***Review Paper:***

# Microbial Biosensors: Design considerations, applications and challenges

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## *Abstract*

*Microbial biosensors have emerged as a cutting-edge breakthrough in sensing and analyte detection, across facets of human life. Essentially, a microbial biosensor integrates a biosensing microbial species that either triggers or limits the expression of a reporter gene in response to an external analyte recognised by a responsive genetic circuit. The signal from the reporter gene is in direct proportion to the levels of target analyte, across a wide range of samples: soil, water, food, clinical samples etc. In the present review, we have focussed on the technical considerations while designing and development of a microbial biosensor: chassis cell, genetically engineered strains, the commonly used transducing elements (reporter genes such as lux/luc, lacZ, gfp).*

*The microbial biosensors have displayed immense potential for detection of heavy metals, toxics, pollutants across environmental samples. Their application is illustrated in ensuring food safety and detection of contaminants such as pesticide residues, bacterial contaminants. Biomedical applications such as utility for detection of diseases' biomarkers for major human diseases (cancer, gut inflammation, colitis) have also been discussed and elaborated, especially the potential for use of engineered commensal/probiotic microbes for real-time monitoring of in vivo disease status. In this comprehensive review, we also discuss the challenges limiting the translational scope of microbial biosensors and discuss potential current efforts to address them.*

**Keywords:** Microbial biosensor, chassis cell, synthetic biology, reporter genes, probiotic biosensors.

## Introduction

Human health care and environmental stewardship demand accurate and fast detection of pathogens (existing and emerging), toxins of biological and chemical origin, pollutants and xenobiotics entering the food chain due to anthropogenic activity etc. There is thus an ever-evolving need for easy to use, cost-effective, sensitive and specific detection systems such as biosensors [2]. A biosensor is an analytical tool that combines a biological recognition component with a physical transducer to generate a detectable signal proportional to the concentration of target analytes[33]. Whole cell or microbial biosensors which utilize microorganisms such as algae, bacteria and unicellular yeasts, present a valuable detection system due to their ease of manipulation, superior viability and large-scale production capabilities through cell culturing methodologies[64].

Microbes work as a vast reservoir for a variety of cofactors, enzymes and other biological components that allow them to respond and sense to a vast range of chemicals. Although microbial metabolism tends to be non-specific, specificity can be enhanced by manipulating metabolic pathways to a targeted approach, making highly selective biosensors possible. With the manipulation of microbial systems, biosensors with higher accuracy in detecting specific analytes can be developed, making them useful for applications in environmental monitoring, food safety and clinical diagnostics. Effectiveness and selectivity of microbial biosensors can be enhanced by adapting the culture via selective cultivation strategies and with specific targeted substrates[83].

The present arena of microbial biosensors-based applications includes: monitoring the food additives, biomolecules and environmental pollution in clinical specimens that facilitate the prevention and diagnosis of diseases, ensuring regulatory compliance, supporting epidemiological studies, risk assessment and advancing research. Further these sensors can also facilitate detection of exposure to infectious agents or substance abuse, to ensure amenability with regulatory standards via effective monitoring and also to play a vital role in maintaining consumer safety and health [84].

Environmental monitoring, fermentation and food industries and clinical diagnostic labs have benefitted immensely from use of microbial biosensors because of their stability, portability, fast response and cost effectiveness. In contrast, traditional detection methods often required specialized equipment, are slow and depend on experience of the personnel besides being cost-intensive. Additionally, the microbial sensors can be used both indoor and outdoor, reliably and durably for varied applications[21,64]. The latest developments in genetic engineering and synthetic biology have taken the potential of microbial biosensors to a new level. In these fields, we are now able to make more precise changes to the pathways that govern the metabolism of the microbes we use. This allows for the tailoring of whole-cell

biosensors with unprecedented accuracy and or diverse applications, utilising one or several specific analytes[84].

For example, the seminal study by Chang and co-workers[20] reported a synthetic receptor platform: Engineered Modularized Receptors activated via Ligand-induced Dimerization (EMeRALD) which allows for a modular assembly of sensing modules into a signaling framework regulating the gene expression in model system. They then applied the EMeRALD technology for detecting levels of bile salts, an established indicator of hepatic dysfunction, by incorporating sensing modules from *Vibrio*. The bactosensor developed had higher sensitivity and lower limit-of-detection achieved via directed evolution. Eventually, the study led to development of a point-of-care colorimetric biosensor for detecting pathological bile salt systemic levels[22].

**Design considerations for a microbial biosensor**

The basic features in the design of a microbial biosensor include the integration of biological sensing elements with a transducer to convert biological responses into measurable signals. Essentially, the analyte is identified by a native/engineered genetic circuit which induces on/off the expression of another genetic circuit carrying a reporter gene. The reporter gene is coupled to the transducer element of the biosensor[64]. The key considerations while designing a microbial biosensor are elaborated hereafter (Figure 1):

- **Characterization of analyte:** The preliminary requirement before designing a microbial biosensor is detailed understanding of physico-chemical features of the analyte that is to be detected: a small molecule (pollutant, antibiotic, chemical byproduct), a whole cell (pathogenic or of industrial use), a biomolecule (protein, antibody, toxin etc.). The understanding of the analyte will lead to the detection elements that will specifically recognize the analyte, will have reduced false positive or false negative responses recorded and will confer reproducibility to the biosensor developed[9,91].

