Applications of immobilized enzymes

Introduction :

The interest that research organizations, industry, and medical teams are currently showing in immobilized enzymes stems from their stability and the ease with which they can be separated from reaction mixtures, making it possible to easily repeat multiple treatment cycles using the same preparation.

1. Enzyme manufacturers :

Company	Country	Estimated Market Share (%)
Novozymes	Denmark	48–50%
BASF	Germany/USA	12–14%
DSM/Firmenich	Netherlands/Switzerland	10–12%
Amano Enzyme	Japan	6–8%
QIAGEN	Germany	4–5%
DuPont (IFF)	USA	3–4%
Huvepharma	Bulgaria	2–3%
Others (Calzyme and Enzyme research/Biolaxi)	USA India	15–20%

2. Distribution of enzyme sales :

Industry	Percentage (in 2005)
Detergents	32 %
Other	29 %
Starch	15 %
Dairy products	14 %
Textiles	10 %

Industry	Percentage (in 2025)
Food and beverages	38 - 40 %
bioethanol	25 %
Animal feed	15 %
Detegents	12 %
Pharmaceuticals and diagnostics	5 - 6 %
Other (Textiles, Leather, Wastewater, Paper, Cosmetics)	4 – 5%

In fact, 75% of the enzymes used in industry are hydrolases that decompose natural substances.

- 40% proteases (dairy products and detergents)
- Carboxylase (starch and textile industries, baking, alcohol production)

3. Industrial applications :

3.1. Applications in the food industry (FI):

The most important ones are :

***Glucose industry** :*

Production of sweeteners (substances capable of imparting a sweet taste to food preparations) from starch, such as glucose and fructose syrups.

- ✓ Bacterial α-amylases : liquefaction of starch
- ✓ Fungal amyloglucosidases : saccharification (amylolytic hydrolysis of gelatinized starch, which primarily provides maltose)
- ✓ Bacterial glucose isomerases : HFCS (high fructose corn syrup : It is a new sweetener produced through the enzymatic conversion of starch).

Sugar Refinery :

Crystallization and refining

- ✓ Fungal melibiase : hydrolysis of raffinose (a highly soluble trisaccharide with no sweet taste, consisting of 2 glucose and 1 fructose molecules; it can be enzymatically broken down into melibiose).

Breadmaking process :

All the steps involved in making bread.

- ✓ Fungal proteases : kneading (through hydration and mechanical action, these enzymes help form a cohesive, viscoelastic gluten network that retains carbon dioxide, ethanol, and the aromas produced during fermentation)
- ✓ Fungal xylanases : viscosity, elasticity;
- ✓ Lipases : strengthen the gluten, resulting in a more stable paste and better bread quality.

Beverage industry :

- ✓ Fungal pectinases : juice clarification (removal of compounds responsible for cloudiness)
- ✓ Pectinases + hemicellulases : pressability of pulp and must (improved juice extraction from pulp);
- ✓ Fungal α-amylases : apple and pear juices;
- ✓ Enzymes are also widely used in wine production.

Maceration/liquefaction :

Maceration is the process of soaking a food product in a liquid to flavor or preserve it; liquefaction, on the other hand, involves converting a compound into a liquid state.

Cellulase, fungal pectinases:

- ✓ Nectars (a beverage made from fruit juice or puree mixed with water and sugar);

- ✓ Coulis (a sauce made from various food ingredients reduced to a puree)

Dairy and Cheese Industries :

- ✓ Animal proteases (chymosin or presure)
- ✓ Lactic acid bacteria : sour milk, yogurt, cheese
- ✓ Lactase : lactose-free milk. It converts milk lactose into glucose and galactose. It is used in dairy products consumed by people with lactose intolerance.

3.2. Applications other than in the agri-food industry :

Detergent industry :

This industry is currently the largest user of industrial enzymes, employing proteases, lipases, amylases, and cellulases, primarily for the production of “biological” laundry detergents.

These enzymes rapidly break down or loosen stains that would normally only be removed at much higher temperatures or through the action of larger quantities of chemical detergents over a longer period of time.

Textile industry :

The use of enzymes in the textile industry is on the rise due to their efficiency, selectivity, and environmental benefits.

- ✓ Amylases / cellulases : jeans fading = lightening the color (stone-washed)
- ✓ Bacterial amylases : starch removal
- ✓ Various cellulases : anti-pilling (preventing the formation of small fuzzy balls on fabric caused by friction)
- ✓ Fungal pectinases : retting (partial removal of pectin bonds from the fiber bundles of certain textile plants such as linen and hemp)

Paper industry (paper manufacturing) :

- ✓ Xylanase : used in pulp bleaching, which significantly reduces the need for chlorine-based bleaching chemicals;
- ✓ Amylase : paper manufacturing (starch modification);
- ✓ Lipases : removal of sticky substances that cause problems in paper machines.
- ✓ Laccase : Degrades lignin to effectively bleach the pulp.

