Essence, Trends and proposed Design for Microbial Sensing

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Abstract— Today there is an emerging need to develop technique and tools for detecting microorganism useful in different sectors including food safety, health, agriculture, defense, etc. Already there are many detection methods, but due to some limitations, these not suitable for commercial applications. However, an intensive research for the commercial biosensor still under progress. In this paper, we intend to present the essence for such sensors with the review of present and future trends. Also, we hereby propose a viable design that could achieve the required goals at low cost.

Keywords: GIT, GDP, DNA

I. INTRODUCTION

Viruses, bacteria and other microorganisms are spread all over the nature and the environment, i.e. soil, water, gastrointestinal tract (GIT) of animals or water contaminated with feces. The human body itself carries more than 150 types of bacteria. Most of these microorganisms perform essential activities associated with both animals and plants. But there are certain microorganisms which could be potentially harmful for human and causes infectious diseases. Such bacterial microbes get easily and rapidly distributed through agricultural products (food, etc.) under favorable conditions like temperature and moisture. About 40% of the total 50 million yearly deaths in the developing countries like India are estimated to be mainly due to infectious diseases caused by dangerous microbes [1]. The pathogen detection utmost important for the healthy society, therefore most of the researchers devoted their concern in this field over the last decade. The three main areas of research concern are food industry, water and environmental quality and clinical diagnosis, the rest of the research is devoted to fundamental studies for the development of new applied methods as depicted in Fig. 1 [2]. The food industry is the key area associated with the detection of pathogenic bacteria.

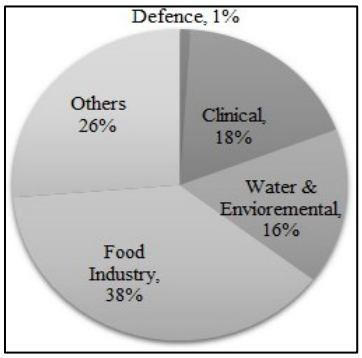

Fig. 1: Areas of Interest for Pathogen Detection

The fact is that India is an agricultural based economy covering about 20% of the GDP (Gross Domestic Products), the pathogen detection become the main thrust of the Indian development story. In the recent years due to advancement of cultivation technologies, the yield of agriculture products have increased tremendously. The food and vegetable productions are registering a year-on-year increase. The indications are that India may lead to major exporter of agricultural products in the global market. Even though ever increasing population and lack of better preservation of food products is a hindrance in achieving this target. In addition to this bio-degradation of food due to pathogenic microbes is another area of concern. Hence, multidisciplinary approach with innovative, advanced technology is required for food safety by contamination monitoring.

A. Conventional Approaches for Pathogen Detection:

The traditional techniques for the detection of pathogenic microbial agents mainly based on specific microbiological and biochemical identification mechanisms. The following sections describe the various traditional methods used commonly for the detection of pathogenic bacteria.

II. CULTURE AND COLONY COUNTING METHODS

The oldest technique for the bacterial detection is culturing and plating methods and still it is the standard method. It requires different selective media for the purpose of detecting particular bacterial species. These media contain different inhibitors for delaying or stopping the multiplication of non-targeted strains, or substrates which degrade targeted bacteria and/or induce a specific color to growing colony. Then the detection could be performed using optical methods by ocular inspection [3]. However, it is excessively time consuming as it needs 4-9 days to obtain negative results while 14-16 days for confirmation of positive results in addition it is labor-intensive therefore, it fails to find the industrial uses and particularly for the food industry.

