

Chapter 11

Using Bioprocesses and Biosystems for Environmental Protection, Microbial Detection, and Prevention in the Food Industry



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Introduction

Biotechnology is defined as “the application of components, organisms, cells thereof, and molecular analogues for products and services through the merger of natural sciences and engineering” (Glick & Patten, 2022; Hernández-Arriaga et al., 2022). Biotechnology is adaptable and has been identified as a critical area that has significantly influenced different technologies which rely on the usage of various approaches in foodstuffs processing, agriculture, pharmaceutical, resource conservation, and environmental preservation (Fig. 11.1) (Goswami et al., 2022). This new era of technological changes has resulted in dramatical improvements in a variety of sectors (production of vitamins, drugs, interferon, steroids, fermentation products used as drink/food, energy from natural sources and waste, and genetic engineering applied to animals, plants, and humans) it can allow a totally original opportunity for the economical creation of existing and new products (Ranawat et al., 2022; Goswami et al., 2022). Besides, ecological worries spur the use of biotechnology not just for contamination treatment (disinfecting of soil, water, and air). yet additionally to forestall waste and contamination in any case, as well with respect to ecological friendly chemical synthesis and bio-monitoring.

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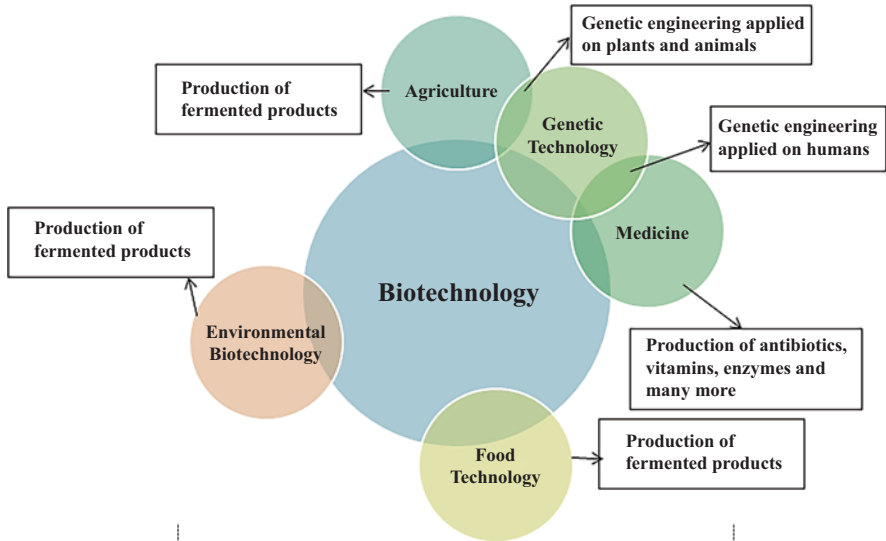


Fig. 11.1 Application of biotechnology in different fields

Nanotechnology is a developing subject in interdisciplinary study, particularly in biotechnology. Research in the nanoparticle is now unavoidable, not only because of its need but also because of the method of synthesis. The production, management, and application of materials in nanotechnology are measured in nanometers (De & Goswami, 2022; Li et al., 2021). Science has learned more and technology has improved as a result of the bridge of nanotechnology in the fields of medicinal plant biology and herbal medicine. There are several uses for nanotechnology, including in agriculture and medicine. Utilizing natural resources, nanotechnology in agriculture can be created to safeguard, produce, and safeguard livestock and crops (Silva et al., 2021; Kumari et al., 2022). However, the preponderance of the chemical processes used to manufacture nanoparticles entail the use of hazardous and damaging compounds that can pose biological dangers, and these chemical processes are not always healthy for the environment. It raises the demand for ecologically friendly improvements by utilizing green synthesis and other biological processes (Salem & Fouda, 2021). Using diverse plant extracts and materials to produce nanoparticles might sometimes be more advantageous than other biosynthetic techniques that need extremely complex procedures for maintaining microbial growth.

Nano-bioanalytical and nano-biosensing systems are certain outcomes of noteworthy advancements in nanotechnology and its applications in pharmaceuticals, foodstuffs, the atmosphere, and energy (Rezaei & Shirani, 2022; Mun'delanji et al., 2015). Nanotechnology contributes significantly to progress and originality by increasing sensitivity and enabling applications based on nanobiosensors and nanosensors (Shang et al., 2019). Traditional bioanalytical techniques are covered by biosensors, but nanobiosensors have fundamentally altered this field by offering

potential alternatives by reducing the need for traditional laboratory procedures and by offering advantages like quicker response times, robustness, improved sensitivity, and portability.

Bioprocessing and Biosensing

Bioprocessing is the process of producing valuable goods by utilizing a living thing—typically cells or cell components, viruses, or a complete organism. End products can range from algae-derived biofuels to medicines derived from mould, such as penicillin (Savchenko, 2017). Beer created from yeast is another instance of bioprocessing in action. This topic requires an understanding of a range of scientific fields, including chemistry, biology, microbiology, biochemistry, and chemical engineering. This is due to the variety of uses for bioprocessing and the complexity of the phases involved (Liu, 2020; Ghosh et al., 2016). Upstream and downstream bioprocessing are the two major components of bioprocessing. The upstream portion of bioprocessing involves the early stages of bioprocessing—identifying the organism to be generated, optimizing the conditions required for growth, and then growing and collecting this organism (John et al., 2020). The upstream process for biopharmaceuticals includes isolating the cell line to be generated, growing those cells to the scale required for the end product, and then harvesting those cells. Downstream bioprocesses include purifying the cells or other organisms collected after the upstream stage to create a finished product that fulfils high safety and quality standards (Rangle et al., 2020). Another important part of bioprocessing is bioprocess engineering, which entails optimizing the environment or system in which the organism inhabits to guarantee it can generate the desired output at the scale and quality required, yet at the lowest possible cost.

