Use of Biosensor Technology in Bacterial Detection

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***Abstract*: This review article demonstrates recent progress in the utilization of biosensors for bacterial determination. Morbidity and mortality are commonly caused by a bacterial infection. Pathogens are causative agents of various infectious diseases. Such diseases growing at an alarming rate and threatens millions of people around the world. Health experts estimated that these diseases cost around 5 to 6 billion per year. Therefore, it is an urgent need for the development of rapid components and reliable methods to deal with harmful bacteria associated with food safety and public health. At present, bacterial detection method relies upon laboratory-based techniques such as cell culture, micro-scope analysis, and biochemical assays. However; these measures are time-consuming, expensive, and require distinctive equipment as well as trained users. Thus, in terms of ease of miniaturisation, lack of reagents, sensitivity, and low cost and rapid detection, biosensors can facilitate all these parameters. Recent developments in bio-sensing technologies that uses electrochemical, piezoelectric, optical, acoustic, thermal and high-density microelectrode array, to detect E-coli, biosensors are used for detection of pathogenic bacteria. This paper attains focus on principle concepts, applications, and examples from analyst to configuration of potential biosensors that have been achieved up until now to detect potential pathogens.**

***Keywords*: Bacteria, Infection, biosensors, pathogen, detection.**

I. INTRODUCTION

Bacterial pathogens detection and identification are prime targets in different fields, such as medicine, food safety, public health, and security. The major root of morbidity and mortality are infectious diseases worldwide, triggering deaths and hospitalizations of millions per year. The infectious and parasitic diseases were recognized by the World Health Organization as the secondary excessive source of deaths worldwide in the year of 2004, which caused deaths all over the world in the year of 2004, along the main sources were like lower respiratory tract infections (third), diarrheal diseases (fifth), and tuberculosis (seventh) in 2011.

Such variety of infective or transmissible diseases are the cause of complications in needy countries with low income, where facilities for medication, diagnosis methods and their treatment is nonexistent. Food-borne pathogens cause grave health danger in profitable countries as well. For example, food-borne bacteria have caused infections of almost 76 million, hospitalizations of 300,000, and deaths of 5,000 yearly, in the United States. Escherichia coli, salmonellae, Campylobacter jejuni, and Listeria monocytogenes are among the main sources to cause infections. Traditional bacterial detection and identification are performed by using biochemical analysis and growth of bacteria on selective and distinctive agar media such methods are conventional, laboratory-based methods which have typically processing times are long, can lack sensitivity and specificity, and also requires specified equipment as well as users who are trained. Therefore, they are expensive and not obtainable in all countries [1]. Commonly, specimens like blood, saliva, urine, or food sample, are forwarded for microbiological analysis using different techniques. Microscopy demands to stain bacteria, then observation of their morphology, it may be quite fast but is not certain, whereas growth of bacteria under certain conditions on the particular media will take the number of days.

In spite of the extensive accessibility of antibiotics, the dominant cause of deaths or serious infection is temporized or incorrect recognition of the bacterial infection. This emphasizes the critical requirement for further specification and quick analytical tests that can be implemented at the concerned point.
In the type of food-borne infections rising from food or beverages that were contaminated, quick and precise identification of these contaminated items is desired for the prevention of additional infections. In the United States, the worst incident of food poisoning which caused 47 deaths around 6 months was reported due to the soft cheese ingesting which was contaminated with Listeria monocytogenes [1].

The utmost requirement for the types of system is one which can provide an advance warning for the contaminated items, such systems which can are successful and overcome the problems like enhanced multiplex capacity, sensitivity, selectivity, speed and economically efficient [2].

Conventional identification methods for bacterial detection usually involves a morphological estimation of the microorganism in addition to the tests for the organism capability to grow in under different conditions on various media. Although standard microbiological techniques enable the detection of single bacteria, signal amplification required through the growth of a single cell into a colony is a time taking process. Series of the test are required prior to any identification can be committed. Normally, no single test yields an absolute identification of an unknown bacterium. In response to this issue, decent attempts are made towards the progress of technologies that can quickly identify low concentrations of pathogens samples. For this cause, numeral instruments have been established using different principles of detection [3]. Biosensors combined with nanotechnology

provides various advantages on the conventional and laboratory-based methods and are also categorized into different groups according to the basic principles of signal transduction and bio-recognition elements [2]-[4]. They provide results in a rapid and cost-effective manner. As per the transducing components, biosensors can be delegated electrochemical, optical, piezoelectric, and thermal sensors. The utilization of biosensors is advanced significantly for environmental and bioprocess observing, quality control of food, agriculture, bioterrorism and medicinal biosensor frameworks. [4].

