Biocatalysis

- **Biocatalysis** refers to the use of living (biological) systems or their parts to speed up (catalyze) chemical reactions. In **biocatalytic** processes, natural catalysts, such as enzymes, perform chemical transformations on organic compounds.
- Enzymes are biocatalysts- the catalysts of life. A catalyst is defined as a substance that increases the velocity or rate of a chemical reaction without itself undergoing any change in the overall process.
- **Enzymes may be defined as biocatalysts synthesized by living cells. They are protein in nature (exception - RNA acting as ribozyme), colloidal and thermolabile in character, and specific in their action**.
- In the laboratory, hydrolysis of proteins by a strong acid at 100 ºC takes at least a couple of days. The same protein is fully digested by the enzymes in gastrointestinal tract at body temperature (37 ºC) within a couple of hours. This remarkable difference in the chemical reactions taking place in the living system is exclusively due to enzymes. The very existence of life is unimaginable without the presence of enzymes.

Historical Background:

- **Berzelius** in 1836 coined the term catalysis (*Greek*: to dissolve).
- In 1878, **Kuhne** used the word enzyme (*Greek*: in yeast) to indicate the catalysis taking place in the biological systems.
- Isolation of enzyme system from cell-free extract of yeast was achieved in 1883 by **Buchner**. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol.
- In 1926, **James Sumner** first achieved the isolation and crystallization of the enzyme **urease** from jack bean and identified it as a protein.

Classification of Biocatalyst:

1. **Oxidoreductases**: Enzymes involved in oxidation-reduction reactions.

$$\text{Oxidation} \longrightarrow \text{Reduction}$$

$$AH_2 + B \longrightarrow A + BH_2$$

2. Transferases: Enzymes that catalyse the transfer of functional groups.

Group transfer

$$AX + B \longrightarrow A + BX$$

3. Hydrolases: Enzymes that bring about hydrolysis of various compounds.

Hydrolysis

$$AB + H_2O \longrightarrow AH + BOH$$

4. Lyases: Enzymes specialised in the addition or removal of water, ammonia, CO_2 etc.

$$\text{Addition} \longrightarrow \text{Elimination}$$

$$A\text{-}B + X\text{-}Y \longrightarrow AX\text{-}BY$$

5. **Isomerases**: Enzymes involved in all the isomerization reactions.

Interconversion of isomers

$$A \longrightarrow A'$$

6. **Ligases**: Enzymes catalysing the synthetic reactions (*Greek*: ligate to bind) where two molecules are joined together and ATP is used.

Condensation (usually dependent on ATP)

$$A + B \longrightarrow A\text{-}B$$

Biocatalyst Specificity:

Biocatalyst (enzyme) are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes. Three types of enzyme specificity are well-recognised:

(1) Stereospecificity,
(2) Reaction specificity,
(3) Substrate specificity

(1) Stereospecificity or optical specificity:

Stereoisomers are the compounds which have the same molecular formula, but differ in their structural configuration. The enzymes act only on one isomer and, therefore, exhibit stereospecificity.

e.g. L-amino acid oxidase and D-amino acid oxidase act on L- and D-amino acids respectively.

Hexokinase acts on D-hexoses;
Glucokinase on D-glucose;
Amylase acts on a-glycosidic linkages;
Cellulase cleaves p-glycosidic bonds.

Stereospecificitiys explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the surface of the enzyme.

- The class of enzymes belonging to isomerases do not exhihit stereospecificity, since they are specialized in the interconversion of isomers.

(2) Reaction specificity: The same substrate can undergo different types of reactions, each catalysed by a separate enzyme and this is referred to as reaction specificity. An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc. The enzymes however, are different for each of these reactions.

(3) **Substrate specificity**: The substrate specificity varies from enzyme to enzyme. It may be either absolute, relative or broad.

Absolute substrate specificity: Certain enzymes act only on one substrate e.g. glucokinase acts on glucose to give glucose-6 -phosphate, urease cleaves urea to ammonia and carbon dioxide.

Relative substrate specificity: Some enzymes act on structurally related substances. This, in turn, may be dependent on the specific group or a bond present. The action of trypsin is a good example for **group specificity**. Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (phenylalanine, tyrosine and tryptophan). Examples of **bond specificity** glycosidases acting on glycosidic bonds of carbohydrates, lipases cleaving ester bonds of lipids etc.

