

CSIR NET Life Science Unit 12

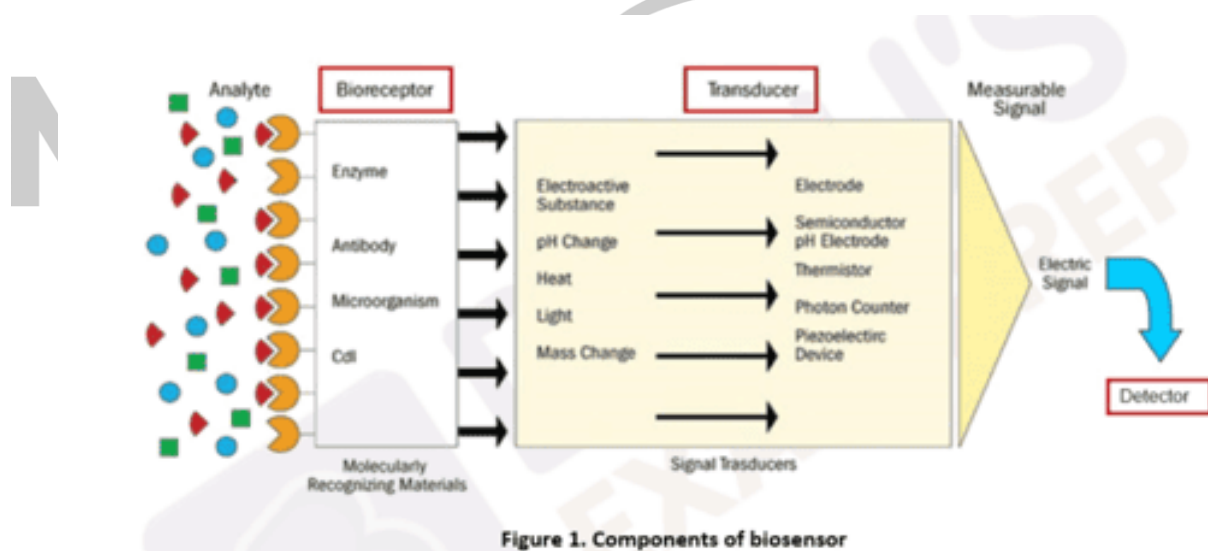
Biosensors

A biosensor is a measurement instrument that contains an immobilized biological material (enzyme, antibody, nucleic acid, hormone, organelle, or complete cell) that can interact with an analyte and produce physical, chemical, or electrical signals. An analyte can be any compound like glucose, urea, drug or pesticide whose concentration has to be measured. Biosensors in general are analytical devices that detect changes in biological processes and convert the data into an electrical signal. Most of the biosensors have immobilized enzymes.

Biosensors have the following characteristics:

- Stability
- Economical
- Sensitivity
- Reproducibility

The biosensor's block diagram is divided into three sections: bioreceptor, transducer, and detector of electric signals (*Figure 1*).



The sensor is a sensitive biological part in the first segment, the detector part in the second segment alters the resulting signal from the analyte contact, and the results are shown in an accessible manner in the third segment. The last section includes a signal conditioning circuit, an amplifier, and a display unit, as well as the CPU. A biosensor is made up of two major

parts: a **biological component** other than a cell or an enzyme, and a **physical component** including an amplifier or a transducer. The biological component communicates with the analyte to generate a signal that can be detected by the transducer. The biological material is properly immobilized over the transducer, and it can be utilized repeatedly for an extended period of time.

Biosensors can be classed according to typical forms of **bioreceptor interactions** including antibodies/antigens, enzymes/ligands, nucleic acids/DNA, cellular structures/cells, or biomimetic materials, but the type of biomolecule utilized can vary widely.

