

Molecular Machines, Biosensor, and Bioremediation

INTRODUCTION

Mankind has advanced through innovations in several fields of science and technology including construction of novel devices. Recent technological advances are mainly due to miniaturization of the components used in the construction of devices and machines especially, in the field of information processing. It is expected that further progress in the process of miniaturization will not only lead to reduction in the size of computers and an increase in processing capabilities but also pave the way for technological advancement in the fields of environment, medicine, agriculture, energy, etc. Nature builds nanodevices and nanomachines from molecules and not atoms as molecules are more stable. There are several such nanomachines in the natural world that have evolved over many millions of years to achieve movement of living biological cells. These molecular motors have few molecular components and are similar to the large-scale man-made devices that convert energy to mechanical work or motion but are regulated by thermal fluctuation of surrounding molecules.

Biosensors are devices, not necessarily on a nanoscale, that uses biological component to detect various components in the environment. The device is a combination of biological element and a transducer that converts changes in the biomolecules in the analyte into an electrical signal. The biological element can be living cells as mentioned in chapter 1, enzymes as discussed in chapter 3 or antibodies as explained in chapter 5. It combines the exquisite specificity of these biological elements (living cells, enzymes, antibodies) and the excellent sensitivity of laser-based optical detection to detect, differentiate, and identify chemical constituents of complex systems for accurate quantification.

Industrialization and mining of minerals have resulted in large-scale environmental contamination and pollution. Bioremediation is a process

through which living microorganisms are used to recover, clean, and/or remedy the contamination of air, soil, and water. In situ and ex situ are two types of bioremediation used to remove contamination in soil, water, and air. Some of the bioremediation techniques involve construction of bioreactors which requires processing of contaminated substances through engineered containment system.

4.1 MOLECULAR MACHINES AND MOTORS

Organisms whether single cell (e.g. bacteria) or multicellular (e.g., animals and human beings) need to be in motion in order to adapt to changes in the environment, search for food or protect themselves from any threat. In turn, cells are not stationary but are active in synthesis, transport, and expulsion of products.

Molecular machines are devices that create movement through the involvement of motor proteins, ATP, and other molecules at the molecular scale of length. Most of them are in the range of nanometer and hence, can be classified under the category of nanomachines. The interactions include ionic and Van der Waal's forces to facilitate movement. There are several natural machines that have evolved over several million years optimizing their performance. Understanding their structure and principles of operation will enable us to devise synthetic ones that will be beneficial to mankind.

Several of the molecular machines in nature are protein-based while DNA-based molecular machines are synthetic (Fig. 4.1). The reason for this is that most of the cellular tasks such as moving substances within and across the cells and catalyzing reactions are carried out by proteins whereas the sole purpose of DNA is the carrier of hereditary information. Hence, most of the molecular machines are made up of proteins. It is important to understand the molecular machinery so that synthetic machines can be created for use in medicine, space exploration, electronics and military.

Molecular machines are divided into three broad categories—protein based, DNA-based, and chemical molecular motors.

Properties of ATP-based protein molecular machines

F0F1-ATP synthase motor and bacterial flagellar motor are examples of ATP-dependent protein molecular machines. The protein-based molecular motors depend on ATP. As already explained, ATP is the energy currency of the cell possessing energy-rich three phosphate molecules that are indispensable to sustenance of life.

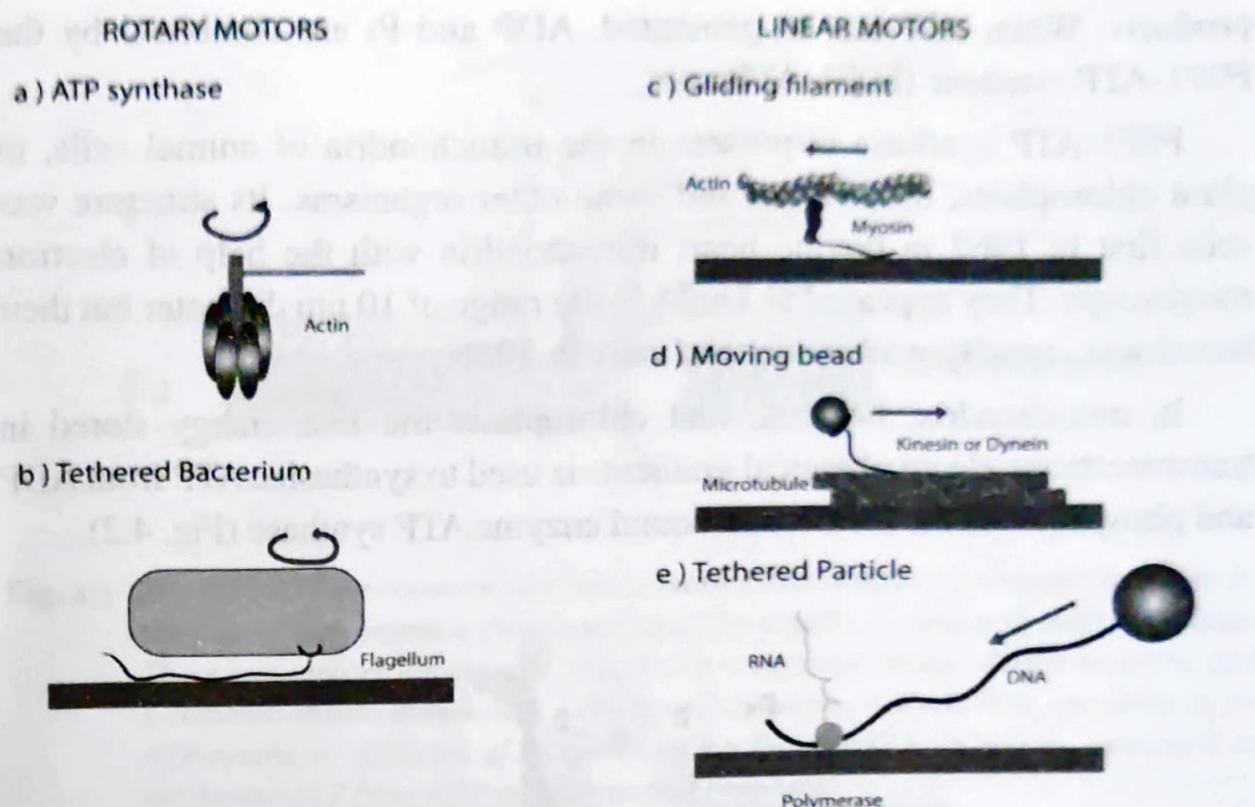


Fig. 4.1 Different types of molecular machines. These machines used by nature for force generation and motion. They convert chemical energy into mechanical force via conformational changes.

The machines, the F0F1-ATPase, the kinesin, myosin, and dynein superfamily of protein molecular machines, and bacteria flagellar motors all depend, directly or indirectly, on ATP for their input energy. The machines have been facilitating various functions within and outside the cell have been studies extensively and are found to possess energy conversion devices to obtain forces, torques, and motion. A disadvantage with these ATP-dependent machines is that the ATP creating machinery is heavier than the other motors that may make the task of assembling these motors difficult. As they have been performing successfully in their natural environment for several million years, it may be possible to create similar machinery provided we gain more knowledge about these molecular machines.

4.2 THE F0F1-ATP SYNTHASE MOTORS

products. When ATP has to be generated, ADP and Pi are combined by the FOF1-ATP synthase (FOF1-ATPase).

FOF1-ATP synthase is present in the mitochondria of animal cells, in plant chloroplasts, in bacteria, and some other organisms. Its structure was seen first in 1962 in bovine heart mitochondria with the help of electron microscope. They appeared as knobs in the range of 10 nm diameter but their functional capacity was understood only in 1966.

In mitochondria, bacteria, and chloroplasts the free energy stored in transmembrane electrochemical gradients is used to synthesize ATP from ADP and phosphate via the membrane-bound enzyme ATP synthase (Fig. 4.2).