While the word “biosensor” was first used by Cammann, and the IUPAC later defined it as Engineering, chemistry, and biology must all work together to develop the materials, transducing devices, and immobilization techniques needed to create biosensors. Based on their processes, the materials employed in biosensors are divided into three groups: the bio-catalytic group, which comprises the set of bio-affinity, which comprised of antibodies, enzymes, nucleic acids, Isolated bacterial cells & microbes fonded group, which includes microorganisms’ biosensors typically consists of biological components like an enzyme, an antibody, an organelle, a transducer, or an analyte (Sinha et al., 2020). These components are highly selective because they can be tailored to interact with an analyte in a particular way thanks to a biological recognition element on the sensor substrate that has a particular affinity for the molecule of interest. The biochemical sign is accordingly transformed into an electrical sign, either nonstop or intermittent, and afterward assessed when the natural material comes into contact with the proper transducer (Saxena et al., 2021; Sinha et al., 2020). A biological reaction is transformed into an electrical signal by biosensors, which are analytical tools. Recognizably, a biosensor must be extremely precise, unaffected by physical factors like pH and temperature, and reusable.

Biosensing and Micro-Nano devices

Frameworks for nano-biosensing and nanobioanalysis are undoubtedly the results of significant headways in the space of nanotechnology and its purposes in the pharmaceuticals, the atmosphere, foodstuffs, and energy fields. The advancement and innovation brought about by nanotechnology, which increases sensitivity and makes it possible to use nanosensors and nanobiosensors in applications, are significant (Table 11.1) (Rezaei & Shirani, 2022). By description, an instrument conveys chemical data of specific percentage value of component of the specimen to an analysis of the sample's whole composition into signals that may be used for analysis comprehended as chemical sensors. Bioactive components like, an bioactive-enzyme, antibodies, or a nucleic acids sequence, are integrated with the relevant bodily transducer to assemble a quantifiable sign that is symmetrical to the amount of a chemical component present in a specimen. This type of analytical device is known as a "biosensor." Nano biosensors, Specifically, are transducers in view of nanomaterials with actual control at the nanoscales. (Malik et al., 2013; Rezaei & Shirani, 2022).

By utilizing continually incorporated, small, multiplexed nanosensors to detect and analyze target molecules, nano-biosensing and nano-bioanalysis can give highly sensitive and selective detection limits. This was made achievable by the disclosure, handling, and utilization of materials for the making of devices, whose morphological qualities give the ideal aversion to locate at the nanoscale. Traditional bioanalytical techniques are covered by biosensors, however, nanobiosensors have fundamentally altered this field by offering viable substitutes by minimizing traditional laboratory procedures as well as benefits including quick reaction times, enhanced sensitivity, resilience, and portability (Srivastava et al., 2018; Rezaei & Shirani, 2022).

Nanotechnology

Nanomaterials are substances with dimensions between 1 and 100 nm, and they are a special gift that nanotechnology has given to society. Nanomaterials have gained significant attraction in contemporary years due to their outstanding electrical, mechanical, and visual capabilities due to their nanoscale size & the best blend of surface and volume components within the general way of behaving (Holzinger et al., 2014; Rezaei & Shirani, 2022).

Table 11.1 The characterization of nanomaterial with its dimensions

S. no	Nanomaterial	Dimension	Size range (nm)
1.	Nanoparticle	3-D	1–100
2.	Nanowire/ nanotubes	2-D	1–100
3.	Nanofibers	2-D	50–300
4.	Nanofilm	1-D	1–100
5.	Nanoplates	2-D	1–100

Fullerene, graphene, carbon nanotubes, and carbon dots are illustrations of metal-composed nanoparticles, whereas the best examples of metal-composed nanoparticles are nanorods, nanowires, quantum dots & oxide nanoparticles. It is ideal to divide nanomaterials used for nano-bioanalytical and nano-biosensing applications into these two main groups (QDs). Fundamental aspects of carbon doped nanomaterials in the zero to three dimensions have made them possibly helpful for the creation of cutting-edge nano-bioanalytical and nano-biosensing technologies. New methods and opportunities for detecting and analyzing target molecules have been made possible by the use of nanomaterials such as carbon in biosensors. (Kour et al., 2020; Rezaei & Shirani, 2022).

A class of functional substances known as metal nanoparticles has distinct chemical and physical characteristics that are especially characterised by their dimensions, form, range, and design. Innovation of metal nanoparticles and their use in numerous fields, including electronics, sensing, catalysis, and medicine, has seen significant advancements. To address the needs for the finding of more accurate and favourably liable biomolecules; metal based nanoparticles plays paramount role in the formation of visionary biosensors and/or modifications in existing biosensing techniques. Multiple biosensors, such as (1) nano-biosensors for illness diagnosis, (2) Probes for in-cell following, Vivo imaging/ detecting, and surveillance of illness aetiology or therapeutic surveillance and Additionally tools have been developed at nanoscale (Baptista et al., 2011; Zhao et al., 2011). In the field of biosensing, such dignified metals as gold and silverware proffer remarkable and concentrated optical attributes (Springer et al., 2017).

