* 2004).

Colorimetric microbial sensor

In this type of biosensor, microbes produce a color due to interaction with target compounds. Colorimetric measurements are correlated with the concentration of compound of interest. In such as study, for the detection of arsenite, a high sensitivity whole-cell biosensor was developed. Appearance of yellowish to reddish color was produced using bacteria, *Rhodovulum sulfidophilum* (Fujimoto *et al.,* 2006). Sphingomonas and Flavobacterium immobilized over the surface of polystyrene microplate and glass fibers have been used for colorimetric detection for methyl parathion (Kumar *et al.,* 2006; Kumar and D'Souza, 2010).

Applications of microbial sensors for the detection of pollutants

The use of biosensor for environmental pollutant monitoring play a significant role in maintaining the health state of the environment as well as a human being (Chong *et al.,* 2008). Different environmental pollutants including soil pollutant can be detected using microbial sensor. Various applications of microbial sensor are as following.

Detection of Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are some non-biodegradable chemical insecticides and herbicides used for the effective control of pests and herbs, respectively. However, these compounds accumulate in the soil and can be further taken up by crops. Consumption of such crop products by humans can cause serious ill effects even deaths in some cases. This is because most of these chemicals are carcinogens. To overcome these problems, a biosensor with a piezoelectric transducers based on antigen-antibody has been developed (Ferrini *et al.,* 2008; Ivask *et al.,* 2009).

Detection of Nitrogenous Compounds

Nitrogenous compound are among the toxic chemicals that cause a threat when taken above certain limit of concentration. These chemicals can affect human health as well as crippling the global economic growth. Nitrates, dioxins are nitrogenous compounds that are among the environmental pollutants. Detection of these compounds has also been achieved by a commercial sensor technique (Bahadır and Sezgintürk, 2015).

Detection of Aromatic Hydrocarbons

The use of sensor for the detection of polycyclic or aromatic hydrocarbons has been reported either using genetically modified microbes with whole cell (Çevik *et al.,* 2016; Lei *et al.,* 2006). This is because; aromatic hydrocarbons such as phenol and catechol are among the environmental toxic chemicals that can be discharged into the environment as a result of industrial and other purposes. An amperometric monooxygenase biosensor was designed for the detection of these compounds. In such a study, E. coli was used as a working organism. *E. coli* was immobilized on the surface of gold electrode using chitosan as biopolymer and iron oxide nanoparticles as signal enhancer. The immobilization procedure was achieved via entrapment method, which covalently entrapped hence due to the presence of nanoparticles large surface area to volume ratio. The iron oxide nanoparticle were used to increase the transfer of electrons thereby achieving a fast and sensitive response of the sensor (Cevik *et al.,* 2016).

Detection of heavy metals

Heavy metals also are among the environmental pollutants that can cause a serious disaster in the ecosystems. Biosensor has been designed to detect heavy metals in wastewater and contaminated soil. An ammonia selective electrode coupled with enzyme urease by the inhibition activity of the enzyme was developed for the measurements of these heavy metals. Likewise biosensors have been designed for sensing of various toxic heavy metals (Ivask *et al.,* 2009; Hou *et al.,* 2009; Chouteau *et al.,* 2005; Kumar and D'Souza, 2010)

Detection of pesticides and derbicides

Biosensors play a vital role in monitoring pesticides, for example, organophosphates are among the most widely used as pesticides like insecticides. Insecticides are chemicals used for the treatment of many insects that can affect the plant growth. Further such chemicals can undergo bioaccumulation in vegetables and grains leading to adverse effects over human growth, development and health. During the processes, these chemicals can also cause environmental changes by altering the soil fertility as such destroying many important microbes and insects that are beneficial, consequently affecting the biodiversity toward negative impacts. Nanobiosensors have also been used to detect presence of pesticides in contaminated water and soil samples (Kumar *et al.,* 2006; Kumar and D'Souza, 2010). Following are some of the pollutants that employed biosensor for their detections. Polychlorinated biphenyls (PCBs) are some non-biodegradable chemical insecticides and herbicides used for the effective control of pests and herbs, respectively. However, these compounds accumulates in the soil which are taken up by crops, when taken by humans can cause serious threats to their life. This is because most of these chemicals are carcinogens. So, biosensor with a piezoelectric transducers base has been designed to overcome this challenge. The biosensor is based on the specificity of antigen-antibody reaction (Ivask *et al.,* 2002). Recently nanobiosensors have been designed to measure and quantify the presence of these chemicals including pesticides in contaminated water and soil samples (Mulchandani *et al.,* 2001; Chouteau *et al.,* 2005; Wei *et al.,* 2015).