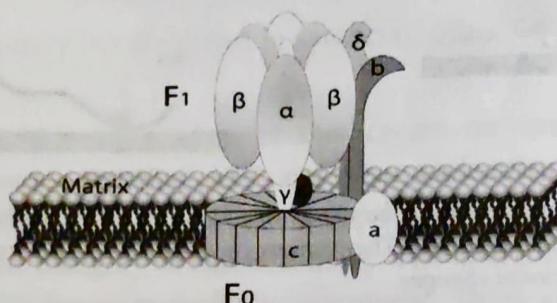


Fig. 4.2 The FOF1-ATPase motors. The Fo motor is embedded in the inner mitochondrial membrane of the mitochondria. Fo is typically composed of a, b, and c subunits as shown. The F1 motor is the soluble region composed of three α-, three β-, one each of γ-, δ- and ε-subunits.

- ATP synthase consists of two portions: a membrane-spanning portion, F0, comprising the ion channel, and a soluble portion, F1, containing three catalytic sites.
- Both F0 and F1 are reversible rotary motors. Possibly, these are the smallest natural motors known to scientists. F0 uses the transmembrane electrochemical gradient to generate a rotary torque to drive ATP synthesis in F1 or, when driven backwards by the torque generated in F1, to pump ions uphill against their transmembrane electrochemical gradient.
- F1 generates a rotary torque by hydrolyzing ATP at its three catalytic sites or, when turned backwards by the torque generated in F0, as a synthesizer of ATP.

Coupling and coordination of motors

The ATP synthase is a combination of two motors functioning together, the hydrophobic transmembrane F0-ATPase motor and the globular F1-ATPase motor (Fig. 4.3). Both motors have distinct structures and functions.

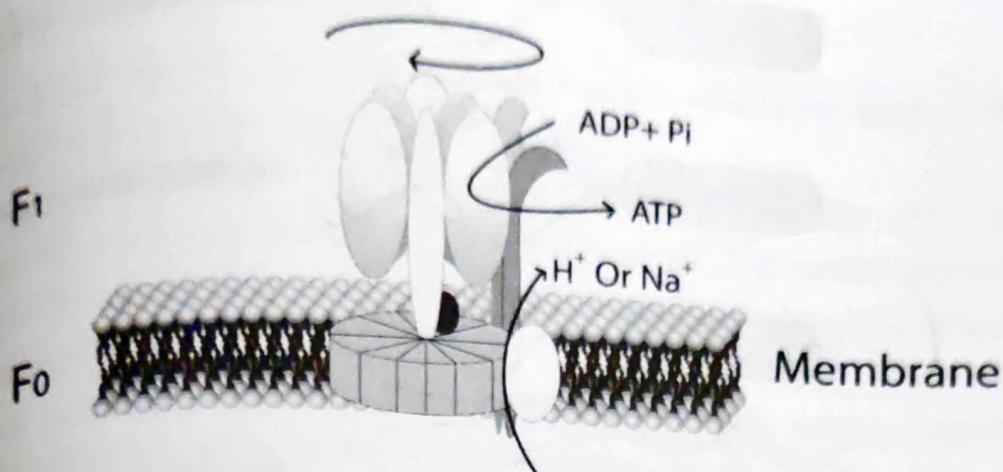


Fig. 4.3 The *FoF1-ATPase* contains two rotary motors: the membrane-bound *F₀*, driven by the flux of ions across a membrane, and the soluble *F₁*, driven by ATP hydrolysis. These two motors are coupled by sharing a common motor, drawn in white, and a common stator, drawn dark. The figure shows the *FoF1-ATPase* operating as an ATP-synthase. Rotation of *F₀* driven by ion flux drives *F₁* in reverse, causing it to synthesize ATP from ADP and inorganic phosphate.

- There are different abbreviations used for the F1-ATPase based on their sources; the heart mitochondrial motors are called mF1, chloroplast motors are cF1, those obtained from *Escherichia coli* are termed EcF1, and the ones from Kagawa's thermophilic bacterium are known as TF1.
- The F0 motor has organism-dependant structural variations.
- In addition, the regulation of catalysis in ATP synthase depends on the organism's source. In animal mitochondria, this motor is embedded in the inner mitochondrial membrane and uses an ion-motive force for its function.
- Initially, however, it was believed that the force was proton-motive only until it was shown that, in some cases, Na^+ ions induce the motive force for the F0 motor; hence the term ion-motive force. The proton-motive force can be defined as the work per unit charge that a proton traveling through a membrane can perform.

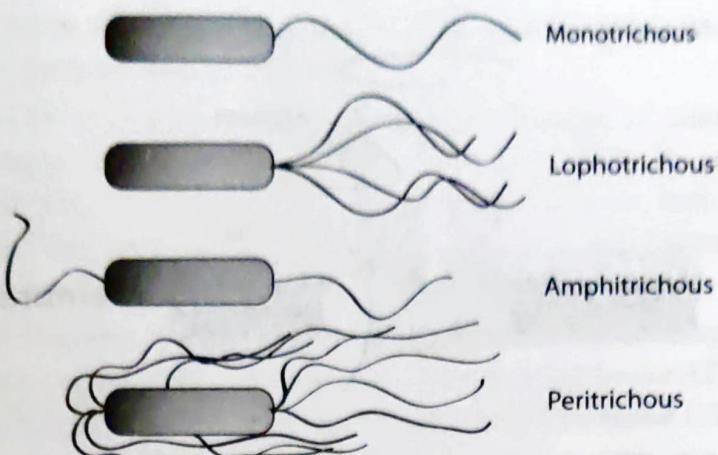


Fig. 4.4 Examples of bacterial flagellar arrangements: *Monotrichous*, *Lophotrichous*, *Amphitrichous*, and *Peritrichous*.

not more than 50 nm in diameter and is assembled with about 20 different kinds of parts.

- It spins clockwise (CW) or counterclockwise (CCW) at speeds on the order of 100 Hz, driving long thin helical filaments that enable cells to swim.
- Peritrichously flagellated cells (*peri*, around; *trichos*, hair), such as *Escherichia coli*, execute a random search, moving steadily at about 30 diameters per second, now in one direction, now in another.
- Steady motion requires CCW rotation. Receptors near the surface of the cell count molecules of interest (sugars, amino acids, dipeptides) and control the direction of flagellar rotation.
- If a leg of the search is deemed favorable, it is extended, i.e., the motors spin CCW longer than they otherwise would. This bias enables cells to actively find regions in their environment where life is better.
- Thus, the flagellar motor is the output organelle of a remarkable sensory system, the components of which have been honed to perfection by billions of years of evolution.
- A number of bacterial species in addition to *E. coli* depend on flagella motors for motility: e.g., *Salmonella enterica* serovar, Typhimurium (*Salmonella*), *Streptococcus*, *Vibrio* spp., *Caulobacter*, *Leptospira*, *Aquaspirillum serpens*, and *Bacillus*.
- The rotation of flagella motors is stimulated by a flow of ions through them, which is a result of a build-up of a transmembrane ion gradient. There is no direct ATP-involvement; however, the proton gradient needed for the functioning of flagella motors can be produced by ATPase.

Flagellar motor structure

- Bacterial flagella are the only biological structures known that use rotation for the purpose of locomotion.
- Flagella consist of a rotary motor embedded in the cell envelope connected to an extracellular helical propeller. The motor is powered by the flow of ions down an electrochemical gradient across the cytoplasmic membrane into the cell.
- The ions are typically H⁺ (protons), although certain marine and alkalophilic species have motors driven by Na⁺.
- The electrochemical gradient (protonmotive force or sodium motive force) consists of a transmembrane voltage and a concentration difference across the membrane, both of which are maintained by various metabolic processes.
- The rotor shown in white (Fig. 4.5), consists of a series of rings spanning the cell envelope and is attached via the flexible hook to the helical propeller or filament. The stator is a ring of particles in the cytoplasmic membrane, containing the proteins MotA and MotB, and anchored to the peptidoglycan cell wall.