The recognition of the pathogenic bacteria is of the sheer importance in the region of health and safety. Which relates it to the food and beverage industry, as it is shown in fig. (a), which requires diagnostic techniques for the assurance of precise parts, for example, sugars, proteins, vitamins and fats for the detection and measurement of chemical contaminants, for example, pesticides, heavy metals, anti-toxins, and pathogenic bacteria. The ongoing notice to food safety and administrative issued concerns towards purchaser wellbeing [4].

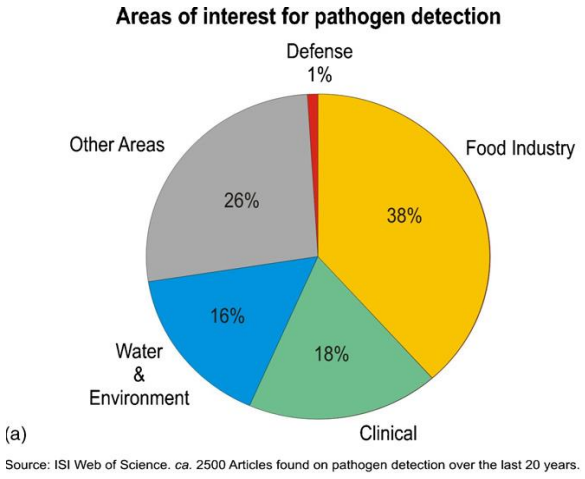

Fig. 1

This paper reviews the recent developments in biosensors for pathogen diagnosis and their introduced technologies and its strategies which constitutes those biosensors with such detecting performances. Current instances of bio-sensors, their benefits and confinements are reviewed and finally, the future outlooks on qualify approaches for the developing the advantageous biosensor devices with quick, exact, and multiplexing potential are delivered. All the illustrations of biosensors, including those defined, also with others that are still developing and are utilized for the identification of pathogenic microorganisms are discussed and their precedence and boundaries, along with the approaches to overpower the problems, are also discussed in this review paper [3].

II. Principles and Configuration of Biosensor

Green innovation in the last decades has become a great target, to achieve the safety and quality of food. Nonetheless, the foodborne pathogens from contaminated food have also intensified. Presence of such pathogens in the food is the principal cause of health issues in humans and animals, which has increased the number of infection from these pathogens. Such problems need to be immediately confirmed or they may lead to severe health issues whereas the common process is too long to process. To prevent such issues biosensors are introduced which acts as an indicator of biological molecules with some selected characteristics [4].

A. Label-free

Various capabilities have been expressed that permit direct, label-free maintenance of cells. Those techniques are established on direct measurement throughout the biochemical reactions on a surface of the transducer. The identified signal is created straightly by the collaboration of the analyzed material with the transducer. Changes in pH, oxygen utilization, ion concentrations, potential distinction, current, obstruction or optical properties which are the signal parameters that can be registered by electrochemical or optical transducers [3].

B. Optical biosensor

Optical biosensor constitutes the most regular type of biosensor. It is the most desired type because of its selectivity and sensitivity which have placed it into this class. It provides benefits like high detailed, descriptive, sensitivity, small size and also economically profitable. Different techniques involving microelectronics, microelectromechanical systems (MEMSs), micro/nano-technologies, molecular biology, biotechnology and chemistry are imposed in the execution of new optical biosensors making multiple advanced conceptions and highly multidisciplinary application. Optical biosensors have met an increasing rate over the past decade in its technology. Most of the applications of the optical biosensor are pointed towards the healthcare, environmental and for industries like biotechnology, each of these applications requires measurement, precision and concentration [7]. Salmonellae aureus is also detected by the optical biosensor [8].
Optical biosensors attain inducted analyte variations in the properties on the exterior of the sensor and then are transduced to a detector. The optical biosensor has introduced various technologies to detect pathogen bacteria [3]. Observation of optical biosensor is executed by utilizing the operation between the optical fields with a biorecognition element. This is also distinguished in label-free mode and label-based model. The optical biosensor is a scientific device embraced with a