- **Selection of suitable microorganisms:** The biological plasticity and adaptability of a microbe are keys to its selection as a sensing element in a microbial biosensor. Choosing microorganisms that will naturally exhibit a particular degree of sensitivity or affinity for the target analyte of interest is the first step [91]. For instance, certain bacteria have receptors or enzymes that allow them to bind to specific chemicals or pollutants, making them suitable for biosensing applications. The ability of the selected organism to endure and flourish under the suggested usage settings is another crucial consideration[1]. Variables such as temperature, pH, and the presence of potential additional considerations such as the presence of interfering substances, must be taken into account to guarantee the reliability and robustness of the biosensor's performance[70]. In addition, the chosen microorganism should be capable of gain-response to match these through maintaining their unit significance. These signals may be detected as changes in the activity of specific enzymes, fluorescence, bioluminescence, or the production of specific metabolites and should be stable for accurate detection. Factors such as feasibility of microbial growth under laboratory, production and storage conditions as well as immobilization onto the biosensor are also critical to select a microbe.

Additionally, while developing microbial biosensors for food industry or healthcare industry in direct contact with humans, that expected the microorganism selected should be Generally Recognized as Safe (GRAS)[71,88]. Finally, the total cost for the use (incubation, maintenance, production etc.) of the microorganism in the biosensor must be considered. Cost-effectiveness represents a major component in the practical and commercial application of the biosensor system[2,68].

**Genetic modifications:** Genetic modifications and ability to engineer bacteria, yeast or fungal cells to identify a target analyte with measurable signal allow for a wider diversity for detection and measurement of analytes in diverse environmental or industrial backdrops. By employing genetic engineering approaches, there have been significant improvements in the detection limits and specificity of analyte detection. The pH based colorimetric sensor developed by de Mora and colleagues[28], detected arsenic in groundwater up to 10µg/l after overnight incubation. Using the arsR–lacZ recombinant gene cassette in a *Escherichia coli* DH5α strain, Chang and co-workers[20] prepared a colorimetric microbial biosensor with detection range of 10 to 500 µg/L of arsenic in mere 3h of incubation time[14,22,29].

Thus, availability of functional genome annotation data, ease of genetic manipulation and availability of compatible genetic modification tools should also be taken into account when selecting a microbe as a sensing unit and designing a microbial biosensor. In a non-engineered microbial cell, the promotor or cis-acting elements regulate the expression of genes encoding the response to a chemical or a protein (analyte). In a microbial biosensor, the cis-acting elements linked to the genes are disrupted and are replaced by reporter genes such as lux/luc (firefly/bacterial luciferase enzyme), lacZ (β-galactosidase) and gfp (green fluorescent protein). The reporter gene when expressed generates a bioluminescent, fluorescent, or colorimetric signal[100].

The expression of the reporter gene can be under two types of regulatory controls and hence two categories of genetically engineered sensing elements exist: constitute expression and inducible expression. The constitutively expressed reporter gene was expressed at high levels, however with exposure to analyte, the signal intensity generated decreased proportional to the intensity of the toxicity of the analyte.

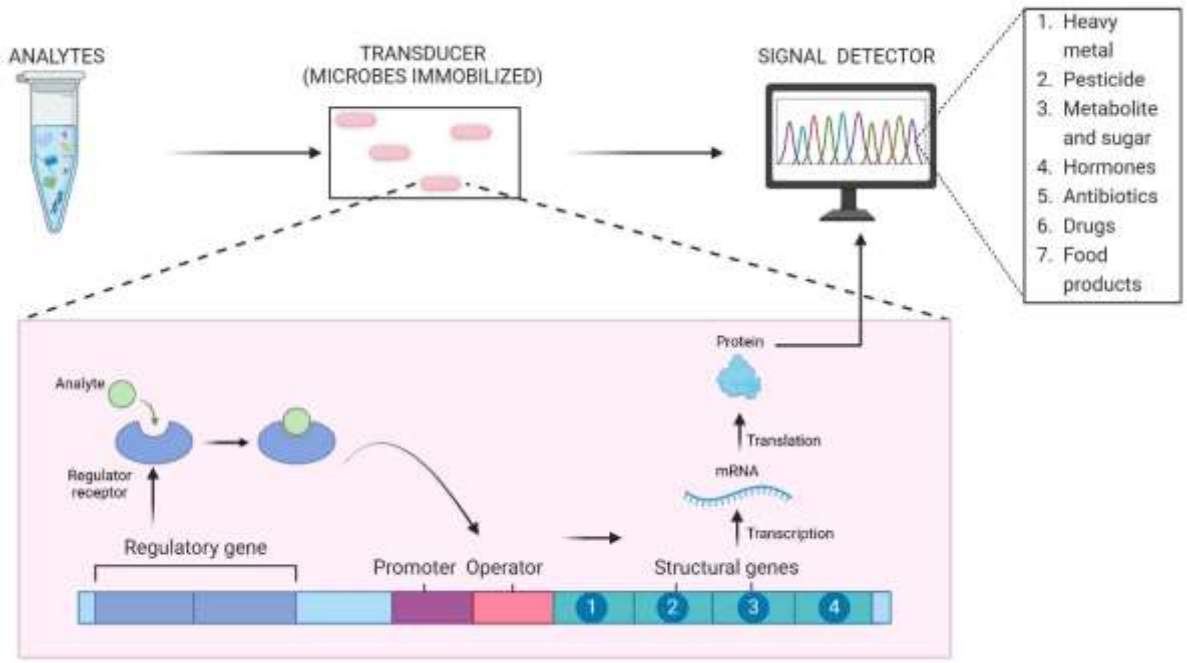


**Figure 1: Schematic of microbial biosensor design**

This format of microbial biosensors was non-specific and non-selective[75]. Despite the shortcomings, the constitutive microbial biosensors were widely used for detection of environmental monitoring and also found application as diagnostics (detection of urinary tract infection pathogens *Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Candida albicans*)[99].