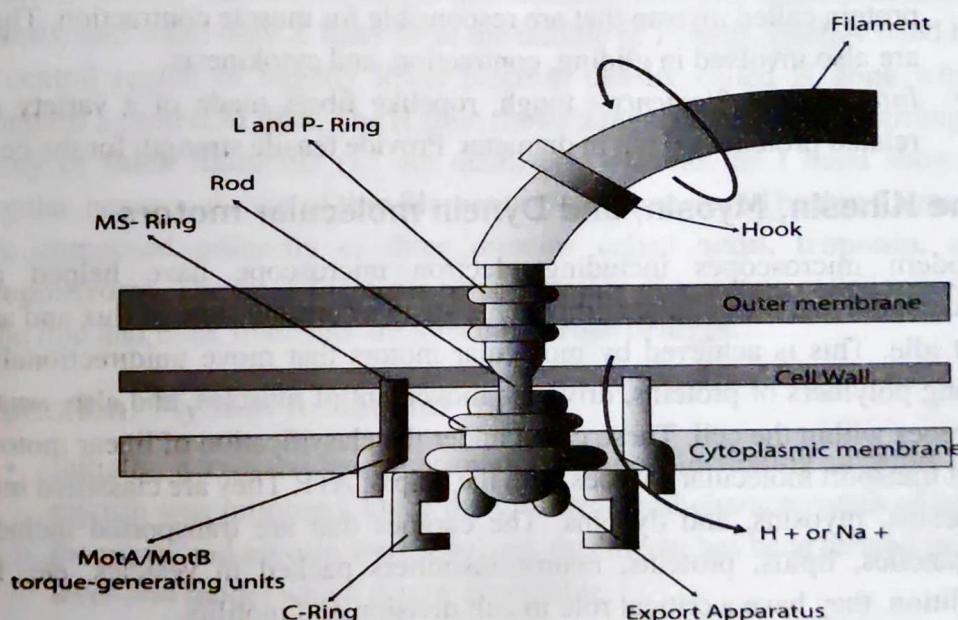


Fig. 4.5 The bacterial flagellar motor. The rotor consists of a series of rings that span the cell envelope and are attached to the extracellular hook and filament. The stator consists of a ring of torque-generating units containing the proteins MotA and MotB and anchored to the cell wall. Ions flowing through the motor generate torque by means of unknown interactions between the rotor and stator in the vicinity of the C-ring. The FoF1-ATPase is also shown, to the same scale.

Cytoskeletons are important structures that give the cell its shape, structure, and physical organization. Motor proteins that were discussed earlier have the capability to rearrange the structural elements or move the cell components around the cytoskeleton. In order to meet the requirements of the cellular function, cytoskeleton is constantly changing its shape and position. The major components of the cytoskeleton are microtubules, microfilaments, and intermediate filaments.

Three major structural elements of the cytoskeleton:

- *Microtubules* - hollow, rigid cylindrical tubes made from tubulin subunits, 20-25 nm in diameter. They are the scaffolds of the cell giving it a shape and also, provide ways through which organelles and vesicles move within the cell. Microtubules form spindle fibers for pulling the chromosomes to different poles during mitosis. Their presence within the cilia and flagella give these structures to facilitate movement.
- *Microfilaments* - solid, thinner structures made of contractile protein called actin, 3-6 nm in diameter. Microfilaments work with another protein called myosin that are responsible for muscle contraction. They are also involved in gliding, contraction, and cytokinesis.
- *Intermediate filaments* - tough, ropelike fibers made of a variety of related proteins, 10 nm in diameter. Provide tensile strength for the cell.

The Kinesin, Myosin, and Dynein molecular motors

Modern microscopes including electron microscope have helped us understand that the cellular components are in a constant state of flux and are not idle. This is achieved by molecular motors that move unidirectionally along polymers of proteins, drive the movement of muscles, and also, small cargoes within the cell. These come under the classification of linear motors that transport molecular cargoes with the help of ATP. They are classified into kinesins, myosins, and dyneins. The cargoes that are transported include organelles, lipids, proteins, neurotransmitters packed in vesicles, etc. In addition, they have a critical role in cell division and motility.

More than 250 kinesin-like proteins have been discovered that are involved in movement of chromosomes and altering the dynamics of cell membranes. The catalytic portion known as the motor domain is common to all kinesins. There are significant differences in their location within the cells, structural organization, and the type of movement generated.

Myosin in the muscle was studied in the 19th century and has been a model system for understanding motility for several years. Kinesin was however discovered within the past 30 years. Kinesin is a highly processive motor that can move on the microtubule for several hundred steps without separating whereas myosin performs only a single stroke and then, disengages. Kinesin is a microtubule-associated whereas myosin is actin-based motor protein.

The Myosin linear motor

There are four different types of muscle in animals: skeletal muscle, cardiac (heart) muscle, smooth muscle, and myoepithelial cells. Skeletal muscles are associated with bones whereas smooth muscles are found in the blood vessels and visceral organs. When observed under the microscope, skeletal and cardiac muscle show alternating light and dark bands and hence, called as striated muscles.

Skeletal muscles consists of 100-mm-diameter **fiber bundles** that in turn contain hundreds of myofibrils. When viewed under electron microscope, myofibrils reveal a banded or striated structure. There is a region of high electron density called as **A bands** that alternate with low electron density, **I bands**, and small dark **Z lines** lie in the middle of I bands. Each A band has a central region of slightly lower electron density called **H zone** which contains a central **M disk**. The H zone shows a regular, hexagonally arranged array of **thick filaments** (15 nm diameter), whereas the I band shows a regular, hexagonal array of **thin filaments** (7 nm diameter). The thin filaments are composed primarily of three proteins called **actin**, **troponin**, and **tropomyosin**. The thick filaments consist mainly of a protein called **myosin**. The thin and thick filaments are joined by **cross-bridges**.

Function: Myosin molecular motor

- Initially, a crossbridge-cycle model for the functioning of actin and myosin was proposed. Once the ultrastructural characteristics of actin monomer and myosin were resolved, this model has been refined into a lever-arm model which is now acceptable.
- During muscle contraction, the thick myosin filaments slide or walk along the thin actin filaments. The molecular events of contraction are powered by the ATPase activity of myosin.
- The myosin heads are dissociated from the actin filaments in the resting muscle when ATP is dissociated to ADP and Pi.
- When there is a signal to contract, the myosin heads move out from the

thick filaments to bind to actin. ATP utilization occurs through the release of phosphate resulting in a **power stroke**. This results in the movement of thick filaments approximately 10 nm across the thin filaments as the myosin head relaxes.

- Myosin heads dissociate from the thin filaments due to binding and hydrolysis of ATP. This causes the myosin heads' long axis to lie nearly perpendicular to the long axis of the thick filaments until next contraction.

The Kinesin linear motor

In contrast to myosin which works with actin, microfilament, kinesin and dynein transport cargo along microtubules. As stated earlier, microtubules are tubes made of protein called tubulin with a diameter of 25 nm. These microtubules have a polarity: one end being the plus (fast-growing) end while the other end is the minus (slow-growing) end. Kinesins move from the minus end to the plus end of the microtubule in the transportation of cargo (Fig. 4.6).

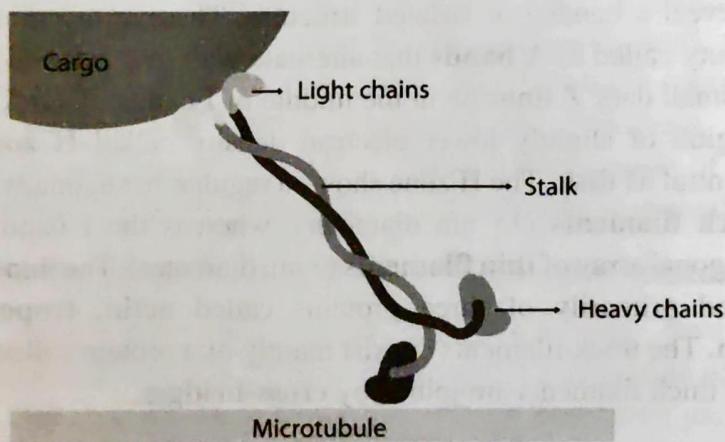


Fig. 4.6 Kinesin linear motor transporting the cargo.