Leather industry (tannery) :

This industry uses proteolytic and lipolytic enzymes. These enzymes (bacterial and fungal) are used to remove unwanted parts and make the leather more supple and easier to dye.

Animal feed industry :

Adding wheat-based xylanases to broiler chicken feed increases the amount of metabolizable energy available.

It reduces viscosity, which improves nutrient absorption, releases nutrients through the hydrolysis of indigestible fiber, and reduces the amount of feces.

Enzymes in personal care products :

These products represent a relatively new area of application for enzymes.

- ✓ Some solutions containing proteases and lipases are used to clean contact lenses.
- ✓ Catalase is used to remove residual hydrogen peroxide after contact lenses have been disinfected.
- ✓ Proteases, amylases, and lipases are used to disinfect medical equipment (in surgery, endoscopy, contaminated instruments, etc.).
- ✓ Some toothpastes contain glucoamylase and glucose oxidase.
- ✓ Enzymes are also being studied for use in skin and hair care products.

4. Therapeutic and pharmaceutical applications :

Some diseases are caused by the absence or deficiency of enzymatic systems, resulting either in a deficiency of an essential metabolite that should be synthesized or, conversely, in the accumulation of a toxic product that is not degraded.

The treatment of certain pathological conditions (caused by enzyme deficiency) through the administration of enzymes faces several challenges : destruction by proteases or stomach acid, or hydrolysis by macrophages. In such cases, the enzyme is bound to a protective molecule (albumin, dextrin, polyethylene glycol) or encapsulated in microcapsules.

The enzymes used in therapy come from a variety of sources : bacteria, fungi, animals, plants, and even humans. Currently, these enzymes are primarily produced using recombinant DNA technology and recombinant proteins.

Examples :

- ✓ Antithrombotic agents : plasmin.
- ✓ Coagulation agents : thrombin, factor III
- ✓ Genetic disorders : adenosine deaminase, glucocerebrosidase, …
- ✓ Cancer : asparaginase, urate oxidase, arginine deaminase, …
- ✓ Infectious diseases : lysozyme, chitinase, ribonuclease, …
- ✓ Certain enzymes can be used as digestive aids (amylase, galactosidase, cellulase, lactase, pepsin, papain, lipase, pancreatin, …).

Certain enzymes are used in the production of drugs (penicillin/β-lactamase, ampicillin and amoxicillin/penicillin amidase, stereospecificity/acylase and esterase, ibuprofen/lipase, etc.)

5. Analytical Applications :

- ✓ As early as the 1960s, researchers recognized the great potential of enzymes for medical diagnostics and for the analysis of food and beverages (biosensors and enzyme test kits).
- ✓ Unlike most industrial enzymes, analytical enzymes must be free of secondary activities; this means that complex purification processes are required.
- ✓ Biosensors represent the most significant advancement in analytical chemistry. The most widely used application is the glucose biosensor, which relies on a reaction catalyzed by glucose oxidase.
- ✓ Several commercial instruments are available that apply this principle to measure molecules such as glucose, lactate, lactose, sucrose, ethanol, methanol, cholesterol levels, and certain amino acids.

6. Biosensors :

6.1. History :

The first biosensor was developed by Leland Clark in **1956** to measure the concentration of dissolved oxygen in blood (Leland, 1956). Leland Clark is known as the “father of biosensors,” and his invention of the oxygen electrode carries his name : the Clark electrode.

In **1962**, this biosensor was improved by the same researcher to measure blood glucose levels (Clark and Lyon, 1962), followed by the discovery of the first potentiometric biosensor capable of detecting urea by Guilbault and Montalvo in **1969**.

The year **1975** saw the discovery of the first immunosensor designed to measure ethanol and lactic acid by Suzuki and colleagues.

Over the past few decades, the field of biosensors has grown remarkably under pressure from various application areas. Their compact and portable nature, as well as their high specificity and sensitivity, make them one of the best alternatives to existing analytical techniques.

