Training Program

Future Perspective of Environmental Biotechnology

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Authors

Sutapa Dutta, Md. Hafizur Rahman, Punarbasu Chaudhury, Shouvik Mahanty, Md. Azizur Rahman, Subhasis Das, Dheeban K Chakravarthi, Suvendu Manna

Coordinators

Arindam Datta & Md. Hafizur Rahman

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For more information

Arindam Datta
T E R I
Darbari Seth Block
IHC Complex, Lodhi Road
New Delhi – 110 003
India

Tel. 2468 2100 or 2468 2111
E-mail: arindam.datta@teri.res.in
Fax 2468 2144 or 2468 2145
Web www.teriin.org
India +91 • Delhi (0)11

Program co-coordinator and Faculty members

Program co-ordinator

Dr. Arindam Datta is Fellow at Centre for Environmental Studies, TERI. He is a Fulbright Scholar and Associate in the National Academy of Agricultural Sciences. He has 15 years research experience in the field of Climate Change and Agriculture. Presently, he is working on different source apportionment studies of ambient air quality, soil carbon sequestration and development of emission factors for different air pollution related activities. Email: arindam.datta@teri.res.in

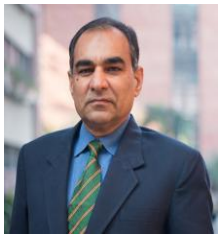
Md. Hafizur Rahman is working as Research Associate at TERI, New Delhi. He completed B.Tech in Biotechnology from West Bengal University of Technology and M.Tech in Environmental biotechnology from Jadavpur University, Kolkata. Email: md.rahman@teri.res.in

Faculty

Prof. Satyabathi Sharma is Professor at Centre for Rural Development and Technology, Indian Institute of Technology Delhi. Her research interests lies in Bio-inoculants and Bio-fertilizer, Rapid composting/Waste management, Tissue culture, Fungal and botanical bio-pesticides, Wasteland Reclamation, Mushroom technology, Vermitechnology, Silvipastoral technology and Biogas slurry management. She has received the 'Iraj Zandi award' in 2013 in recognition of her contribution to the field of Solid Waste Technology and Management by Widener University, Philadelphia, USA. Email: satyawatis@hotmail.com

Dr. Vibha Dhawan is one of the Senior Directors in TERI and Fellow of National Academy of Sciences, India. She has also completed one term as Vice-Chancellor, TERI-School of Advanced Studies. Dr Dhawan is actively involved in research as well as policy development, both at the national and international level She was an Advisor, Bio-resources & Biotechnology to the former Chief Minister of Assam (2007–2016). Her major achievements includes developing e-contents for PG programme in Biotechnology, an initiative of University Grants Commission, establishing Micropropagation Technology Park and Biofuel research at TERI with support from DBT, India. Email: vibhad@teri.res.in

Dr. Sangita M Kasturi is Joint Director/Scientist-E in the Department of Biotechnology, Govt. of India. She is the Program Officer in the Bioergy Program: Biofuel Technology of DBT.
Email: sangita.kasture@nic.in

	**Dr. Banwari Lal** is one of the senior directors in TERI. He is an eminent biotechnologist of the country. He is a member of the Scientific Panel of the Food Safety and Standards Authority of India, Ministry of Health & Family Welfare His area of interest is Petroleum Biotechnology and has developed three technologies namely "Oilzapper ", "Microbial Enhanced Oil Recovery" and "Prevention of paraffin deposition in oil well tubing and flow lines". He is the founding director of the ONGC-TERI Biotech Ltd. which is a start-up company. All the three technologies are patented and commercialized. Email: banwari@teri.res.in
	**Dr. Punarbasu Chaudhury** is an Environmental Microbiologist and Associate Professor at the Department of Environmental Science, University of Calcutta, West Bengal. Dr. Chaudhuri did his post doctoral research on the effects of costal pollution on mangroves from University of Sydney as Endeavour Fellow of Government of Australia. He participated Canadian Sub-Arctic expedition as Shastri Indo-Candian fellow to study the impact of climate change on polar environment. He received Young Investigator Fund from Department of Science and Technology, Govt of India for Climate change studies in Hooghly estuary. He has more than fifty research publication in different international journals. Email: punarbasu_c@yahoo.com
	Dr. Nupur Bahadur
	**Dr. Sutapa Dutta** is Plant Biotechnologist, from the ICAR-National Research Centre on Plant Biotechnology, New Delhi. She was a Post-doc and demonstrator in different courses of molecular biology at the University of Aberdeen, United Kingdom. She works in the development of molecular markers for climate resilient crop improvement. She has publications in different international scientific journals. Email: koyeldatta@gmail.com
	**Dr. Md. Azizur Rahman** is working as post-doctoral fellow at department of Chemical Engineering, Jadavpur University, Kolkata with National Post-Doctoral Fellowship (NPDF), awarded by SERB-DST, India. He did his B.Tech and M.Tech in Biotechnology from West Bengal University of Technology, Kolkata. He was awarded Doctor of Philosophy in Engineering from Indian Institute of Technology Delhi. He has research and constancy experience in the field of resource recovery from waste streams, faecal sludge management, sanitation, appropriate technology for rural development and nanotechnology. Email: azizur.md.rahman@gamil.com

	**Dr. K. Dheeban Chakravorthi** is Fellow at TERI. He is in algal biofuel research. His focus areas are photobioreactor design, algal harvesting and lipid recovery. He has previously been a Senior Research Engineer at the algal biofuel company, OriginOil in Los Angeles, USA. He has publications on algal growth system design, algal nutrient uptake, lipid recovery, engineering aspects of algal biofuels and phase equilibria and patent applications on outdoor algal growth system and wet algal lipid recovery. Email: dheeban.kannan@teri.res.in
	**Dr. Sanjukta Subudhi** is a molecular microbiologist and working as senior fellow at TERI. Her major research achievements include development of pilot scale microbial process for bio-hydrogen production. Research explorations of her group span the domain of; (i) microbial production of clean fuel, (ii) cyanobacterial based PUFA production, (iii) use of microbes/algae for production of value added chemicals and enzymes, biopolymers. Email: ssubudhi@teri.res.in
	**Dr. K. Nanthakumar** is Fellow at TERI. He has specialization in Environmental biotechnology: Bioenergy, Bioremediation of wastewater and soil, emerging pollutants management. His main research areas include the role of microorganisms in remediation of polluted environments including total petroleum hydrocarbon, emerging micro-pollutants, development of specialized micro electrochemical reactor system for the treatment of various wastewater and recovery of energy. Email: k.nanthakumar@teri.res.in
	**Dr. Subhasis Das** is a molecular microbiologist and Fellow at TERI and was a team member of the soil remediation project of TERI at Kuwait. His main areas of research is bioremediation of Xenobiotics, flame retardant compounds and other contaminants. He is a member of scientific society, Associate Editor of National and International Journal and examiner of Indian University. Email: subhasis.das@teri.res.in

Content

Preface – Importance of the Training program

One of the greatest problems that the world is facing today is that of environmental pollution, increasing with every passing year and causing grave and irreparable damage to the earth. Environmental pollution has existed for centuries but only started to be significant following the industrial revolution in the 19th century. Pollution occurs when the natural environment cannot destroy an element without creating harm or damage to itself. The elements involved are not produced by nature, and the destroying process can vary from a few days to thousands of years (that is, for instance, the case for radioactive pollutants). In other words, pollution takes place when nature does not know how to decompose an element that has been brought to it in an unnatural way. Environmental pollution consists of five basic types of pollution, namely, air, water, soil, noise and light.

Environmental Biotechnology is a rapidly developing, increasingly important branch of science that has implications for both the prevention and clean-up of pollution in different strata of the environment. One notable example is bioremediation, i.e., the use of microorganisms to clean up contaminated environments, including contaminated soils, sediments and water. Environmental biotechnology is at the interface of biology and engineering, which presents both significant opportunities and limitations. Effective application of environmental biotechnology requires professionals who have a background in both areas.

However, environmental engineers have considerable engineering skills required for the design of processes *per se*, have only a rudimentary knowledge of general biology and microbiology in particular. The development of a training program on Environmental Biotechnology satisfies an urgent need in terms of professional preparation, and is timely as biology and engineering are becoming more integrated to solve our future problems related to environment.

There are opportunities in Environmental Biotechnology to provide sustainable solutions to diverse environmental problems. The Pollution Control Boards in India was established to control the environmental pollutions particularly in water and air. However, at present, Pollution Control Boards across the nation working on industrial waste management, municipal waste management etc. apart from controlling the pollution of the air and water.

This training program will help to develop the understanding on the application of molecular biology and biotechnology to solve environmental problems as noted in diverse areas across the country. Additionally, this short duration training program will support the participants to develop professional networks which will help them to develop future plans to crack environmental pollution problems with sustainable application of biotechnology.

Basics of Molecular Biology & Biotechnology

What is Biotechnology?

The exploitation of biological processes for industrial and other purposes, especially the genetic manipulation of microorganisms for the production of antibiotics, hormones, etc. In other word Biotechnology is the broad area of biology involving living systems and organisms to develop or make products.

Cell: Unit of life

When you look around, you see both living and non-living things. You must have wondered and asked yourself – 'what makes an organism living, or what an inanimate thing does not have but a living thing does'? The answer to this is the presence of the basic unit of life – the cell in all living organisms. All organisms are composed of one or multiple numbers of cells. Single cell organisms are called unicellular organisms while others, composed of more than one cell, are known as multicellular organisms.

Unicellular organisms are capable of (i) independent existence and (ii) performing the essential functions of life. Anything less than a complete structure of a cell does not ensure independent living. Hence, cell is the fundamental structural and functional unit of all living organisms. In 1665, the cell was first discovered by Robert Hook. Anton Von Leeuwenhoek first saw and described a live cell. Robert Brown later discovered the nucleus. The invention of the microscope and its improvement leading to the electron microscope revealed all the structural details of the cell.