- Antigen-antibody interaction – Serological testing (covid testing)
- Artificial binding proteins - Recombinant binding fragments (Fab, Fv or scFv) or domains (VH, VHH) of antibodies. Antigen Binding Proteins (AgBP) are capable of selective binding to different target proteins while keeping the parent molecule's beneficial qualities. Display techniques such as phage display, ribosome display, yeast display, or mRNA display are frequently used in vitro to select family members that specifically bind to a given target antigen.
- Enzymes/ligands - Here the enzyme converts the analyte into a product that is sensor-detectable, and enzyme inhibition or activation is detected by the analyte or monitoring modification of enzyme properties resulting from interaction with the analyte.
 - Nucleic acids/DNA - Nucleic acid-based receptors can be either based on complementary base pairing interactions referred to as genosensors or specific nucleic acid-based antibody mimics (aptamers) as aptasensors.
 - Cellular structures/cells – Here for example cells of microalgae are entrapped on a quartz microfiber and the chlorophyll fluorescence modified by herbicides is collected at the tip of an optical fiber bundle and transmitted to a fluorimeter.
 - Microbial biosensors – These exploit the response of bacteria to a given substance eg: arsenic detection by ars operon.
- The following characteristics are used to classify biosensors in a broad sense:

- **Bio affinity devices** - These rely on the selective binding of a surface-restricted ligand partner (antibody, oligonucleotide, DNA, cell) to a target analyte.
- **Bio catalytic devices** - Here, an immobilized enzyme is used to aid in the identification of the target substrate. Sensor strips with immobilized GOX, for example, have proved used for monitoring diabetes mellitus at home.

Biosensors can also be classified based on transducer type. The most relevant ones are noted below.

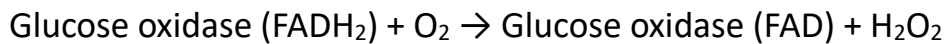
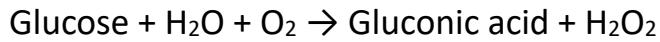
Calorimetric Biosensors

Many enzyme-catalyzed processes emit "heat," which can be detected with biosensors (exothermic). Calorimetric biosensors assess the temperature change in the solution containing the analyte as a result of enzyme action and interpret the temperature change in terms of the analyte concentration in the solution. This is the most widely used type of biosensor, and it can detect turbid and brightly colored liquids. The most significant disadvantage is maintaining the temperature of the sample stream at a consistent temperature, say + 0.01°C. For most applications, the sensitivity (10^{-4} M) and range (10^{-4} - 10^{-2} M) of such biosensors are insufficient. The sensitivity of the biosensor can be improved by linking multifunctional enzymes. The use of glucose oxidase for glucose determination is one example.

- **Electrochemical transducer** - Here chemical reaction between immobilized receptors and the target analyte produces ions or electrons, which are used to measure the electrical properties of the solution. Amperometric and potentiometric transducers are the most commonly used types.

1. **Amperometric transducer** - The current resulting from the electrochemical oxidation or reduction of an electroactive species is measured using amperometry. The Clark oxygen electrode, which determines the decrease of O₂ present in the sample (analyte) solution, is used in the simplest amperometric biosensors and is grouped under first-generation Redox reactions that can be analyzed by amperometric biosensors. The measurement of glucose using glucose oxidase is an example of these biosensors.

- i. **Enzyme-based glucose transducer** - Leland C. Clark developed this enzyme-based electrochemical biosensor. Glucose oxidase catalyzes the oxidation of glucose to gluconolactone, which is hydrolyzed to gluconic acid and hydrogen peroxide.



The enzyme glucose oxidase acts as a bioreceptor molecule and once it binds with the glucose molecule, it oxidizes glucose to gluconic acid and hydrogen peroxide. For the measurement of glucose in an aqueous solution, three different transducers are used.

- An oxygen sensor for determining O_2 concentration - converts O_2 to electric current.
- A pH sensor for measuring gluconic acid – converts pH change to voltage change.
- A peroxidase sensor that measures H_2O_2 concentration - converts peroxidase concentration to electric current. Biosensor is made of dialysis membrane using oxygen electrode a thin layer of glucose oxidase entrapped on it. In this Amperometric electrochemical glucose biosensor the amount of glucose was calculated by reduced dissolved O_2 concentration, which is proportional to glucose concentration. Glucose dehydrogenase instead of glucose oxidase can be also used, but its uses are practically restricted due to the oxidation of NAD^+ to NADH , NADH increase voltage and it has to be mediated.