However, for an inducible format, the biosensing framework involves constitutive expression of a regulatory protein which recognizes the analyte, the analyte-sensing protein complex and then control the expression of a reporter gene under the control of an inducible promoter[58,74]. Synthetic genetic circuits using a two component regulatory system (TCRS) from prokaryotic and eukaryotic systems exemplify the case of inducible biosensors: TCRS is composed of a histidine kinase sensor and a response regulator. The histidine kinase is a homodimer localized in the plasma membrane containing a sensor loop on the extracellular side sandwiched between two transmembrane domains and a transmitter domain on the intracellular side.

On sensing the external stimuli, the histidine kinase auto-phosphorylates conserved histidine residues. Hereafter the phosphate moiety is transferred to the response regulator on specific aspartate residues, bringing a conformational change activating it. The response regulators are usually transcription factors which activate or repress genes by binding at their promotor sites[78]. Synthetic TCRS genetic circuitry has been employed for detection of heavy metals, organic pollutants etc.[74]

- **Chassis cell:** Chassis cells provide the "hardware" for a sensing genetic circuit. They are essentially simple cells (SimCells) with genetic encoding only for basic functions of viability and non-essential genetic networks are obliterated. The "software" encodes the sensing function for target analyte and the genetic elements needed for functioning in the target environment. Some widely used chassis cells are *E. coli, Bacillus subtilis, Lactococcus lactis, Mycoplasma mycoides,* Chinese hamster ovary cells etc.[13,76] Whole cell biosensors are developed for detection of low levels of pollutants, in a cyanobacterial chassis cell. These cyanobacterial biosensors offer high sensitivity, broad dynamic range, are cost-effective and stable for long durations[60].

- **Transducer element:** A transducer converts the interaction of the target molecule and the recognition element into a signal that can be detected. The earliest microbial cell was developed by employing an electrochemical sensor for detection and measurement of electroactive moieties[69]. The present technologies rely on colorimetric, fluorescent, or bioluminescent proteins for transduction of analyte-sensing element interaction. As mentioned earlier, lux/luc (firefly/bacterial luciferase enzyme), lacZ (β-galactosidase) and gfp (green fluorescent protein) are widely preferred and reported genes. Bacterial bioluminescence uses a bacterial luciferase gene cassette - luxCDABE(G) that codes for proteins that produce bioluminescence.

  Essentially, bacterial luciferase are heterodimeric proteins that intracellularly synthesize luxAB, which in turn reacts with $FMNH_2$ and $O_2$ to generate a light signal emitted at 490 nm. The eukaryotic luciferases are single subunit enzymes utilizing luciferin and ATP-$Mg^{2+}$ in the presence of oxygen to generate bioluminescent signal at 562 nm[16,52]. JMP134-32, a genetically modified bacterial derivative of *Ralstonia eutropha* JMP134 harboring a

tfdRPDII-luxCDABE fusion construct, was employed in a whole cell biosensor to generate a bioluminescent signal to 2,4-dichlorophenoxyacetic acid in between concentrations of 2µM - 5mM[40].

Fluorescent microbial biosensors are extensively utilized in analytical procedures for their ability to emit fluorescent light that is directly proportional to the concentration of analytes, even at low levels. These biosensors rely on the fusion of an inducible promoter with a reporter gene, encoding a fluorescent protein that can produce detectable fluorescence in genetically engineered microorganisms[32]. Among the fluorescent proteins used, Green Fluorescent Protein (GFP) is the most common because of its stability and sensitivity. The chromophore in GFP is constituted of the triplet of Ser, Tyr, Gly with excitation maxima at 385 nm and emission maxima at 509 nm.

The signal is stable, fast response time and non-toxic. Derivatives of GFP exist, namely blue fluorescent protein, yellow fluorescent protein etc.[94]. Recombinant *Escherichia coli* carrying three consecutive copies of the *ars* promoter/operator and the GFP gene encoded in a plasmid was used for detection of arsenic between the range of 7.5-20 mg/L. Due to the use of fluorescent transduction, the signal-to-noise ratio was observed to be doubled[36]. Colorimetric reporter genes are also widely used, despite the need for addition of substrate externally. Common colorimetric genes used are *Mjdod* coding for DOPA 4,5-dioxygenase (yellow pigment), *vioABCDE* gene cassette coding for violacein (blue/green/purple), Crt operon coding for carotenoid synthesis (red/orange/ yellow pigments) etc.

The most sought-after colorimetric reporter gene is lacZ coding for beta-lactamase activity (blue color). Colorimetric detection allows for easy and stable signal detection, although it may cost sensitivity[66]. Essentially, the transducers amplify and transform the weak biological signals produced by the microorganisms into measurable output signals. This integration greatly enhances sensitivity, accuracy and efficiency in signal detection and measurement[94].

- **Signal and data interpretation:** Finally, the output signal is then analyzed under the electronic system for the analyte concentration detected by the sensor. This information is displayed in a readable format, for example, a digital readout, or it can be transmitted for further analysis[55].

Hence, the accuracy and sensitivity of the readout given by the sensor are largely governed by the microbial chassis used, the sensing genetic circuitry, resistance of the microbial cell (native or engineered) to the concentration of analyte being detected and the molecular/metabolic burden imposed by reporter gene output molecule, though is not limited to these factors.