Prokaryotic Cells

The organisation of the prokaryotic cell is fundamentally similar even though prokaryotes exhibit a wide variety of shapes and functions. All prokaryotes have a cell wall surrounding the cell membrane except in mycoplasma. The fluid matrix filling the cell is the cytoplasm. There is no well-defined nucleus. The genetic material is basically naked, not enveloped by a nuclear membrane. In addition to the genomic DNA (the single chromosome/circular DNA), many bacteria have small circular DNA outside the genomic DNA. These smaller DNA are called plasmids. The plasmid DNA confers certain unique phenotypic characters to such bacteria. One such character is resistance to antibiotics. In higher classes you will learn that this plasmid DNA is used to monitor bacterial transformation with foreign DNA. Prokaryotes have something unique in the form of inclusions. A specialized differentiated form of cell membrane called mesosome is the characteristic of prokaryotes. They are essentially infoldings of cell membrane.

Cell Envelope and its Modifications

Most prokaryotic cells, particularly the bacterial cells, have a chemically complex cell envelope. The cell envelope consists of a tightly bound three layered structure i.e., the outermost glycocalyx followed by the cell wall and then the plasma membrane. Although each layer of the envelope performs distinct function, they act together as a single protective unit. Bacteria can be classified into two groups on the basis of the differences in the cell envelopes and the manner in which they respond to the staining procedure developed by Gram viz., those that take up the gram stain are Gram positive and the others that do not are called Gram negative bacteria.

Glycocalyx differs in composition and thickness among different bacteria. It could be a loose sheath called the slime layer in some, while in others it may be thick and tough, called the capsule. The cell wall determines the shape of the cell and provides a strong structural support to prevent the bacterium from bursting or collapsing. A special membranous structure is the mesosome which is formed by the extensions of plasma membrane into the cell. These extensions are in the form of vesicles, tubules and lamellae. They help in cell wall formation, DNA replication and distribution to daughter cells. They also help in respiration, secretion processes, to increase the surface area of the plasma membrane and enzymatic content. In some prokaryotes like cyanobacteria, there are other membranous extensions into the cytoplasm called chromatophores which contain pigments.

Bacterial cells may be motile or non-motile. If motile, they have thin filamentous extensions from their cell wall called flagella. Bacteria show a range in the number and arrangement of flagella. Bacterial flagellum is composed of three parts – filament, hook and basal body. The filament is the longest portion and extends from the cell surface to the outside. Besides flagella, Pili and Fimbriae are also surface structures of the bacteria but do not play a role in motility. The pili are elongated tubular structures made of a special protein. The fimbriae are small bristle like fibres sprouting out of the cell. In some bacteria, they are known to help attach the bacteria to rocks in streams and also to the host tissues.

Ribosomes and Inclusion Bodies

In prokaryotes, ribosomes are associated with the plasma membrane of the cell. They are about 15 nm by 20 nm in size and are made of two subunits - 50S and 30S units which when present together form 70S prokaryotic ribosomes. Ribosomes are the site of protein synthesis. Several ribosomes may attach to a single mRNA and form a chain called polyribosomes or polysome. The ribosomes of a polysome translate the mRNA into proteins. Inclusion bodies: Reserve material in prokaryotic cells are stored in the cytoplasm in the form of inclusion bodies. These are not bound by any membrane

system and lie free in the cytoplasm, e.g., phosphate granules, cyanophycean granules and glycogen granules. Gas vacuoles are found in blue green and purple and green photosynthetic bacteria.

Eukaryotic Cells

In eukaryotic cells there is an extensive compartmentalization of cytoplasm through the presence of membrane bound organelles. Eukaryotic cells possess an organised nucleus with a nuclear envelope. In addition, eukaryotic cells have a variety of complex locomotory and cytoskeletal structures. Their genetic material is organised into chromosomes. All eukaryotic cells are not identical. Plant and animal cells are different as the former possess cell walls, plastids and a large central vacuole which are absent in animal cells. On the other hand, animal cells have centrioles which are absent in almost all plant cells (Figure 1).

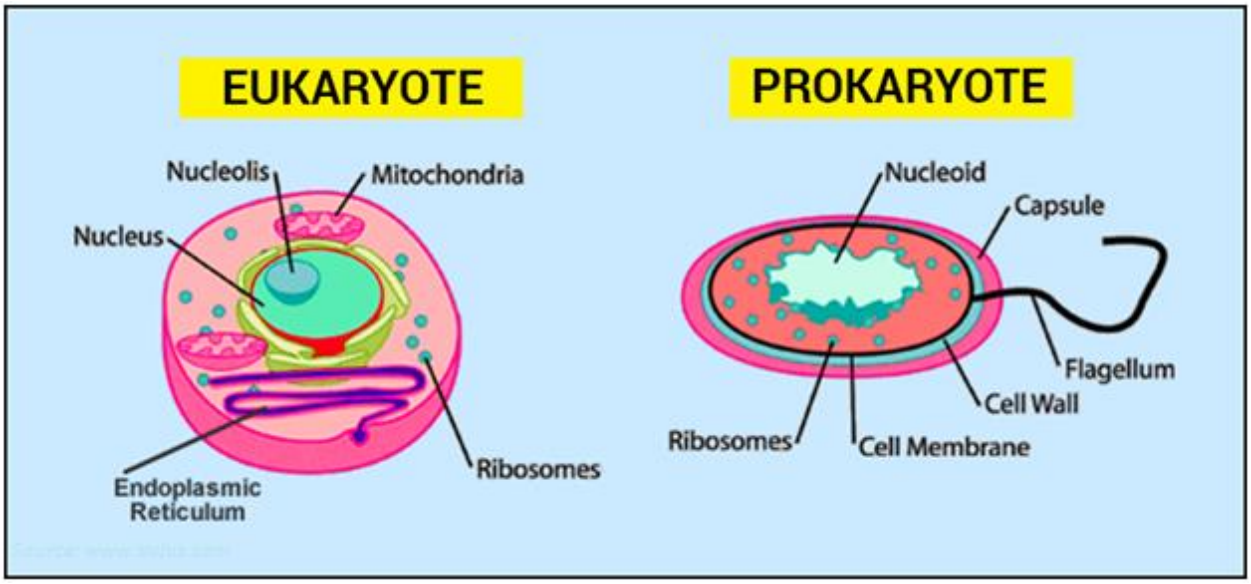


Figure-1 Difference between Prokaryotic and Eukaryotic Cells:

Cell membrane

The cell membrane is mainly composed of lipids and proteins. The major lipids are phospholipids that are arranged in a bilayer. Also, the lipids are arranged within the membrane with the polar head towards the outer sides and the hydrophobic tails towards the inner part. This ensures that the non polar tail of saturated hydrocarbons is protected from the aqueous environment (Figure 2). In addition to phospholipids membrane also contains cholesterol. The lipid component of the membrane mainly consists of phosphoglycerides. Cell membranes also possess protein and carbohydrate. The ratio of protein and lipid varies considerably in different cell types. In human beings, the membrane of the erythrocyte has approximately 52 per cent protein and 40

per cent lipids. An improved model of the structure of cell membrane was proposed by Singer and Nicolson (1972) widely accepted as fluid mosaic model (Figure 2). According to this, the quasi-fluid nature of lipid enables lateral movement of proteins within the overall bilayer. This ability to move within the membrane is measured as its fluidity. The fluid nature of the membrane is also important from the point of view of functions like cell growth, formation of intercellular junctions, secretion, endocytosis, cell division e

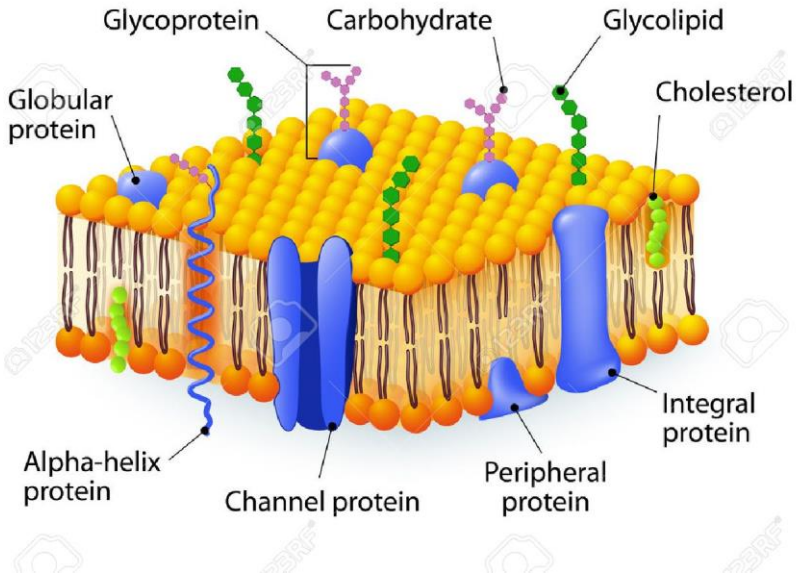


Figure-2 Fluid mosaic model of a cell membrane

Cell Wall

As you may recall, a non-living rigid structure called the cell wall forms an outer covering for the plasma membrane of fungi and plants. Cell wall not only gives shape to the cell and protects the cell from mechanical damage and infection, it also helps in cell-to-cell interaction and provides barrier to undesirable macromolecules. Algae have cell wall, made of cellulose, galactans, mannans and minerals like calcium carbonate, while in other plants it consists of cellulose, hemicellulose, pectins and proteins. The cell wall of a young plant cell, the primary wall is capable of growth, which gradually diminishes as the cell matures and the secondary wall is formed on the inner (towards membrane) side of the cell.

Endomembrane System

While each of the membranous organelles is distinct in terms of its structure and function, many of these are considered together as an endomembrane system because their functions are coordinated. This includes endoplasmic reticulum (ER), golgi complex, lysosomes and vacuoles. Since the functions of the mitochondria, chloroplast and peroxisomes are not coordinated with the above components, these are not considered as part of the endomembrane system.

