SEMINAR REPORT

ON

BIOSENSOR

Submitted in partial fulfillment for the award of the degree of

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IN

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Submitted By

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STUDENT DECLARATION

We hereby certify that the work being presented in the seminar entitled **"BIOSENSOR"** submitted in the **Department of Electronics and Communication Engineering, Faculty of Engineering and Technology, Gurukula Kangri (Deemed to be University),Haridwar,** is my work carried out under the guidance of **Mr. Amrish, Department of Electronics and Communication Engineering** in partial fulfilment of the award of the degreeof **Bachelor of Technology**, **Faculty of Engineering and Technology, Gurukula Kangri (Deemed to be University), Haridwar.**

The matter presented in this report has not been submitted by us anywhere for the award of any degree or to any other institute.

Aniket Pandey
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<u>CERTIFICATE</u>

This is to certify that the seminar report entitled "**BIOSENSOR**" submittedby **Aniket Pandey (226320062), Bachelor of Technology** in **Electronics and Communication Engineering**, **Faculty of Engineering and Technology, Gurukula Kangri (Deemed to be University), Haridwar,** is a work carried out under the guidance of **Mr. Amrish (ASSISTANT PROFESSOR).**

Under the Guidance of	**H.O.D**
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Mr. Amrish	**Prof. Tanuj Garg**
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Chapter 1

1.1 INTRODUCTION:

A biosensor is a device that measures biological or chemical reactions by generating signals proportional to the concentration of an analyte in the reaction. Biosensors are employed in applications such as disease monitoring, drug discovery, and detection of pollutants, disease-causing micro-organisms and markers that are indicators of a disease in bodily fluids (blood, urine,saliva, sweat). The simplest definition of a Biosensor is given here: A Biosensor is an analytical device that detects changes in biological processes and converts them into an electrical signal. The term biological process can be any biological element or material like enzymes, tissues, microorganisms, cells, acids, etc. Biosensors are nowadays ubiquitous in biomedical diagnosis as well as a wide range of other areas such as point-of-care monitoring of treatment and disease progression, environmental monitoring, food control, drug discovery, forensics and biomedical research. A wide range of techniques can be used for the development of biosensors. Their coupling with high-affinity biomolecules allows the sensitive and selective detection of a range of analytes. We give a general introduction to biosensors and biosensing technologies, including a brief historical overview, introducing key developments in the field and illustrating the breadth of biomolecular sensing strategies and the expansion of nano technological approaches that are now available . Biosensors like tissue based, DNA biosensors, thermal and piezoelectric biosensors are used in food industry to check in its quality. And play an important role in medical sciences like heart diseases. Biosensors are portable, un-expensive tools for detecting proteins and other analytes, biosensors are techniques which can often used in existence with alive system to decrease cost and increase the profit oriented process rate. This review has comprised a cursory meta-analysis to grow a working visibility for biosensor regeneration. Some of the popular fields implementing the use of biosensors are food industry to keep a check on its quality and safety, to help distinguish between the natural and artificial; in the fermentation industry and in the saccharification process to detect precise glucose concentrations; in metabolic engineering to enable in vivo monitoring of cellular metabolism. Biosensors and their role in medical science including early stage detection of human interleukin-10 causing heart diseases, rapid detection of human papilloma virus, etc. are important aspects. Fluorescent biosensors play a vital role in drug discovery and in cancer. Biosensor applications are prevalent in the plant biology sector to find out the missing links required in metabolic processes. Other applications are involved in defence clinical sector, and for marine applications.

Chapter 2

History of Biosensor:

The history of biosensors dates back to as early as 1906 when M. Cremer demonstrated that the concentration of an acid in a liquid is proportional to the electric potential that arises between parts of the fluid located on opposite sides of a glass membrane. However, it was only in 1909 that the concept of pH (hydrogen ion concentration) was introduced by Soren Peder Lauritz Sorensen and an electrode for pH measurements was realised in the year 1922 by W.S. Hughes. Between 1909 and 1922, Griffin and Nelson first demonstrated immobilisation of the enzyme invertase on aluminium hydroxide and charcoal. The first 'true' biosensor was developed by Leland C. Clark, Jr in 1956 for oxygen detection. He is known as the 'father of biosensors and his invention of the oxygen electrode bears his name: 'Clark electrode'. The demonstration of an amperometric enzyme electrode for the detection of glucose by Leland Clark in 1962 was followed by the discovery of the first potentiometric biosensor to detect urea in 1969 by Guilbault and Montalvo, Jr. Eventually in 1975 the first commercial biosensor was developed by Yellow Spring Instruments (YSI).
Table 1 shows the historical overview of biosensors in the period 1970–1992. Ever since the development of the i-STAT sensor, remarkable progress has been achieved in the field of biosensors. The field is now a multidisciplinary area of research that bridges the principles of basic sciences (physics, chemistry and biology) with fundamentals of micro/nano-technology, electronics and applicatory medicine. The database 'Web of Science' has indexed over 84000 reports on the topic of 'biosensors' from 2005 to 2015.