The arrangements of the microtubules vary depending on the cell types. In nerve axons, they are arranged longitudinally such that their plus ends point away from the cell body and into the axon. In epithelial cells, their plus ends point toward the basement membrane. They extend radially out of the cell center in fibroblasts and macrophages with the plus end protruding outward. The energy-driven process of transport requires ATP just like the other motors. One unique characteristic of the kinesin family proteins is their processivity; they bind to microtubules and literally walk on it for many enzymatic cycles before detaching.

The Dynein motor

The dynein superfamily of proteins was discovered in 1965. Dyneins exist in two isoforms: cytoplasmic and axonemal (Fig. 4.7). Cytoplasmic dyneins are involved in cargo movement, whereas axonemal dyneins are involved in producing bending motions of cilia and flagella. In contrast to kinesin, dyneins move from the plus end to the minus end.

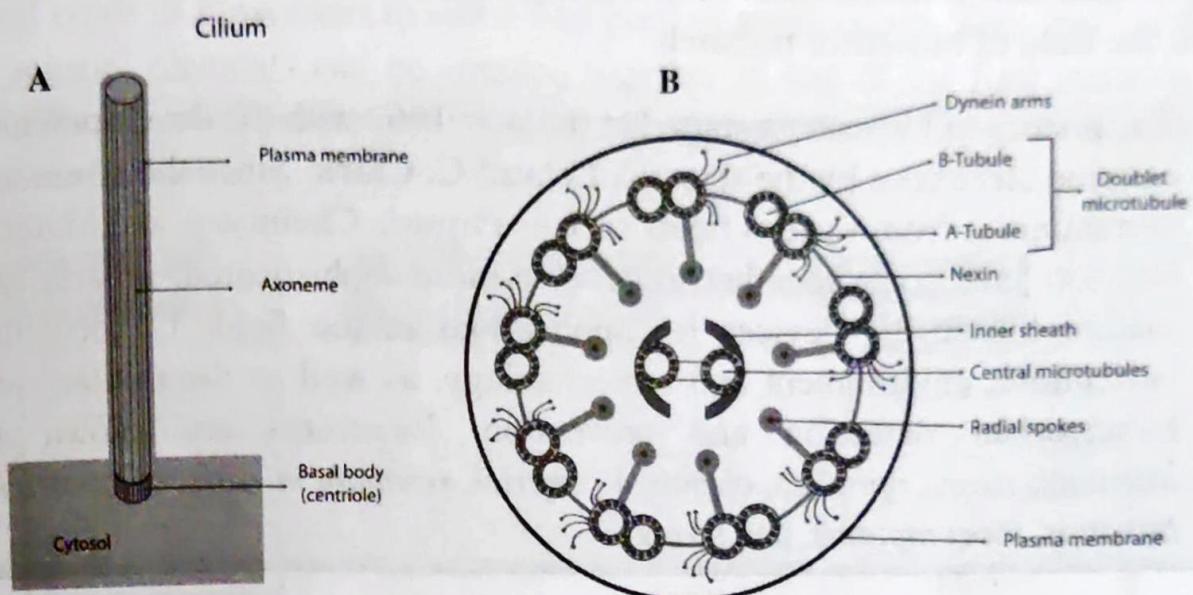


Fig. 4.7 (A) Cilia and flagella have a core axoneme, a complex of microtubules and associated proteins. (B) A cross-section of an axoneme, with axonemal dynein arms

Because dynein is a larger and more complex structure than other motor proteins, its mode of operation is not as well known. However, electron microscopy and image processing was used to show the structure of a flagellar dynein at the start and end of its power stroke, which gives some insight into its possible mode of force generation.

Nanomachines carrying drugs have been developed to effectively deliver the drug to the cancer cells and selectively cause their death. When magnetic-field stimulus is applied, the valves in the nanomachines open and release the drugs into the cells. Advancement of this nature is critical to avoid the side-effects and reduce the dosage of the chemotherapeutic agents used in cancer therapy.

advantages of using biological components in sensors are their good specificity, sensitivity, and portability.

The real-time and *in situ* quantitation of biologically and environmentally important analytes has long been a goal of analytical research. The need for continuous monitoring of substrates in fermentation broths, pesticides and environmental contaminants in natural waters, and biochemicals or pharmaceutical metabolites in living organisms has led to extensive activity in the field of biosensor research.

The history of biosensors started in the year 1962 with the development of enzyme electrodes by the scientist **Leland C. Clark**. Since then, research communities from various fields such as Physics, Chemistry, and Material Science have come together to develop more sophisticated, reliable and mature biosensing devices for applications in the fields of medicine, agriculture, environment and biotechnology, as well as the military and bioterrorism detection and prevention. Biosensors are known as: *immunosensors, optrodes, chemical canaries, resonant mirrors, glucometers, biochips, biocomputers*, and so on.

What is a biosensor? Various definitions and terminologies are used depending on the field of application. A biosensor is a sensing device comprised of a combination of a specific biological element and a transducer. A “specific biological element” recognizes a specific analyte and the changes in the biomolecule are usually converted into an electrical signal (which is in turn calibrated to a specific scale) by a transducer.

The name “biosensor” signifies that the device is a combination of two parts: (i) a bio-element, and (ii) a sensor-element. The basic concepts of a biosensor’s operation can be illustrated with the help of Figure 4.8. A specific “bio” element (e.g., enzyme) recognizes a specific analyte and the “sensor” element transduces the change in the biomolecule into an electrical signal. The bioelement is very specific to the analyte to which it is sensitive. It does not recognize other analytes.

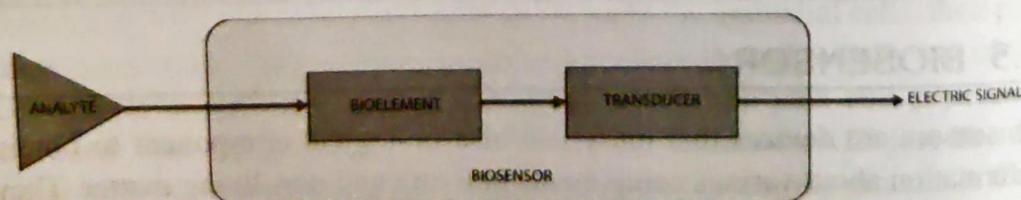


Fig. 4.8 Schematic representation of biosensors

Working principle/basic concepts

As shown in Fig. 4.9, a biosensor consists of a bio-element and a sensor-element. The bioelement 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 bio-elements and sensor-elements is shown in Fig. 2.

Different combinations of bio-elements and sensor-elements constitute several types of biosensors to suit a vast pool of applications. The "bio" and the "sensor" elements can be coupled together in one of the four possible ways : *Membrane Entrapment*, *Physical Adsorption*, *Matrix Entrapment*, and *Covalent Bonding*.