Broad specificity: Some enzymes act on closely related substrates which is commonly known as broad substrate specificity, e.g. hexokinase acts on glucose, fructose/mannose and glucosamine and not on galactose. It is possible that some structural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

Applications of Biocatalyst:

Biocatalyst	Application
Therapeutic Applications	
Streptokinase/urokinase	To remove blood clots
Asparaginase	In cancer therapy
Papain	Anti-inflammatory
α_1-Antitrypsin	To treat emphysema ((breathing difficulty due distension of lungs)
Analytical Application Reagents (for estimation)	
Glucose oxidase and peroxidase	Glucose
Urease	Urea
Cholesterol oxidase	Cholesterol
Uricase	Uric acid
Lipase	Triacygylcerols
Luciferases	To detect bacterial contamination of foods
Alkaline phosphatase/ horse radish peroxidase	In the analytical technique ELISA
Applications in Genetic Engineering	
Restriction endonucleases	Gene transfer, DNA finger printing
Taq DNA Polymerase	Polymerase chain reaction
Industrial Applications	
Rennin	Cheese preparation
Glucose isomerase	Production of high fructose syrup
α-Amylase	In food industry to convert starch to glucose
Proteases	Washing powder

Applications of Biocatalyst

In Textile Industry:

In olden times, textiles were treated with acid, alkali, or oxidizing agents or soaked in water for several days to breakdown the starch without knowing the role of microorganisms in this process. This practice was difficult to control and some times also led to damage or discoloration of the material. Crude enzyme extracts in the form of malt extract, or later, in the form of pancreas extract, were first used to carry out designing, which was followed by enzymes from other sources.

There are a large number of microorganisms that produce a variety of biocatalyst helpful in carrying out many processes. The Textile industry, particularly the chemical processing sector, always has a major share in the global pollution. Biocatalyst play a key role in such alternative processes. The use of biocatalyst in textiles started as long as a century ago. Bacterial amylase derived from *Bacillus subtilis* was used for desizing for the first time by **Boidin** and **Effront** in 1917.

Detergent Biocatalyst:

Breakthrough in detergents was made in 1959, when Dr. Jaag, a chemist, developed a new product called **Bio 40** that contained a bacterial protease instead of trypsin. Currently, these biocatalyst are manufactured commercially in large quantities through fermentation by common soil bacteria *Bacillus subtilis* or *Bacillus licheniformis*. This was made possible in the last two decades by the rapid advances in enzymology and fermentation technology. Although numerous other microorganisms produce proteases and amylases, the types secreted by the above strains have the advantage that they work best at the warm alkaline conditions prevailing in washing liquids. They also must not lose their activity in an environment that contains a multitude of potentially inhibitory chemicals routinely formulated into laundry detergents, such as surface active agents, magnesium or calcium ions, builders (sodium tripolyphosphate), perfumes, and other additives.

Biocatalyst to Convert Sugars from Starch:

Initially, fungal amylase was used in the manufacture of specific types of syrup, i.e., those containing a range of sugars, which could not be produced by conventional acid hydrolysis. This practice was changed in the 1960s when an enzyme glucoamylase was launched for the first time; this enzyme was capable of completely breaking down starch into glucose. Later, heat stable alpha amylase development led to further improvement in this process.

A large number of cellulose-, starch-, and sugar-containing plants can be processed to produce sugars and alcohols, such as sugarcane, sweet sorghum, and nipa palm, which are the candidates for the high yield production of alcohol fuel. Likewise, the starch-containing crops such as cassava, sweet potatoes, yams, taro, and tannia are good candidates, but require an additional step to breakdown starch to sugar, the major part of biomass containing cellulose and which, therefore, needs special treatment before it can be used to produce glucose and alcohols.

Years of research in biochemistry and biotechnology have boosted knowledge of biocatalyst for industries as well as research and led to the development of new techniques to modify and discover new application of enzymes in medicine, research, and industries. Thus, it has become the need of the day to device efficient methods for enzyme extraction as well as production for commercial purpose.

Production of Biodiesel:

The idea of using biodiesel as a source of energy is not new, but it is now being taken seriously because of the escalating price of petroleum and, more significantly, the depletion of fossil fuels (oil and gas) within the next 35 years and the emerging concern about global warming that is associated with burning fossil fuels. Biodiesel is much more environmentally friendly than burning fossil fuels, to the extent that governments may be moving toward making biofuels.

The global market survey of biodiesel has shown a tremendous increase in its production. Biodiesel is made by chemical combination of any natural oil or fat with an alcohol such as methanol and a catalyst (e.g., lipases) for the transesterification process. Trans esterification is catalyzed by acids, alkalis, and lipase enzymes. Use of lipases offers important advantages as it is more efficient, highly selective, involves less energy consumption (reactions can be carried out in mild conditions), and produces less side products or waste (environmentally favorable). However, it is not currently feasible because of the relatively high cost of the catalyst. The immobilized lipases most frequently used for biodiesel production are lipase B from *Candida antarctica*. This is supplied by Novozymes under the commercial name Novozym 435$^{®}$ (previously called SP435) an is immobilized on an acrylic resin.