Table 1. Important cornerstones in the development of biosensors during the period 1970–1992

Year	Milestone
1970	Discovery of ion-sensitive field-effect transistor (ISFET) by Bergveld
1975	Fibre-optic biosensor for carbon dioxide and oxygen detection by Lubbers and Opitz
1975	First commercial biosensor for glucose detection by YSI
1975	First microbe-based immunosensor by Suzuki et al.
1982	Fibre-optic biosensor for glucose detection by Schultz
1983	Surface plasmon resonance (SPR) immunosensor by Liedberg et al.
1984	First mediated amperometric biosensor: ferrocene used with glucose oxidase for glucose detection
1990	SPR-based biosensor by Pharmacia Biocore
1992	Handheld blood biosensor by i-STAT

Chapter 3

Basic Concepts of Biosensors:

A biosensor consists of a bio-element and a sensor-element. The bio-element of the may be an enzyme, antibody, living cells, tissue, etc., and the sensing element may be electric current electric potential, and so on. A detailed list of different possible bio-elements and sensor-elements is Fig 1 The most commonly used sensing elements and transducers are described below.

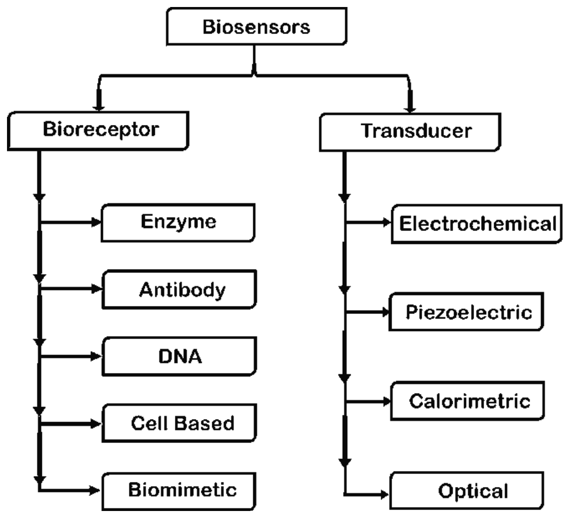

Fig:3.1 Basic Concepts of Biosensors

1.Enzymes

Enzymes are proteins with high catalytic activity and selectivity towards substrates. They have been used for decades to assay the concentration of diverse analytes. Their commercial availability at high purity levels makes them very attractive for mass production of enzyme sensors. Their main limitations are that pH, ionic strength, chemical inhibitors, and temperature affect their activity. Most enzymes lose their activity when exposed to temperatures above 608C. Most of the enzymes used in biosensor fabrication are oxidases that consume dissolved oxygen and produce hydrogen peroxide.

2.Microbes

The use micro-organisms as biological elements in biosensors is based on the measurement of their metabolism, in many cases accompanied by the consumption of oxygen or carbon dioxide, and is, in most cases, measured electrochemically. Microbial cells have the advantage of being more stable, and can carry out several complex reactions involving enzymes and cofactors. Conversely, they are less selective than enzymes; they have longer response and recovery times and may require more frequent calibration. Micro-organisms have been immobilized, for example, in nylon nets, cellulose nitrate membranes, or acetyl cellulose.

3.1 Transducer elements

1.Electrochemical, Amperometric and potentiometric transducers

Electrochemical, Amperometric and potentiometric transducers are the most commonly used electrochemical transducers. In Amperometric transducers, the potential between the two electrodes is set and the current produced by the oxidation or reduction of electro active species is measured and correlated to the concentration of the analyte of interest. Most electrodes are made of metals like platinum, gold, silver, and stainless steel, or carbon-based materials that are inert at the potentials at which the electrochemical reaction takes place. However, because some species react at potentials where other species are present, either a selective membrane is used or an electron mediator that reacts at lower potential is incorporated into the immobilization matrix or to the sample containing the analyte.