## Current applications of microbial biosensors

Owing to the renewability, environment friendliness, low cost of detection and easy deployment as point-of-care devices, the 21st century has seen an evolving interest towards the development of whole microbial cell biosensors. Whole microbial cell biosensors can be instrumental to global endeavors for achievement of Sustainable Development Goal 3 (Good Health and Well-being) by allowing for early and accurate disease diagnosis and Sustainable Development Goal 6, 12, 13, 14, 15 (Clean water and sanitation, responsible consumption and production, climate action, life below water, life on land) by facilitating detection of environmental pollutants, heavy metals, adulterants in food and other xenobiotics at risk for human/plant/animal exposure (Figure 2)[67]. Hereafter, we discuss the versatile applications of microbial biosensors:

**1. Environmental Pollutants Monitoring:** The dominant application of microbial biosensors is exploited in real-time environmental monitoring of metallic ions, organic pollutants and their byproducts (Table 1) [42.81].

**Detection of metal ions:** Environmental contamination by metal ions (including heavy metals) is an outcome of rapid industrialization and increasing anthropogenic activity. Chronic exposure to high levels of metal ions has direct health risks to human, plant and animals[17,51]. Mercury is a major contaminant in the aquatic ecosystems, introduced in the water bodies due to surface run-offs and disposal of industrial effluent. Measurement/detection of mercury (II) is crucial to estimate conversion to methyl mercury. Methyl mercury is a known neurotoxin in humans that can cause severe developmental delays, Minamata disease etc. via bioaccumulation and biomagnification[39]. For detection of mercury, largely two types of biosensors are known: protein based (antibody mediated detection, merR mercury (II) binding transcription mediated and enzymatic detection) and whole microbial cell biosensors.

One of the earliest mercury (II) biosensor utilized a combination of promoter less luxCDABE from *Vibrio fischeri* and Tn21 mer operon by Selifonova and co-workers[81] in 1993. The working range of this biosensor was between 1-20nM[85]. Shortly after, Virta and co-workers[93] reported a whole cell microbial biosensor where the sensing system was merR Tn21 operon combined with a firefly luciferase reporter gene and *E. coli as* the chassis cell for determination mercury (II) in aquatic environments with lowest detectable limit of 0.1 fM [97]. Recently, advancing the sensitivity of mercury (II) detection in infected aquatic environments was demonstrated using whole-cell biosensors relying on firefly luciferase (LucF) as reporter, as well as using a cell-free biosensor, with detection limit of 1 ppb[37].

An interesting host microbial cell used was *Chlorella* sp. allowing for detection of mercury (II) in agricultural and industrial run-offs/effluents, allowing for detection between $10^{-14}$ M to $10^{-6}$ M[24,89]. Lead (II) is another heavy metal

posing immense health risk to biosphere, it is enlisted in the top ten chemicals causing public health concerns, compromising lifespan and costing up to 4,800,000 disability-adjusted life years[101]. The permissible upper limit of lead, as prescribed by WHO, in potable water is 10 ppb and there exists a high dependence on expensive and labour intensive instrumentation like atomic absorption spectroscopy for quantitation[31]. The MerR family member PbrR - a metalloregulatory protein of the pbr operon responsible for lead (II) detoxification system, was first discovered in *Cupriavidus metallidurans* CH34.

The pbr operon components have been used in part or entirety for design and development of lead sensing whole microbial cell biosensors, employing violacein biosynthetic pathway to enable a colorimetric output. Most significant contributions were made by Hui and co-workers[42,43] who initially demonstrated the proof-of-concept of assembling the violacein biosynthetic pathway from *Chromobacterium violaceum* under the inducible control of lead sensing PbrR into *E.coli.* The whole cell biosensor had a lower limit of detection of 0.1875 µM Pb (II)[23]. Eventually, the group integrated metabolic engineering and synthetic biology approaches to produce violacein and its derivatives in *E.coli.* Pb(II)-switchable metabolically-active enzyme clusters were engineered to produce violaecin, prodeoxyviolacein, proviolacein and deoxyviolacein.

The deoxyviolacein-based biosensor demonstrated a linear dose-response in the range (2.93–6000 nM) and additionally was non-toxic, preserving the reusability of the biosensor[44,45]. An innovative multiplexing approach is to detect multiple toxic metal ions by constructing a lux reporter array sensor via transformation of the lux genes in differentially specific microbial host cells, overriding the technical deficits of using metal ion responsive promoters (laborious, slow response, compromised selectivity). The developed sensor array was easy to implement on field, selective, fast and the concept could be extended to other scenarios where a complex presence of analytes is present[91].

**Monitoring of organic pollutants:** Organic pollutants are a human health hazard and also compromise the natural ecosystem. Organic pollutants persist in the environment for long duration, bioaccumulate and biomagnify via the food chain and manifest their toxic effects on the reproductive, neurological and endocrine system, besides being a positive risk factor for cancers[49,62]. There exist optical, electrochemical, mass based- and calorimetric biosensors for the detection of organic pollutants, however, they are plagued with high manufacturing cost, limited sensing functions and are not sustainable. Whole cell microbial biosensors offer an advantageous alternative, especially with incorporation of synthetic biology approaches.