biorecognition sensing element combined with a system of optical transducer. A trend is progressing in the detection of the pathogen at the point of care (POC). The first commercialized optical biosensor was fibre optic, its optical concept lies behind fluorescently labelled pathogens [4]. One of the pleasing systems of an optical biosensor is colourimetric biosensors because of its appealing nature that person can effortlessly and immediately notice the existence of pathogenic microorganisms with the naked eye in the test with simple colour change without requiring any analytical instrument. The patient-centred system requires quick results at or close the position of the patient so that the treatment for the patient can be instantly applied. For such requirement, optical biosensors are combined with the micro fluids, which will be advantageous in the sense of time-saving, multiple tests, and can be easily carried out and nominal supervising for hazardous materials. Integration of biosensors with micro fluids will enable the conveyance, segregation, composition, and inspection of the sample on a single device. A handheld thermal cycler has been designed for the detection of various bacteria altogether, under the limited time of 30 minutes [3]-[4]. Because of their sensitivity, fluorescence and surface plasmon resonance, SPR, based methods have grown motion in recent years [9].

B.1 Methodology

Optical biosensors changes in the properties of the optical because of the analyte induced in the surface of the sensor then transduces to the detector. Generally, these biosensors are distributed into two classifications, fluorescence based or label-free. The easiest way of optical biosensors is by determining a fluorescence change, or, upon analyte recognition surface absorbance. Such methods are now advanced from the previous practice of sandwiching immunoassays, immobilized antibodies as biorecognition component that will offer the particular detection of the analyte. The fluorescently labelled antibody as a reagent which binds to the capture an analyte on the sensor surface. And then will produce an optical signal, whose strength is proportional to particular analyte binding. To exchange and provide a stage for smaller and portable system, optical fibres are engaged for the identification of the bacteria. The light source passed through the optical fibers that comprises immobilized bio receptors to a photon detector. Binding of an analyte and Analyte binding and following the accumulation of a suitable labelled reagent which will increase signal alter at the detector. Fluorescence-based biosensors can offer exceptional sensitivity. Though, the main drawback of this technique is that it requires for labelled sample with fluorescent reagents, which makes this method costly and time-consuming.

Surface plasmon resonance (SPR) technique is a label-free method of optical identifying which has been engaged for the identification of analytes. SPR systems consist of a plane-polarized light source which is passed through a glass prism and then contacts with the bio receptor-functionalized transducer surface at its bottom, which is usually a gold thin film. Because of the binding of the analyte to the transducer surface, it causes changes in the refractive index, which will then modifies the light angle that was causing the prism to excite (the SPR angle). Several of the development are made to the techniques of SPR-based biosensors for identifying bacteria by utilizing the range of the bio receptors, with antibodies also [15].

C. Piezoelectric biosensors

Another category of biosensors used for the identification of the pathogenic bacteria is the piezoelectric biosensors of biosensors used in pathogenic. Direct label-free might be used for the detection of bacteria. Because of an oscillating crystal, acoustic waves are caused and conveyed by these sensors. These sensors resonate at the principle of the natural resonance frequency. Such sensors common knowledge depends on the coating with bacterial definite antibodies on the surface, for instance, antibodies to bacteria, and after that inserting it in a bacteria comprised solution. The expanded mass of quartz crystal and a proportionate lessening in the recurrence of oscillation are resulted because of the binding of the bacteria with antibodies, which is identified by the quartz crystal microbalance (QCM) on the surface transducer. This oscillation frequency depends on the frequency of electrical that is applied to the crystal and also additionally on the mass of crystal. For foodborne pathogen recognition, it is very appealing, uncomplicated and actual phase method. The revised form of probes with the protein-A antibody for the detection of the highly piezoelectric bacterium Salmonella typhimurium existence on the surface of the piezoelectric biosensor.

The food industry, environmental monitoring, clinical diagnostics and biotechnology are applications of piezoelectric sensors which are discussed in many reports that have been issued, in the last span of time.

One of the drawbacks of piezoelectric sensors is that it takes reasonably the process of incubation of the bacteria is time-consuming, the various washing and drying steps, and the difficulty in the restoration of the crystal surface and is also not fit presently for the onsite applications. This technology is more appreciated for laboratory-based methods. These problems may become less problematic if small crystals are manufactured reasonably low rate because the disposable transducers are economically feasible. Lack of precision, sensitivity and intrusions from the liquid media where the analysis takes place, are some of the other restrictions of this technology [3]-[8].