Biosensor

The study and development of nanobiosensors are based on an understanding of the biosensing concept. A biosensor is a sensing device or measurement system that is intended to measure a substance using interactions biologically and to translate the results into a readable form using electromechanical interpretation and transduction (Rezaei & Shirani, 2022). There are three fundamental parts to every biosensor: a bioreceptor, a transducer, and a detector (Fig. 11.2). During activity, the bioreceptor, which is situated outwardly of the biosensor, comes into contact with the objective analyte. Target analytes are captured by bioreceptors with great selectivity and specificity (Koyun et al., 2012). Bioactive-Enzymes (Zhao et al., 2017), Antibodies (Kim et al., 2008), Total cells (Han et al., 2018), Aptamers (Kim et al., 2016), and Deoxyribonucleic acid (Li et al., 2010) are a few of the typical bioreceptors utilized to create biosensors. The biorecognition component is typically absorbed into the sampling panel of the biosensor to bring out the preparation. Sensitivity and selectivity must therefore be maintained by the techniques used to connect the biorecognition component to the biosensor. The most widely recognized strategies for immobilizing biorecognition parts are adsorption, capture, covalent holding, micro-encapsulation, and cross-connecting (Luong et al., 2008; Sassolas et al., 2012; Datta

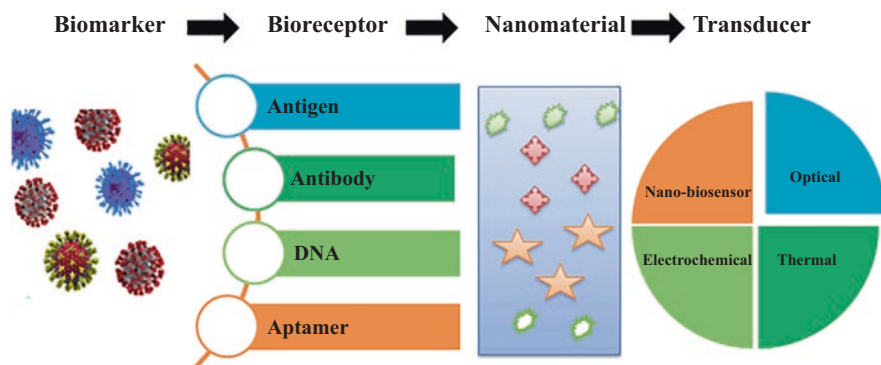


Fig. 11.2 Pictorial representation for working the biosensor

et al., 2013). The purpose of immobilization is to (1) repeatedly employ the biosensor; (2) Consistently screen the analytes in streaming examples, for example, natural liquids, ecological examples containing follow levels of target atoms, or bioreactor liquids; (3) enhance the sensitivity and reproducibility of biosensor performance by developing the biorecognition unit, and (4) make the immobilization strategy straightforward and versatile. The collaboration between a bio-analyte and its matching bioreceptor is generally changed over into an electrical structure continuously part of a biosensor, the transducer framework. A transducer, as its name suggests, effectively transforms one type of energy into the other. The first source of energy is biological in origin, whereas the second is often electrical due to the exact connection between the bioanalyte and the bioreceptor. The electronic indication from the transducer is shipped off the third component of a biosensor which is the indicator, and enhances adequately with the goal that the going with reaction can be perused and accurately examined.

Bioanalyses System

The preparation and detection of samples both heavily utilize bioanalysis systems. A bioanalysis system will first pre-treat and/or modify the recognition elements, then it will modify and/or treat the substrate surface before adding the biological recognition element, and last it will add the target analyte. The bioanalysis approach considers the classification of tools utilized & the construction of individually merged sophisticated segments for the analysis. (Rezaei & Shirani, 2022). Recently, bioanalytical researchers' nanomaterials are used for sample preparation. In extraction methods like micro-extraction, solid-phase extraction, and filtration, several nanomaterials have been used. An alternate sample preparation method to liquid-liquid extraction is solid-phase extraction (SPE), which can use less solvent overall. SPE has been used for many years to remove target analytes by pre-concentrate

from various matrices. In this methodology, the sorbent is allocated within discs, micro-columns, cartridges. Compounds based on silica, such as C18 bound silica, are typical SPE sorbents. The shrinking of SPE cartridges directed towards the preface of a brand-new microextraction procedure comprehended as microextraction by packed sorbent (MEPS) (Abdel-Rehim, 2004). Microsyringes are used in MEPS in place of SPE cartridges. The adsorbent material can be employed within the needle as a cartridge or a plug and closely compacted in the needle's barrel. This approach is specifically valuable for completely automatic online analyses. The integral specimen volume also vastly diminished to a fewer ml. For bioanalytical uses, much more advanced sorbents can be packed and used. This strategy has an equal chance of being used online as other absorbents, including established adsorbent materials.

QDs' distinctive optical & electrical aspects have led to surface modification for usage in bioimaging and biomedicine (Clapp et al., 2006). In general, bonding (covalent and non-covalent) is the two categories into which the methods for altering QD surfaces are divided. Specifically, in a non-covalent procedure, that comprises electrostatic interchanges. Organophosphorus hydrolase (OPH) was proficiently bioconjugated with adversely charged CdSe/ZnS QDs via electrostatic cooperations, which likewise created emphatically charged protein side chains and NH₂ end groups linkages (Ji et al., 2005).