Conclusion

Pollutions bring about a disastrous effect in the ecosystems, hence leading to the crippling economic growth. Pollutants are also a threat to the lives of humans and other living organisms. The use of biosensors for environmental monitoring and industrial applications for this purpose plays an important role in the field of biotechnology. Therefore, there is an urgent need for the improvement of these methods for the detection of environmental pollutants by modifying the earlier reported methods. In addition, a multiplex biosensor needs to be employed largely that allow detection of multiple analyte in a single phase or run. The use of multiplex sensor can highly reduce the time consuming and manages the use of much manpower as well as cost-effective. Use of nanoparticles in various kinds of biosensor design is a good future option.

References

Abd-El-Haleem, D., S. Ripp, C. Scott, and G.S. Sayler (2002). A luxCDABE-based bioluminescent bioreporter for the detection of phenol. J. Ind. Microbiol. Biotechnol., 29(9), p. 233-237.

Alamri, S. A (2009). Use of microbiological and chemical methods for assessment of enhanced hydrocarbon bioremediation. J. Biolog. Sciences, 9(1), p. 37-43.

Bahadır, E.B. and M.K. Sezgintürk (2015). Applications of commercial biosensors in clinical, food, environmental, and biothreat/biowarfare analyses. Anal. biochem., 478(2015), p. 107-120.

Cayron, J., E. Prudent, C. Escoffier, E. Gueguen, M. A. Mandrand-Berthelot, D. Pignol and A. Rodrigue (2017). Pushing the limits of nickel detection to nanomolar range using a set of engineered bioluminescent *Escherichia coli*. Environ. Sci. Poll. Res., 24(1), p. 4-14.

Çevik, E., M. Dervisevic, A.R. Gavba, K.C. Yanik-Yildirim, M.F. Abasiyanik and G. Vardar-Schara (2016). Amperometric monooxygenase biosensor for the detection of aromatic hydrocarbons. Sens. Letts., 14(3), p. 234-240.

Chakraborty, T., P.G. Babu, A. Alam and A. Chaudhari (2008). GFP expressing bacterial biosensor to measure lead contamination in aquatic environment. Curr. Sc., 94(2008), p. 00113891.

Chaubey, A. and B. Malhotra (2002). Mediated biosensors. Biosens. Bioelectron., 17(6-7), p. 441-456.

Chong, K. F., K.P. Loh, K. Ang and Y.P. Ting (2008). Whole cell environmental biosensor on diamond. Analyst, 133(6), p. 739-743.

Chouteau, C., S. Dzyadevych, C. Durrieu, and J.M. Chovelon (2005). A bi-enzymatic whole cell conductometric biosensor for heavy metal ions and pesticides detection in water samples. Biosen. Bioelectron., 21(2), p. 273-281.

Crawford, R. L., A. Paszczynski, Q. Lang, D. P. Erwin, L. Allenbach, G. Corti and R. Wells (2002). Measurement of microbial activity in soil by colorimetric observation of in situ dye reduction: an approach to detection of extraterrestrial life. BMC Microbio. 2(1), p. 22.

Dai, C. and S. Choi (2013). Technology and applications of microbial biosensor. Open J. App. Biosen., 2, p. 83.

Davila, D., Esquivel, J. P., N. Sabate and J. Mas (2011). Silicon-based microfabricated microbial fuel cell toxicity sensor. Biosens. Bioelectron., 26(5), p. 2426-2430.

Ferrini, A. M., V. Mannoni, G. Carpico and G.E. Pellegrini (2008). Detection and identification of β-lactam residues in milk using a hybrid biosensor. J. Agri. Food Chem., 56(3), p. 784-788.

Fujimoto, H., M. Wakabayashi, H. Yamashiro, I. Maeda, K. Isoda, M. Kondoh and K. Yagi (2006). Whole-cell arsenite biosensor using photosynthetic bacterium

Rhodovulum sulfidophilum. Appl. Microbio. Biotechnol., 73(2), p. 332-338.

Geetha, S. J., S. J. Joshi and S. Kathrotiya (2013). Isolation and characterization of hydrocarbon degrading bacterial isolate from oil contaminated sites. APCBEE Proced., 5, p. 237-241.

Hou, Q. H., A.Z. Ma, Y. Li, X.L. Zhuang, Z.H. Bai, X.K. Zhang and G.Q. Zhuang (2014). Assessing the effect of phosphate and silicate on Cd bioavailability in soil using an Escherichia coli cadAp: luc-based whole-cell sensor. Envir. Sci. Process. Impact., 16(4), p. 890-896.