The Endoplasmic Reticulum (ER)

Electron microscopic studies of eukaryotic cells reveal the presence of a network or reticulum of tiny tubular structures scattered in the cytoplasm that is called the endoplasmic reticulum (ER) (Figure). Hence, ER divides the intracellular space into two distinct compartments, i.e., luminal (inside ER) and extra luminal (cytoplasm) compartments. The ER often shows ribosomes attached to their outer surface. The endoplasmic reticulum bearing ribosomes on their surface is called rough endoplasmic reticulum (RER). In the absence of ribosomes they appear smooth and are called smooth endoplasmic reticulum (SER). RER is frequently observed in the cells actively involved in protein synthesis and secretion. They are extensive and continuous with the outer membrane of the nucleus. The smooth endoplasmic reticulum is the major site for synthesis of lipid. In animal cells lipid-like steroidal hormones are synthesized in SER.

Golgi apparatus

Camillo Golgi (1898) first observed densely stained reticular structures near the nucleus. These were later named Golgi bodies after him. They consist of many flat, disc-shaped sacs or cisternae of 0.5µm to 1.0µm diameter (Figure). These are stacked parallel to each other. Varied number of cisternae are present in a Golgi complex. The Golgi cisternae are concentrically arranged near the nucleus with distinct convex *cis* or the forming face and concave *trans* or the maturing face. The *cis* and the *trans* faces of the organelle are entirely different, but interconnected. The golgi apparatus principally performs the function of packaging materials, to be delivered either to the intra-cellular targets or secreted outside the cell.

Lysosomes

These are membrane bound vesicular structures formed by the process of packaging in the golgi apparatus. The isolated lysosomal vesicles have been found to be very rich in almost all types of hydrolytic enzymes (hydrolases – lipases, proteases, carbohydrases) optimally active at the acidic pH. These enzymes are capable of digesting carbohydrates, proteins, lipids and nucleic acids.

Vacuoles

The vacuole is the membrane-bound space found in the cytoplasm. It contains water, sap, excretory product and other materials not useful for the cell. The vacuole is bound by a single membrane called tonoplast. In plant cells the vacuoles can occupy up to 90 per cent of the volume of the cell. In plants, the tonoplast facilitates the transport of a number of ions and other materials against concentration gradients into the vacuole, hence their concentration is significantly higher in the vacuole than in the cytoplasm. In *Amoeba* the contractile vacuole is important for excretion. In many cells, as in protists, food vacuoles are formed by engulfing the food particles.

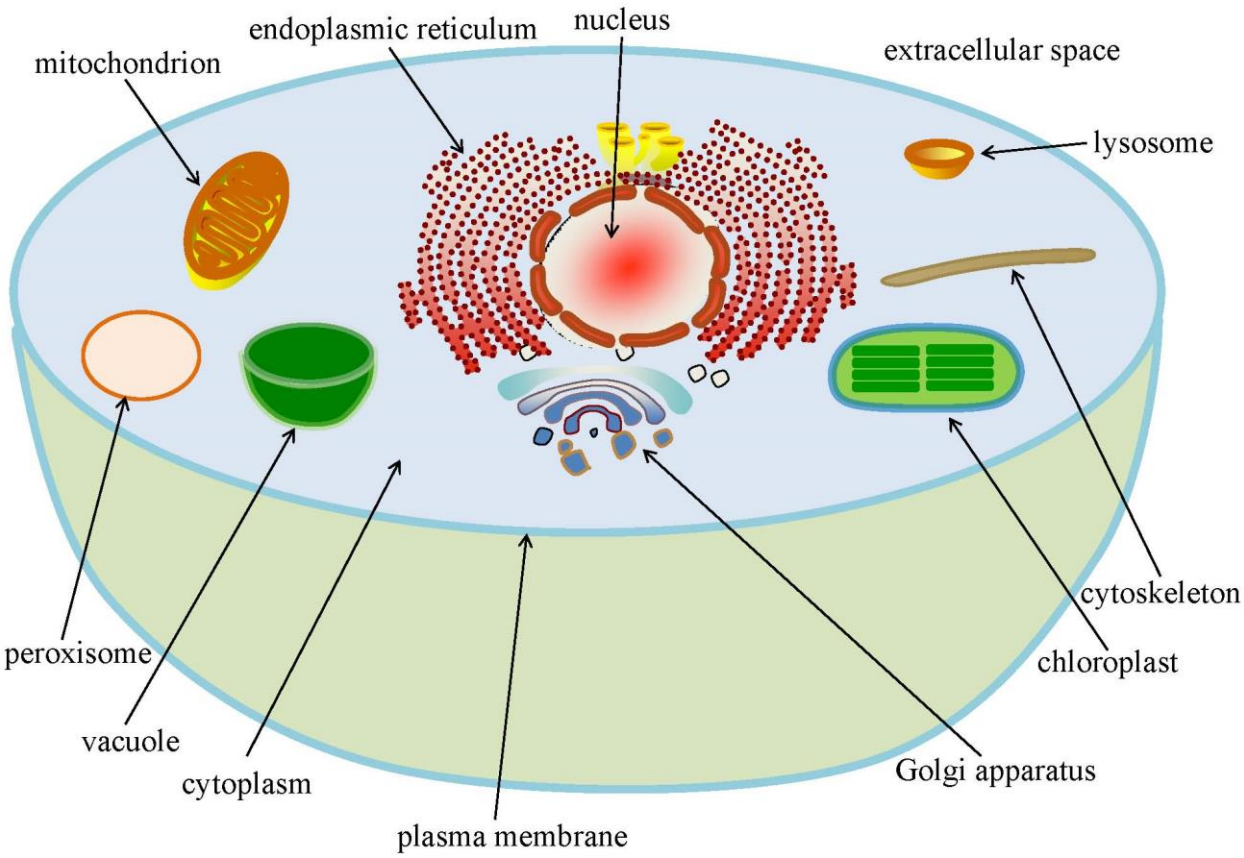


Figure 3 Major organelles in a typical eukaryotic cell

Mitochondria

Survival of the cells requires energy to perform different functions. The mitochondria are important as the fact that these organelles supply all the necessary biological energy of the cell, and they obtain this energy by oxidizing the substrates of the Krebs cycle. In 1890, mitochondria was first described by Richard Altmann and he called them as bioblasts. Benda in the year 1897 coined the term mitochondrion. In the 1920s, a biochemist Warburg found that oxidative reactions takes place in most tissues in small parts of the cell (Figure 4).

The components of mitochondria are as follows:

Outer membrane

I. It is smooth and is composed of equal amounts of phospholipids and proteins.
II. It has a large number of special proteins known as the porins.
III. The porins are integral membrane proteins and they allow the movement of molecules that are of 5000 daltons or less in weight to pass through it. The outer membrane is freely permeable to nutrient molecules, ions, energy molecules like the ATP and ADP molecules.

Inner membrane

I. The inner membrane of mitochondria is more complex in structure.
II. It is folded into a number of folds many times and is known as the cristae.
III. This folding help to increase the surface ares inside the organelle.
IV. The cristae and the proteins of the inner membrane aids in the production of ATP molecules.
V. Various chemical reactions takes place in the inner membrane of the mitochondria.
VI. Unlike the outer membrane, the inner membrane is strictly permeable, it is permeable only to oxygen, ATP and it also helps in regulating transfer of metabolites across the membrane.

Chloroplasts

This is the site of photosynthesis in eukaryotic cells. They are only present in photosynthetic cells like plant cells and algae. There are no chloroplasts in animal or bacterial cells. Plastids are found in all plant cells and in euglenoides. These are easily observed under the microscope as they are large. They bear some specific pigments, thus imparting specific colours to the plants. Based on the type of pigments plastids can be classified into chloroplasts, chromoplasts and leucoplasts. The chloroplasts contain chlorophyll and carotenoid pigments which are responsible for trapping light energy essential for photosynthesis. In the chromoplasts fat soluble carotenoid

pigments like carotene, xanthophylls and others are present. This gives the part of the plant a yellow, orange or red colour. The leucoplasts are the colourless plastids of varied shapes and

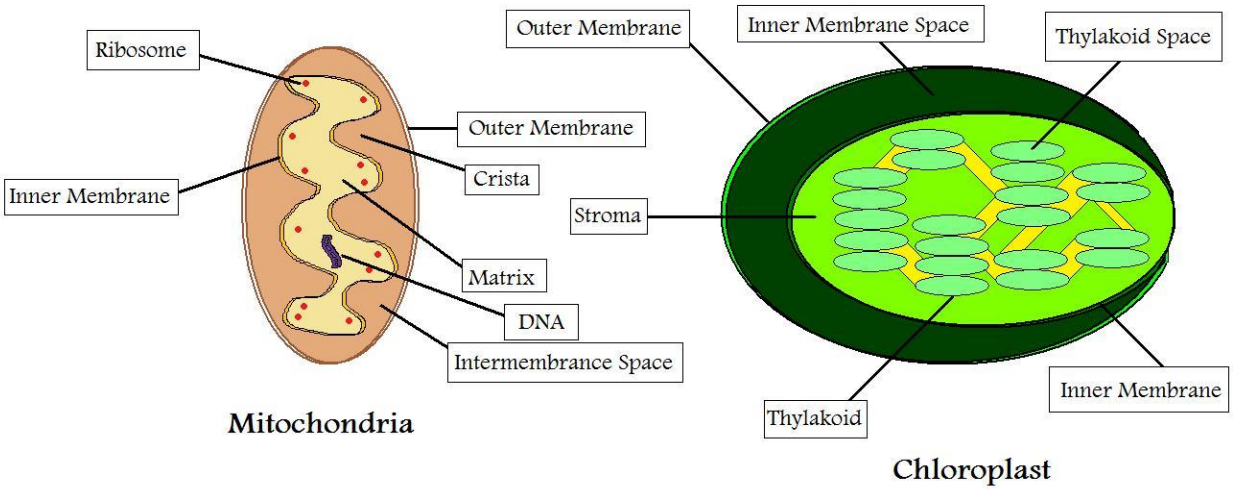


Figure 4 Outline of Mitochondria and Chloroplast

sizes with stored nutrients: Amyloplasts store carbohydrates (starch), e.g., potato; elaioplasts store oils and fats whereas the aleuroplasts store proteins. These are lens-shaped, oval, spherical, discoid or even ribbon-like organelles having variable length (5-10µm) and width (2-4µm). Their number varies from 1 per cell of the *Chlamydomonas*, a green alga to 20-40 per cell in the mesophyll. The membrane of the thylakoids enclose a space called a lumen. The stroma of the chloroplast contains enzymes required for the synthesis of carbohydrates and proteins. It also contains small, double stranded circular DNA molecules and ribosomes. Chlorophyll pigments arepresent in the thylakoids. The ribosomes of the chloroplasts are smaller (70S) than the cytoplasmic ribosomes (80S).