III. POLYMERASE CHAIN REACTION (PCR)

This method was developed in mid 80s and is based on nucleic acid amplification for the bacterial detection. It targets the genetic material of bacteria by isolation, amplification and then quantification of a short deoxyribonucleic acid (DNA) sequence. PCR bacteria detection is performed in various cycles of denaturation of extracted and purified DNA by heat followed by an extension phase using specific primers and a thermo-stable polymerization enzyme. After these each double standard DNA acts as targets for new cycle, thus an exponential amplification is attained. The presence of amplified sequence is then detected by gel electrophoresis technique. Many PCR variant have been developed in mid-2000, some of these are real time PCR, multiplex PCR, reverse transcriptase PCR etc. In addition, there are other techniques which couples PCR such as surface acoustic sensor or evanescent wave biosensor. The PCR method suffers disadvantage of being very time consuming, requiring 5 to 24 h to produce results. However the multiplex PCR is appreciated since, it performs simultaneous detection of various organisms by introducing respective primers to amplify DNA regions coding for particular genes of each bacteria. The real time PCR on the other hand, can obtain faster results without too much manipulation as it is based on the fluorescent emission by particular dye which binds itself to targeted amplicon and the fluorescence intensity is proportionate to amount of amplified product. Thereby, it eliminates the laborious post-amplification process such as gel electrophoresis. Similar approaches have been obtained to make different kinds of probes [4].

IV. IMMUNOLOGY BASED METHODS

The most powerful method for bacterial detection is immunology based which cover a wide range of targets. In this method an immunomagnetic separation (IMS) step is performed wherein antibody coated magnetic beads introduced in the bacterial suspension, which help in capturing and extracting the targeted pathogen [5]. This method can be combined with any other detection techniques (i.e. optical, magnetic force microscopy, magneto-resistance, hall effect, etc.). A number of companies offer customized magnetic beads which vary in sizes from nanometres to tens of microns and can be selected based on the requirements. Generally, larger beads are suited for measurement of intermolecular forces, smaller particles and are best for detection of small analytes where precise sensitivity is critical while the low micrometer range used in case of whole bacteria which provides the accurate balance between time and sensitivity. Perhaps immunological detection method is the only tool successful for detection of bacterial cells, spores, metabolites, viruses, etc. Many other detection methods are only based on immunological techniques that includes enzyme immunoassay (EIA), enzyme-linked immunosobent assay (ELISA), enzyme-linked immunomagnetic chemiluminescence (ELIMCL), flow injection immune-assay, immunochromatography (ICG) strip test, immuno-precipitation assay, radio-immunoassays modified western bolt include the line immunoassay (LIA) and the recombinant immunobolt assay (RIBA) [6].

These conventional methods are sensitive, inexpensive and capable of giving both qualitative and quantitative information on the examined microbes. However, they are limited by assay time and also due to the requirement of initial enrichment of detectable pathogen which typically come low in number in the food products. Therefore, there is an urgent need for new technology that is fast, reliable, simple, sensitive and specific. Also such method should be designed which perform in situ real time monitoring at low cost. An active research is done in the recent years for the same and various biosensors have been devised for detecting pathogen microorganisms.

V. BIOSENSORS FOR PATHOGEN DETECTION

Most of the microbial tests toady is localized in the stationary laboratories due to complex instruments and requires skilled technical staff. But intensive research has been under way for the decentralization of such tests so that these can be executed virtually anywhere and under field conditions. The development of portable, rapid and sensitive biosensor technology with immediate on the spot result is the current requirement. The biosensor is a device which has the ability to convert the biological response to an electrical signal. The main components of typical biosensor are 1) bioreceptor (or biorecognition element) which could recognize the target analyte and, 2) transducer that can convert a recognition event into a measurable electrical signal. A bioreceptor can be a microorganism, tissue, organelle, enzyme, cell antibody, nucleic acid or biomimic etc. while the transducer could be optical, magnetic, electrochemical, thermometric, piezoelectric or micromechanical or any blends of one or more of these.