Optimised use of reporter genes and regulatory protein combinations, can allow for amplification of sensitivity, selectivity and sustainability of the biosensor[10,42]. Whole microbial cell biosensors have been developed to detect and monitor presence of organic pollutants in the soil, air and water. An interesting, cost effective, sustainable and easy to fabricate bioluminescent nanopaper device was prepared by combining a bacterial nanocellulose scaffold with luminescent bacteria *Aliivibrio fischeri*[97]. Here the uses of synthetic biology and genetic engineering of the sensing bacteria were overridden and luminescence inhibition of bioluminescent nanopaper indicated the quantity of the toxicity level of the pollutant analysed. The bioluminescent paper was tested with contaminants like diuron, tributyltin and polybrominated diphenyl ether in spiked seawater and freshwater, displaying high sensitivity, reusability up to 10 cycles and storability for long term usage[34,57].

Benzene is a major air pollutant in the vicinity of oil refineries. To monitor benzene levels, two genetically engineered strains of *E. coli* were used: recombinant strains carrying genes coding for enzyme benzene dioxygenase and benzene dihydrodiol. Dehydrogenase originally isolated from *P. putida*. The benzene dioxygenase transformed benzene to dihydrodiol, dihydrodiol was dehydrogenated to catechol by the catalytic activity of benzene dihydrodiol dehydrogenase. The microbial sensor had a sensitivity to detect the benzene vapor in air samples up to 0.01 mM within a span of 30 minutes. A major advantage of this whole microbial sensor was that it was compatible with monitoring benzene levels across air, soil and water[30,55].

Lindane, an organochlorine pesticide, also known as gamma-hexachlorocyclohexane, causes severe health hazard risks. Lindane is neurotoxic and carcinogenic. Prathap and coworkers developed a sensitive whole microbial biosensor for detecting lindane, based on genetically modified strains of *E.coli*. The enzyme responsible for lindane biotransformation, the -HCH dehydrochlorinase (LinA2) coded by the linA2 gene were overexpressed in *E. coli*. The recombinant cells were immobilized on a polyaniline film. The LinA2 enzyme degraded lindane to release HCl leading to reduction of polyaniline matrix which enhanced its conductivity, measured via amperometry. The biosensor could detect part-per trillion-concentration range, with a linearity in the range of two to forty-five parts per trillion.

Additionally, the biosensor was specific and did not recognise the degradation products of lindane or other similar aromatic compounds[3,73]. Chemical processing industries release a vast amount of organic pollutants in nearby water bodies and hence monitoring the levels of respective pollutants is critical to comply with health safety standards. Patel and coworkers[69] developed two biosensing bacterial strains to facilitate online detection of aromatic hydrocarbons. Benzene, toluene, ethylbenzene and xylenes were detected using a *E. coli* DH5α 2296 has *tbuT* promoter-operator, which is capable of detecting. Naphthalene, di-methylnaphthalene, phenanthrene and other polyaromatic hydrocarbons were detected using *E. coli* DH5α 2301 as a phn promoter-operator. Both the biosensing bacteria

employed the luxAB reporter gene and could detect the aromatic pollutants up to micromolar range[72].

Lifshitz and co-workers[51] developed a. *E. coli*-based bioluminescent microbial cell strain for the detection of 1,3,5-trinitro-1,3,5-triazine or RDX (military explosive) contamination in soils. The sensor strains are based on fusing promoters of *hmp* (nitric oxide dioxygenase) or *hcp* (a high-affinity nitric oxide reductase) from *E. coli* gene, to the bioluminescent reporter gene cassette - *luxCDABEG*. The researchers were successfully able to detect 1.67 mg of RDX /kg, with the biosensing strains encapsulated in calcium alginate beads. The biosensing beads can be useful in detecting explosive devices and landmines in war zones or conflicted regions to ensure public safety[54,98].

Summarily, microbial biosensors have demonstrated immense applicability in detection and monitoring of diverse environmental pollutants and advancements in technology will facilitate cheaper, faster and sensitive devices that can facilitate human life.

**2. Food Additives Detection:** Microbial biosensors are also used for the detection of nutritional additives in food and food contaminants (toxins, allergens or chemical moieties), thus ensuring food safety and quality. The process involves isolating microorganisms that can interact with specific additives, followed by potential genetic modification to improve their sensitivity and selectivity. The microorganisms generate a particular reaction after the chemicals are added to the bioassay[12]. This response can range from enzyme production to changes in gene expression, ultimately generating a detectable signal. The signal is then captured using transducer systems such as optical sensors or electrodes. Microbial biosensors allow for real-time monitoring, are sensitive and selective, thus being of utmost utility for detection and quantification of food additives to guarantee compliance with regulations and to ensure food safety for end users[56].

Lactic acid is a valuable organic acid with application in the food, cosmetic and pharmaceutical industry. Augustiniene and research group[5] developed a transcription factor-based microbial biosensor for determination of production of enantiomers L- or D-lactic acid during fermentation. The efficacy of biosensor was cross validated with chromatographic and enzymatic methods, the response being reported to be faster and more sensitive. The sensor strains were *E. coli* and *P. putida*-based named BLA1 (for detecting L-lactic acid) and BLA2 (for detecting L/D-lactic acid) harbouring L- and D-lactate-inducible systems EcLldR/PlldP derived from plasmid pEA015 and PfPdhR/PlldP derived from plasmid pEA025. The detection level for this biosensor system was 0-001–0.5 mM[48,54].
The challenge of antibiotic residues in food has hazardous health implications for end users. Unnecessary exposure to antibiotics via the food consumed can accelerate development and spread of antibiotic resistance, can trigger allergies (penicillin), or cause off target pathologies: cancers, anaphylactic shock, nephropathy, spontaneous mutations, reproductive toxicity etc.[4] A bioluminescent whole microbial cell biosensing strain TetLux was developed for detecting tetracyclines in poultry meat. The *E. coli* biosensing strain harbors a plasmid with luciferase operon under control of the tetracycline responsive elements from Tn10. Repressor protein TetR binds tetracycline and hence loses affinity to the operator sequence upstream of ptetA, allowing transcription from the promoter.