Due to their exceptional optical and electrical capabilities, Quantum dots, which are metal doped Nanoparticles, have been engaged in multiplexing examination, fluid and solid stage designs & trace investigation of inorganic substances. Based on the changes in the fluorescence characteristic, several techniques have been devised to identify Ag (I). Relying on the concentration of Ag (I), various fluorescence responses are witnessed (Xia et al., 2008). According to reports, QD fluorescence is enhanced by low Ag (I) concentrations and vice versa. According to the findings, particle size is important because most trapping flaws in tiny particles originate on surfaces that may passivate to increase fluorescence. In systems based on QDs, fluorescence quenching-based techniques are the most popular ones for detecting Pb (II).

Use of Bioprocesses and Biosystems

Two key instruments for promoting social welfare and economic prosperity are biotechnology and bioprocesses. As they create processes employing genetics biology, synthetic biology, molecular biology, and competitive biotechnological goods, as alternatives to chemical-based applications, the academic, industrial, and governmental sectors are certain to run into technical issues. The interaction between a bioanalyte and its matching bioreceptor is mostly transformed into an electrical state by the dual element of a biosensor, the transducer (Croughan et al., 2015; Shong et al., 2012). Improved bioprocesses are constantly needed in the biopharmaceutical sector to handle new regulatory requirements, quality control requirements, manufacturing issues with cell culture titration, biological products, and the

creation of biosimilars (Whitford, 2013; Cramer & Holstein, 2011). Biotechnology has many purposes, for example, food handling, planning, and enhancement to raise supplement input; process advancement for monoclonal antibody response filtration for the treatment of different cases; assessment of host cell proteins (HCPs) and improvement of microorganisms for the handling and transformation of biomass into biofuels, the making of helpful antibodies, and the production of hematopoietic stem cells (HSCs) for restorative applications (Barragán-Ocaña et al., 2020).

Environmental Protection

By using biotechnology to (bio)treat/(bio)remediate historic pollution as well as address it through pollution prevention and control techniques, environmental dangers and hazards brought on by accumulated hazardous chemicals or other waste and pollutants might be diminished or eliminated. Pollution stemming from present industrial practices could also be minimized. The US Environmental Protection Agency (USEPA) characterizes bioremediation as “a directed or spontaneous approach wherein microbiological exercises are exploited to breakdown or change pollutants into nontoxic forms, ultimately remediating or removing environmental pollution” (Gavrilescu, 2010). For the monitoring and detection of diverse environmental contaminants, biosensors such as genosensors, aptasensors, immunosensors, and enzymatic biosensors have been described shown in Table 11.2. These biosensors use nucleic acids, antibodies, enzymes, and aptamers as the appropriate recognition components (Justino et al., 2017).

Identification of Pesticides

Because of their broad use in the environment, pesticides are among the harmful substances. For example, the group of pesticides comprehended as organophosphorus insecticides, which are ordinarily utilized in agriculture, represent a critical risk to the environment because of their high harmfulness. Thus, insightful techniques without extensive sample pre-treatment, including biosensors, have been made for their observation and identification (Justino et al., 2017). For the finding of organophosphorus pesticides, dispensable amperometric enzymatic (acetylcholinesterase) biosensors with cysteamine self-gathered surface on gold screen printed cathodes were proposed utilizing paraoxon as the prototype (Lang et al., 2016; Arduini et al., 2013). The detection limit of disposable biosensors falls between 2 ppb to 40 ppb with a sensitivity of 113 A mM cm⁻². The scientific presentation was significant because of the profoundly orientated enzyme immobilization employing oneself collected monolayer. Recovery yields of 98.3% (n = 3) were kept after tests in stream water examinations spiked with 10 ppb of paraoxon, demonstrating the proficiency of such enzymatic biosensors (Guo et al., 2017). Other biosensors for the identification of paraoxon in actual water specimens have been examined, including

Table 11.2 Application of various types of biosensors and its role in environment protection

S. No	Pollutant detection	Type of biosensor	Recognizing element	Detection limit	References	
1.	Paraoxon	Electrochemical	Enzymes	Approx. 40 ppb	Arduini et al. (2013)	
2.				Approx. 30 μgL^{-1}	Hassani et al. (2017)	
3.				1 nm–5 μM	Lang et al. (2016)	
4.				Methyl parathion	0.5–1000 μgML^{-1}	Zhao et al. (2013)
5.					0–1500 μgML^{-1}	Mishra et al. (2017)
6.				Chlorpyrifos	0.01–0.1 μM	Mayorga-Martinez et al. (2014)
7.				Carbaryl	1–9 μM	Santos et al. (2015)
8.	<i>E. coli</i>	Optical	Histidine	–	Yilmaz et al. (2015)	
9.	<i>Bacillus subtilis</i>	Electrochemical	Antibodies	10^9 – 10^{10} CFU/mL	Yoo et al. (2017)	
10.	Okadaic acid	Optical	Antibodies	–	McNamee et al. (2013)	
11.	Bisphenol	Optical	Aptamers	1–10000 ngmL^{-1}	Ragavan et al. (2013)	

an amperometric acetylcholinesterase biosensor assembled from gold nanoparticles. The most extreme buildup level showed in the European Association pesticides data set was not exactly the identification furthest reaches of amperometric and colourimetric biosensors, which were 4.8 ppb (18 nM) and 0.8 nM. The amperometric biosensor was utilized to recognize paraoxon in specimens of stream water and ocean water with recuperations of 96–98%, while the colourimetric biosensor was utilized to distinguish paraoxon in agriculture irrigation system, water with recuperations rate of 88–100%. (Laws of the EU, 2017).