Substrate	Bioreceptor	Product obtained
Choline	Choline oxidase	H_2O_2
Ethanol	Ethanol oxidase	H_2O_2

Hypoxanthine	Hypoxanthine	H ₂ O ₂
L-glutamate	Glutamate oxidase	H ₂ O ₂
Sucrose	Invertase	H ₂ O ₂
Oligosaccharides	Glucosylase / glucose oxidase	H ₂ O ₂

1. **Potentiometric transducer** - These biosensors transform a biological reaction into an electrical signal using ion-selective electrodes. pH meter glass electrodes (for cations), glass pH electrodes coated with a gas selective membrane (for CO₂, NH₃, or H₂S), or solid-state electrodes are the most frequent electrodes used. Many reactions generate or consume H⁺, which the biosensor detects and measures; in these circumstances, extremely weakly buffered solutions are utilized. The amount of gas produced is detected and measured with gas sensing electrodes. Urease, which catalyzes the processes below, is one example of such an electrode.



Following are examples of potentiometric biosensors.

Substrate	Bioreceptor	Product detected
Aspartamine	L-aspartase	NH ₃
Fats	Lipase	Fatty acids
Glucose	Glucose oxidase	Gluconic acid

Urea	Urease	NH_4^+ , CO_2
Nitrite	Nitrite reductase	NH_4^+
Penicillin	Penicillinase	H^+
Sulfate	Sulfate oxidase	HS

Enzyme Electrodes

Enzyme electrodes are a new form of biosensor that has been devised for amperometric and potentiometric assays of substrates such as urea, amino acids, glucose, alcohol, and lactic acid. A given electrochemical sensor is in close contact with a thin permeable enzyme membrane capable of reacting with the given substrates to form the electrode. The enzyme is implanted in the membrane and, depending on enzymatic processes, produces O_2 , H^+ , NH_4^+ , CO_2 , or other tiny molecules. The specialized sensor picks up on this. The concentration of substrates is determined by the magnitude of the response.

Optical Biosensors

These biosensors are capable of measuring both catalytic and affinity processes. The products created by catalytic processes induce a change in fluorescence or absorbance, which they monitor. Alternatively, they assess changes in the intrinsic optical characteristics of the biosensor surface as a result of dielectric molecules such as protein being loaded on it (in case of affinity reactions). The firefly enzyme luciferase is an example of this type of biosensor that uses luminescence to identify bacteria in food or clinical samples. The bacteria are lysed to release ATP, which is used by luciferase to create light in the presence of oxygen, which is monitored by the biosensor.

Bio affinity Sensors

Bio affinity sensors have only lately been developed. It uses equilibrium binding to determine the concentration of the determinants, i.e. substrates. This demonstrates a high level of sensitivity. It uses

- **Third-generation instruments** - Here, the reaction creates a response without the usage of intermediaries. The biochip can be used in such equipment when electrochemistry happens on a semiconductor. The progression from the use of a freely diffusing mediator (O₂ or artificial) to a system where the biocatalyst and mediator are immobilized at an electrode surface, thereby making the recognition component an integral part of the electrode transducer, is characterized by the third generation sensors that do not have reagents. Redox mediator label the enzyme followed by enzyme immobilization could be used to achieve immobilization of enzyme and mediator.
- A successful biosensor must have some of the following features:
 - High specificity/sensitivity for the analyte
 - The reaction used should be independent of factors like stirring, pH, temperature etc.,
 - The response time should be linear over a useful range of analyte concentrations,
 - Tiny and bio-compatible in case it is to be used for analyses within the body.
 - Cheap, durable, and easy to use
 - Range and detection limit

Applications of Biosensor

- Uses in Medicine and Health (Glucose biosensor)
- Uses in Pollution Control (a biosensor coupled with oxygen electrode and immobilized *Trichosporon cutaneum* is used for measuring biological oxygen demand (BOD))
- Uses in Industry (spectrophotometer and auto-analyzer are used to estimate the substrates utilized and the products formed in the fermented broth)
- The biosensor in Military biosensor application is to provide support to the military with such a biosensor that can detect toxic gases including chemical warfare agents.