The bacterial biosensors were rapid and sensitive to detect 5 ng/g doxycycline, 7.5 ng/g chlortetracycline and 25 ng/g tetracycline and oxytetracycline[96]. An advanced whole microbial cell-based biosensor system was reported by Lu et al[58] where they built a smartphone-based whole microbial biosensing system - LumiCellSense. The LumiCellSense comprises of a sixteen well biochip containing bioluminescent *E coli* and an image capture system (lens, barrel etc). The biosensing *E. coli* contains a plasmid with recA gene promoter (*E.coli* origin) and *Photorhabdus luminescens* luxCDABE operon.

The bacteria emit luminescence in response to the presence/absence of target antibiotic which is captured in an image via the phone's camera and a compatible application - LCS_Logger in real time. The utility of LumiCellSens was demonstrated by detection of ciprofloxacin in dairy products with a detection threshold of 7.2 ng/mL[61]. Thus, whole microbial cell biosensors have revolutionized real-time monitoring, high specificity and notable sensitivity in detecting food contaminants to assist preserving food integrity and guarantee quality. In order to maintain strict quality control and regulatory compliance in the food business, advancements in such technologies will pave the way for safer food products.

**3. Whole Microbial biosensors for detection of biomolecules and pathogens in clinical Specimens:** Prokaryotic cells possess an array of molecular signaling pathways that are responsive to diverse extrinsic analytes: receptors, enzymes and ion channels. Whole cell microbial biosensors can be used to detect the variations in physiological changes, metabolic disturbances and changes in action potential associated with onset or progression of a disease [35]. Hereafter we discuss State-of the Art applications of whole cell microbial biosensors in disease diagnosis.

**Detection of Biomarkers for diseases:** A diagnostic biomarker confirms the presence of a disease or pathological state of interest in a given set of subjects. Biomarkers can be of diverse nature: physiological, cellular, biochemical or molecular [18]. Whole cell biosensors employing living cells offer a responsiveness to range of analytes in comparison to standard chemistry-based sensors. Biosensing cells are compatible with broad temperature/pH and offer fast and reproducible results in complex body fluids like urine/blood/serum/saliva [24]. An interesting case-in-point was

an ultrasensitive biosensing bacterial platform developed to detect hematuria. Standard methods to detect hematuria are based on laboratory testing by sedimenting RBCs and detection via microscopy or use of dipstick tests. The conventional testing methods are plagued by a high false positive rate.

The researchers developed a synthetic gene circuit that detects heme and generates a bioluminescent signal coupled to a single-photon avalanche photodiode. The genetically engineered *E.coli* chassis includes a heme responsive promoter and the luxCDABE split luxCDE, regulated by heme responsive promotor and luxAB under constitutive expression. The end product was cost effective. User-friendly hematuria detection device could detect $5 \times 10^4$ to $5 \times 10^5$ RBC per mL of urine samples. This device can find applications for several diseases - urinary tract infections, stones, cancers etc.[7,86]

Proteins are popular analytes, especially in agglutination-based assays. A recent study reported a biosensing platform utilizing agglutination of biosensing *E.coli* cells with surface-displayed nanobodies (single-domain antibodies produced by the *Camelidae* family) that are selective to target analyte. Biosensing engineered bacteria display an anti-GFP (dummy protein analyte) nanobody through a β-intimin anchor on their cell surface to recognise the GFP simulated samples. The bacterial cells displayed multiple copies of the anti-GFP nanobody leading to generation of a multivalent bacterial sensor for target analyte. Exposure to multiple epitopes of an antigen cross-linking takes place between bacterial cells and protein analyte leading to aggregation reaction.

The visual output is a concentrated bacterial pellet or a membranous structure at the base of a well when the interaction was allowed in a 96-well plate. Eventually this biosensing format was developed to detect human fibrinogen, a biomarker for risk of cardiovascular diseases (high levels) or clotting disorders (low levels). The platform could detect human fibrinogen as low as 10 pM plasma samples [53,87].

**Infectious pathogenic microbial cells:** Early and on-site detection of pathogenic bacterial growth in environmental sites, water/soil is an important step towards ensuring limitation of waterborne/food borne infections. The standard techniques used for detecting bacterial outgrowth are reliant on culturing and staining. Such methods are time consuming, with low specificity and potential interference from commensals. Molecular techniques such as PCR require sample pre-processing, expensive equipment and trained technical personnel[104]. Use of whole microbial cell biosensor for detection of pathogenic bacteria *Pseudomonas aeruginosa* and *Burkholderia pseudomallei* was achieved by exploiting the QscR quorum sensing system from *P. aeruginosa*. Quorum sensing allows for bacteria-bacteria communication used by bacterial consortia to detect and regulate population density via gene expression regulation. In *P. aeruginosa*, N-acylhomoserine lactone is the key molecule controlling the quorum sensing response via the LasI–LasR gene circuit. LasI synthase is constitutively switched on to produce N-3-oxododecanoyl-homoserine lactone that binds the transcriptional regulator LasR leading to formation of LasR–3OC12-HSL complex triggering gene expression. Hence, a whole cell biosensor was assembled by using the QscR sensing element and GFP as well as lycopene, as the reporter element, with high sensitivity. The authors also put forth a prototype of a paper-based assay by immobilizing the microbial biosensor on paper to facilitate on-site use[98].