C.1 Methodology

The operating principle relies on the usage of piezoelectric crystals that can be vibrated with an electric signal at a particular frequency under the impact of an electrical. The frequency of the oscillation simply depends on the applied electrical frequency to the crystal and also on the mass of crystal. Thus, when chemicals bind on the crystal surface, the mass of the crystal increases due to the reaction of the antibody that takes place and also the oscillating frequency of the crystals alterations can be determined by the electrical signals to estimate the further mass of the crystal. The significant type of the piezoelectric biosensor is the quartz which normally utilized. According to a linear association between the deposited mass and response of its frequency, the quartz crystal microbalance is an appealing method that is skilled in identifying the small amounts of analytes.

D. Mechanical biosensors

These biosensors present various benefits for usage at the point of care; they can give high sensitivity and speedy preparing times without the requirement for test handling or additional reagents. The two fundamental classifications of mechanical biosensors depend on quartz crystal microbalance (QCM) or cantilever technology. QCM sensors are without label piezoelectric biosensors which examine the resonance frequency variation that concludes from raised mass on the sensor surface due to analyte assembly. QCM sensors have been created for the identification of entire bacterial cells. The advancement of sandwich-type examines which utilize nanoparticles for signal amplification has permitted for the identification of very little bacteria. Microcantilever sensor technology is a rising label-free procedure that compromises high sensitivity, less time taken for the reaction and simplicity of scaling down for the advancement of point-of-care sensors. Cantilever sensors normally involve a bioreceptor which is functioned for microcantilever that fluctuates at a specific resonant frequency. The full recurrence of the cantilever changes because of prompted mechanical bending upon expansion in mass on the sensor surface. Microcantilever sensors are created to identify the different microscopic organisms, involving EscherichiacoliO157:H7, Salmonella Typhimurium, Vibriocholerae, and the biowarfare specialist Francisellatularensis. The lately advanced piezoelectric-energized millimetre-size cantilevers (PEMC) utilizing antibodies as bio receptors had distinguished that in the buffer the presence of one E. coli cell and in milk one hundred Listeria monocytogenes were identified.

E. Acoustic biosensors

The working of such type of sensors is the alterations in physical properties of an acoustic wave is monitored. To attain medical, biochemical, and biophysical knowledge in the concern of analyte, an acoustic wave biosensor makes use of acoustic or mechanical waves for the identification mechanism. They are generally engaged with piezoelectric materials, which will motivate the acoustic waves in compact constituents using electrical fields. The acoustic waves are then detected by the charge made because of the induction of the mechanical deformation. Generally, quartz is used, because some of the benefits like they are plentiful, agreeable to economically in its manufacture and also is linked with exceptional mechanical properties and decent chemical constancy. It depends on quartz crystal cut because distinct properties can then be gathered. It will then permit to transmit the acoustic waves in distinct directions proficiently. Moreover either beside the surface of the sensor or apart from the surface of the sensor towards the bulk substrate. The difference is pretty significant and basic for the categorizing of acoustic sensors into surface acoustic wave (SAW) or bulk acoustic wave (BAW) sensors.

Alterations in mass, elasticity, conductivity, and dielectric properties are by varying mechanical or electrical properties are detected.

Such procedures are characteristically sensitive. Several of the benefits and drawbacks are concerned with all of the wave mechanisms subjected to their particular applications [10].

E.1 Methodology

E.1.1. Bulk Acoustic Wave

These biosensors utilize both longitudinal or shear waves, in spite of the fact that the second one is frequently favoured to condense acoustic radiation in the concerning medium. Still, these devices are the eldest and least difficult ones. Such devices comprise a corresponding electrode placed on both sides of the crystal thin piece. Employed with any piezoelectric component, quartz is normally utilized because it is a low-cost material readily obtainable in nature and simply synthesizable in profuse measures. Additionally, quartz thin disks are steadier at high temperatures than other piezoelectric components. Once applied the substituting electric field, it gives the outcome indifference of potential between the two cathodes and the shear crystal distortion.

As an outcome, wave across the bulk of the quartz will have mechanical vibration. The frequency of the oscillation depends on quartz properties which involves density, size, and the crystal surface contact [10].

E.1.2. BAW Device: Thickness Shear Mode (TSM) Resonator

It is one of the simplest and common types of an acoustic wave device that is used presently. It also is known as quartz microbalance (QCM). Normally a TSM consists of the quartz plate that is placed in between the electrodes on opposing faces. Electrodes, as soon as the voltage is applied, it experiences an electric field which passes through this plate and all this results in the outcome of a shear mechanical strain or the quartz displacement. The extreme displacement of crystal arises at the surfaces that were because of the generation of the mechanical resonance and this all can be done by vibrating the voltage frequency.