Parts of Chloroplasts

I. **Outer membrane –** It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

II. **Intermembrane Space –** It is usually a thin intermembrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.
III. **Inner membrane –** The inner membrane of the chloroplast forms a border to the stroma. It regulates passage of materials in and out of the chloroplast. In addition of regulation activity, the fatty acids, lipids and carotenoids are synthesized in the inner chloroplast membrane.
IV. **Stroma-** Stroma is a alkaline, aqueous fluid which is protein rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA chlroplast ribosomes and the thylakoid sytem, starch granules and many proteins are found floating around the stroma.
V. **Thylakoid System-** The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacks called thylakoids. The chlorophyll is found in the thylakoids and is the sight for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. Each granum contains around 10-20 thylakoids (Figure 4).

Functions of Chloroplast

I. Absorption of light energy and conversion of it into biological energy.
II. Production of NAPDH2 and evolution of oxygen through the process of photosynthesis of water.
III. The chloroplasts with the nucleus and cell membrane and ER are the key organelles of pathogen defense.
IV. The most important function of chloroplast is to make food by the process of photosynthesis.
V. Food is prepared in the form of sugars. During the process of photosynthesis sugar and oxygen are made using light energy, water, and carbon dioxide.
VI. Light reactions takes place on the membranes of the thylakoids.
VII. The dark reactions also known as the Calvin cycle takes place in the stroma of chloroplast.

Ribosomes

Ribosomes are the granular structures first observed under the electron microscope as dense particles by George Palade (1953). They are composed of ribonucleic acid (RNA) and proteins and are not surrounded by any membrane. The eukaryotic ribosomes are 80S while the prokaryotic ribosomes are 70S. Each ribosome has two subunits, larger and smaller subunits (Fig 8.9). The two subunits of 80S ribosomes are 60S and 40S while thatof 70S ribosomes are 50S and 30S. Here 'S' (Svedberg's Unit) stands for the

sedimentation coefficient; it is indirectly a measure of density and size. Both 70S and 80S ribosomes are composed of two subunits.

Cytoskeleton

An elaborate network of filamentous proteinaceous structures present in the cytoplasm is collectively referred to as the cytoskeleton. The cytoskeletons in a cell are involved in many functions such as mechanical support, motility, maintenance of the shape of the cell.

Cilia and Flagella

Cilia (sing.: cilium) and flagella (sing.: flagellum) are hair-like outgrowths of the cell membrane. Cilia are small structures which work like oars, causing the movement of either the cell or the surrounding fluid. Flagella are comparatively longer and responsible for cell movement. The prokaryotic bacteria also possess flagella but these are structurally different from that of the eukaryotic flagella.

Centrosome and Centrioles

Centrosome is an organelle usually containing two cylindrical structures called centrioles. They are surrounded by amorphous pericentriolar materials. Both the centrioles in a centrosome lie perpendicular to each other in which each has an organisation like the cartwheel. They are made up of nine evenly spaced peripheral fibrils of tubulin protein. Each of the peripheral fibril is a triplet. The adjacent triplets are also linked. The central part of the proximal region of the centriole is also proteinaceous and called the hub, which is connected with tubules of the peripheral triplets by radial spokes made of protein. The centrioles form the basal body of cilia or flagella, and spindle fibres that give rise to spindle apparatus during cell division in animal cells.

Nucleus

Nucleus as a cell organelle was first described by Robert Brown as early as 1831. Later the material of the nucleus stained by the basic dyes was given the name chromatin by Flemming. (Figure 5). Electron microscopy has revealed that the nuclear envelope, which consists of two parallel membranes with a space between (10 to 50 nm) called the perinuclear space, forms a barrier between the materials present inside the nucleus and that of the cytoplasm. The outer membrane usually remains continuous with the endoplasmic reticulum and also bears ribosomes on it. At a number of places the nuclear envelope is interrupted by minute pores, which are formed by the fusion of its two membranes. These nuclear pores are the passages through which movement of RNA and protein molecules takes place in both directions between the nucleus and the

cytoplasm. Normally, there is only one nucleus per cell, variations in the number of nuclei are also frequently observed.

The nuclear matrix or the nucleoplasm contains nucleolus and chromatin. The nucleoli are spherical structures present in the nucleoplasm. The content of nucleolus is continuous with the rest of the nucleoplasm as it is not a membrane bound structure. It is a site for active ribosomal RNA synthesis. Larger and more numerous nucleoli are present in cells actively carrying out protein synthesis. The interphase nucleus has a loose and indistinct network of nucleoprotein fibres called chromatin. Chromatin contains DNA and some basic proteins called histones, some non-histone proteins and also RNA. A single human cell has approximately two metre long thread of DNA distributed among its forty six (twenty three pairs) chromosomes. Every chromosome (visible only in dividing cells) essentially has a primary constriction or the

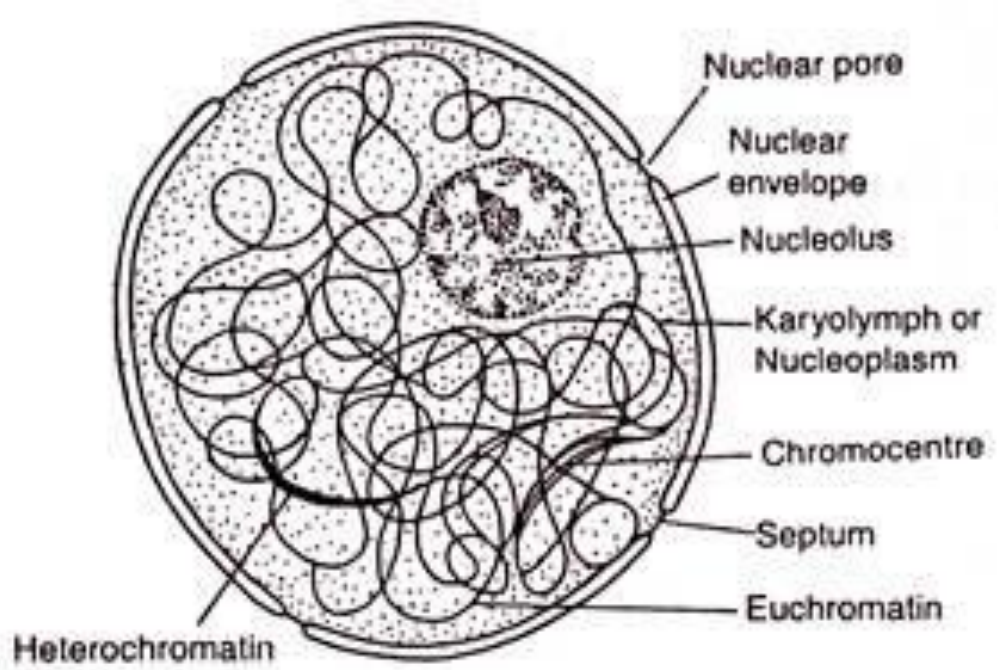


Fig. 8.1 Structure of a nucleus.

Figure 5 Structure of a nucleus

centromere on the sides of which disc shaped structures called kinetochores are present (Figure 5). Centromere holds two chromatids of a chromosome.

Examples of Eukaryotic Cells

Plant Cells

Plant cells are unique among eukaryotic cells for several reasons. They have reinforced, relatively thick cell walls that are made mostly of cellulose and help maintain structural support in the plant. Each plant cell has a large vacuole in the center that allows it to maintain turgor pressure, which is pressure from having a lot of water in the cell and helps keep the plant upright. Plant cells also contain organelles called chloroplasts which contain the molecule chlorophyll. This important molecule is used in the process of photosynthesis, which is when a plant makes its own energy from sunlight, carbon dioxide, and water.

Fungal Cells

Like plant cells, fungal cells also have a cell wall, but their cell wall is made of chitin (the same substance found in insect exoskeletons). Some fungi have septa, which are holes that allow organelles and cytoplasm to pass between them. This makes the boundaries between different cells less clear.

Animal Cells

Animal cells do not have cell walls. Instead, they have only a plasma membrane. The lack of a cell wall allows animal cells to form many different shapes, and allows for the processes of phagocytosis "cell eating" and pinocytosis "cell drinking" to occur. Animal cells differ from plant cells in that they do not have chloroplasts and have smaller vacuoles instead of a large central vacuole.

Protozoa

Protozoa are eukaryotic organisms that consist of a single cell. They can move around and eat, and they digest food in vacuoles. Some protozoa have many cilia, which are small "arms" that allow them to move around. Some also have a thin layer called a pellicle, which provides support to the cell membrane.

Biomolecules

There is a wide diversity in living organisms in our biosphere. Now a question that arises in our minds is: Are all living organisms made of the same chemicals, i.e., elements and compounds? You have learnt in chemistry how elemental analysis is performed. If we perform such an analysis on a plant tissue, animal tissue or a microbial paste, we obtain a list of elements like carbon, hydrogen, oxygen and several

others and their respective content per unit mass of a living tissue. If the same analysis is performed on a piece of earth's crust as an example of non-living matter, we obtain a similar list. What are the differences between the two lists? In absolute terms, no such differences could be made out. All the elements present in a sample of earth's crust are also present in a sample of living tissue. However, a closer examination reveals that the relative abundance of carbon and hydrogen with respect to other elements is higher in any living organism than in earth's crust (Table 1).