2. Potentiometric transducers

.Potentiometric transducers measure the cheaper than enzymes or antibodies, potential of electrochemical cells with very low current. Field effect transistors (FET) are potentiometric devices based on the measurement of potential at an insulator– electrolyte interface. The metal gate of a FET can be substituted by an ion selective membrane to make a Ph transducer (pH ISFET). Enzymes have been immobilized on the surface of such pH ISFET to produce enzyme sensitized field effect transistors (ENFET).

3.Optical Fiber

Optical Fiber, optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wave length range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength Surface Plasmon resonance transducers, which measure minute changes in refractive index at and near the surface of the sensing element, have been proposed.

4.Surface plasmon resonance (SPR) transducers

Surface plasmon resonance (SPR) transducers have been proposed. SPR measurement is based on the detection of the attenuated total reflection of light in a prism with one side coated with a metal. When a p-polarized incident light passes through the prism and strikes the metal at an adequate angle, it induces a resonant charge wave at the metal/dielectric interface that propagates a few microns. The total reflection is measured with a photodetector, as a function of the incident angle. For example, when an antigen binds to an antibody that is immobilized on the exposed surface of the metal the measured reflectivity increases. This increase in reflectivity can then be correlated to the concentration of antigen

Chapter4

Components of Biosensors:

A typical biosensor is represented in Figure 2; it consists of the following components

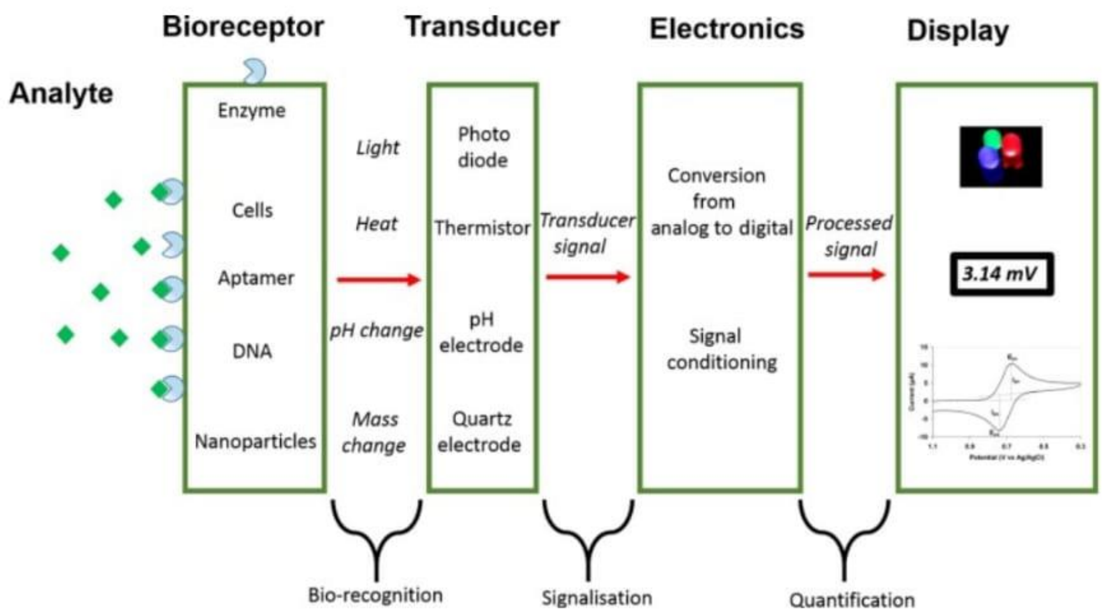

Fig:4.1 Components of Biosensors

Analyte: A substance of interest that needs detection. For instance, glucose is an 'analyte'in a biosensor designed to detect glucose.

Bioreceptor: A molecule that specifically recognises the analyte is known as a bloreceptor. Enzymes, cells, aptamers, deoxyribonucleic acid (DNA) and antibodies are some examples of bioreceptors. The process of signal generation (in the form of light, heat, pH, charge or mass change, etc.) upon interaction of the bioreceptor with the analyte is termed bio- recognition.

Transducer: The transducer is an element that converts one form of energy into another. In a biosensor the role of the transducer is to convert the bio-recognition event into a measurable signal. This process of energy conversion is known as signalisation. Most transducers produce either optical or electrical signals that are usually proportional to the amount of analyte-bioreceptor interactions.