Photolithography is generally used for fabrication of the TSM resonator. It relies on the shear modulus, density, and material thickness. Placement of the photoresist and the device is then subjected to exposure to UV light by mask, it is done after polishing the quartz. Position of the electrode plates is represented by the TSM resonator. Molecules present in the liquid, can bound and can be identified, to the remaining crystal surface a chemical interface is added.

E.1.3. BAW Device: Shear Horizontal Acoustic Plate Mode (SH-APM)

These sensors utilize a thin piezoelectric substrate, or a plate, to lead the acoustic wave and to confining its energy within the plate's top and bottom surfaces. Rather than the electrode plates, the sensor engages interdigital transducers (IDT). These transducers are placed at the surface on the opposite end, where one IDT produces displacement waves by applying a vibrating voltage. The electrodes plates face the corrosion problems in the biological solution that is why the surface without IDT is engrossed in the targeted liquid which performs as the sensor so that the device does not have to suffer. Energy radiation is lessened in the form of compression waves into the liquid. Although, similar plate thickness are provided to an SH-APM and a TSM device of, the previous one usually offers higher mass sensitivity than the last since IDT is much small in size and more exact than the plates of an electrode on TSM.

However the crystals and substrates may be distinct, fabrication of these sensors have the almost same procedure as TSM resonator. The only difference that is counted is the usage of the IDTs in SH-APM fabrication. The optimized performance can be gained from the modifications in the length, width, position, and thickness of the IDTs [10].

E.1.4. Surface Acoustic Wave Sensors (SAW)

These sensors have been utilized for years in determining temperature, pressure, viscosity, acceleration, concentration, and chemical/biological entities. The signal-processing operations are used for such sensors performance and are delicate to their environment. The basic constituents that this type consists of such as piezoelectric substrate (crystals such as quartz), interdigital transducers (IDT), and active thin films. Rather like BAW devices that simply interrelates with the opposed surface environment of the material by crossing through it, whereas SAW devices simply travel along or nearby the surface of the piezoelectric material. The piezoelectric device identifies minute mass changes of the sensor at its surface as frequency response, when the electrodes present on the surface of the piezoelectric substrate convey and accept acoustic waves. The acoustic wave is confined to the piezoelectric substrate surface, and the exhilarating wave is spread on the crystal surface. The speed of surface wave alters because of the mass, or the viscosity changes because of the binding reactions taking place on the surface of the sensor. The variety of these devices relies on the acoustic velocity of the crystal substrate and wavelength of IDT [10].

F. label based

Colourimetric, fluorescent or luminescent methods produce the optical signal, label-based sensing includes the usage of a label and the optical signal. Enzymatic oxidation using label-assisted sensing can be used to identify simple molecules like glucose [11].

G. Electro-Chemical Biosensors

These biosensors mostly depend on the inspection of current or potential changes because of the arising interface at the sensor [9]. The present prevalence of scientific biosensors is because of critical preferences controlled by these biological sensors. They are profoundly delicate in miniaturized identifiers and can work well in turbid media. These highlights permit extremely sample free measure of test natural food samples. One reason for their prominence is the straightforward utilization of systematic strategies and their low efficient expense. These biosensors are exceptionally touchy and are also been used to identify salmonella and e.Coli O157:H7 in under an hour and a half. Depends on the sort of transducer utilized, these are delegated amperometric, potentiometric, conductometric, or impedimetric biosensors. In addition, the development of electrochemical immune and DNA stages has prepared to recognize the objective pathogens explicitly in a multi organism's matrix, the flexibility to identify particular analytes, the affectability to distinguish bacteria online barring difficult pre-advancement step, and the strength to give ongoing outcomes [4]. To detect pathogenic bacteria in food and water, numerous biosensors have been established on electrochemical technology [7]. Moreover, these biosensors can be unified into one simple device, automated, and low cost because of their existing state-of-the-art methods which are used in the fabrication of electronics [8].