TABLE 1 A Comparison of Elements Present in Non-living and Living Matter*

Element	**% Weight of**	
	Earth's crust	**Human body**
Hydrogen (H)	0.14	0.5
Carbon (C)	0.03	18.5
Carbon (C)	46.6	65.0
Nitrogen (N)	very little	3.3
Sulphur (S)	0.03	0.3
Sodium (Na)	2.8	0.2
Calcium (Ca)	3.6	1.5
Magnesium (Mg)	2.1	0.1
Silicon (Si)	27.7	negligible

* Adapted from CNR Rao, *Understanding Chemistry*, Universities Press, Hyderabad.

Analysis of biomolecules

We can continue asking in the same way, what type of organic compounds are found in living organisms? How does one go about finding the answer? To get an answer, one has to perform a chemical analysis. We can take any living tissue (a vegetable or a piece of liver, etc.) and grind it in trichloroacetic acid (Cl3CCOOH) using a mortar and a pestle. We obtain a thick slurry. If we were to strain this through a cheesecloth or cotton we would obtain two fractions. One is called the filtrate or more technically, the acid-soluble pool, and the second, the retentate or the acid-insoluble fraction. Scientists have found thousands of organic compounds in the acid-soluble pool. There is one feature common to all those compounds found in the acid soluble pool. They have molecular weights ranging from 18 to around 800 daltons (Da) approximately.

The acid insoluble fraction, has only four types of organic compounds i.e., proteins, nucleic acids, polysaccharides and lipids. These classes of compounds with the exception of lipids, have molecular weights in the range of ten thousand daltons and

above. For this very reason, biomolecules, i.e., chemical compounds found in living organisms are of two types. One, those which have molecular weights less than one thousand dalton and are usually referred to as biomolecules while those which are found in the acid insoluble fraction are called biomacromolecules.

Proteins

Proteins are polypeptides. They are linear chains of amino acids linked by peptide bonds as shown in Figure. Each protein is a polymer of amino acids. A homopolymer has only one type of monomer repeating 'n' number of times. Amino acids can be essential or non-essential. The latter are those which our body can make, while we get essential amino acids through our diet/food. Proteins carry out many functions in living organisms, some transport nutrients across cell membrane, some fight infectious organisms, some are hormones, some are enzymes, etc. (Table). Collagen is the most abundant protein in animal world and Ribulose bisphosphate Carboxylase-Oxygenase (RuBisCO) is the most abundant protein in the whole of the biosphere.
The size of the proteins also varies greatly. It actually depends on the number of polypeptide molecules it contains. One of the smallest protein molecules is insulin, and the largest being Titin which consist of 34,350 amino acids. Let us now look at the four types of protein structure that make up a protein molecule.

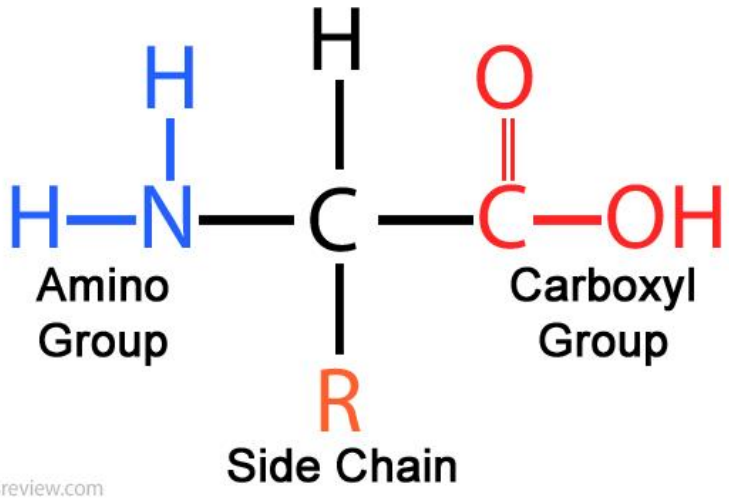


Figure 6 Amino acid Structure

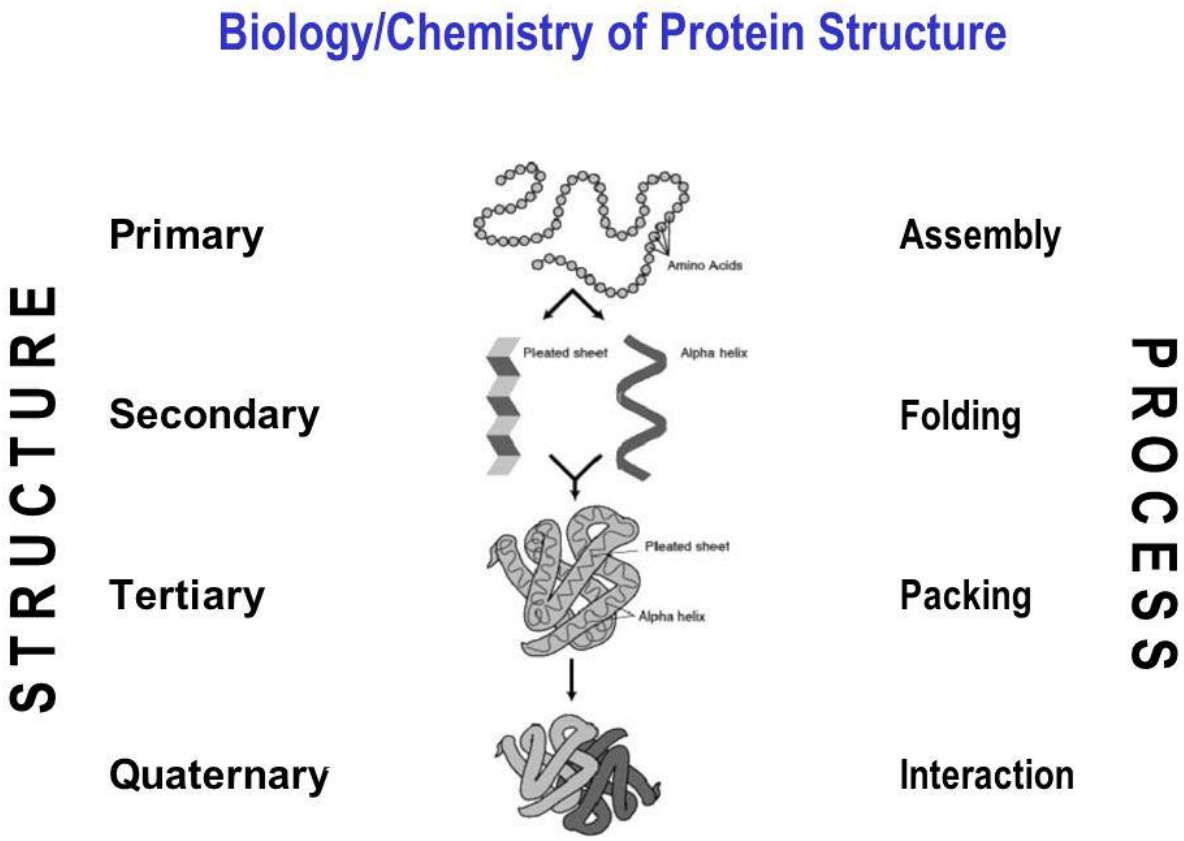


Figure 7 Biology of Protein structure

1] Primary Protein Structure

The primary structure is the unique formation and order in which the amino acids (the building blocks) combine and link to give us a protein molecule . Protein gets all its properties from its primary structure. There are in all twenty amino acids in the human body. All of these have a carboxyl group and an amino group. But each has a different variable group known as the "R" group. It is this R group that lends a particular protein its unique structure (Figure-6). Every protein is determined by the sequencing of the amino acids. The formation and ordering of these amino acids in proteins are extremely specific. If we alter even one amino acid in the chain it results in a non-functioning protein or what we call a gene mutation.

2] Secondary Protein Structure

After the sequencing of amino acids, we now move on to the secondary structure. This is when the peptide backbone of the protein structure will fold onto itself, to give proteins their unique shape. This folding of the polypeptide chains happens due to the interaction between the carboxyl groups along with the amine groups of the peptide chains (Figure-7).

There are two kinds of shapes formed in the secondary structure. These are

- α-helix: The backbone follows a helical structure. The hydrogen bonds with the oxygen between the different layers of the helix, giving it this helical structure.
- β-pleated sheet: here the polypeptide chains are stacked next to each other and their outer hydrogen molecules form intramolecular bonds to give it this sheet-like structure

3] Tertiary Structures

This is the structure that gives protein the 3-D shape and formation. After the amino acids form bonds (secondary structure) and shapes like helices and sheets, the structure can coil or fold at random. This is what we call the tertiary structure of proteins. If this structure is disrupted or disturbed a protein is said to be denatured which means it is chemically affected and its structure is distorted.

4] Quaternary Structure

Finally, we come to the fourth structure. The spatial arrangement of two or more peptide chains leads to this structure. It is important to note it is not necessary for proteins to have quaternary structures. Primary, secondary and tertiary structures are present in all natural proteins, but the same is not true for quaternary structure. Hence if a protein has only the first three structures it is considered to be a protein.

Carbohydrates

What are the three basic necessities of any human being? Food, shelter and clothing? And did you know that carbohydrates are the source of all three? It will be safe to say that without carbohydrates life as we known it would not exist on planet earth. Now let us learn a bit more about the chemical properties of carbohydrates.

Importance of Carbohydrates

Now as we previously discussed, carbohydrates are absolutely essential for life on the planet. Let us take a more detailed look at the importance of carbohydrates.

Carbohydrates are responsible for storing chemical energy in living organisms. You must hear all the time when athletes carbo-load before a game. This is so they can provide themselves with extra energy. They are also an important constituent for supporting tissues in plants and even in some animals.