Bioremediation: It involves the cleaning of environment with the help of microorganisms or plants. A complete biodegradation results in detoxification by mineralizing pollutants to carbon-dioxide, water and harmless inorganic salts. Incomplete biodegradation will yield breakdown products which are less toxic than the original pollutants. A number of microbial groups are known to be used in the process (Table 7.1). *Bacillus subtillis* has the ability to degrade crude oil. Similarly, *Vibrio cholera* can degrade heavy metals present in the soil or water. Fungi play a role in the decomposition of cellulose and lignin. A number of plants, like *Brassica juncea*, Tomato and alpine pennycress also have the potential to accumulate contaminants (arsenic) into their roots and aboveground shoots or leaves. Some can evaporate/transpire selenium, mercury, and other volatile hydrocarbons from soil and water

Material	Biocatalyst	Source

Cellulose Materials	Cellulase	*Penicillum funiculosum*
Chitin	Chitinase	Actinobacteria
Keratin	Keratinase	*Chysosporium keratinophilum*
Kraft pulp	Xylanase, b-Xylosidase	*Sreptomyces thermoviolaceus*
Sewage sludge	Protease, Phosphatase	Sulphate reducing bacteria
Nylon	Nylon-degrading enzyme (MnP)	Un-named white-rot Fungus (+ Mn and lactate)

Bioindicators*:* These include the species/organisms used to indicate the quality of the environment and it can be done by showing changes in different ways like behaviour, physiology or chemical changes. Lichens are one of the plant species which are found on rocks and tree trunk. Their disappearance from the forest indicate the environmental stress like high sulphur-dioxide pollution. Some microorganisms will produce new proteins, called stress proteins, when exposed to contaminants like cadmium and benzene. These stress proteins can be used as an early warning system to detect high levels of pollution.

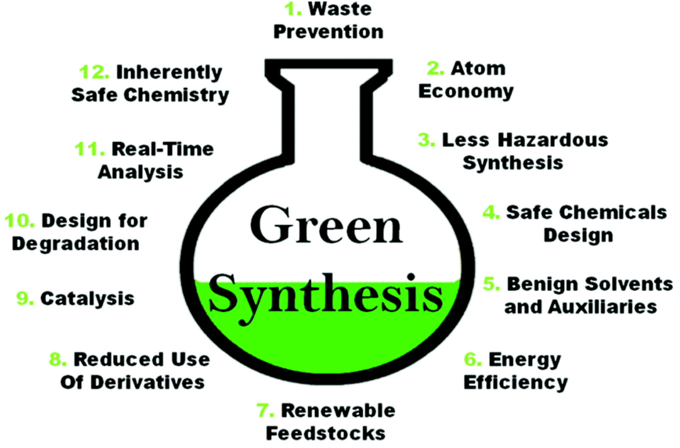
Waste management*:* Use of microbes in waste management is a major application of biotechnology and involves large-scale treatment of municipal wastes as well as industrial wastes. Bacteria digest wastes in order to produce CO_2 and methane gases. Waste treatment by this process converts about 93 percent of wastes into gases, leaving only about 3 percent as sludge.

Biomining*:* Biomining is the extraction of specific metals from their ores through biological means usually bacteria. A variety of mineral oxidizing bacteria are found in nature which can easily oxidize iron and sulfur containing minerals. These include the iron-oxidizing bacteria –*Thiobacillus ferrooxidans*, and the sulfur-oxidizing bacteria –*Thiobacillus thiooxidans*. This helps in reducing the environmental pollution caused due to the mining operations.

In Food Industry:

Applications in food industries are based on the use of enzymes obtained from various microorganisms. Number of daily used products like cheese, curd, beer and bread are the result of biotechnology. Cheese is traditionally prepared using calf rennet, a protease. Recently, genetically modified microorganisms (*E. coli, A. niger*) containing calf rennet gene have been developed. In baking and brewing industries, strains of *Saccahromyce scerevisae* are used for making bread soft , and producing beer. Lactic acid bacteria (*Lactobacillus sp.*) are used in producing dairy products like curd. Similarly, different species of *Penicillium* are employed to produce different flavoured cheese Pectinases are added into the canned juices to make them clear. Ethanol currently produced by fermenting grain (old technology). Cellulose enzyme technology allows conversion of crop residues (stems, leaves and hulls) to ethanol. Results in reduced CO_2 emissions by more than 90% (compared to oil). Allows for greater domestic energy production and it use a renewable feedstock. In Brazil, molasses (a dark syrup obtained from sugarcane) containing fructose and glucose can be fermented into alcohol by yeast. The alcohol is distilled, and mixes with petrol and burned as fuel in motor vehicles. It is known as gasohol. Domestic wastes or biodegradable wastes can be fermented by microorganisms like anaerobic bacteria *Methanobacterium* spp. to convert it into biogas.