Electrochemical biosensors are the biosensors that change biochemical data, for example, analyte fixations into a scientifically helpful signal, current or voltage. Additionally, this kind of sensor does not require different transduction component, it straightly identifies analyte concentration from the measured electrical reaction that was gained directly from the electrode. Electrochemical biosensors consistently depend upon the enzymatic catalysis reaction that takes place between the immobilization of the biomolecules and the concentrated on analyte that produces electrons or ions, which impacts the electrical properties of the solution, such an electric potential. There are three anodes required for electrochemical detecting: a reference terminal, a working cathode, and a counter cathode. Working the electrodes the combination of a bimolecular acknowledgement structure and the physiochemical transducer, which functions as the transduction segment, known as the redox, or recognizing and the cathode. Evading the reference anode no matter what form the reaction site keeps up a consistent potential. In the counter anode terminal, current can be passed to the working cathode by developing up a relationship with the electrolytic constitution. Gold, platinum, carbon, and silicon compounds are ordinarily used anodes, as they are conductive and are chemically stable.

G.1. Potentiometric Biosensors

Ion-selective electrodes can determine the ionic concentration changes in this biosensor, based on precise exchanges with ions in the solution. Hence when hydrogen ions are discharged a great amount of enzymatic reactions is included. Some of the significant electrodes are ammonia-selective and carbon dioxide selective. Potential difference can help in estimating the potentiometric and the reference electrode and the substrate concentration have direct proportionality to it. The potentiometric biosensors are the sensitivity of enzymes to ionic concentration. The ion-selective field-effect transistors are devices with low cost. Its example is to monitor intra-myocardial for open-heart surgery.

G.2. Amperometric Biosensors

This type of biosensors relies on the motion of the electrons, which is that the enzyme-catalyzed redox reaction as a reaction determines the electronic current. Normally, for analyzing voltage is passed through the electrodes. In the enzymatic reaction which yields the substrate or product can transmit the electrons with the surface of electrodes to be reduced. As an outcome, an alternative current flow can be estimated. The concentration of the substrate is directly proportional to the magnitude current. The oxygen reduction is attained through the oxygen electrodes and it is the simplest approach to form an amperometric biosensor. The above depiction is about the start of amperometric biosensor and it faces some of the problems when electrodes release direct transfer of electrons. After that generation, amperometric biosensors are developed in a moderator which takes the electrons and transmit to the electrodes. Certainly, the vital benefits of these biosensors are their

qualified effortlessness and comfort of miniaturization. They also usually converse tremendous sensitivity. Some of the confinements involve low specificity subjected to the concerned potential, which if high may cause interferences in the signal due to the involvement of other redox-active species and principal to impreciseness in outcomes. Salmonella can also be detected by an enzyme-linked amperometric immunosensor in a time of 4 hours [7].

G.3.ImpedimetricBiosensor

Impedimetric strategies are used from time to time to recognize the substrate or result of an enzyme reaction. They are favored for the portrayal of enzyme-based biosensors. Numerous researches on impedimetric biosensors are centered on the observing of affinity reactions. These biosensors are planned for checking biological reaction at the surface of the transducer. An expendable immunomagnetic electrochemical sensor is dependent on functionalized magnetic beads on the surface of the gold for the discovery of atrazine. Various biomolecules, for example, enzymes, antibodies, cells, and microorganisms are the essential recognition components. Aimed at the improvement of an electrochemical biosensor, the significant necessity is reproducible the immobilization of the biomolecules on the surface of the sensor keeping their natural movement. A new sensitive doxorubicin impedimetric immunosensor dependent on a particular monoclonal antibody modified electrode. As per the literature, different methodologies are been utilized to develop the impedimetric biosensors. Among these techniques is the arrangement of surface functional groups (carboxyl, amino, and so on). By different chemistries biomolecules can be connected to the surfaces, for example, silane on glass or indium tin oxide, and alkanethiol monolayers on auto covalently connect biomolecules to surfaces; the physical capture of biomolecules by different electrochemically designed polymers (i.e., polypyrrole and polyaniline) or gel coatings; immobilizing biomolecules inside Langmuir–Blodgett (lb) films dependent on amphiphilic polyelectrolytes; and the layer-by-layer get together of oppositely charged polyions [6].

G.4. Methodology

The electrochemical biosensor bio-recognition component that is secured on the electrode surface by the physical or chemical method is the basic constituent. Such a biosensor technique can detect the aimed molecule and arrested onto the surface of the electrode, owing to the particular detection purpose of the bio-recognition component with the element to be verified. As the fundamental body of the signal converter, the electrode can originate the detection signal that was created on the electrode surface and alter it into an electrical signal, involving current, voltage, and resistance, that can be determined and analyzed in order to attain a qualitative or quantitative analysis of the analysis target. The Figure below shows the working principle of the electrochemical biosensor.