## Engineered probiotic strains for monitoring human gut health

The human gut harbors a consortium of microbial population (30 trillion - 400 trillion cells) which is in direct contact and in several cases the source of biomarkers of health or pathology. Any changes in the composition of gut microbiota or the metabolite signatures of microbiota are well documented by several research groups to be associated with several human diseases such as metabolic syndrome and diabetes, cancer, neurological and cognitive diseases and behavioral disorders[15,63,84]. Probiotics - live microorganisms conferring health benefits to the host organism on administration in defined amounts, are valuable interventions for preserving human health. Probiotics can eliminate pathogens by hampering colonization and reign the frequency and severity of disease incidence.

Synthetic biology advances facilitating engineering of probiotic strains, evolving understanding of host–pathogen/probiotic and pathogen–probiotic interactions in the human gut indicating potential for tapping probiotics as whole microbial cell biosensors[47,82]. A building body of literature reports engineering of sensing modules in probiotic chassis to generate multi-functionalized diagnostics. Riglar et al[76] modified the phage λ CI/Cro bi-stable switching for detection of analyte tetrathionate ($S_4O_6^{2-}$) ion, an emerging indicator of gut inflammation.

The chassis used was a commensal *E. coli* from mice engineered to detect the tetrathionate ion from memory of exposure in the gut and the analysis was performed in fecal matter from streptomycin treated *Salmonella* colitis model as well as IL-10 knockout mice simulating gut inflammation[80]. In an advancement Daeffler and his research group[26] designed a commensal gut adapting *E.coli* strain to detect colon inflammation induced thiosulfate production in mice.

During *S. typhimurium* infection, ROS was produced as a byproduct of host inflammation converting thiosulfate ($S_2O_3^{2-}$) to tetrathionate, which in turn feeds the *S. typhimurium* to aggravate the infection. Colonic thiosulfate and tetrathionate are thus positive predictors of pro-inflammatory in the gut.

As thiosulfate responsive genetic circuitry was unknown previously, the researchers through *in silico* approaches, identified the thiosulfate responsive genetic elements as well as refined tetrathionate sensing elements from marine *Shewanella.* The two-component system elements were then engineered into probiotic strain *E. coli* Nissle, administered in dextran sodium sulfate treated mice, to facilitate a non-invasive live biosensor for colitis[27]. Such probiotic chassis are now gaining favor to develop "smart" biosensing microbes' therapeutic functions. Especially, in the backdrop of cancers, probiotic strains or commensals have been engineered to sense the hypoxic or the acidic tumor core and make thus specifically aid targeted delivery of therapeutic protein (tumstatin, tumstatin-p53 fusion)[41].