6.2. Definition :

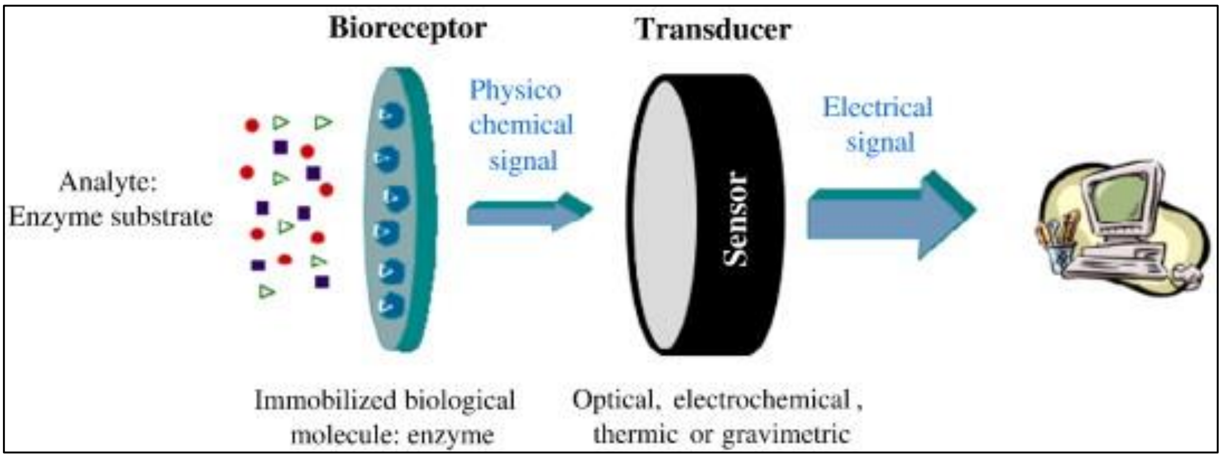
A ***biosensor*** is a detection tool or system that contains specific immobilized biochemical components (enzymes, antibodies, bacteria, or yeast), known as “ligands,” combined with electronic components that transmit and convert a biochemical signal into a physical signal. This system enables the measurement of a physical, chemical, or biological parameter.

6.3. Principle :

This analytical device is, therefore, the result of the synergy between an immobilized biological recognition element (bioelement) and a physical sensor: the transducer.

Biological recognition elements can be of very different types (antibodies, enzymes, DNA, cells). These elements interact with their environment, and this interaction generates a specific analog signal (emission of photons, changes in pH, mass, respiratory activity, etc.).

The role of the transducer is to convert the biological signal into an analyzable digital signal. The nature of the biological signals directly determines the type of transducer used. Consequently, the development of such systems requires advanced expertise in the fields of biology, physical chemistry, and signal processing, thereby necessitating close interdisciplinary collaboration.



6.4. Components of a biosensor :

- **The bioreceptor (ligand) :**

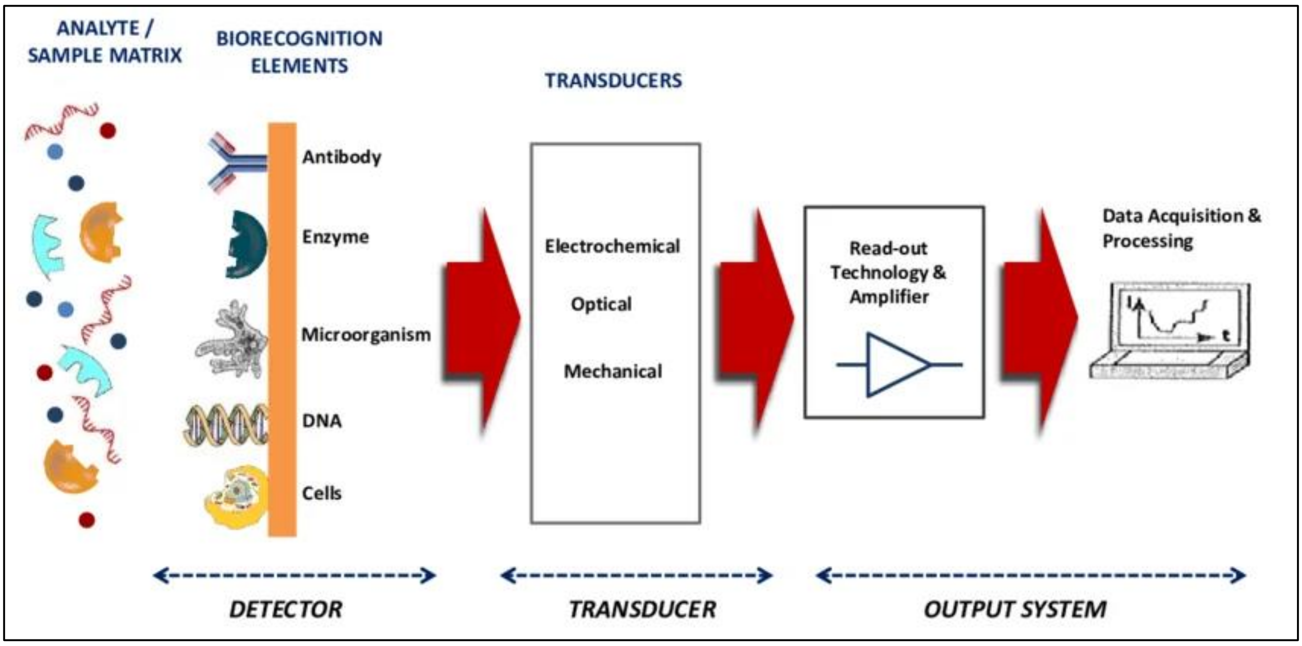
It constitutes the specific capture component of the biosensor, and in theory, any biochemical or biological structure possessing this specific recognition capability can be used. Examples: enzymes, animal or plant cells, bacteria, membrane protein receptors, DNA, …

- **The transducer :**

The transducer is the physical component that converts the biochemical change resulting from the interaction between an analyte and the bioreceptor into an electrical signal.