Electronics: This is the part of a biosensor that processes the transduced signal and prepares it or display. It consists of complex electronic circuitry that performs signal conditioning such as amplification and conversion of signals from analogue into the digital form. The processed signals are then quantified by the display unit of the biosensor.

Display: The display consists of a user interpretation system such as the liquid crystal display of a computer or a direct printer that generates numbers or curves understandable by the user. This part often consists of a combination of hardware and software that generates results of the biosensor in a user-friendly manner. The output signal on the display can be numeric, graphic, tabular or an image, depending on the requirements of the end user.

Biological Element

$\Downarrow$

Biological Response

$\Downarrow$

Electrical Response

$\Downarrow$

Measurement

Oxidation acts as a catalyst and alters the pH of the biological material. The change in pH will directly affect the current carrying capacity of the enzyme, which is once again, in direct relation to the enzyme being measured. Output of the transducer i.e the current, is a direct representation of the enzyme being measured. The current is generally converted into voltage so that it can be properly analyzed and represented.

1. Biosensors works on the principle of signal transduction and biorecognition of element.

2. All the biological materials including-enzyme, antibody, nucleic acid, hormone, organelle or whole cell can be used as sensor or detector in a device. But the desired bio-receptor is usually a specific deactivated enzyme.

3. The deactivated enzyme is placed in proximity to the transducer.

4. The tested analyte links to the specific enzyme (bio-receptor) and inducing a change in biochemical property of enzyme. The change in in turn gives an electronic response through an electroenzymatic approach.

5. Electroenzymatic process is the chemical process of converting the enzymes into corresponding electrical signals with the aid of transducer.

6. Now, the outcome from transducer i.e. electrical signal is a direct representation of the biological material (i.e. analyte and enzyme in this case) being measured.

7. The electrical signal is usually converted into physical display for its proper analysis and representation.

Chapter 5

Working Principle of Biosensors:

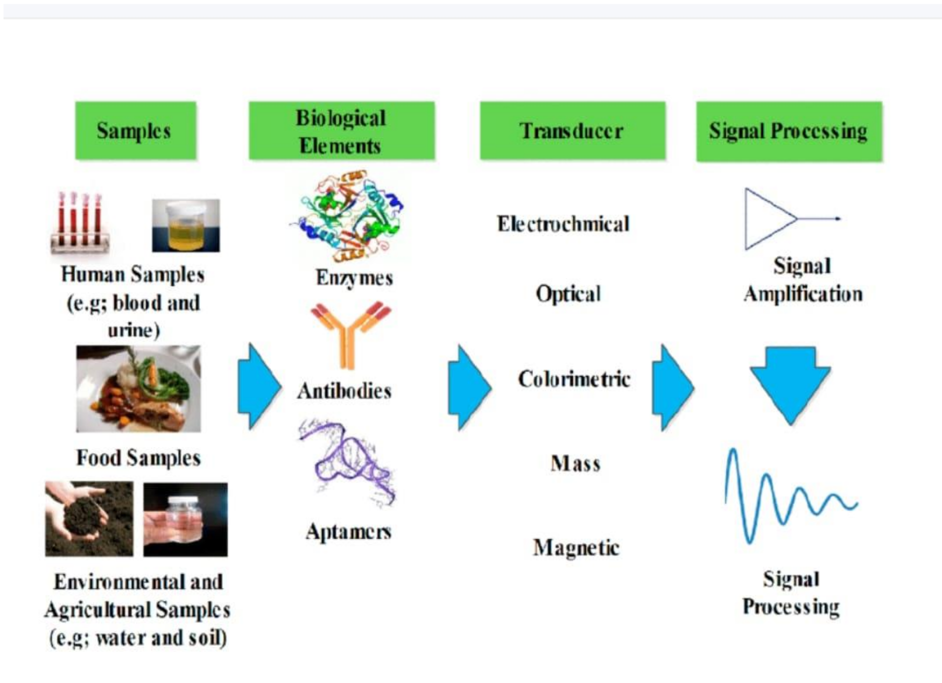

Fig: 5.1 Working Principle of Biosensors

1. The union of biological sensitive element and a transducer is responsible to convert the biological material into a corresponding electrical response in form of signal.