Identification of Toxins

Since cyanobacteria bloom caused by eutrophication of aquatic systems creates harmful toxins such as microcystins and brevetoxins, reliable and affordable technologies are required for the early identification of such toxins. Utilizing electrodes assembled of gold, operationalized with a cysteamine self-gathered monolayer, an electrochemical aptasensor was utilized to delicately recognize brevetoxin-2, a marine neurotoxin (Eissa et al., 2015). A constraint of identification of 106 $\mu\text{g mL}^{-1}$ was accomplished, and brevetoxin-2 exhibited high selectivity against diverse toxins from a periodic class, including okadaic corrosive and microcystins. By breaking down shellfish, the reasonability of the aptasensor to identify brevetoxin-2 in

genuine specimens was conducted. Great recuperations rate of 102–110% was accomplished, showing that there were no obstructions from the shellfish framework on the aptasensor sensors. (McNamee et al., 2013). Furthermore, the utilization of biosensors for the observation of toxin okadaic acid in algal, saltwater and shellfish specimens were conveyed. A multiplex surface plasmon biosensor was implied for okadaic investigation in algal and saltwater specimens, as well as for the observation of saxitoxin and domoic acid. Okadaic acid was observed in algal cells utilising a concise specimen preparation approach that affected employing glass beads to lyse the cells and release the toxins, heeded by centrifuging and purifying the extract. Toxins Saxitoxin (0.82 ng/ml), okadaic acid (0.36 ng/ml), and domoic acid (1.66 ng/ml) had been detected in the observation respectively (Pan et al., 2017).

Identification of Pathogens

Pathogens can designate a significant hazard to human health when present in enveloping matrices, particularly water compartments, and certain biosensors have lately been offered for their environmental monitoring. For example, surface plasmon resonance based optical biosensors that can identify metabolically dynamic *Legionella pneumophila* in experimenting natural water trials have been recommended (Foudeh et al., 2015; Enrico et al., 2013). The uncovering of bacterial RNA by the probe employed to identify RNA is mounted on the biochip gold surface was the basis for the detection concept in one study (Foudeh et al., 2015). The biosensing gadget seems suitable for the effective discovery of microorganisms in the possibility of 10^4 – 10^8 CFU/ml, as shown by the utilization of streptavidin-formed quantum dots (Foudeh et al., 2015). In the second experiment a self-assembled protein a monolayer and an anti-*L. the pneumophila* antibody solution was used to functionalize the gold substrate (Enrico et al., 2013). Because no labelling was required and antibody immobilization on the biosensor surface by protein, a limit of proportions of 103 CFU mL⁻¹ was acquired and the biosensor was capable of determine *L. pneumophila* in tainted water specimens in 30–45 min (Enrico et al., 2013).

The results showed strong selectivity, a good detection limit (10^4 CFU mL⁻¹), and acceptable stability. *Salmonella*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Aeromonas* were observed to see if the selectivity interfered with them. During the shelf-life study, detection of *L. pneumophila* was observed (10 CFU mL⁻¹) by plasmon resonance (Meneghello et al., 2017).

Others

The requirement for novel, fast, and reliable insightful methodologies have emerged because of the continuous event of risky algal blooms. Biosensors for recognizing algal RNA have been created because of the unprecedented particularity and responsiveness of nucleotide tests to their related restricting accomplices (Orozco et al., 2016; McPartlin et al., 2017). Lately, it was proclaimed that an electrochemical

genosensor casted on a screen-mounted gold electrode could inspect the RNA of 13 unsafe algae species. The genosensor was competent to isolate RNA targets from ocean water specimens. (Orozco et al., 2016). Environmental biosensors have also been utilised to determinate halogenated combinations. For example, a fluorescence-based enzymatic biosensor was designed to observe 1,2-dichloroethane, 1,2,3-trichloropropane, and hexachlorocyclohexane in ocean water specimens within pH ranges of 3–10 and controlled temperatures 5–50 °C (Bidmanova et al., 2016). Detection limits for beforementioned halogenated compounds were found to be 2.7, 1.4, and 12.1 mg/L, respectively. Biosensor was operated for speedy observation of 1,2-dichloroethane contamination in seawater specimens during real-world testing and can map the distribution of the contamination using GPS (Bidmanova et al., 2016).

Microbial Detection

The most prevalent foodborne pathogens, which yearly infect millions of people, are *Salmonella species*, *Campylobacter species*, *Listeria monocytogenes*, and *E Coli* O157 (Finn et al., 2013; Alvarez-Ordóez et al., 2018; Lamas et al., 2018; McCabe et al., 2019). Fever, cerebral pain, queasiness, regurgitating, stomach inconvenience and infrequently organ failure and demise are among the mild to severe symptoms. Responsive joint pain and Guillain Barré condition are two extreme sequelae that some foodborne diseases, for example, *Campylobacter* spp., can produce (Keithlin et al., 2014). Foods like eggs, poultry, and other commodities with animal origins, as well as those with them as the major ingredients are frequently connected to outbursts of foodborne illness (Ma et al., 2014). Heterogeneous distribution of illnesses rendering microorganisms, stress experienced by the microorganisms during food handling, and nontargeted microorganisms' presence from the ordinary microbiota (*Staphylococcus*, *Pseudomonas*, *Enterobacteriaceae*, *Bacillus*, *Acinetobacter*, and yeasts), particularly in natural food varieties, make fostering a strategy to screen food tests especially problematic (Varghese et al., 2016).