Hou, Q., A. Ma, T. Wang, J. Lin, H. Wang, B. Du, and G. Zhuang (2015). Detection of bioavailable cadmium, lead, and arsenic in polluted soil by tailored multiple Escherichia coli whole-cell sensor set. Anal. Bioanal. Chem., 407(22), p. 6865-6871.

Ivask, A., T. Rolova and A. Kahru (2009). A suite of recombinant luminescent bacterial strains for the quantification of bioavailable heavy metals and toxicity testing. BMC Biotechnol., 9(1), p. 41.

Ivask, A., M. Virta, and A. Kahru (2002). Construction and use of specific luminescent recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium, zinc, mercury and chromium in the soil. Soil Biol. Biochem., 34(10), p. 1439-1447.

Jarque, S., M. Bittner, L. Blaha and K. Hilscherova (2016). Yeast biosensors for detection of environmental pollutants: current state and limitations. Trend. Biotechnol., 34(5), p. 408-419.

Kumar, J. and S.F. D'Souza (2010). An optical microbial biosensor for detection of methyl parathion using Sphingomonas sp. immobilized on microplate as a reusable biocomponent. Biosen. Bioelectron., 26(4), p. 1292-1296.

Kumar, J. and S.F. D'Souza (2011). Immobilization of microbial cells on inner epidermis of onion bulb scale for biosensor application. Biosens. Bioelectron., 26(11), p. 4399-4404.

Kumar, J. and S.F. D'Souza, (2011b). Microbial biosensor for detection of methyl parathion using screen printed carbon electrode and cyclic voltammetry. Biosens. Bioelectron., 26(11), p. 4289-4293.

Kumar, J., S. K. Jha, and S. F. D'souza, (2006). Optical microbial biosensor for detection of methyl parathion pesticide using Flavobacterium sp. whole cells adsorbed on glass fiber filters as disposable biocomponent. Biosen. Bioelectron., 21(11), p. 2100-2105.

Kumar, R., K. Ojha, M. H. Ahmadi, R. Raj, M. Aliehyaei, A. Ahmadi and N. Nabipour, (2019). A review status on alternative arrangements of power generation energy resources and reserve in India. International Journal of Low-Carbon technologies., ctz066, p. 1-17.

Kumar, S., S. Kundu, K. Pakshirajan and V.V. Dasu, (2008). Cephalosporins determination with a novel microbial biosensor based on permeabilized Pseudomonas aeruginosa whole cells. Appl. biochem. Biotechnol., 151(2-3), p. 653-664.

Lei, Y., W. Chen and A. Mulchandani (2006). Microbial biosensors. Anal. Chim. Acta, 568(1-2), p. 200-210.

Lei, Y., Mulchandani, P., Chen, W., & Mulchandani, A. (2005). Direct determination of p-nitrophenyl substituent organophosphorus nerve agents using a recombinant *Pseudomonas putida* JS444-modified Clark oxygen electrode. J. Agri. Food Chem., 53(3), p. 524-527.

Monosik, R., M. Stredansky and E. Sturdík (2012). Biosensors-classification, characterization and new trends. Acta Chim. Slov., 5(1), p. 109-120.

Mulchandani, A., Mulchandani, P., Chauhan, S., Kaneva, I., and Chen, W. (1998). A potentiometric microbial biosensor for direct determination of organophosphate nerve agents. Electroanalysis: Int. J. Devot. Fund. Pract. Aspect. Electroanal., 10(11), p. 733-737.

Mulchandani, P., W. Chen, A. Mulchandani, J. Wang, and L. Chen (2001). Amperometric microbial biosensor for direct determination of organophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. Biosens. Bioelectron., 16(7-8), p. 433-437.

Nigam, V.K and P. Shukla (2015). Enzyme based biosensors for detection of environmental pollutants-A review. J. Microbiol. Biotechnol., 25(11), p. 1773-1781.

Pakshirajan, K., Rene, E.R., and Ramesh, A. (2015). Biotechnology in environmental monitoring and pollution abatement 2015. BioMed Res. Int., 2015(2015), p. 963803.

Raja, C.E. and G.S. Selvam (2011). Construction of green fluorescent protein based bacterial biosensor for heavy metal remediation. Int. J. Environ. Sci. Technol., 8(4), p. 793-798.