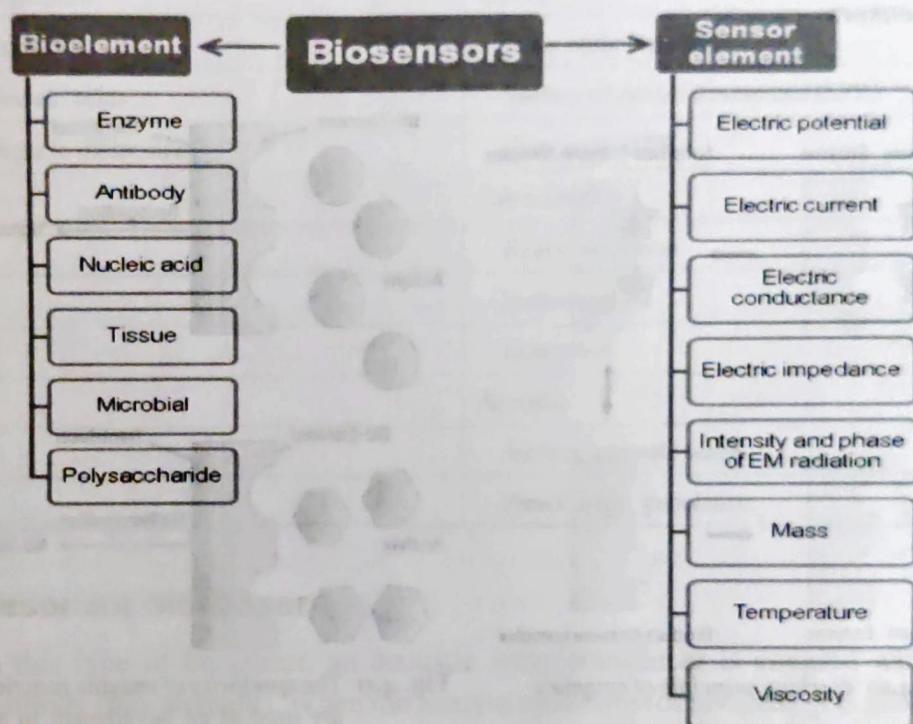


Fig. 4.9 Components of a biosensor

In the membrane entrapment scheme, a semi permeable membrane separates the analyte and the bioelement, and the sensor is attached to the bioelement. The physical adsorption scheme is dependent on a combination of van der Waals forces, hydrophobic forces, hydrogen bonds, and ionic forces to attach the biomaterial to the surface of the sensor. The porous entrapment scheme is based on forming a porous encapsulation matrix around the biological material that helps in binding it to the sensor. In the case of the covalent bonding the sensor surface is treated as a reactive group to which the biological materials can bind. The typically used bioelement, enzyme is a

large protein molecule that acts as a catalyst in chemical reactions, but remains unchanged at the end of reaction.

Working principle of enzymes

The Fig. 4.10 shows the working principle of enzymes which has already been dealt in detail in chapter 3. An enzyme upon reaction with a substrate forms a complex molecule which under appropriate conditions forms the desirable product molecule releasing the enzyme at the end. ***The enzymes are extremely specific in their action:*** an enzyme X will change a specific substance A (not C), to another specific substance B (not D), as illustrated in Fig. 4.11. ***This extremely specific action of the enzymes is the basis of biosensors.***

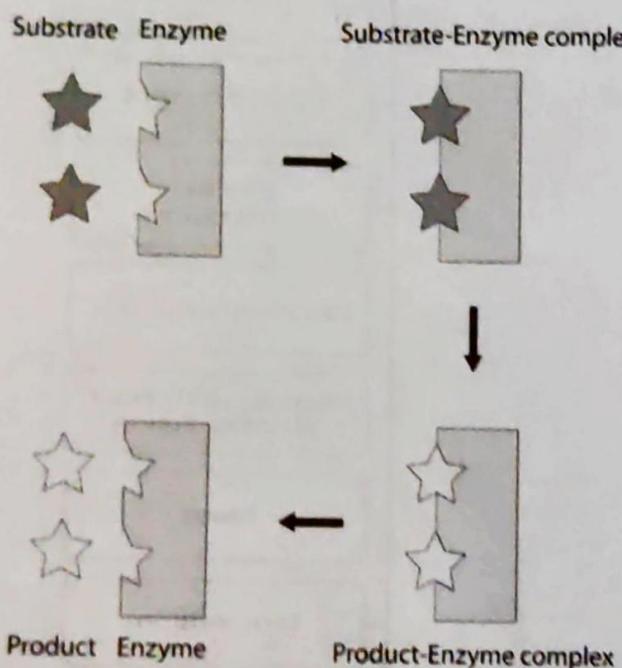


Fig. 4.10 Working principle of enzymes

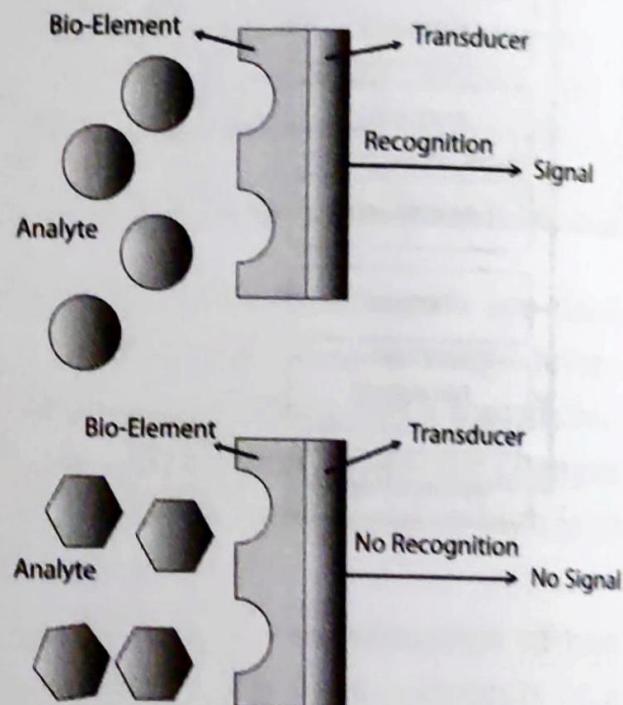


Fig. 4.11 The specificity of enzyme functions are used as an bio-element in bio-sensor

Biological Elements	Transducers
Enzymes	Electrochemical
Antibodies	Amperometric
Receptors	Potentiometric
Cells	Ion-selective
Membranes	Field effect transistors
Tissues	Conductimetric
Organisms	Optical
Organelles	Fiber optic (optrode)
Nucleic acids	Surface Plasmon Resonance (SPR)
Organic molecules	Fiber optic SPR
	Calorimetric
	Heat conduction
	Isothermal
	Isoperibol
	Acoustic
	Surface acoustic wave
	Piezocrystal imbalance

antibody bindings are formed in the active regions, thus creating a diffraction grating. This grating produces a diffraction signal when illuminated with a light source such as laser. The resulting signal can be measured or can be further amplified before measuring for improved sensitivity.

Thermal-detection biosensors

This type of biosensor is exploiting one of the fundamental properties of biological reactions, namely absorption or production of heat, which in turn changes the temperature of the medium in which the reaction takes place. They are constructed by combining immobilized enzyme molecules with temperature sensors. When the analyte comes in contact with the enzyme, the heat reaction of the enzyme is measured and is calibrated against the analyte concentration. The total heat produced or absorbed is proportional to the molar enthalpy and the total number of molecules in the reaction. The measurement of the temperature is typically accomplished via a thermistor, and such devices are known as enzyme thermistors. Their high sensitivity to thermal changes makes thermistors ideal for such applications. Unlike other transducers, thermal biosensors do not need frequent recalibration and are insensitive to the optical and electrochemical properties of the sample. Common applications of this type of biosensor include the detection of pesticides and pathogenic bacteria.

Ion-sensitive biosensors

These are semiconductor FETs having an ion-sensitive surface. The surface electrical potential changes when the ions and the semiconductor interact. This change in the potential can be subsequently measured. The Ion Sensitive Field Effect Transistor (ISFET) can be constructed by covering the sensor electrode with a polymer layer. This polymer layer is selectively permeable to analyte ions. The ions diffuse through the polymer layer and in turn cause a change in the FET surface potential. This type of biosensor is also called an ENFET (Enzyme Field Effect Transistor) and is primarily used for pH detection.

Electrochemical biosensors

Electrochemical biosensors are mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration, etc. The underlying principle for this class of biosensors is that many chemical reactions produce or consume ions or electrons which in turn cause some change in the electrical properties of the solution which can be sensed out and used as measuring

parameter. Electrochemical biosensors can be classified based on the measuring electrical parameters as: (1) conductimetric, (2) amperometric and (3) potentiometric.

4.7 GLUCOSE BIOSENSORS

The most commercially successful biosensors are amperometric glucose biosensors. These biosensors have been made available in the market in various shapes and forms such as glucose pens, glucose displays, etc. The first historic experiment that served as the origin of glucose biosensors was carried out by Leland C. Clark. He used platinum (Pt) electrodes to detect oxygen. The enzyme *glucose oxidase* (GOD) was placed very close to the surface of platinum by physically trapping it against the electrodes with a piece of dialysis membrane. The enzyme activity changes depending on the surrounding oxygen concentration.