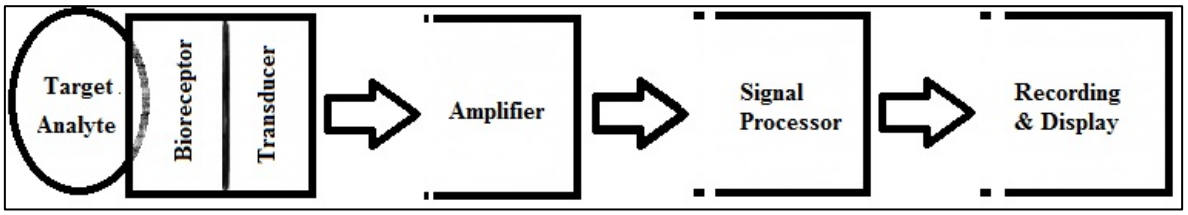

Fig. 2: Schematic of a Typical Biosensor

The schematic representation of a typical biosensor is depicted in Fig. 2. First bioreceptor recognize the target analyte which stimulate the biological response, which then gets converted into an equivalent electrical signal from the transducer. The small electrical signal further gets amplified using amplifier and deliver a large output signal that contains the essential waveform to signal processor, wherein it could be processed to display and store the meaningful result. Biosensors can be classified according to their bioreceptors and transducer types as depicted in Fig. 3.

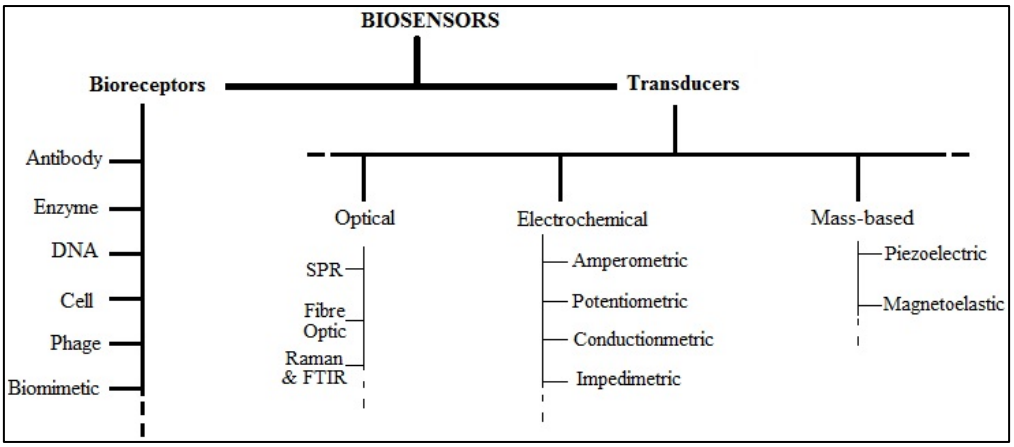

Fig. 3: Classification of Biosensors

A close contact is necessary between microorganism and transducer in any biosensor, therefore immobilization on transducer is necessary. The immobilization strategy plays an important role in business and could be achieved by either chemical or physical methods. The chemical methods include covalent binding between microorganisms' cell wall components, i.e. amine, carboxylic, or sulphydryl and transducer, i.e. as amine, epoxy or tosyl. The cross linking is another chemical method which involves linking between functional groups on the outer membrane of cells by multifunctional reagents such as glutaraldehyde and cyanuric chloride, to form a network. The covalent bonding method has the disadvantage that cells get exposed to dangerous chemicals due to adverse reactions which damages the cell membrane and decreases its biological activity. In physical method for microbial immobilization includes adsorption and entrapment. These methods are preferred over chemical methods as it does not involve covalent bond formation with microbial cells and only a small perturbation of microorganism is required. The physical adsorption is simplest method in microbial suspension is incubated with the electrode or an immobilization matrix like alumina or glass bead subsequently by washing with buffer to remove unabsorbed cells. Entrapment of microorganisms is accomplished by retention of cells in close vicinity of the transducer surface using dialysis or filter membrane. It however, induces an additional diffusion resistance which limits the sensitivity and detection limit.

VI. FUTURE TRENDS FOR BIOSENSORS

In the past decade the extensive research work has been performed by various researchers in the field of microbial biosensors. In fact, some electrochemical and optical microbial businesses have already been commercialized for the environmental purposes. Still, there are some intrinsic disadvantages associated with biosensors such as slow response, little sensitivity, poor selectivity, etc. That pushes great amount of interest in the academic research on microbial sensing. The advent of biotechnology with the ability to genome sequencing of more microorganisms, it is possible to genetically engineer microbes with specific metabolic pathways. This enhances the macrobiotic selectivity to specific targets. The selectivity also could be enhanced by developing macrobiotic sensor arrays.