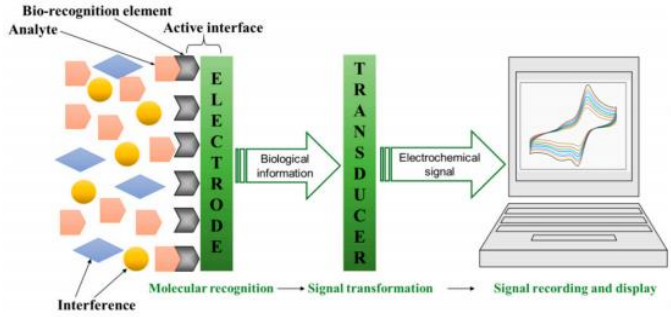

Fig. 2: Working Principle of the Electrochemical Biosensors

According to the examined data type, for instance, current, impedance, potential and conductance, electrochemical biosensors are then classified into amperometric, impedimetric, potentiometric and conductometric biosensors respectively [14]-[18]. The planning of electrochemical-active interference is the core of the better-stated biosensors established to date. Though, electrochemical biosensors indeed own some of the shortcomings like other biosensors. Amongst the confinement points of the electrochemical biosensor, the immobilization of bio-recognition component without denaturation or irregular direction is the most undefeatable. Furthermore, numerous biosensor catches the benefits form the self-assembled monolayer (SAM) technology, it is improved with gold electrode surfaces due to the fact that they could provide favorable substrate and binding sites for a bio-recognition component with the help of chemical groups such as salines, thiols, acid, disulphides, or amines at the electrode surface. Various publications have proved electrochemical technology to be one of the capable techniques in the field of pathogen detection [8].

H. Thermal biosensors

Less amount of consideration is concerned with thermal biosensors. But some of the inappropriate remarks such as it is complicated thermostats, very weak sensitivity or indefinite heating properties have caused in an unfortunate reputation. In fact, thermal biosensors have inclined the total of biosensor research again and again. The information for signal amplification of immobilized multi-enzyme, utilizing immobilized coenzymes and distinct methods for immobilization have been improved by the enzyme thermistor (ET). Such type of biosensors have numerous benefits like they have no connections of chemical between transducer and sample, they are also economically feasible and there is no need to vary ionic or optical sample characteristics [10].

I. High-density microelectrode array

This type is utilized to identify distinct concentrations of E. coli O157:H7 in solution. Its working is that the microelectrode array is fabricated from a silicon substrate to form an electrical transducer. The surface of the biosensor is functioned and is also attached with the particular analyte antibodies through crosslinkers to form an arrangement for the biological transducer. antibodies immobilised to the surface will cause to bound the target analyte, when the biosensor is experienced in solution. The impedance measured across the electrodes to change because of the existence of bacteria on the surface of the sensor and then can it be measured and correlated to find analyte concentration. The fabrication process of the biosensor is done by fabricating from 4" silicon wafers (p-type 100, thickness 500–550 μm). In between the electrodes and the substrate a thick layer of thermal oxide grown over the silicon of 2μm to serve as an insulator. The interconnected electrode array was developed by the procedure of lift-off, for the purpose of the adhesion, metal electrodes were deposited by the evaporation of 5 nm of titanium, under 50 nm of gold and for the arrangement of photoresist photolithography was used. To form a large active area of 9.6mm2 all sensors chip had a total of 1700 electrodes, with all electrode finger length was 750 μm, width of 3μm and spacing in-between was of 4μm. For the dimension of the sensor, all of the sensors were diced to 12mm × 8 mm.
The biological substrates were immobilised to the oxide on the surface of the sensor for the following step in the fabrication of the biosensor and then antibodies were committed to the surface of the sensor through crosslinkers. The biosensor was then cleaned and activated, concisely. On the surface of the chip, the silanizing was done. After the silanizing an ester was attached to the surface. An antibody polyclonal was coated with a crosslinker, then placed on the surface and then was permitted to incubate. The biosensor was ready for testing after the antibody was immobilized [7].