Due to their great heterogeneity and potential compatibility issues with the analytical procedures, food samples frequently need pre-treatments. Further, the mark microorganisms are generally present at remarkably inferior concentrations; in these occurrences, pooling of specimens may be feasible to boost up the analysis. Preparing food specimens for preanalytical testing has the subsequent goals: withdrawing mark pathogens/impurities from the food matrix, stimulating their ratios (in a few cases), isolating them from microbes matrix that are not targeted, and excluding inhibitory chemicals. Sequencing & molecular techniques, which are marked susceptible to inhibitory substances in the specimen, the later operation is extremely paramount. Food is oftentimes solely partly analysed during a microbiological review; just a representative specimen is taken into account for the analysis. The subsequent available measures must be taken into account when constructing a unexplored detection technique:

Microbial detection	Conventional method	ISO method
	Rapid method	Immunological method Molecular method Sequencing method
	Spectroscopic techniques	Near infrared spectroscopy Raman spectroscopy Biosensors Meta data profiling

Fig. 11.3 Different methods for detection of microbial load

- (1) Enhanced specificity, detection at the lower limit, and sensitiveness; (2) Adaptable, making it functional by non-professional; (3) Improved observation time; (4) affordable; (5) Improved capacity and industrialisation, and (6) validation against tried-and-true methods. The detection practices are conferred below and illustrated in Fig. 11.3.

Conventional Method

Traditional culture techniques, such as preparation of specimens, enrichment, serial dilution, count, and separateness of individual species colonies for succeeding reports, are the foundation of the traditional procedure utilised to specify & detect foodborne bacteria. According to the sampling/characteristic targets, the label established on the different characteristics using a range of strategies, as further explained below.

Commonly, total plate count or any agar-based medium can be utilised for the total viable count of bacteria in non-specific cultures (TVC). For the enumeration of certain groups of bacteria and pathogens, selective and/or differential media are employed (Gracias & McKillip, 2004). Utilizing a mixture of combinations, for example Gram stain & biochemical or serological tests, each morphologically diverse colony retrieved from the specimen, in particular catalase or potentially oxidase activity (distinct colony, dimension & colour as assessed by a human spectator) and additionally inspected for pathogenicity or potential role in food spoilage. (Castro et al., 2017; Gracias & McKillip, 2004). Because of their reliability, usefulness, sensitiveness, and broad-spectrum applications, traditional culturing methods

nevertheless labelled as the most acceptable. They are yet a prerequisite for count and detection, defining validating, and viability phenotype projections established on genomic breakdown. The prevalence of ISO measures for estimating security and sanitary indicators (moulds, *Enterobacteriaceae*, yeasts & lactic acid bacteria) and exposing pathogens (*Listeria*, *E. coli*, *Staphylococcus*, *Salmonella*) are founded on standard culturing approaches, and the regulatory boards specified permissible limits for individual category.

Rapid Method

Rapid testing may be qualitative, identifying the presence of microbial contamination, for instance, by measuring changes in pressure, impedance, or CO₂ concentration (Bancalari et al., 2016; Dheilily et al., 2008). The impedimetric approach uses conductance changes caused by bacterial growth to quickly identify microorganisms in samples. In distinction to execute impedance technology, which utilises the shift in conductivity of a fluid culture medium as a determining framework, indirect impedimetry estimates the transformation in electrical conductivity of a retort solution, which emerges as a consequence of the absorption of gases from the immunised bacterial culture.

In the depth of food microbiology, impedance has mostly been utilised to determine and measure *Enterobacteriaceae*. This procedure's primary limitation is that it can only be effectively used when working with extremely identical specimens, as it brings a lot of effort to optimize the process and calibrate individual category. (Ferone et al., 2020). Furthermore, if the microorganisms have been exposed to aspects that drive sub-lethal damage to the bacteria, this method is not fit for estimating the total bacterial count.

Spectroscopic Methods

The investigation of matter established on how it interacts with electromagnetic radiation is comprehended as spectroscopy. There are numerous distinctive spectroscopic techniques available to manage a sort of analytical issues. The approaches vary relying on the species being investigated (atomic/ molecular spectroscopy), radiation-matter interaction noticed (like emission, absorption), and the extent of the electromagnetic range being analysed. For analytical investigations, spectroscopic approaches have been employed in practically all technological sectors of engineering, including life sciences (Ferone et al., 2020). Diverse absorption spectra can be created by the distinct macromolecular makeup of bacterial cells (i.e., proteins, nucleic acids, Fats & Carbohydrates). Nevertheless, because of the majority of microbes have extremely identical spectra due to the slight differences in their chemical makeup. To quantify and differentiate microorganisms, spectroscopic methods must be paired with spectral preprocessing and other chemometric methods. Partial least square regression (PLSR), artificial neural network (ANN) and

support vector machine (SVM) and stepwise multiple linear regression (SMR) are the most frequently utilized chemometric approaches for quantitative investigation.