Glucose reacts with glucose oxidase (GOD) to form gluconic acid while producing two electrons and two protons, thus reducing GOD. The reduced GOD, surrounding oxygen, electrons and protons (produced above) react to form hydrogen peroxide and oxidized GOD (the original form). This GOD can again react with more glucose. The higher the glucose content, more oxygen is consumed. On the other hand, lower glucose content results in more hydrogen peroxide. Hence, either the consumption of oxygen or the production of hydrogen peroxide can be detected by the help of platinum electrodes and this can serve as a measure for glucose concentration. Disposable amperometric biosensors for the detection of glucose are also available.

The typical configuration is a button-shaped biosensor consisting of the following layers: metallic substrate, graphite layer, isolating layer, mediator modified membrane, immobilized enzyme membrane (GOD), and a cellulose acetate membrane. This biosensor uses graphite electrodes instead of platinum electrodes (as originally used by Clark). The isolating layer is placed on the graphite electrodes which can filter out certain interfering substances (ascorbic acid, uric acid) while allowing the passage of hydrogen peroxide and oxygen. The mediator-modified membrane helps in keeping the GOD membrane attached to the graphite electrode when the electrochemical reaction takes place at a specific applied potential. The cellulose acetate outer layer placed over the GOD membrane also provides a barrier for interfering substances. The amperometric reading of the biosensor (current versus glucose concentration) shows that the relationship is linear up to a specific

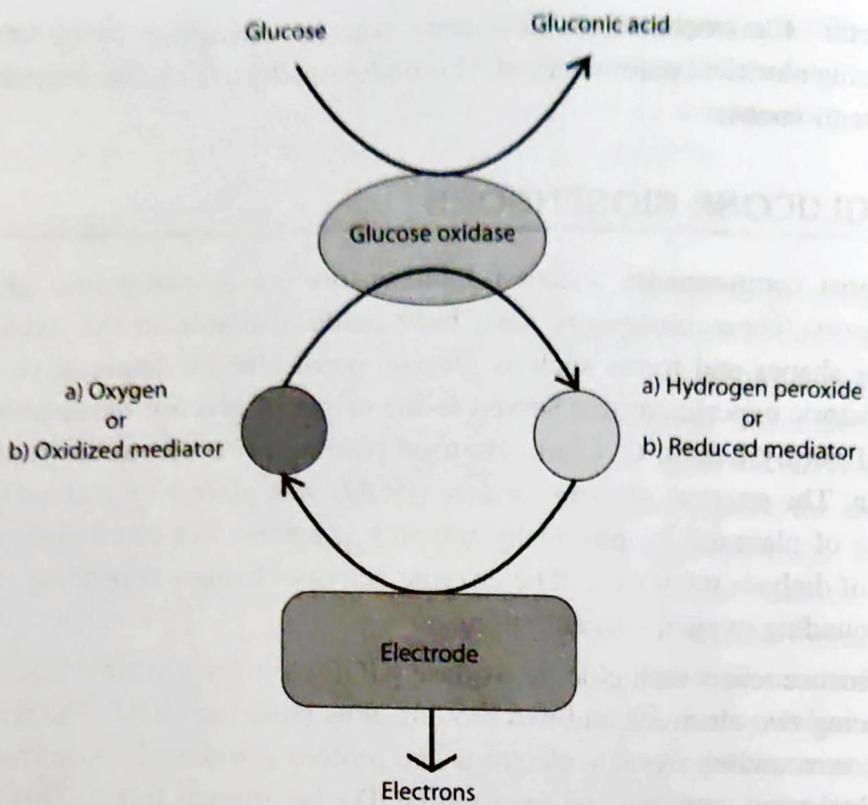


Fig. 4.12 The working principle of a glucose biosensor

glucose concentration. In other words, current increases linearly with glucose concentration, hence it can be used for detection. The current and future applications of glucose biosensors are very broad due to their immediate use in diabetic self-monitoring of capillary blood glucose. These types of monitoring devices comprise one of the largest markets for biosensors today and their existence has dramatically improved the quality of life of diabetics.

4.8 BIODETECTORS: DNA DETECTION

The category of biosensors used for DNA detection is also known as biodeTECTors. The objective is to isolate and measure the strength of single DNA–DNA or antibody–antigen bonds, which in turn helps in detecting and characterizing single molecules of DNA or antigen. In one method, multiple copies of the sample DNA are created using polymerase chain reaction (PCR). On the other hand, FABS (Force Amplified Biological Sensor), BARC (Bead Array Counter), and FDA (Force Differentiation Assay) biosensors can perform many such measurements in a single easy operation. In these cases magnetic micro-beads are used to pull on DNA–DNA or antibody–antigen bonds with a known force, and the strengths of the presumed bonds are tested

by observing with a micromechanical sensor (FABS), or with a magneto-resistive sensor (BARC) whether the beads detach from the surface. This kind of biosensor is extremely useful in the detection of Anthrax, Ricin, Botulinum, and other harmful substances.

4.9 BIOSENSOR DETECTION OF POLLUTANTS

Traditionally, the monitoring of environmental pollutants and the contaminants they produce has involved physicochemical analytical techniques. Spectrometry (UV, Visible, IR) and chromatography (GC, TLC, HPLC) represent increasingly powerful analytical tools that are capable of detecting, quantifying, and identifying xenobiotic pollutants in parts per million(ppm) and even parts per billion (ppb). These powerful analytical techniques are now being supplemented by biosensors, which are biological systems based on reporter genes or immunoassays that can detect specific pollutants.

Reporter genes can be readily detected when they are expressed. These genes can be joined through recombinant DNA technology to the genes that code for specific metabolic pathways that are inducible. When the pathway is induced, so is the reporter gene. Thus, a reporter gene that codes for production of light, such as the lux gene that codes for luminescence, can be coupled with various inducible genes. If light is emitted, the pathway has been induced, indicating the presence of a specific inducer substance. If that inducer is a pollutant, the reporter gene can be used as a sensitive indicator of that pollutant. Biosensor based on the lux gene has been genetically engineered to detect naphthalene.

Immunoassay biosensors have been developed to detect a variety of organic molecules, including benzopyrene and parathion. These assays are based on the specificities of immunological reactions. Because antibodies react only with specific antigens, immunoassays can be used to detect substances that are both pollutants and antigenic that is capable of reacting with a specific diagnostic antibody. Monoclonal antibodies, which react only with a single specific antigen, are particularly useful as diagnostic biosensors for the immunological detection of environmental pollutants.

4.10 BIOSENSOR IN FOOD INDUSTRY

Biosensors for the measurement of carbohydrates, alcohols, and acids are commercially available. These instruments are mostly used in quality assurance laboratories or at best, on-line coupled to the processing line through a flow injection analysis system. Their implementation in-line is

limited by the need of sterility, frequent calibration, analyte dilution, etc. Potential applications of enzyme based biosensors to food quality control include measurement of amino acids, amines, amides, heterocyclic compounds, carbohydrates, carboxylic acids, gases, cofactors, inorganic ions, alcohols, and phenols. Biosensors can be used in industries such as wine beer, yogurt, and soft-drinks producers. Immunosensors have important potential in ensuring food safety by detecting pathogenic organisms in fresh meat, poultry, or fish.

- Antibody-based fluoroimmunosensors to detect carcinogens, Biochips using DNA probes to detect tumor suppressor genes, and nanosensors for studying the components of cells are some of the biosensors that can be potential useful to mankind.
- Combination of nanotechnology and biosensor has yielded a novel biosensor that can detect very low concentrations of typhoid-causing organism, *Salmonella typhi*.
- Using antibodies and FET based on nanotubes, researchers have developed a biosensor that can detect the fungi, *Candida albicans*, that can cause simple mycosis to severe infection, candidiasis.
- A biosensor capable of detecting explosives, sarin gas, and landmines has been developed by genetically modifying yeast cells that has been incorporated with rat olfactory cells.
- A fusion of antibodies and electronic sensors has resulted in a novel biosensor that can detect marine pollutants such as oil in a fast manner and aid in cleanup efforts.

which creates a bad odor. Soil contamination in turn leads to contamination of ground water due to percolation of leachate. Therefore, proper scientific management of waste is required to tackle the huge amount of waste generated at a faster rate for safe disposal.