Principles of Green Chemistry



1. It is better to prevent waste than to treat or clean up waste after it has been created.
2. Synthetic methods should be designed to maximise the incorporation of all' materials used in the process into the final product.
3. Whenever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and environment.
4. Chemical products should be designed to affect their desired functions while minimising their toxicity.
5. The use of auxiliary substances *(e.g.,* solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. Energy requirements of chemical processes should be recognised for their environmental and economic impacts and should be minimised. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. Unnecessary derivatization should be minimised or avoided if possible because such steps require additional reagents and can generate waste.
9. Catalytic agents as selective as possible should be used.
10. Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. Substances and the form of a substance used in a chemical process should be chosen to minimise the potential for chemical accidents, including releases, explosions and fires.
12. Real time analysis

Green Chemistry as an Alternative Tool for Reducing Pollution:

Innovative "green" chemical methods already have made an impact on a wide variety of chemical manufacturing processes by decreasing or eliminating the use or creation of toxic substances. For example cheaper, less wasteful and less toxic methods have been developed to produce ibuprofen, pesticides, new materials for disposable diapers and contact lens, new dry cleaning methods and recycled silicon wafer for integrated circuits. Some examples of green chemistry are given below:

(i) Halons are greenhouse gas compounds composed of carbon, fluorine and bromine. These are used in fire-fighting and other applications. Using a green chemistry approach, Pyrocool Technologies has synthesised a halon substitute. The product, called Pyrocool, is an environmentally benign foam that is more effective than halons in fire-fighting, even large scale fires such as those on oil tankers and jet airplanes can be extinguished in a short time.

(ii) Chlorofluorocarbons have been in use in refrigerators and air conditioning plants. However, these compounds have been found to be unfriendly to environment. These compounds are responsible for depletion of ozone layer in stratosphere and for global warming. Substitutes to these compounds such as HFC-134a ($CF_3 \cdot CH_2F$) have been discovered which are considered to be more friendly to environment.

(iii) Solvents used to dry clean clothes are usually chlorinated compounds such as tetrachloroethylene C_2Cl_4 which is a potential human **carcinogen.** These materials also have serious environmental consequences. **Dr. Joe Desimone** has discovered a substitute for chlorinated compounds by synthesising cleaning detergents that work in liquid' carbon dioxide. The detergents are such that one end of the molecule is soluble in non-polar substances like grease and oil stains, while the other end dissolves in liquid CO_2. The breakthrough process is paving the way for designing replacements for conventional halogenated solvents currently used in manufacturing and in industries making coatings.

(iv) Switching from air (78% nitrogen) to pure oxygen eliminates NO production and also saves fuel by more efficient burning. This has been achieved by glass manufacturers in the U.S.

(v) Scientists are engineering biodegradability into some synthetic polymers. Certain bonds or groups are introduced into the molecules to make them susceptible to fungal or bacterial attack or to decomposition by moisture. Recently, a biodegradable polymer called **Biomax** has been developed which decomposes in about eight weeks in a landfill. Biomax could be used in a variety of applications such as lawn bags, bottles, liners of disposable diapers, disposable eating utensils and cups.

(vi) The current commercial method for producing ibuprofen is a stunning application of green chemistry. Conventional methods of ibuprofen production required six steps, used large amounts of solvents and generated significant quantities of wastes. By using a catalyst that also serves as a solvent, BHC company has discovered a method of ibuprofen production in just three steps with a minimum of solvents and waste.

(vii) Urea is a major fertilizer used worldwide. It provides nitrogen to soil by decomposing to ammonia and CO_2 when acted on by urease, an enzyme in soils. The ammonia is then taken up by plants. $NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO2$

However, the efficiency of urea as a fertilizer is reduced because of 30% ammonia is lost by evaporation before it can be taken up by plant roots. To overcome this loss, a formulation named **Agrotrain** has been developed which acts as urease inhibitor, *i.e.*, this formulation reduces the rate at which urease decomposes urea so that aminonia is released more slowly and efficiently.