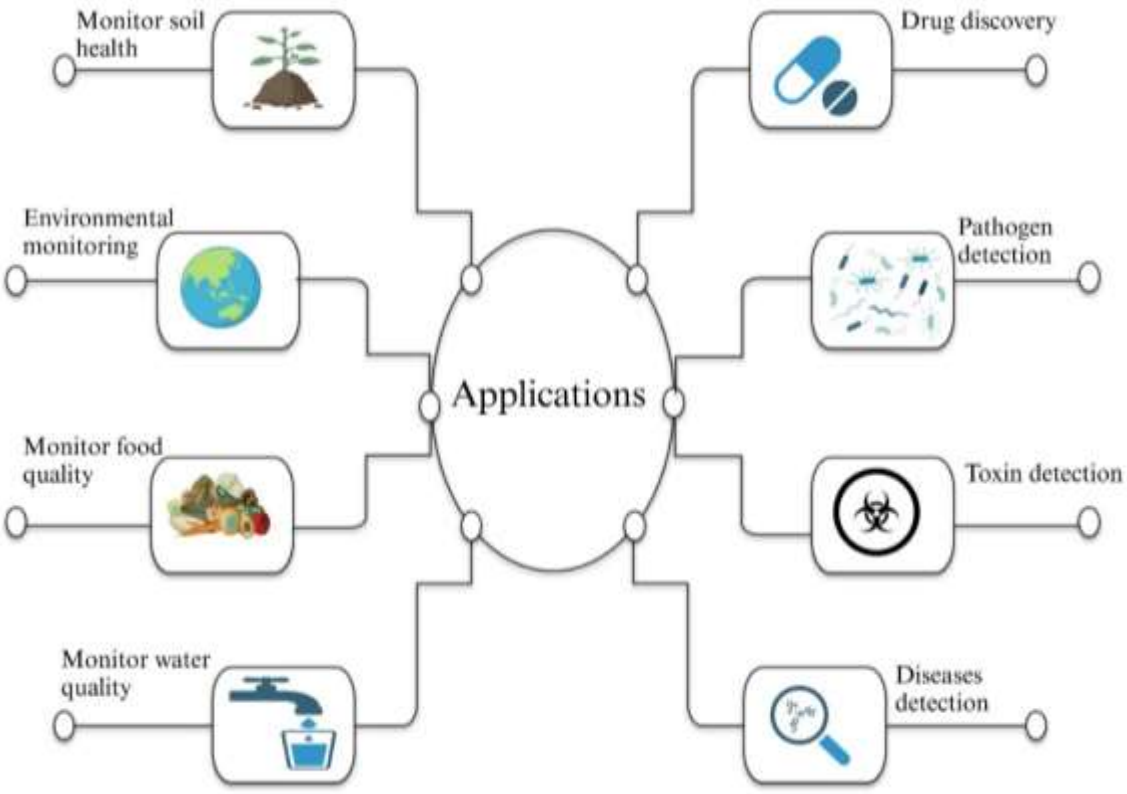


**Figure 2: Different applications of microbial biosensor in different fields**

**Table 1**
**Whole cell microbial biosensors used for environmental monitoring**

| S.N. | Microorganism (Genetically Engineered) | Analyte | Transducer | Target |
|---|---|---|---|---|
| 1. | *Escherichia coli DH5α* | Lead (II) ion | Fluorescent | Environment monitoring[6] |
| 2. | *Escherichia coli* | Mercury | Bioluminescence | Organic-Inorganic toxicities[8] |
| 3. | *Shewanella oneidensis* | Arsenic (III) | Electrochemical | Environment monitoring[99] |
| 4. | *Pseudomonas putida X4* | Zinc | Fluorescent | Organic-Inorganic toxicities[59] |
| 5. | *Saccharomyces cerevisiae S288C* | Cupric ion<br>Lead (II) ion<br>Nickel (II) ion | Amperometric | Wastewater[50] |
| 6. | *Dictyosphaerium chlorelloides Dc1M* | Simazine | Luminescent | Drinking Water[38] |
| 7. | *Bacillus megaterium VR1* | Cadmium ion<br>Zinc ion<br>Cupric ion | Fluorescent | Soil[37] |
| 8. | *Escherichia coli* | Parathion<br>Paraoxon | Amperometric | Environment monitoring[92] |
| 9. | *Anabaena variabilis* | Atrazine | Amperometric | Environment monitoring[93] |
| 10. | *Escherichia coli XL1-Blue* | Zinc and copper | Fluorescent | Organic-Inorganic toxicities[79] |

Additionally, "smart" microbes have also been evaluated in models of infection, metabolic diseases and inflammatory conditions[82]. A gainful scope exists for probiotic chassis that can be engineered for dual action of diagnostic as well as aiding recuperation from pathological state. The field stands to reap also from advancements in synthetic biology to discover novel sensing pathways and cognate responsive transcription factors or proteins to expand the biosensing capabilities. Additionally, success of *in vitro* simulated biosensing does not always correlate with success *in vivo* due to the complex physiology in the gut and endeavors should be directed to refine the *in vitro* testing.

## Challenges and future perspectives

Biosensing microbial platforms, though an innovative solution to a multitude of fields where detection and quantitative sensing of analytes is unavoidable, find limited translational scope. The major challenges plaguing the microbial biosensors are discussed hereafter.

Functionality of the microbial biosensors *in vivo* is a major issue as sensitivity, selectivity and robustness are very often compromised due to complex and dynamic conditions. In an *in vivo* setting diverse variable, diet, native microbiome, physiology and responses of host cells, stability of engineered microbial cells, all influence biosensor performance[65,95]. Genetic robustness of engineered circuits in *in vivo* settings is imperative to reliable and reproducible output. If the engineered sensing circuit mutates, the biosensing response is limited temporally. Use of strong, inducible promotors or having multiple copies of genes coding for the biosensing element can address this issue [26,71]. The hesitancy of the general public over exposure to genetically modified cells is a viable challenge, ensuring stringent biocontainment of the engineered strain only to the biosensing device/platform.

A promising approach to this challenge is use of generally recognized as safe genetically modified microorganisms for the development of microbial biosensors[9]. An excellent alternative is Sim cells, deficient in native chromosomes, these cells lack the ability to self-replicate or carry out horizontal gene transfer to the native microbes in vicinity[76]. The present State-of-the Art in microbial biosensors is limited to detection of one analyte. Conventional diagnostics are gaining immensely in terms of selectivity and utility of output as the number of analytes detected is increased. However, microbial biosensors with multi-input genetic circuits will bring them at par with conventional detection platforms.

For instance, a biosensing microbial platform that responds to low oxygen conditions in tumors as well as chemoresistance markers will give insight into the aggressiveness of the cancer[25,28]. A cross-disciplinary approach that can accelerate applicability of microbial biosensors in real life scenarios is integrating whole microbial cell biosensors with microfluidics technology. Microfluidics technology entails precise fluid handling and on integration with biosensing platforms can facilitate detection in small sample volumes, with efficient sensitivity, as well as potential for multiplexing[19,20].

## Conclusion

The standard detection methods/biosensing platforms are expensive, lack user friendliness and have limitations in sensitivity/specificity, reproducibility, portability. Microbial biosensors are an innovative platform employing a whole microbial cell as a sensing unit in place of enzymes, antibodies or aptamers. The native biosensing capacity of the microbial cells can be harnessed or the microbial cells can be engineered to harbour a genetic circuit comprising of a gene encoding the sensing protein coupled with a reporter gene, generating a robust signal (optical/fluorescent/ luminescent).

Advances in synthetic biology have in fact yielded in standard and well characterised chassis cells compatible for developing microbial biosensors. The microbial biosensors have been employed across diverse applications to ease human quality of life, environmental monitoring and detection of toxic substances/pollutants, food contaminants, disease biomarkers and probiotic/commensal strains for real-time monitoring gut microbiota.

Despite advances, translational applicability of microbial biosensors is limited by the deficits in *in vivo* functionality, mutation susceptibility of the biosensing genetic circuit, biocontainment of the engineered strain used in the biosensor to ensure public health safety and adaptability for multiplexing for detection of multiple analytes. Overriding the challenges will facilitate translational scope of whole microbial biosensors.

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