Prevention in Food Industries

With the development of nanotechnology, it is now possible to influence matter at the supramolecular or atomic level, leading to a wealth of discoveries and uses for nanoscale materials. As a result, key international industries have experienced a progressive innovation leap thanks to nanotechnology. Despite being a multi-trillion-dollar business, the food and beverage industry meets the basic needs of the majority of people. The Industrial Revolution 4.0 is coming, and with it will come a speed-up toward an autonomous and effective process. To make use of the extended versatility of nanoscience to improve the production, packaging, security, aroma, and quality management of their produce. Food conglomerates are drawn to and facilitated to engage in nanotechnological study (Jideani et al., 2020). As a outcome, In recent years, Numerous nanotech products are utilised in the food supply and are demonstrated in Table 11.3. The preponderance of the measures is intended for use by others. These possess biodegradable and environmentally friendly nanomaterials, as well as novel packaging techniques that preserves & monitors the food process until it gets consumes. Antimicrobial packaging, nano based sensors and active packaging techniques for barrier protection against microorganisms employed for protection. Nanoparticles are also incorporated into the food products such as the encapsulation of functional materials in supplements. Nanomaterials-based techniques, such as nanosensors and nanopipsticks, are innovations that combine and miniaturize complementing approaches. These have flared an increase in research into and the innovation of mobile smartphone-based sensor arrays for usefulness in the lab and outdoors. As a consequence, nanotechnology is incredibly valuable for the food industry and can suffice the gap as a feasible explanation to unsolved food research problems. Nonetheless, there remains a significant transitional obstacle for underdeveloped nations that depend heavily on food sources. Economic conditions have an mark on domestic and multinational aids for analysis and development, which are yet in their premature phases (Chaudhry & Castle, 2011). Safety concerns are another major obstacle to their deployment since nanoparticles' physiochemical characteristics differ greatly from those of their macro-counterparts.

Nano-material Based Sensors in Food Analysis

Nanomaterials have measured between 1 and 100 nm. The tested nanomaterial systems, according to Mustafa & Andreescu (2020), possess organic-based (mainly biosensors, with periodic cases of biosynthesized nanoparticles and organic

Table 11.3 Application of biosensors in various food segments

S. No	Sample	Application	Biosensor	References
1.	Apple	Pesticides and heavy metals	Electrochemical biosensor	Chen et al. (2021)
2.	Apple, broccoli, and cabbage	Pesticides and heavy metals	Electrochemical biosensor	Cesarino et al. (2012)
3.	Mandarin	Infection and diseases	Electric nose	Park et al. (2015)
4.	Apples, kiwi, and pear	Ripening and maturity	Electrochemical ethylene sensor	Jia et al. (2016)
5.	Peaches	Infection and diseases	Electric nose	Liu et al. (2018)
6.	Fruits	Pesticides and heavy metals	Photo-electrochemical biosensor	Li et al. (2015)
7.	Vegetables	Pesticides and heavy metals	Conductometric Biosensor	Mulyasuryani and Dofir (2014)
8.	Apple	Defect detection	HSI and MSI	Zhang et al. (2018)
9.	Tomato	Insect infection	MSI	Mireei et al. (2017)
10.	Apple	Quality determination and control	Optical biosensor	Saeyns et al. (2008)
11.	Apple	Shelf-life assessment	MOS (prototype)	Trirongjitmoah et al. (2015)
12.	Peanut	Allergen	Aptamer based sensors	Peeters et al. (2014)
13.	Wheat	Allergen	Electrochemical	Ontiveros et al. (2017)
14.	Chicken	<i>Salmonella</i>	Optical	Zhang et al. (2018)
15.	Pork	Allergen	Calorimetric	Kuswandi et al. (2017)

product), inorganic (oxides metal, and compounded metal), and hybrid composites that merge both organic and inorganic substances (Majeed et al., 2013). In spans of how they operate, conventional sensor displays lean on molecular pattern recognition with customisable selectivity (biochemical) or electrochemical transduction, which mainly aims at volatile substances (Mustafa & Andreescu, 2020). The latest and progressing improvements in sensors are in optical biosensors established on surface plasmon resonance and colour change (SPR). A more careful history of the improvement of nanomaterial-based optical biosensors, with an emphasis on the joining of refractometric plans into the sensors, might be tracked down in this significant subject here (Kobun et al., 2015). According to reports, allergies, pollutants, pathogens, adulterants, minerals, and other foreign compounds that are judged dangerous for human consumption can be identified and detected using nanotech analytical procedures. Gold nanoparticles (AuNPs) are much of the time utilized in frameworks due to their momentous SPR abilities & silver nanoparticles (AgNPs)

because of their additional antibacterial property. The calibrating system of nanoparticle characteristics has been expanded over the course of the years by progressions nearby, prompting different structures and dimensions of Au/AgNPs for a more successful restricting cooperation towards target particles. Albeit metal oxides are all the more often found in the advanced food business as endorsed added substances, (for example, TiO₂ for food colouring), they are additionally presumed to be significant for examination (Fe₂O₃ for its ferromagnetic and electromagnetic acceptance abilities) (He et al., 2019).

Detection of Allergen

Allergens in food are biological substances (mainly proteins) that can unintentionally provoke immune responses and display symptoms in a range of life-threatening degrees and can induce immunological reactions in people who consume or come into contact with them (Flanagan, 2014). The presence of food allergens in the final stage of production can be attributed to two main factors: (1) naturally found proteins in the raw ingredients, such as glycinin in soybeans, ovalbumin in eggs, and sometimes dairy goods, etc. (2) small amounts of allergens in connection with different foodstuffs in the exact production line of industry (Rai & Bai, 2017).

In newly published modified biosensors, fullerene nanoparticles and protease enzymes were merged for a brief and rapid identification of allergic substances in which kernels contains gluten (Ontiveros et al., 2017). Gliadin is the offender after gluten allergies, which direct to Celiac disorder, an untreatable illness (Checkin et al., 2016). A biosensor and electrochemical chronoamperometry are combined. Protease enzymes are entrapped on carbon electrodes, while the latest modifications of fullerene nanoparticles in the detector raise both selectivity and sensitiveness. The modified biosensor has detection limit up to 0.56 mg/L and can identify gluten with a limit up to 8.4 mg/L under ideal conditions.

