

organic compounds produced by plants. Do not play any role in growth. of plant. Required for plant survival. Help in response to stress.

10.Genetic diversity is a crucial biochemical characteristic of bioresources, influencing their adaptability, resilience, and potential for use in various applications. Here's how genetic diversity relates to biochemistry and bioresources.

11.Biotechnological Applications

- **Crop Improvement:** Genetic diversity in plants can be harnessed for breeding programs aimed at enhancing traits like yield, disease resistance, or nutritional content.
- **Microbial Diversity in Industry:** In industrial biotechnology, diverse microbial strains can be selected for specific biochemical processes, such as enzyme production or biofuel generation.

Bioenergetics

Bioenergetics is the field of biochemistry concerned with the transformation and use of energy by living cells. So, basically Bioenergetics is "the study of energy changes in biological reactions".

Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical processes underlying these transductions. The goal of bioenergetics is to describe how living organisms acquire, transform and utilize energy in order to perform biological work. The study of metabolic pathways is thus essential to bioenergetics.

Biological Energy Transformations Obey the Laws of Thermodynamics

The **first law** is the principle of the conservation of energy: for any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed. The **second law** of thermodynamics, which can be stated in several forms, says that the universe always tends toward increasing disorder: in all natural processes, the entropy of the universe increases. Living organisms consist of collections of molecules much more highly organized than the surrounding materials from which they are constructed, and organisms maintain and produce order, seemingly oblivious to the second law of thermodynamics. But living organisms do not violate the second law; they operate strictly within it.

The reacting system is the collection of matter that is undergoing a particular chemical or physical process; it may be an organism, a cell, or two reacting compounds. The reacting system and its surroundings together constitute the universe. Living cells and organisms, however, are open systems, exchanging both material and energy with their surroundings; living systems are never at equilibrium with their surroundings, and the constant transactions between system and surroundings explain how organisms can create order within themselves while operating within the second law of thermodynamics.

Three thermodynamic quantities that describe the energy changes occurring in a chemical reaction.

Gibbs free energy, G, expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure. When a reaction proceeds with the release of free energy (that is, when the system changes so as to possess less free energy), the free-energy change, ΔG , has a negative value and the reaction is said to be exergonic. In endergonic reactions, the system gains free energy and ΔG is positive.

Enthalpy, H, is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products. When a chemical reaction releases heat, it is said to be exothermic; the heat content of the products is less than that of the reactants and ΔH has, by convention, a negative value. Reacting systems that take up heat from their surroundings are endothermic and have positive values of Enthalpy, ΔH , is the heat content of the reacting system. It reflects the number and kinds of chemical bonds in the reactants and products.

Entropy, S, is a quantitative expression for the randomness or disorder in a system. When the products of a reaction are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.

Under the conditions existing in biological systems (including constant temperature and pressure), changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation

$$\Delta G = \Delta H - T \Delta S$$

By convention, ΔS has a positive sign when entropy increases and ΔH , as noted above, has a negative sign when heat is released by the system to its surroundings. Either of these conditions, which are typical of favorable processes, tend to make ΔG negative. In fact, ΔG of a spontaneously reacting system is always negative

The second law of thermodynamics states that the entropy of the universe increases during all chemical and physical processes, but it does not require that the entropy increase take place in the reacting system itself. The order produced within cells as they grow and divide is more than compensated for by the disorder they create in their surroundings in the course of growth and division. In short, living organisms preserve their internal order by taking from the surroundings free energy in the form of nutrients or sunlight, and returning to their surroundings an equal amount of energy as heat and entropy

Cells Require Sources of Free Energy Cells are isothermal systems—they function at essentially constant temperature (they also function at constant pressure). Heat flow is not a source of energy for cells, because heat can do work only as it passes to a zone or object at a lower temperature. The energy that cells can and must use is free energy, described by the Gibbs free-energy function G , which allows prediction of the direction of chemical reactions, their exact equilibrium position, and the amount of work they can in theory perform at constant temperature and pressure. Heterotrophic cells acquire free energy from nutrient molecules, and photosynthetic cells acquire it from absorbed solar radiation.

The Standard Free-Energy Change Is Directly Related to the Equilibrium Constant

The composition of a reacting system (a mixture of chemical reactants and products) tends to continue changing until equilibrium is reached. At the equilibrium concentration of reactants and products, the rates of the forward and reverse reactions are exactly equal and no further net

change occurs in the system. The concentrations of reactants and products at equilibrium define the equilibrium constant, K_{eq} . In the general reaction



where a, b, c, and d are the number of molecules of A, B, C, and D participating, the equilibrium constant is given by

$$K_{eq} = \frac{[C]^c [D]^d}{[A]^a [B]^b}$$

where [A], [B], [C], and [D] are the molar concentrations of the reaction components at the point of equilibrium.

When a reacting system is not at equilibrium, the tendency to move toward equilibrium represents a driving force, the magnitude of which can be expressed as the free-energy change for the reaction, ΔG

Actual Free-Energy Changes Depend on Reactant and Product Concentrations

The free-energy change, ΔG , and the standard free-energy change, ΔG^0 . Each chemical reaction has a characteristic standard free-energy change, which may be positive, negative, or zero, depending on the equilibrium constant of the reaction. The standard free energy change tells us in which direction and how far a given reaction must go to reach equilibrium when the initial concentration of each component is 1.0 M, the pH is 7.0, the temperature is 25 °C, and the pressure is 101.3 kPa.

Moreover, the ΔG of any reaction proceeding spontaneously toward its equilibrium is always negative, becomes less negative as the reaction proceeds, and is zero at the point of equilibrium, indicating that no more work can be done by the reaction.

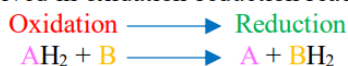
Biocatalysis

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.

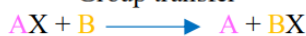
Classification of Biocatalyst:

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



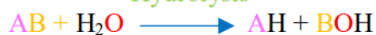
2. **Transferases:** Enzymes that catalyse the transfer of functional groups.

Group transfer



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

Hydrolysis

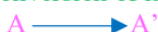


4. **Lyases:** Enzymes specialised in the addition or removal of water, ammonia, CO₂ etc.



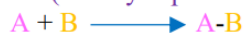
5. **Isomerases:** Enzymes involved in all the isomerization reactions.

Interconversion of isomers



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)



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.

Stereospecificity 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 exhibit 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.

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	Triacylglycerols
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 devise 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. Transesterification 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).

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 subtilis* 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

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 CO₂ 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 *Saccharomyces cerevisiae* 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 CO₂ emissions by more than 90% (compared to oil). Allows for greater domestic energy production and it uses 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 mixed 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.

Kinetics of product formation

For growth-linked products and non-growth-linked products formation by microbial cultures

Growth-linked products may be considered equivalent to primary metabolites which are synthesized by exponentially growing cells and

Non-growth-linked products may be considered equivalent to secondary metabolites which are synthesized by stationary phase of culture.