2. The output of the transducer will be either current or voltage relying on the type of enzyme.

3. If the output is voltage, then it is fine. But if the output is current, then this current needs to be converted into equivalent voltage (using an Op-Amp based current to voltage converter) before proceeding further.

4. The output voltage signal is generally very low in amplitude and is superimposed on a high frequency noise signal.

5. Thus, the signal is amplified (using an Op-Amp based Amplifier) and then it is passed through a Low Pass RC Filter.

6. Signal Processing Unit or a Signal Conditioning Unit is accountable for performing the this process of amplifying and filtering the signal.

7. The output of the signal processing unit is termed as an analog signal. This output is equivalent to the biological quantity being measure.

Chapter 6

Characteristics of a biosensor:

There are certain static and dynamic attributes that every biosensor possesses. The optimisation of these properties is reflected on the performance of the biosensor. Selectivity is perhaps the most important feature of a biosensor.

1.Selectivity:

Selectivity is the ability of a bioreceptor to detect a specific analyte in a sample containing other admixtures and contaminants, The best example of selectivity is depicted by the interaction of an antigen with the antibody. Classically, antibodies act as bioreceptors and are immobilised on the surface of the transducer. A solution (usually a buffer containing salts) containing the antigen is then exposed to the transducer where antibodies interact only with the antigens. To construct a biosensor, selectivity is the main consideration when choosing bioreceptors.

2.Reproducibility:

Reproducibility is the ability of the biosensor to generate identical responses for a duplicated experimental set-up. The reproducibility is characterised by the precision and accuracy of the transducer and electronics in a biosensor. Precision is the ability of the sensor to provide alike results every time a sample is measured and accuracy indicates the sensor's capacity to provide a mean value close to the true value when a sample is measured more than once. Reproducible signals provide high reliability and robustness to the inference made on the response of a biosensor.

3.Stability:

Stability is the degree of susceptibility to ambient disturbances in and around the biosensing system. These disturbances can cause a drift in the output signals of a biosensor under measurement. This can cause an error in the measured concentration and can affect the precision and accuracy of the biosensor. Stability is the most crucial feature in applications where a biosensor requires long incubation steps or continuous monitoring. The response of transducers and electronics can be temperature-sensitive, which may influence the stability of a biosensor.

4.Sensitivity:

The minimum amount of analyte that can be detected by a biosensor defines its limit of detection (LOD) or sensitivity. In a number of medical and environmental monitoring applications, a biosensor is required to detect analyte concentration of as low as ng/ml or even fg/ml to confirm the presence of traces of analytes in a sample. For instance, a prostate-specific antigen (PSA) concentration of 4 ng/ml in blood is associated with prostate cancer for which doctors suggest biopsy tests. Hence, sensitivity is considered to be an important property of a biosensor.

5. Linearity:

Linearity is the attribute that shows the accuracy of the measured response (for a set of measurements with different concentrations of analyte) to a straight line, mathematically represented as $y=mc$, where c is the concentration of the analyte, y is the output signal, and mis the sensitivity of the biosensor. Linearity of the biosensor can be associated with the resolution of the biosensor and range of analyte concentrations under test. The resolution of the biosensor is defined as the smallest change in the concentration of an analyte that is required to bring a change in the response of the biosensor.

6.1 Types of Biosensors:

On the basis of sensor device as well as the biological material the biosensors are classified as:
1. Electrochemical biosensors
2. Calorimetric/Thermal detection biosensors
3. Optical biosensors
4. Piezo-electric biosensors
5. Resonant biosensors

1. Electrochemical biosensors:
Generally, electrochemical biosensor works on the principle that many enzyme catalysis reactions consumes or generates ions or electrons causing some change in electrical properties of the solution which can be detected and used as a measuring parameter.
An electrochemical biosensor uses an electrochemical cell with electrodes of different dimension and modifications.
Three kinds of electrodes are generally used-
a) Working electrode
b) Reference electrode
c) Counter or Auxilary electrode

2. Calorimetric/Thermal detection biosensors:
Calorimetric biosensors measure the change in temperature of analyte solution following enzyme action and interpret it in terms of analyte concentration in the solution.
The analyte solution is passed through a small packed bed column consisting immobilized enzyme.
The temperature of the solution is measured just before the entry of the solution into the column, and just as it leaves the column using separate thermistors.
It is the most usually applicable type of biosensor and can also be used for turbid and colourful solutions.