III. Critical Analysis

Well, sensitivity is offered by the optical techniques rather than the electrochemical techniques, yet their cost and complications in working have made them unpleasant to a lot of the users. However technologies like electrochemical biosensors, considerably relax the user to use but then again their working still needs to enhance in detecting pathogens in the food samples due to the complex pattern of food, suitable sample collection and its preparation is required for it. Biosensors technologies still demand to reach to the skilled results conventional methods give with the intention of converting into appealing technology, without overseeing cost [9]. Some of the times the biosensor experience complications when low levels of bacteria are detected because of the sample size. The time taken for incubation of the bacteria is long enough, to process the sample, it requires several times washing and drying steps and due to the interference of the food matrix sometimes presents some other problems like lack of sensitivity when the sensor systems are being developed [7]. The table below shows the detection of different pathogen bacteria in different samples and their analysis time [9].

Table 1: The detection of different pathogen bacteria in different samples and their analysis time

Bacteria for detection	Technique Used for Detection	Type of sample	Time is taken for analysis
E. coli	Fibre optic immunosensor	Culture	10 hour
	SPR biosensor	Culture	Not quoted
	QCM Immunosensor	Culture/Water	170 minute
	Impedimetric immunosensor	Culture/Water	10 minute
	Conductimetric Biosensor	Mixed Culture containing up to five different microorganisms water	10 minute
		Vegetable wash water	6 minute
Legionella pneumophila	SPR	Culture	2 hour 20 minute
Campylobacter jejuni	Total Internal Reflection fluorecente biosenser	Culture	More then 2 hour
	Amperometric immunosensor	Culture and chiken carcass wash water	2-3 hour
Salmonellae	Electrochemical sandwich ELISA		
	QCM	Phosphate buffer	60 mnute
	Amperometric biosensor	Culture and water	1-2 hour

IV. Conclusion

It has been taken under the caution that the detection of infectious bacteria in less time is mandatory to avert further consequences. This then requires a system to work in less time that can take cautionary measures and can aware of the problem so that the biosensor system is developed to prevent health troubles [4]. This review paper has discussed generally used types of biosensors, their principles and configuration that depends on the properties of the transducer, including optical, electrochemical, amperometric, impedimetric, potentiometric, piezoelectric, mechanical, acoustic, high-density microelectrode array and thermal biosensors and their applications in the food industry and their detection methods. Various techniques are established to enhance the working of the biosensor system and their benefits can then be joined to establish the progressive system of diagnostics [2-4]. These systems provide an amazing substitute over the conventional methods for the detection of the microorganism, biosensors permits for less time, quick on-site, and numerous analysis which are required for the revealing and distinguishing of bacteria specifically in food [8]

In the past recent years some of the informative researches are done in the field of the biosensor system, for its development in the food industry and to make it commercially acceptable and significant [8]. The type of biosensor used for the detection of the foodborne pathogen requires an appropriate biorecognition element such as antibodies [4].
Optical biosensors have estimated the detection of the pathogen are becoming progressively significant when implemented in the applications of clinical research, forensics, biodefense, food safety, animal healthcare, pathology, and drug discovery [4]. But when linked to the electrochemical biosensors, it has been seen that for pathogen identification electrochemical methods are broadly explored because of their theoretic background, quick responsive properties, economically feasible arrangement and for their portability. They have revealed the ability to miniaturization and fabrication potential, as biosensor has arisen as practical devices for detection of the foodborne pathogen, due to their advantages [8].
Biosensor systems with extreme sensitivity and accuracy can have excessive applications in the field of medical diagnosis, quality control of food, environmental monitoring, protection and other industries, mainly if biosensors systems can be planned that numerous analytes can be sensed at once. The diagnosis in the field of medical deals actual prospects for the utilization of biosensors for bacterial detection. Actually, the biosensors may have the chance to come into the market of clinical diagnosis [3].

But it has become an ultimate challenge to construct biosensors with the properties like reliability and effectiveness practice in routine applications. Biosensor system must have the property to differentiate between the target bacteria in a multi-organism matrix, different detecting analytes flexibility, detecting bacteria with sensitivity and giving outcomes quickly.Biosensors should reasonably simple and cheap configurations [3].

Low-level adulteration in food is required to be detected but biosensor has lacked in this property. Upcoming development now focuses on the increasing immobilized antibody to increase the sensitivity of the biosensor [7].

Some of the other significant traits of the biosensor are the sensor surface regeneration and multiplexing, where at the same time many bacteria can be examined. The process of regeneration can be cost-effective. Yet again large-scale optimization is demanded these progressions. Suitable miniaturization, optimization has to be done beforehand any product goes commercially [1].

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