Renella, G. and L. Giagnoni (2016). Light dazzles from the black box: whole-cell biosensors are ready to inform on fundamental soil biological processes. Chem. Biol. Technol. Agricul., 3(1), p. 8.

Sassolas, A., L.J. Blum and B.D. Leca-Bouvier (2012). Immobilization strategies to develop enzymatic biosensors. Biotechnol. Adv., 30(3), p. 489-511.

Segura, A., V. Hernández-Sánchez, S. Marques and L. Molina (2017). Insights in the regulation of the degradation of PAHs in Novosphingobium sp. HR1a and utilization of this regulatory system as a tool for the detection of PAHs. Sci. Tot. Environ., 590, p. 381-393.

Stocker, J., D. Balluch, M. Gsell, H. Harms, J. Feliciano, S. Daunert, and J. R. VanderMeer, (2003). Development of a set of simple bacterial biosensors for quantitative and rapid measurements of arsenite and arsenate in potable water. Envir. Sci. Technol., 37(20), p. 4743-4750.

Su, L., W. Jia, C. Hou and Y. Lei, (2011). Microbial biosensors: a review. Biosens. Bioelectron., 26(5), p. 1788-1799.

Tauriainen, S., M. Karp, W. Chang, M. Virta (1997). Recombinant luminescent bacteria for measuring bioavailable arsenite and antimonite. Appl. Environ. Microbiol., 63(11), p. 4456-4461.

Tauriainen, S., M. Karp, W. Chang, M. Virta (1998). Luminescent bacterial sensor for cadmium and lead. Biosens. Bioelectron., 13(9), p. 931-938.

Timur, S., U. Anik, D. Odaci and L. Gorton (2007). Development of a microbial biosensor based on carbon nanotube (CNT) modified electrodes. Electrochem. Comm., 9(7), p. 1810-1815.

Valdman, E., F. Battaglini, S.G.F. Leite and B. Valdman (2004). Naphthalene detection by a bioluminescence sensor applied to wastewater samples. Sens. Actuat. B, 103(1-2), p. 7-12.

Van Dorst, B., J. Mehta, K. Bekaert, E. Rouah-Martin, W. DeCoen, P. Dubruel and J. Robbens (2010). Recent advances in recognition elements of food and environmental biosensors: a review. Biosens. Bioelectron., 26(4), p. 1178-1194.

Velasquez-Orta, S. B., D. Werner, J. C. Varia, and S. Mgana (2017). Microbial fuel cells for inexpensive continuous in-situ monitoring of groundwater quality. Water Res., 117, p. 9-17.

Verma, N. and M. Singh (2006). A Bacillus sphaericus based biosensor for monitoring nickel ions in industrial effluents and foods. J. Anal. Method. Chem., 2006: 83427

Wang, G.H., C.Y. Cheng, M.H. Liu, T.Y. Chen, M.C. Hsieh and Y.C. Chung (2016). Utility of Ochrobactrum anthropi YC152 in a microbial fuel cell as an early warning device for hexavalent chromium determination. Sensors, 16(8), p. 1272.

Wei, M., J. Rakoczy, C. Vogt, F. Harnisch, R. Schumann and H.H. Richnow (2015). Enhancement and monitoring of pollutant removal in a constructed wetland by microbial electrochemical technology. Biores. Technol., 196, p. 490-499.

Yuce, M., H. Nazır and G. Donmez (2010). A voltammetric Rhodotorula mucilaginosa modified microbial biosensor for Cu (II) determination. Bioelectrochem., 79(1), p. 66-70.

Zeng, L., Li, X., Shi, Y., Qi, Y., Huang, D., Tade, M., & Liu, S. (2017). $FePO_4$ based single chamber air-cathode microbial fuel cell for online monitoring levofloxacin. Biosens. Bioelect., 91, p. 367-373. Kumar, P. Purnima *et al*., 2018e. "Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity" *International Journal of Current Microbiology and Applied Sciences*, vol. 7(10), pp. 1635-1639.

Kumar, P. Pathak, S. 2019f. "Responsiveness index of sorghum (Sorghum bicolor (l.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza" *Bangladesh J. Bot.* vol.48 (1).

Kumar, P. Siddique, A. *et al.* (2019g). "Role of Polyamines and Endo-mycorrhiza on Leaf Morphology of Sorghum Grown under Cadmium Toxicity" *Biological Forum – An International Journal.* vol.11 (1) pp. 01-05.

Siddique, A. Kumar, P. (2018h). "Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview" *Plant Archive*, 18(2), pp. 1933-1937.