3.Optical biosensors:
Both catalytic and affinity reactions are measured by this biosensor.The products generated during the catalytic reactions cause a change in fluorescence that is measured by the biosensor. In the presence of 02, luciferase takes up the ATP released from the lysis of bacteria to produce light which is detected and measured by biosensor.

4. Piezo-electric biosensors:
In these biosensors, the surface is coated with antibodies which binds to the complementary antigen present in the sample solution.
This results in increased mass which decreases their vibrational frequency, this alteration/change is used to determine the amount of antigen present in the sample solution.

5.Resonant biosensors:
The vibrations of the electron cloud in a molecule is termed as resonant biosensors. The resonant angle depends on the refractive index of the medium.The refractive index in turn is determined by the local mass density on the metal surface, • If the surface of the metal film is modified with the antibody/receptor i.e. capture molecule, then specific binding occurs between the capture molecule on addition of the sample and its ligand leading to an alteration in mass and hence change in resonant.

6.2 Applications of biosensors in food industries:

Biosensors in food quality

It is obvious that many batch operations in the food industry are being replaced by continuous processing and high degree of automation. Accordingly, there is an increasing demand for instruments suitable for automatic quality control through the process and at the end of the line so that the real time state of the process can be described. Biosensors obviously offer food industry monitoring of specific analyte at real-time and a feedback control. This will not only increase the food safety but also provide less effective control, less employment, time and energy saving (Velasco-Garcia and Mottram, 2003).
Biosensors can be used as analytical tools in some food industries, especially applied to the determination of the composition, degree of contamination of raw materials and processed foods, and for the on-line control of the fermentation process. Despite the enormous diversity of research involving biosensors for the food industry, its application in this area for any analyte is still restricted (Mello and Kubota, 2002).

Some important applications of biosensors to the area of food quality control are as follows:

1.Detection of microorganisms:
Conventional methods to determine and specify microorganisms are time consuming and laborious. They are based on so-called colony counts on solid media and often include different enrichment and isolation steps on selective media. The confirmation of the identity of the isolated microorganism is achieved by microscope, biochemical and immunological characteristics. This leads to total detection times of several days which are the major disadvantage of conventional plating methods. However, improved analytical methods have been developed which predominantly use the advantages supplied by immunological or t-based methods for the past decade biosensors have become more and more important for the determination of microorganism. leads to total detection times of several days which are the major disadvantage of conventional plating methods. However, improved analytical methods have been developed which predominantly use the advantages supplied by immunological or t-based methods for the past decade biosensors have become more and more important for the determination of microorganism.
Very specific antibodies can be produced against surface antigens of various microorganisms. In this way, an immunosensor can discriminate between different organisms. In combination with different transducers (e.g. piezoelectric materials or optical fibers) antibodies have been successfully employed for the detection of microorganisms. Most applications focus on confiming the absence of pathogenic organism like Salmonella species and Escherichia coli species. It is worth to mention that many strains of Escherichia coli are known to be dangerous human pathogens that can cause life-threatening conditions including bloody diarrhea, hemorrhagic colitis, renal failure and meningitis.
In recent years, various types of biosensors have been developed which could help in overall quality control in food processing plants by detecting pathogens within minutes of sampling. If pathogens are found with on- or near-line bios sensors, then food processors can make decisions more quickly about applying treatments, minimizing the chance of a contaminated final product.

2.Quality control of modified atmosphere packages:

Improper package design or temperature abuse during handling may cause fruits and vegetables in modified atmosphere packages to be exposed to low, injurious 02 levels associated with the production of fermentation volatiles quality loss and eventually product breakdown (Velasco- Garcia and Mottram, 2003). Excessively low package 02 also may promote growth of dangerous pathogens. The detection of ethanol would provide a sensitive technique for low-02 injury identification. A commercial ethanol biosensor composed of a chromagen and immobilized enzyme: alcohol oxidase and peroxidase have been tested. Alcohol oxidase catalyses oxidation of ethanol into acetaldehyde and H202 in the presence of O2 and peroxidase (an H202 decomposing enzyme) catalyses oxidation of the chromagen causing a colour change. The biosensor detects ethanol to levels below the human olfactory threshold (10µl) ethanol in gas phase at 50°Cwith a 15s exposure. The onset of low 02 injury was detected in lightly processed lettuce, cauliflower, broccoli und cabbage modified atmosphere packages as measured by accumulation of headspace ethanol (Smyth et al., 1996).