As I am sure you are already aware of photosynthesis. It is the process by which plants utilize solar energy to generate energy for themselves and food for us. Through this process, plants fix CO2 and synthesize carbohydrate. Let us take a look at the chemical reaction occurring during photosynthesis.

$x(CO2) + y(H2O) + \text{Solar energy} \Rightarrow Cx\ (H2O)y + O2$

So carbohydrates due to photosynthesis are the repository of solar energy in plants, Then when plants or animals metabolize the said carbohydrate this energy releases. The metabolizing equation is just the reverse of the photosynthesis equation

$Cx\ (H2O)y + O2 \Rightarrow x(CO2) + y(H2O) + \text{Energy}$

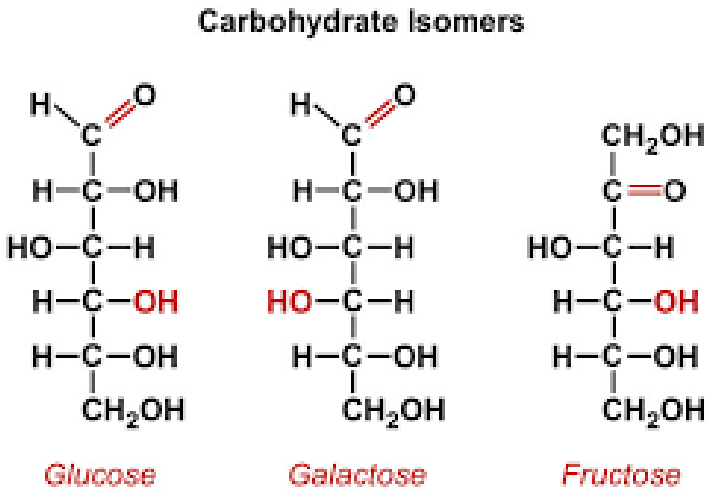


Figure 8 Basic structure of Carbohydrate

Classification of Carbohydrates

The main classification of carbohydrate is done on the basis of hydrolysis. This classification is as follow:

1. *Monosaccharides*: These are the simplest form of carbohydrate that cannot be hydrolyzed any further. They have the general formula of $(CH_2O)_n$. Some common examples are glucose, Ribose etc (Figure-8).

2. *Disaccharides*: A further classification of oligosaccharides, these give two units of the same or different monosaccharides on hydrolysis. For example, sucrose on hydrolysis gives one molecule of glucose and fructose each. Whereas maltose on hydrolysis gives two molecules of only glucose,

3. *Trisaccharides*: Carbohydrates that on hydrolysis gives three molecules of monosaccharides, whether same or different. An example is Raffinose.

4. *Tetrasaccharides*: And as the name suggests this carbohydrate on hydrolysis give four molecules of monosaccharides. Stachyose is an example.

5. *Polysaccharides*: The final category of carbohydrates. These give a large number of monosaccharides when they undergo hydrolysis, These carbohydrates are not sweet in taste and are also known as non-sugars. Some common examples are starch, glycogen etc.

Polysaccharides are classified into two parts, namely

i. *Homopolysaccharide*: These molecules are made up of only one type of monosaccharides. A homopolysaccharide made up of only glucose molecules is named Glucans. One with only galactose molecules earns the name Galactus. In this given topic we will be focussing only on Glucans.

ii. *Heteropolysaccharide*: These are polysaccharide molecules consisting of more than one type of monosaccharides.

Now let us focus on the three main polysaccharides commonly found in nature. They are the ones that we see every day in our day to day lives.

Starch

Starch is an element present in all photosynthetic plants. We generally find starch in the plant's roots and seeds. All plants when they synthesize glucose, the extra glucose is stored in the form of starch. Starch is a glucan, meaning it only consists of glucose molecules all linked together.

The general molecular formula for starch is $(C_6H_{10}O_5)n$. The 'n' denotes the number of molecules linked together. We find starch in the seeds of plants as granules. On heating these granules in water we form a *colloidal suspension.*. We obtain two components from this process. These two components are Amylose and Amylopectin.

Amylose- Amylose themselves are also polysaccharides constitute about 10-20 % of a starch molecule. They are made up of D-glucose units that connect with each other with the help of a α-glycosidic linkage.

Amylopectin-They have the same basic structure that Amylose does which is D-glucose units combining in a {α (1-40} form and constituent about 80-90% of a starch molecule

.

Glycogen

Glycogen is also a Glucon i.e. it is made up exclusively of D-glucose units. It is a reserved carbohydrate source for animals as well as plants. Let us now see the structure and the functions of Glycogen.

Structure

The structure of glycogen is similar to that of Amylopectin. The only exception being that glycogen is very highly branched. In a glycogen molecule, the branching happens more frequently, almost after every six glucose units. This is the reason glycogen behaves differently to Amylopectin. This is the reason a glycogen molecule has a very high molecular weight. It is not compact in size either, it is a big molecule. A hydrolysis experiment will suggest that in a glycogen molecule, one end group occurs after every ten to twelve units of glucose.

Functions

Glycogen performs some very important functions in plants and animals, It is able to perform these functions due to its unique structure and formation.

- Now as you are aware, glucose is found in the cell membranes of cells of plants and animals. These glucose molecules are very small and compact. They can easily diffuse out of a cell membrane. But glycogen is a big and complex molecule, so it will not diffuse out of the cell membrane. Hence it is an important function of glycogen, the storage of glucose within cells.

- If a large number of glucose cells are present inside the cells, the osmotic pressure in the cell will be very high. This can cause the cell membrane to burst. But if glucose combines into one big molecule of glycogen, the problem does not occur.

- As mentioned earlier glycogen is a glucose reserve for the cells of our body. If the glucose concentration is low, enzymes present in the cells can easily hydrolyze the end groups of glycogen to make glucose. This detachment process is made easy due to the structure of glycogen. The reverse of the above is true as well. If the glucose concentration is high, enzymes can attach glucose molecules to form glycogen.

Cellulose

Cellulose is an important structural element of the cell walls of all photosynthetic plants. It is a fibrous kind of polysaccharide which is highly insoluble in water. Here again, Cellulose is a glucan. The D-glucose units connect in (1→4) fashion.

The connection though is different from starch and glycogen, it is a beta linkage. So the linkage is β-glucosidic linkage. The structure is not helical since the beta linkage confines the polysaccharide to a straight-chain form.

In the structure of cellulose -OH groups point outside the chain structure. Whenever two chains come close to each other they tend to form a stack on each other due to hydrogen bonding between these hydroxyl groups. As a result, we get a fibrous insoluble structure which is suitable for the functions of cellulose in the cell walls.

Nucleic acid

The other type of macromolecule that one would find in the acid insoluble fraction of any living tissue is the nucleic acid. These are polynucleotides. Together with polysaccharides and polypeptides these comprise the true macromolecular fraction of any living tissue or cell. For nucleic acids, the building block is a nucleotide. A nucleotide has three chemically distinct components. One is a heterocyclic compound,

the second is a monosaccharide and the third a phosphoric acid or phosphate. As you notice in Figure 9, the heterocyclic compounds in nucleic acids are the nitrogenous bases named adenine, guanine, uracil, cytosine, and thymine. Adenine and Guanine are substituted purines while the rest are substituted pyrimidines. The skeletal heterocyclic ringis called as purine and pyrimidine respectively. The sugar found in polynucleotides is either ribose (a monosaccharide pentose) or 2'deoxyribose. A nucleic acid containing deoxyribose is called deoxyribonucleic acid (DNA) while that which contains ribose is called ribonucleic acid (RNA).