- **The conditioner :**

This component is capable of amplifying, acquiring, and processing signals to convert them into information in a format suitable for the user.



Classification of biosensors :

Biosensors can be classified according to various criteria :

- ✓ By type of biosensor : enzymatic, immunological, etc.
- ✓ By type of transducer : optical, electrochemical, thermal, etc.

In fact, enzymatic biosensors are the most widely used and commercially available. While they offer numerous advantages, including batch reproducibility, they can also exhibit operational instability and may require the use of a cofactor or multiple associated enzymes for a single biosensor.

There are various methods for immobilizing enzymes :

- ~ Immobilization on porous glass beads ;
- ~ Covalent bonding to dialysis membranes ;
- ~ Cross-linking with glutaraldehyde ;
- ~ Immobilization on agarose gel.

6.5. Characteristics of biosensors :

Specificity (selectivity) :

Specificity is determined by the bioreceptor, which is most often an enzyme. Specificity is an inherent property of an enzyme and cannot be modulated in practice. Some enzymes have very narrow specificity.

Stability :

This ability to maintain catalytic activity over time is also an inherent property of a given enzyme. However, certain experimentally controllable parameters such as pH, the nature of the buffer, or temperature influence stability.

Sensitivity :

This parameter represents the ratio between the increase in the sensor's response and the corresponding change in the measured quantity.

Reuse :

The reuse of the biosensor's sensing element is made possible by immobilization.

Reproducibility :

This is one of the most important parameters. It indicates the biosensor’s ability to produce results that are very close to one another when the same quantity of the measured variable is measured repeatedly.

Accuracy :

This is the match between the measurement result and the true value of the measured quantity, and the difference is called the absolute error.

Detection limit (resolution) :

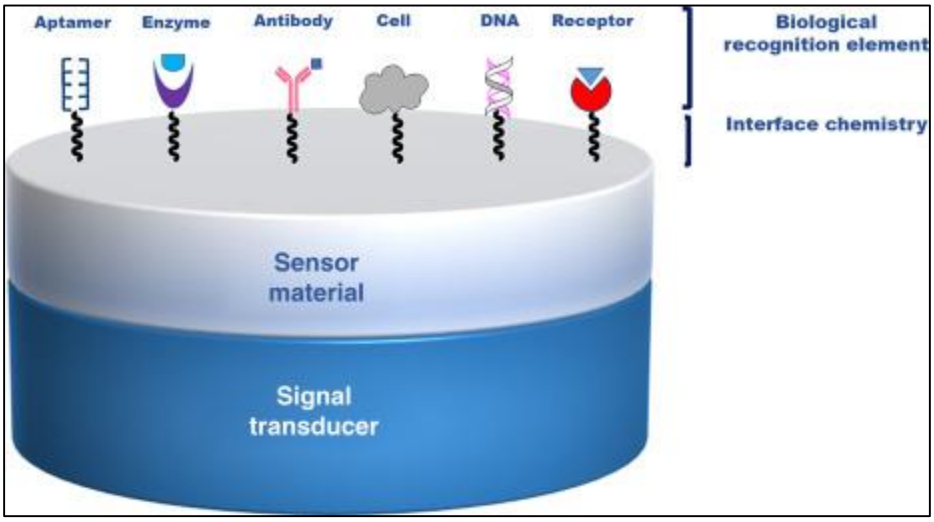
This is the smallest value of the quantity being measured that can be significantly detected by the biosensor.

Linearity :

In the linear region, the change in the output value is proportional to the change in the measured quantity.

Response time :

Response speed is defined by the time it takes for the biosensor to respond to a sudden change in the measured parameter. Knowing a biosensor's response time is essential when taking measurements.



6.6. Applications of Enzymatic Biosensors :

Domain of application	Usage
Food industry	✓ Detection and quantification of nutrients ✓ Detection of pathogens ✓ Detection of toxins, toxic chemicals, and heavy metals ✓ Monitoring fermentation processes
Health	✓ Measurement of glucose, cholesterol, cardiac troponin, and CRP ✓ Detection of ethanol in human blood ✓ Analysis of biomarkers for certain diseases, such as cancer ✓ Detection of viral agents (HIV, COVID-19, etc.)
Environment	✓ Heavy metal testing ✓ Organic pollutant testing ✓ Detection of toxic compounds ✓ Detection of pathogens