3.Fish freshness analysis:

various ions. Except ethanol and glycerol, other aliphatic and aromatic alcohols, amino acids and phenolic compounds are present at much lower concentrations.

The main enzyme substrate (glucose for GDH, glycerol for GIDH and ethanol for ADH) is firstly oxidized while the enzyme"s cofactor is simultaneously reduced. The active form of the enzyme is regenerated via the interaction with the electrochemical mediator (Os modified redox polymer), which is maintained in its oxidized form by the positive potential applied at the electrode.

4.Ethanol determination in alcoholic beverages:

Microbial biosensor was used to determine ethanol concentration in alcoholic beverages. Different dilutions between 40 and 500 times were performed. Results were compared with the enzymatic spectrometric method. The correlation coefficient of experimental data of 0.9983 shows a good correlation between biosensor and spectrometric method. The variation coefficient was no more than 5% for biosensor and 2.5% for spectrometric method, for determination realised in the same day.

5.Free radicals and antioxidants:

Antioxidants are one of the main ingredients that protect food attributes by preventing oxidation that occurs during processing, distribution and end preparation of food. Different types of antioxidants are added to food products to increase the attributes of food product. Amperometric biosensors are generally preferred for the determination of antioxidants in various food products (Mello and Kubota, 2007).

6. Fresh fruits and vegetables:

The fresh produce industry illustrates many of the problems encountered across the whole food industry in terms of still generally employing archaic QC methodologies (Manikas, 2002)

Chapter 7

Advantages of Biosensors:

1.Using biosensors we can easily detect the harmful chemicals or biological agents inside a human body.

2. A biosensor can convert the biological signal into an electrical or electronic signal which can be easily measured, quantified and amplified.

3. A biosensor can work just utilizing a specific biochemical reaction, there is no more complexity in the working of the biosensor.

4. Biosensor technology not so costly, day by day the uses of biosensor technology increases. 5. Biosensors are highly specific for the analyte.

6. Biosensor devices are very smaller in size and they are biocompatible.

7. Biosensors are very reliable

7.1 Impact of Biosensors:

Looking at the never-ending literature related to biosensors over the last few decades, it undoubtedly reveals that biosensors are attractive not only in academia but also in industry. Biosensor technology exploits the unique properties of a biological recognition event on a transducing device. In such an event, the interaction of the analyte with the bioreceptor is converted into a suitable output that is easily readable by the user. This approach not only exploits the molecular binding event, but also brings researchers from different areas of science and engineering to bridge their skills. Similar practices have created an immense impact on early-stage researchers in the field of biosensors. A simple example is a pregnancy test biosensor where researchers from biology highlighted the biological aspects and co-operated with engineers to work on the electronics of the system for the read-out. Finally, research from the laboratory is being transferred to customers worldwide because of management professionals. It would be naïve to think that biosensor research is confined to a niche-this can be seen clearly by the rapid increase in biosensors available in the market in recent times. Recently, there has been a gradual increase in start-up companies based on biosensor technology worldwide, which is having a profound impact on the healthcare industrial sector. In general, it can be said that biosensors have found an important place in our society as they aim to improve the quality of life in diverse areas such as homeland defence and security, agriculture, food safety, environment, medicine and pharmacology.

Conclusion:

Biosensor is a rapidly growing field encompassing various fields like medicine, agriculture and environmental science. Biosensors application in medical field is highly advanced especially in the area of medical diagnostics. In the food industry quality control is a major thrust area, the need for fast methods to monitor the quality of food is urgent. Conventional methods are expensive, time consuming and labour intensive. Development of efficient sensors will not only speed up the process but will be also cost effective. Biosensor is an interdisciplinary field involving many areas; research in material science, microfabrication and nanofabrication will enhance the development of suitable sample preparation steps, such as extraction, concentration, and isolation. Future sensors developments must focus on provide multi-analyte detection combined with signal transmitters for remote sensing. Biosensor technology is in its infancy but there is unlimited potential to reduce costs and still provide a respectable quality of care. Intel and other companies are taking steps in the right direction to provide products and services that will benefit patients and help retain their dignity. “According to the U.S. Healthcare Financing Administration, home care is the largest growing segment of health care. Experts believe that care for the elderly will increasingly be provided in homes and in low-skill care facilities as opposed to institutions.” (Intelligent Biosensors to Monitor Seniors Movements, 2007) These statements solidify the viability of biosensors and other telehealth devices.

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