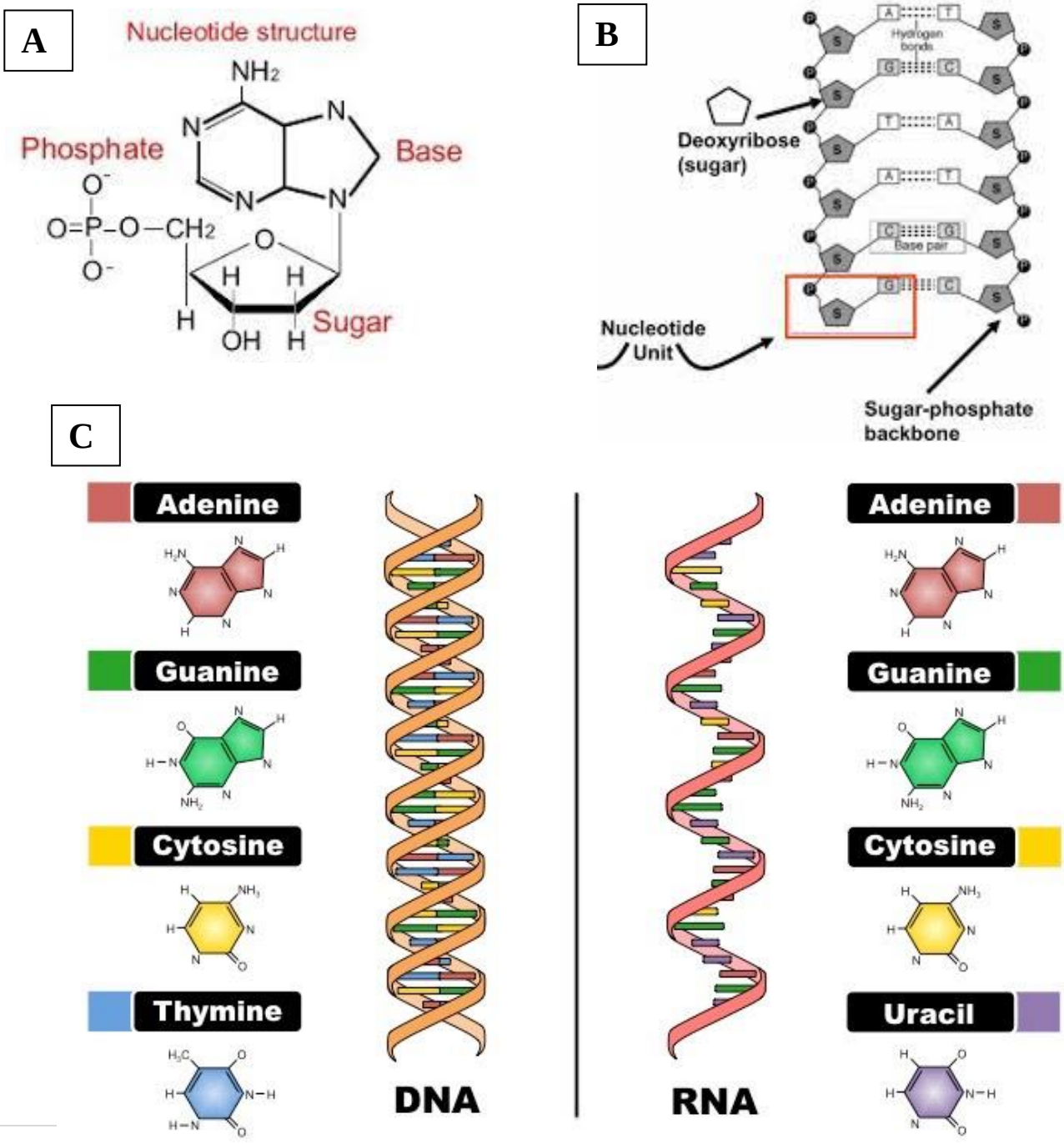


Figure 9 A. Structure of Nucleotide, B. DNA double Helix, C. Nucleobases of DNA

In a polypeptide or a protein, amino acids are linked by a peptide bond which is formed when the carboxyl (-COOH) group of one amino acid reacts with the amino (-NH2) group of the next amino acid with the elimination of a water moiety (the process is called dehydration). In a polysaccharide the individual monosaccharides are linked by a glycosidic bond. This bond is also formed by dehydration. This bond is formed between two carbon atoms of two adjacent monosaccharides. In a nucleic acid a phosphate moiety links the 3'-carbon of one sugar of one nucleotide to the 5'-carbon of the sugar of the succeeding nucleotide. The bond between the phosphate and hydroxyl group of sugar is an ester bond. As there is one such ester bond on either side, it is called phosphodiester bond (Figure 9)

Enzymes

What we have learnt till now is that living organisms, be it a simple bacterial cell, a protozoan, a plant or an animal, contain thousands of organic compounds. These compounds or biomolecules are present in certain concentrations (expressed as mols/cell or mols/litre etc.). One of the greatest discoveries ever made was the observation that all these biomolecules have a turn over. This means that they are constantly being changed into some other biomolecules and also made from some other biomolecules.

An enzyme like any protein has Primary, secondary and the tertiary structure. When you look at a tertiary structure (Figure 10) you will notice that the backbone of the protein chain folds upon itself, the chain criss-crosses itself and hence, many crevices or pockets are made. One such pocket is the 'active site'. Thus enzymes, through their active site, catalyse reactions at a high rate. Enzyme catalysts differ from inorganic catalysts in many ways, but one major difference needs mention. Inorganic catalysts work efficiently at high temperatures and high pressures, while enzymes get damaged at high temperatures (say above 40°C). However, enzymes isolated from organisms who normally live under extremely high temperatures (e.g., hot vents and sulphur springs), are stable and retain their catalytic power even at high temperatures (upto 80°-90°C). Thermal stability is thus an important quality of such enzymes isolated from thermophilic organisms.

Chemical Reactions

How do we understand these enzymes? Let us first understand a chemical reaction. Chemical compounds undergo two types of changes. A physical change simply refers

to a change in shape without breaking of bonds. This is a physical process. Another physical process is a change in state of matter: when ice melts into water, or when water becomes a vapour. These are also physical processes. However, when bonds are broken and new bonds are formed during transformation, this will be called a chemical reaction. For example:

$$Ba(OH)_2 + H_2SO_4 \longrightarrow BaSO_4 + 2H_2O$$

is an inorganic chemical reaction. Similarly, hydrolysis of starch into glucose is an organic chemical reaction. Rate of a physical or chemical process refers to the amount of product formed per unit time. It can be expressed as:

$$\text{rate} = dP/dt$$

Rate can also be called velocity if the direction is specified. Rates of physical and chemical processes are influenced by temperature among other factors. A general rule of thumb is that rate doubles or decreases by half for every 10°C change in either direction. Catalysed reactions proceed at rates vastly higher than that of un-catalyzed ones. When enzyme catalysed reactions are observed, the rate would be vastly higher than the same but un-catalyzed reaction. For example

CO2	+	H2O	⟷		H2CO3
carbon dioxide		water		**Carbonic anhydrase**	carbonic acid

In the absence of any enzyme this reaction is very slow, with about 200 molecules of H2CO3 being formed in an hour. However, by using the enzyme present within the cytoplasm called carbonic anhydrase, the reaction speeds dramatically with about 600,000 molecules being formed every second. The enzyme has accelerated the reaction rate by about 10 million times. The power of enzymes is incredible indeed!
There are thousands of types of enzymes each catalysing a unique chemical or metabolic reaction. A multistep chemical reaction, when each of the steps is catalysed by the same enzyme complex or different enzymes, is called a metabolic pathway. For example,

Glucose	⟶	Pyruvic acid
$C_6H_{12}O_6 + O_2$		$2C_3H_4O_3 + 2H_2O$

This is actually a metabolic pathway in which glucose becomes pyruvic acid through ten different enzyme catalysed metabolic reactions.

Nature of Enzyme Action

Each enzyme (E) has a substrate (S) binding site in its molecule so that a highly reactive enzyme-substrate complex (ES) is produced. This complex is short-lived and dissociates into its product(s) P and the unchanged enzyme with an intermediate formation of the enzyme-product complex (EP). The formation of the ES complex is essential for catalysis.

$$E + S \leftrightarrow ES \longrightarrow EP \longrightarrow E + P$$

The catalytic cycle of an enzyme action can be described in the following steps:

1. First, the substrate binds to the active site of the enzyme, fitting into the active site.
2. The binding of the substrate induces the enzyme to alter its shape, fitting more tightly around the substrate.
3. The active site of the enzyme, now in close proximity of the substrate breaks the chemical bonds of the substrate and the new enzyme- product complex is formed.
4. The enzyme releases the products of the reaction and the free enzyme is ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

Factors Affecting Enzyme Activity

The activity of an enzyme can be affected by a change in the conditions which can alter the tertiary structure of the protein. These include temperature, pH, change in substrate concentration or binding of specific chemicals that regulate its activity. Enzymes generally function in a narrow range of temperature and pH.

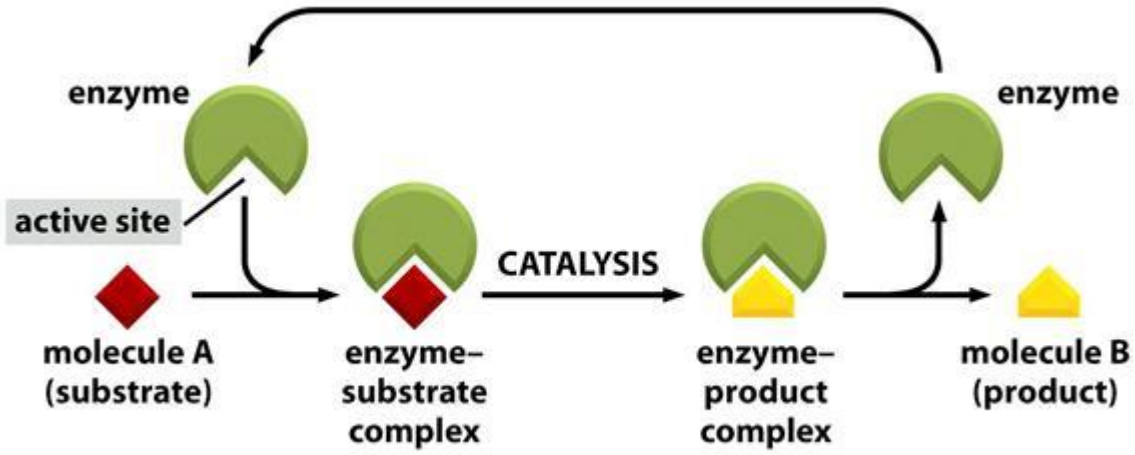


Figure 10 How enzymes work

Each enzyme shows its highest activity at a particular temperature and pH called the optimum temperature and optimum pH. Activity declines both below and above the optimum value. Low temperature preserves the enzyme in a temporarily inactive state whereas high temperature destroys enzymatic activity because proteins are denatured by heat. Concentration of Substrate with the increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately reaches a maximum velocity (Vmax) which is not exceeded by any further rise in concentration of the substrate. This is because the enzyme molecules are fewer than the substrate molecules and after saturation of these molecules, there are no free enzyme molecules to bind with the additional substrate molecules.

Enzyme Inhabitation

The activity of an enzyme is also sensitive to the presence of specific chemicals that bind to the enzyme. When the binding of the chemical shuts off enzyme activity, the process is called inhibition and the chemical is called an inhibitor. When the inhibitor closely resembles the substrate in its molecular structure and inhibits the activity of the enzyme, it is known as competitive inhibitor. Due to its close structural similarity with the substrate, the inhibitor competes with the substrate for the substrate binding site of the enzyme. Consequently, the substrate cannot bind and as a result, the enzyme action declines, e.g., inhibition of succinic dehydrogenase by malonate which closely resembles the substrate succinate in structure. Such competitive inhibitors are often used in the control of bacterial pathogens.

Types of Inhibition

Reversible and irreversible inhibitors are chemicals which bind to an enzyme to suppress its activity. One method to accomplish this is to almost permanently bind to an enzyme. These types of inhibitors are called irreversible. However, other chemicals can transiently bind to an enzyme. These are called **reversible.** Reversible inhibitors either bind to an active site (competitive inhibitors), or to another site on the enzyme (non-competitive inhibitors)(Figure 11)..