Different methods of treatment like physical, chemical and biological methods are currently available. But the major concern in waste management is high cost and scarcity of land required for treatment and disposal. Bioremediation offers a promising solution in treatment of specific wastes.

Bioremediation can be defined as any process where organic wastes are biologically degraded by living organisms, primarily microorganisms into less toxic forms. It generally employs the use of naturally occurring bacteria, fungi or plants to degrade the environmental contaminants. The ability of microorganisms to degrade a wide range of contaminants has made it an acceptable technology, which can be applied to different conditions. Bioremediation is safer and more economical than traditional methods such as incineration. Some pollutants can be treated onsite thereby reducing the risk of transportation accidents. Since bioremediation is mainly based on natural attenuation it is more accepted than other technologies.

Role of microorganisms

Bioremediation has been occurring in nature for millions of years where dead vegetation and dead animals are degraded by a kind of bioremediation. It is a part of the carbon, nitrogen, and sulfur cycles.

Bacteria and other microorganisms play a key in the biodegradation process. These microorganisms are universal in nature. They have been isolated even from extreme environments like hot springs and salt lakes. Therefore they have the ability to survive in hostile environmental conditions. Hence they are exploited for treatment of wastes. Bioremediation consists of using these natural indigenous organisms native to the contaminated site or laboratory cultivated organisms for waste treatment. In any case, the chemical energy present in waste materials is utilized by the microorganisms to grow and convert organic carbon and hydrogen to carbon dioxide and water. These reactions occur as part of their metabolic process by producing enzymes that degrade the contaminants and use it as food which helps them to survive in the extreme conditions. Due to its size and rapid growth they can easily contact the contaminants and help in its removal.

Removal efficiency generally depends on the nature of the pollutant and ability of the microorganisms. Some of the pollutants are completely degraded by the microorganisms into basic elements like water and carbon dioxide by

a process called mineralization. Some are partially or incompletely degraded into less complex compounds. Further some of the pollutants are transformed into less toxic forms. In some cases even immobilization occurs where they are not altered or eliminated by the microorganisms. In certain scenario, microorganisms are also imported to a contaminated site to enhance biodegradation. This process is called bioaugmentation.

Different types of microbes are being used depending on the type of pollutant present in the environment. Microbes employed in bioremediation can divided into the following groups:

Aerobes: These microbes use atmospheric oxygen. Examples of aerobic microorganisms are *Pseudomonas*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium*. Most of these microbes are used in the degradation of pesticides and hydrocarbons. For example bacterial species like *Pseudomonas putida* has been used in the bioremediation of hydrocarbons in petroleum contaminated soil. *Dechloromonas aromatica* is used in degrading perchlorate and aromatic compounds in soil. *Nitrosomonas europa* and *Nitrobacter hamburgensis* help in treatment of waste water generated by industrial process. They mainly help in removal of unwanted nitrogen compounds by nitrification in aerobic conditions. *Deinococcus radiodurans* a radiation-resistant extremophile bacterium has been genetically engineered for the bioremediation of solvents and heavy metals in radioactive mixed waste environment.

Anaerobes: These microbes survive in the absence of oxygen. For example, in anaerobic conditions nitrate is removed by microbes like *Paracoccus denitrificans* by a process called denitrification. Anaerobes are mostly used in bioremediation of chlorinated biphenyls (PCB's), dechlorination of trichloroethylene and chloroform.

Ligninolytic fungi: Fungal species like *Phanerochaete chrysoporum*, a lignin-degrading fungi, has the potential to degrade a wide range of pollutants like pesticides, polycyclic aromatic hydrocarbons, dyes, explosives, cyanides etc.,. Some of the substrates used for fungal growth are straw, saw dust and corn cobs.

Methylo trophs: These are aerobic bacteria that utilize methane as its carbon and energy source. Methane monooxygenase, an enzyme produced initially in the pathway of aerobic degradation is active against a wide range of compounds such as chlorinated aliphatics.

Bioremediation is mostly carried out as a result of action of multiple microorganisms. Most bioremediation technologies are operated under

For bioremediation to be more effective environmental conditions has to be optimized for microbial growth and activity. Therefore it involves manipulation of these conditions to improve microbial growth and enhance the degradation process. The major factors influencing bioremediation include bioavailability of the contaminant to the microbes and environmental factors such as type of soil, pH, nutrients, temperature, and presence of oxygen or other electron acceptors.

Bioavailability: To increase the rate of degradation it is necessary to increase the contact between the bacteria and the contaminant. This is difficult to achieve since both the bacteria and the pollutant are not uniformly spread in soil. Some motile bacteria sense the contaminant by chemotactic response and move towards it. In case of fungi it grows in a filamentous form towards the contaminant. Some organic compounds such as petroleum hydrocarbons which are insoluble in water float on top of the water bodies thereby reducing the surface area available for active degradation. In such cases, surfactants like sodium dodecyl sulfate are used to enhance degradation.

Nutrients: Whenever a contaminant is added to the environment, some microorganisms will die whereas others capable of transforming this contaminant will use it for its own growth and reproduction. Bioremediation basically involves biostimulating the growth and activity of these microbes by addition of nutrients. Most of the organic contaminants serve as a source of carbon which is the basic building blocks of the cell. Apart from carbon nitrogen, phosphorus, sulfur and other nutrients are required for microbial growth. Therefore fertilizers or nutrients (e.g., nitrates) are added in optimum levels to accelerate the natural biodegradation process.

Type of soil and moisture content: Soil structure controls the effective delivery of air, water and nutrients to the indigenous microorganisms. High permeability is required to allow nutrients and oxygen to reach these microbes. Therefore *in situ* bioremediation might not be well suited for soil with low permeability (e.g., fine clay). To improve soil structure, gypsum or other organic matter are being used. Soil moisture should also be from 50-70% of the water holding capacity of the soil to improve microbial activity. Irrigation is needed to achieve optimum moisture levels in contaminated soil.

Temperature and pH: Though microorganisms are isolated from extreme conditions, most bioremediation techniques work over a narrow range. Microbial growth and activity are readily affected by temperature and pH and therefore, it is necessary to optimize these conditions to accelerate biodegradation. The best pH range is from 6.5-7.5. If the soil has too much acid, pH can be adjusted by adding lime. Temperature can be from 15-45°C for microbial activity. Temperature affects the biochemical reaction rates of the microbes. The rate of the reaction increases with rise in temperature, but above a certain limit the cell dies. Plastic covering can be used to reduce heat in summer.

Oxygen and other electron acceptors: Microorganisms obtain energy by catalyzing chemical reactions that involve breaking of chemical bonds and transferring electrons away from the contaminant. These chemical reactions are called oxidation-reduction reactions where the organic contaminant is oxidized, losing electrons and correspondingly the chemical that gains electron is reduced. The contaminant is the electron donor. The energy gained from these electron transfers along with the carbon from the contaminant is used to increase cell biomass. Therefore, electron donor and acceptor are important for cell growth. Many microbes, especially aerobes use oxygen as electron acceptors. They use oxygen from aerobic respiration to oxidize part of the carbon in the contaminant to carbon dioxide while rest of the carbon is used to produce new cells. In this process, the oxygen gets reduced producing water. Therefore, the byproducts of aerobic respiration are carbon dioxide, water, and cell biomass. To increase aeration in soil, tilling or sparging of air is done. In case of ground water pollution, sometimes instead of pumping oxygen dilute solutions of hydrogen peroxide or magnesium peroxide is added.

In anaerobic respiration, inorganic chemicals such as nitrate, sulfate and metals (e.g., Iron, manganese) serve as electron acceptors. The major byproducts of anaerobic respiration apart from cell biomass are nitrogen gas, hydrogen sulfide, reduced form of metals and methane depending on the electron acceptor.

4.13 TYPES OF BIOREMEDIATION

Depending on the nature of the site, type of contaminant, degree of saturation, carrying capacity of the microbial population and aeration of an area different treatment technology are being employed. It can be generally classified as *in situ* or *ex situ* bioremediation. *In situ* bioremediation involves treating the contaminant on site with minimal disturbance to the surrounding environment.