For security of customers who are allergic, peanuts have drawn a lot of attention for the identification of their allergens (such as arachin h1, arachin h2, and arachin h6). The detection of arachin h1 has become more accurate recently as more reported nanosensors. Peeters et al. (2014) investigated and designed aptamer-based detectors augmented with gold nanoparticles for the prosperous application of carbodiimide chemistry and covalently bound for examination of arachin h1. Impedance spectroscopy has detection range of 1–250 nM and is utilised to carry out real-time measurement of arachin h1's binding affinity. By employing second nanoparticle alteration Fish parvalbumin & Pen (a-1) were analysed (Jiang et al., 2015). To build the sign from the limiting connections of antibodies-allergen; silicon dioxide covering was applied to Fe₃O₄ nanoparticles encased in liposomes. Fish parvalbumin and shellfish Pen a 1 both had lower limits of detection up to 0.17 g/mL and 0.02 g/mL.

Identification of Pathogens

Food- and drink-borne illnesses are well known for being spread by tainted products containing germs and parasites. As indicated by the information from the WHO Foodborne Infection board and by the study of disease transmission Reference Group (FERG), 48 million people have become sick because of different microbes, such as *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* (Havelaar et al., 2015). Three criteria can be used to classify the difficulties and restrictions associated with integrating modern nano-sensing of harmful pollutants into the analytical climate: Real-time PCR must be sensitive enough to identify pathogens in low concentrations, and it must also incorporate pretreatment steps for future large-scale sensing. Ultimately, it must undoubtedly differentiate between living and non-living pathogens in edibles to dodge overestimating sickness hazards, which can have severe repercussions (Shen et al., 2021).

MnFe₂O₄ magnetic NPs-AuNP surface composite (AuMNPs) has been utilised to identify *S. aureus* and its toxicants (Wang et al., 2018). The adversely charged AuNPs were then electrostatically adsorbable on the outer layer of the magnetic NP centers after the polyethyleneimine coating and the magnetic NP centers had been formed. Great band intensity was visible in SERS data with a LOD of 10 cells/mL. Another work used a paper-based biosensor to combine magnetic nanobeads with a selected *S. aureus* peptide sequence (Suaifan et al., 2017). With the bare eye, observable colour differences induced by nanobead-peptide template dissociations could be witnessed, and the consequences were said to bring place fast. When incorporating AuNPs with a enduring magnet to exaggerate the magnetic signs of a screen-bounded carbon electrode, separate subspecies of *Salmonella enterica* was also recognized. (Afonso et al., 2013). A LOD of 143 cells/mL of bacteria was successfully separated and isolated thanks to the conjugation of the capture antibodies in skimmed milk.

Detection of Adulterant

Consumers are exposed to substantial health risks when foods and drinks are adulterated. This happens when essential ingredients are removed or when inexpensive, subpar ones are added, which reduces the quality and safety of the product (Jha, 2016). Nearly all adulterants formerly used in the food sector have been categorized and outlawed by their harmful nature. Real-time applications are constrained by the high cost and complexity of techniques like HPLC, ELISA, and RT-PCR that need pre-treatments. Consequently, there is an enormous opportunity for novel countermeasures research using nanotechnology. Meatballs and other processed meat items are popular in both Asian and European cuisines. As per study conducted by Stephen and Chen (2016), hog meat may have been employed as an adulteration in processed beef and chicken meatballs to diminish the cost of the uncooked materials. When it comes to health, consuming too much-processed meat raises cholesterol and fat levels, which causes chronic illnesses including type I diabetes and

myocardial infarction. Due to ingesting swine serum albumins and subsequent IgE-mediation, unusual situations may result in allergic responses to pork (Wilson & Platts-Mills, 2018). On the other side, from a religious perspective, such as the Halal dietary rules, the adulteration and ingestion of hog flesh generate controversy and unease.

Future Prospective and Conclusions

The use of Bioprocesses and Biosystems for Environmental Protection, Microbial Detection, and Prevention in the Food Industry is discussed in this chapter. Although more effort must be done to progress toward a wide sustainable framework, the scientific and technological revolution exemplifies how sustainability & bioprocesses are topics of substantial welfare and are continuously evolving. Since it enables prematurely understandings and errors intervention in production lines at an accelerated pace with authentic specimen tests starting in this era. Nanotechnology has advanced as the foundation of real-time environment and foodstuffs surveying. As another choice, the current pattern uncovers that carbon-based and crossover nanocomposite (GO, GQDs), miniaturization for onsite investigation, and 3D chip engraving are being concentrated on as the business future bearings. This is a outcome of increased automation, data accessibility, and data handling convergence. Big data directs to how the pervasive amount of information has progressed further standard databanks. To investigate, narrative, and construct the data surplus with inferior latency in authentic term, environment and food security monitoring. Adulteration detection, content assurance, Freshness indicators, and foodstuff processing monitoring are some potential uses for biosensors. In the food sector and environment, competent workers frequently conduct routine chemical and microbiological testing. The extraction or pre-treatment of materials makes this research expensive and time-consuming. Biosensors, which offer rapid, non-destructive, and affordable quality control solutions, can address all of these shortcomings. To solve the issues the food business and environment are experiencing, biosensors have the potential to spark an analytical revolution. This chapter gives a general review of the many biosensor types used in the food industry and explores their future possibilities.

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