Siddique, A., Kandpal, G., Kumar P. (2018i). "Proline accumulation and its defensive role under Diverse Stress condition in Plants: An Overview" *Journal of Pure and Applied Microbiology*, vol.12 (3) pp.1655-1659.

Pathak, S., Kumar, P., P.K Mishra, M. Kumar, (2017j). "Mycorrhiza assisted approach for bioremediation with special reference to biosorption", *Pollution Research*, Vol. 36(2).

Prakash, A., P. Kumar, (2017k). "Evaluation of heavy metal scavenging competence by in-vivo grown Ricinus communis L. using atomic absorption spectrophotometer" *Pollution Research*, vol.37(2), pp.148-151.

Kumar, P., Mandal, B., (2014L). Dwivedi, "Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants" *vegetos*, vol.26(2), pp. 416-425.

Kumar, P., Mandal, B., Dwivedi P., (2014m). "Phytoremediation for defending heavy metal stress in weed flora" *International Journal of Agriculture, Environment & Biotechnology*, 6(4), pp. 587-595.

Kumar, P., Kumar, P.K., Singh, S. (2014n). "Heavy metal analysis in the root, shoot and a leaf of psidium guajava l. by using atomic absorption spectrophotometer" *Pollution Research*, .33 (4) pp.135-138.

Kumar, P. (2013o). "Cultivation of traditional crops: an overlooked answer. *Agriculture Updat*e, vol.8 (3), pp.504-508.

Kumar, P., Dwivedi, P. (2015p). "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" *Journal of Scientific Research*, B.H.U., 59, pp.121-148.

Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. (2014q). "Cloning and molecular characterization of an active gene from garlic (Allium sativum L.)" *International Journal of Agriculture, Environment and Biotechnology*, vol.7 (1), pp.1-10.

Kumar, P., (2014r). "Studies on cadmium, lead, chromium, and nickel scavenging capacity by in-vivo grown Musa paradisiacal. using atomic absorption spectroscopy" *Journal of Functional and Environmental Botany*, vol.4(1), pp.22-25.

Kumar, P., Dwivedi, P., Singh, P. (2012s). "Role of polyamine in combating heavy metal stress in stevia rebaudiana Bertoni plants under in vitro condition" *International Journal of Agriculture, Environment and Biotechnology*, 5(3) pp.185-187.

Mishra, P.K., Maurya, B.R., Kumar, Pp. (2012t). "Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season" Journal of Functional and Environmental Botany, 2(2): 1-6.

Kumar, P., Mandal, B., Dwivedi, P. (2011u). "Heavy metal scavenging capacity of Mentha spicata and *Allium cepa*" Medicinal Plant-*International Journal of Phytomedicines and Related Industries* vol. 3(4) pp. 315-318.

Kumar, P., Mandal, B., Dwivedi, P. (2011v). "Screening plant species for their capacity of scavenging heavy metals from soils and sludges. *Journal of Applied Horticulture*, 13 (2), 144-146.

Kumar, P., Pathak, S. (2016w). "Heavy metal contagion in seed: its delivery, distribution, and uptake" *Journal of the Kalash Sciences, An International Journal*, 4(2), 65-66.

Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. (2016x). "Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for a cure". *Journal of the Kalash Sciences, An International Journal*, 4(2): 61-65.

Kumar, P., Harsavardhn, M. *et al.*, (2018y). "Effect of Chlorophyll a/b ratio in Cadmium Contaminated Maize Leaves Treated with Putrescine and mycorrhiza" *Annals of Biology* 34(3)-281-283.

Kumar, P., Yumnam, J. *et al*., (2018z). "Cadmium Induced Changes in Germination of Maize Seed Treated with Mycorrhiza" Annals of *Agri-Bio Research*, 23(2); 169-170.

Kumar, P., Pandey, A.K., *et al*., (2018aa). "Phytoextraction of Lead, Chromium, Cadmium, and Nickel by Tagetes

Plant Grown at Hazardous Waste site" *Annals of Biology*, 34(3): 287-289.

Kumar, P., Kumar, S. *et al.*, (2018bb). "Evaluation of Plant Height and Leaf Length of Sorghum Grown Under Different Sources of Nutrition" *Annals of Biology*, 34(3): 284-286.

Kumar, P., Krishna, V., *et al.*, (2018cc). "Assessment of Scavenging Competence for Cadmium, Lead, Chromium and Nickel Metals by in vivo Grown Zea mays L. using Atomic Absorption Spectrophotometer, *Annals of Ari-Bio Research*, 23(2): 166-168.