The advances of nanotechnology provide nanostructured materials that could be applied in biosensing mechanism because of their good biocompatibility, enhanced surface areas and better electron transfer properties. The nanoparticles together with microbes get co-immobilized on nanostructured electrodes that possibly improve the sensitivity of microbial biosensors. It is now possible to perform microfabrication in laboratories which permits to design a single microbe based devices which can provide results for how specific microbes target the given sample. It is also possible to integrate microbe on a microfluidic chip using the advances of lab-on-a-chip technology.

The Caulobacter species can tightly attach itself to a surface unlike other microbes with its very unique adhesive feature and form a high density monolayer. This can act as advantage in making simple immobilization method without losing the biological identity. Moreover genome sequence for Caulobacter with its intracellular surface expressions of protein or enzyme is also available.

All these advances being under research and still conventional methods remain the most reliable for microbial detection [7].

VII. PROPOSED DESIGN FOR BIOSENSORS

Our group proposed to design a sensor that will detect and monitor the quality of raw/pasteurized milk at farm level/market or even at home. In addition, the microbial contamination level in street vended foods such as Paani-Puri can also be monitored. On the basis of the data acquisition, we will be able to determine the microbial quality of dairy and street vended food samples. The developed proposed device will be much more useful for farmers, housewife and food vendors to know their products keeping quality and storage time limit. The proposed sensor is using microorganism as biosensing elements. Since, enzymes used for biological sensing elements have very high specificity for their substrate and handling it is more time consuming, costly. Enzymes based sensors detects a large number of chemicals and hence its selectivity is not too good. The purpose of this sensor is to meet the requirement of small size, multi sensor platform light weight, low power consumption and wireless communication. The schematic diagram of the proposed biosensor is shown in Fig. 4.

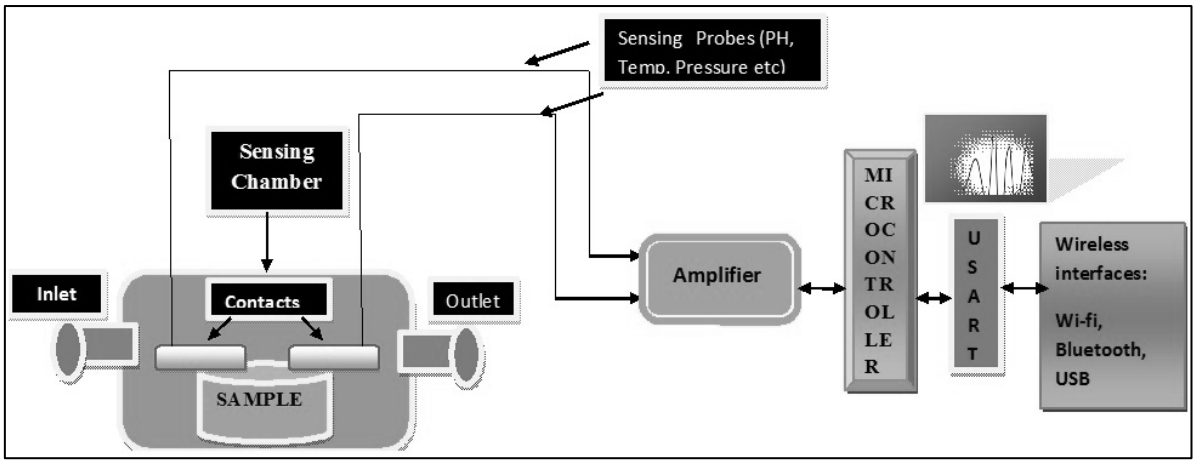

Fig. 4: Schematic Block Diagram of Proposed Biosensor

The measurement circuit consists of four sections i.e. sensing chamber having vacuum in it, a sample holder from which two sensing probes are connected to measure required sensing parameter such as pressure, temperature, conductivity, impedance and pH, etc. The sensing probe is connected with an amplifier circuit to enhance the strength of natural signals (sinusoidal) received from the sample. The microcontroller the heart of this unit is responsible for collecting environmental information and does analog to digital conversion. It is also responsible for controlling and managing the entire wireless system attached with this unit.

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