Ex situ bioremediation involves removal of the contaminated material from the site via excavation of soil or pumping of ground water to be treated elsewhere.

I. In situ Bioremediation: Since *in situ* techniques offers to treat the contaminant at the site it may be less expensive, cause less release of contaminants and also possible to treat large volume of the contaminant at once. It also avoids excavation and transport of the contaminant. It speeds the natural biodegradation process where diffusion of air might be a limiting factor. It is more effective at sites with permeable soil. Examples are bioventing, biosparging, bioaugmentation, and injection of hydrogen peroxide.

Bioventing: It involves pumping of nutrients (e.g., nitrogen and phosphorus) and air from the atmosphere into the contaminated soil through injection wells to stimulate the growth of indigenous microorganisms. The air flow rates are regulated to provide oxygen necessary for degradation and minimize volatilization and release of contaminants to the atmosphere. This method works efficiently in case of soil contaminated with petroleum hydrocarbons where contamination is deep under the surface.

Biosparging: It involves injection of air under pressure below the water table to treat ground water contamination *in situ* by enhancing biodegradation of indigenous microorganisms. Biosparging also increases mixing in the saturated zone. The ease and low cost of installing small diameter injection points has increased the flexibility in the design and construction of the treatment technique.

Injection of hydrogen peroxide: This process involves supply of oxygen by circulating hydrogen peroxide through contaminated soil. It stimulates biodegradation by increasing the growth of naturally-occurring microorganisms. It is employed at sites where the ground water is already contaminated and the injected hydrogen peroxide seeps to the ground water. In case of shallow contaminated soils, a system of pipes or sprinkler system is used to supply hydrogen peroxide and injection wells are used for deeper contaminated soils.

Bioaugmentation: Bioaugmentation involves addition of specially selected or genetically engineered organisms with higher degradation abilities to contaminated soil or water. The major limiting factor in addition of microbial cultures is that the non-indigenous microorganisms rarely compete well with indigenous organisms to develop and sustain useful population levels.

II. Ex situ Bioremediation: This technique requires excavation of contaminated soil or pumping of ground water before treatment. However, this technique can be faster, easier to control and treats a wide range of contaminants and soil types than *in situ* techniques. It includes (A) slurry-phase and (B) solid-phase treatments.

A. Slurry-Phase bioremediation: It involves initial combination of water with the contaminated soil followed by degradation. Bioreactor is an example of this type of bioremediation.

Bioreactor: Bioreactor involves processing of the contaminated soil or water through an engineered containment system. The contaminated soil is mixed with water and other additives in a large tank (bioreactor) to enhance the activity of native microorganisms present in the soil. Nutrients and oxygen are added and the conditions are controlled to provide an optimum environment for the microorganisms. The rate and extent of degradation is relatively rapid than other biological treatments because the contained environment is more controllable and predictable.

B. Solid-phase bioremediation: In this process the contaminated soil is treated above ground by adding required amounts of nutrients, oxygen and moisture for degradation. It is also equipped with collection systems to prevent any contaminants being let out without treatment. It is relatively simple to operate and maintain but requires more time for treatment and large amount of space than slurry-phase treatment. Examples of solid-phase treatment are land farming, biopiles, and composting.

Land farming: Land farming is a relatively simple technique in which the contaminated soil is excavated and spread over pre-prepared beds with systems to collect leachate that seep out of the contaminated soil. Moisture and nutrients are added in required amounts and the soil is periodically tilled for proper mixing to enhance aerobic degradation by native microorganisms. It is widely used since it has the potential to reduce monitoring and maintenance cost.

Composting: It is a traditional method which is widely used to recycle nutrients in garden and yard waste. The contaminant is mixed with non-hazardous amendments such as manure, agricultural waste or bulking agents like straw, hay or corncobs to optimize aeration and moisture content for degradation. It generates odors and gases which has to be controlled. Composting supports rich microbial population and its activity is characterized by elevated temperature.

Biopiles: The technique involves piling of contaminated soil in heaps of

several meters high over an air distribution system. Aeration is provided by pulling air through the heap with a vacuum pump. Nutrient and moisture levels are controlled to optimize bioremediation. Biopiles provides favorable environment for the activity of both indigenous aerobic and anaerobic microorganisms. Physical loss of the contaminant by volatilization and leaching is controlled. Volatile contaminants are usually part of the air steam being pulled through the heap. It is mostly used for treatment of surface contamination with petroleum hydrocarbons.

4.14 ADVANTAGES AND DISADVANTAGES OF BIOREMEDIATION

Advantages of bioremediation:

- Bioremediation provides an environment-friendly cleanup strategy by just enhancing the naturally occurring biodegradation process. The microorganisms capable of degradation flourish by utilizing these contaminants and once the contaminants are completely used up, the biodegradative microbial population slowly declines.
- Depending on the site and type of contaminants, bioremediation may be safer and cost-effective than other methods like incineration or land filling.
- In case of *in situ* bioremediation, it has the advantage of treating the contaminants in place which does not require excavation or pumping and transport of large quantities of soil, sediment or water. This causes fewer disturbances to the surrounding environment.
- Bioremediation is a useful tool in the treatment of many types of organic wastes. Most of the organic wastes are completely degraded by these microorganisms into end-products such as water and carbon dioxide. Some of the hazardous compounds are transformed into harmless products.
- In bioremediation, most of the organic wastes are completely degraded or destroyed instead of concentrating or transferring the contaminant from one environmental medium to another like transfer from land to water or air.

Disadvantages of bioremediation:

- Bioremediation requires manipulation of conditions for enhancing microbial activity. The diversity of the natural ecosystem leads to uncertainty in results.

- Bioremediation is a slow process and it takes longer time than other treatment methods like excavation and removal or incineration.
 - Sometimes, the intermediates or the breakdown products of biodegradation may be more toxic and persistent than the original contaminant.
 - Bioremediation is limited to those compounds that are biodegradable or susceptible to complete and rapid degradation like non-halogenated volatile and semi-volatile organics and fuel. It is limited at sites with high concentration of metals, chlorinated organics, and inorganic salts.
 - Very high contaminant concentration can be toxic to the microorganisms and there is a threshold up to which biodegradation can be effective.
 - Lower temperature and soil with less permeability are not well suited for bioremediation.
 - Difficulty lies in extrapolation of results from bench- and pilot-scale to field operation.
 - Bioremediation techniques have to improve for treating complex mixture of contaminants that are not evenly dispersed in the environment.
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- In spite of these limitations, bioremediation has been successfully employed in the treatment of oil spills, soil and ground water contaminated with volatile and semi-volatile organic compounds, perchlorates and pesticides.
 - ‘Oilzapper’ and ‘Oilivorous-S’, a consortium of oil degrading microbes, developed by The Energy and Resources Institute (TERI) is commercially available for treatment of oil contaminated soil and water and has been successful in the treatment of recent oil spills off the coast of Mumbai.
 - Bioremediation is also used in treatment of municipal waste to decompose suspended solids, reduce pathogenic organisms and pollutants like perchlorates and petrochemicals.

facilitating various functions within and outside the cell have been studies extensively and are found to possess energy conversion devices to obtain forces, torques, and motion.

- Cytoskeletons are critical components of the cell that give the cell its shape, structure, and physical organization. The major components of the cytoskeleton are microtubules, microfilaments, and intermediate filaments.
- Linear motors that transport molecular cargoes with the help of ATP are classified into kinesins, myosins, and dyneins.
- Biosensor is a combination of biological element and a man-made sensor that can detect small fractions of compounds with accuracy. The biological element can be enzymes, antibody, nucleic acid, etc while the sensor element can possess electrical properties such as conductance, impedance, etc.
- Bioremediation is a process through which organic wastes are biologically degraded by microorganisms into less toxic materials in an efficient manner. Aerobic and anaerobic organisms are used to decontaminate air, soil, and water depending on the nature of the pollutants.
- Bioremediation can be done at the site of contamination using bioventing, biosparging, bioaugmentation, and injection of hydrogen peroxide. It can be also done by transporting the contaminants to another site by slurry-phase and solid-phase bioremediation.