Competitive Inhibitors

Competitive inhibitors compete with the substrate at the active site, and therefore increase Km (the Michaelis-Menten constant). However, Vmax is unchanged because, with enough substrate concentration, the reaction can still complete. The graph plot of enzyme activity against substrate concentration would be shifted to the right due to the increase of the Km, whilst the Lineweaver-Burke plot would be steeper when compared with no inhibitor.

Non-Competitive Inhibitors

Non-competitive inhibitors bind to another location on the enzyme and as such decrease V^{max}. However, KM is unchanged. This is demonstrated by a lower maximum on a graph plotting enzyme activity against substrate concentration and a higher y-intercept on a Lineweaver-Burke plot when compared with no inhibitor.

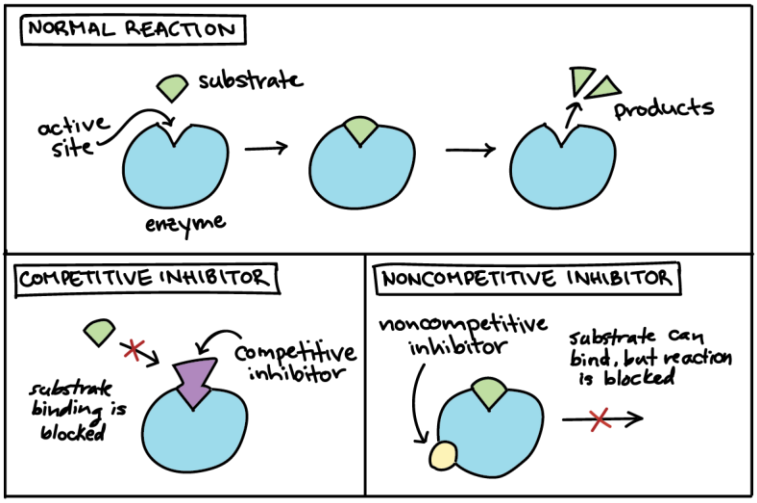


Figure 11 Types of Inhibitors

Allosteric Inhibition

Allosteric enzymes display a **sigmoidal** curve in contrast to the hyperbolic curve displayed by Michaelis-Menten Enzymes. This is because most allosteric enzymes contain multiple sub-units which can affect each other when the substrate binds to the enzyme. Inhibition can affect either K0.5, which is the substrate concentration for half-saturation, Vmax or both. This results in a shift of the curve to the right, and in the case of reducing Vmax, shifts the curve down.

Allosteric enzymes have two states: a low affinity state dubbed the "T" state and the **high affinity** "R" state. Inhibitors work by preferentially binding to the T state of an allosteric enzyme, causing the enzyme to maintain this low affinity state.

This is extremely useful to limit the amount of an enzyme's product, as the product can then go on to inhibit the same type of enzyme to ensure the amount of product is not excessive. This is known as **feedback inhibition.** For example, ATP allosterically inhibits pyruvate kinase to prevent increased formation of pyruvate, so less ATP is eventually formed. Additionally, phosphofructokinase is allosterically inhibited by citrate, an intermediate of the Kreb's cycle. This means that glycolysis will be limited when there is high ATP generation from the Kreb's cycle.

Enzyme Kinetics

Kinetics are concerned with the rate of Reactions. Ths is very important for the living organism which maintain it steady state by adjusting steady rates in response to the environment and to hormonal controls. The study of the rate at which an enzyme act is called enzyme kinetics.

Classification and Nomenclature of Enzymes

Thousands of enzymes have been discovered, isolated and studied. Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. Enzymes are divided into 6 classes each with 4-13 subclasses and named accordingly by a four-digit number.

Oxidoreductases/dehydrogenases: Enzymes which catalyse oxidoreduction between two substrates S and S′ e.g.,

$$S \text{ reduced} + S' \text{ oxidised} \longrightarrow S \text{ oxidised} + S' \text{ reduced}$$

Transferases: Enzymes catalysing a transfer of a group, G (other than hydrogen) between a pair of substrate S and S′ e.g.,

$$S - G + S' \longrightarrow S + S' - G$$

Hydrolases: Enzymes catalysing hydrolysis of ester, ether, peptide, glycosidic, C-C, C-halide or P-N bonds.

Lyases: Enzymes that catalyse removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

$$\begin{array}{c} X\ \ Y \\ |\ \ | \\ C\text{-}C \end{array} \longrightarrow X\text{-}Y + C{=}C$$

Isomerases: Includes all enzymes catalysing inter-conversion of optical, geometric or positional isomers.

Ligases: Enzymes catalysing the linking together of 2 compounds, e.g., enzymes which catalyse joining of C-O, C-S, C-N, P-O etc. bonds.

Co-factors

Enzymes are composed of one or several polypeptide chains. However, there are a number of cases in which non-protein constituents called cofactors are bound to the the enzyme to make the enzyme catalytically active. In these instances, the protein portion of the enzymes is called the apoenzyme. Three kinds of cofactors may be

identified: prosthetic groups, co-enzymes and metal ions. Prosthetic groups are organic compounds and are distinguished from other cofactors in that they are tightly bound to the apoenzyme. For example, in peroxidase and catalase, which catalyze the breakdown of hydrogen peroxide to water and oxygen, haem is the prosthetic group and it is a part of the active site of the enzyme. Co-enzymes are also organic compounds but their association with the apoenzyme is only transient, usually occurring during the course of catalysis. Furthermore, co-enzymes serve as co-factors in a number of

different enzyme catalyzed reactions. The essential chemical components of many coenzymes are vitamins, e.g., coenzyme nicotinamide adenine dinucleotide (NAD) and NADP contain the vitamin niacin. A number of enzymes require metal ions for their activity which form coordination bonds with side chains at the active site and at the same time form one or more cordination bonds with the substrate, e.g., zinc is a cofactor for the proteolytic enzyme carboxypeptidase. Catalytic activity is lost when the co-factor is removed from the enzyme which testifies that they play a crucial role in the catalytic activity of the enzyme.

Cell Cycle

The cell cycle is an ordered series of events involving cell growth and cell division that produces two new daughter cells. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and division that produces two identical (clone) cells. The cell cycle has two major phases: interphase and the mitotic phase (Figure 1). During interphase, the cell grows and DNA is replicated. During the mitotic phase, the replicated DNA and cytoplasmic contents are separated, and the cell divides.

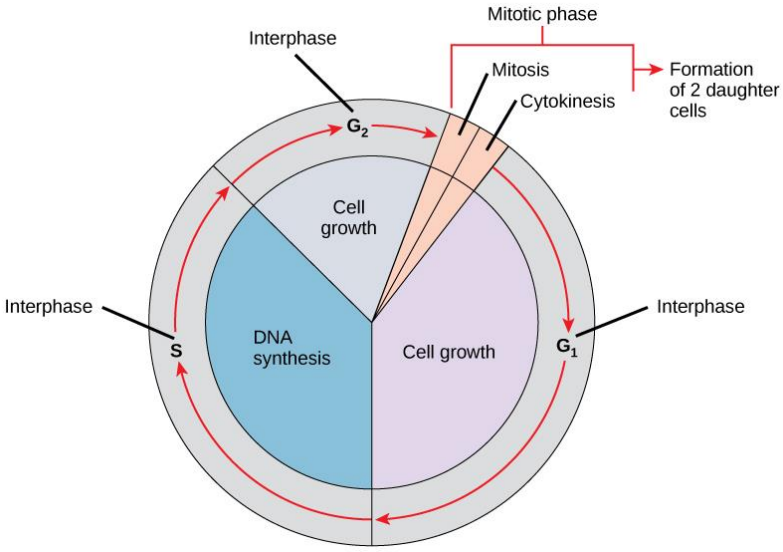


Figure 13 The cell cycle consists of interphase and the mitotic phase.

The cell cycle is divided into two basic phases:

- Interphase
- M Phase (Mitosis phase)

Interphase

During interphase, the cell undergoes normal growth processes while also preparing for cell division. In order for a cell to move from interphase into the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G_1, S, and G_2.

i) G_1 Phase (First Gap)

The first stage of interphase is called the G_1 phase (first gap) because, from a microscopic aspect, little change is visible. However, during the G_1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins as well as accumulating sufficient energy reserves to complete the task of replicating each chromosome in the nucleus.

ii) S Phase (Synthesis of DNA)

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the S phase, DNA replication can proceed through the mechanisms that result in the formation of identical pairs of DNA molecules—sister chromatids—that are firmly attached to the centromeric region. The centrosome is duplicated during the S phase. The two centrosomes will give rise to the mitotic spindle, the apparatus that arrange the movement of chromosomes during mitosis. At the center of each animal cell, the centrosomes of animal cells are associated with a pair of rod-like objects, the centrioles, which are at right angles to each other. Centrioles help organize cell division. Centrioles are not present in the centrosomes of other eukaryotic species, such as plants and most fungi.

iii) G_2 Phase (Second Gap)

In the G_2 phase, the cell replenishes its energy stores and synthesizes proteins necessary for chromosome manipulation. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic phase. There may be additional cell growth during G_2. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis.

The Mitotic Phase

The mitotic phase is multistep processes during which the duplicated chromosomes are aligned, separated, and moves into two new, identical daughter cells. The first portion of the mitotic phase is called karyokinesis, or nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into the two daughter cells.

Karyokinesis (Mitosis)

Karyokinesis, also known as mitosis, is divided into a series of phases—prophase, metaphase, anaphase, and telophase—that result in the division of the cell nucleus (Figure 13). Karyokinesis is also called mitosis

Prophase

Characteristic events of Prophase are:

- Chromosomal material condenses to form compact mitotic chromosomes. Chromosomes are seen to be composed of two chromatin attached together at the centromere.
- Centrosome which had undergone duplication during interphase begins to move towards opposite poles of the cell.

Each centrosome radiates out microtubules called asters. The two asters together with spindle fibres form mitotic apparatus. Cells at the end of prophase, when viewed under the microscope, do not show golgi complexes, endoplasmic reticulum, nucleolus and the nuclear envelope.

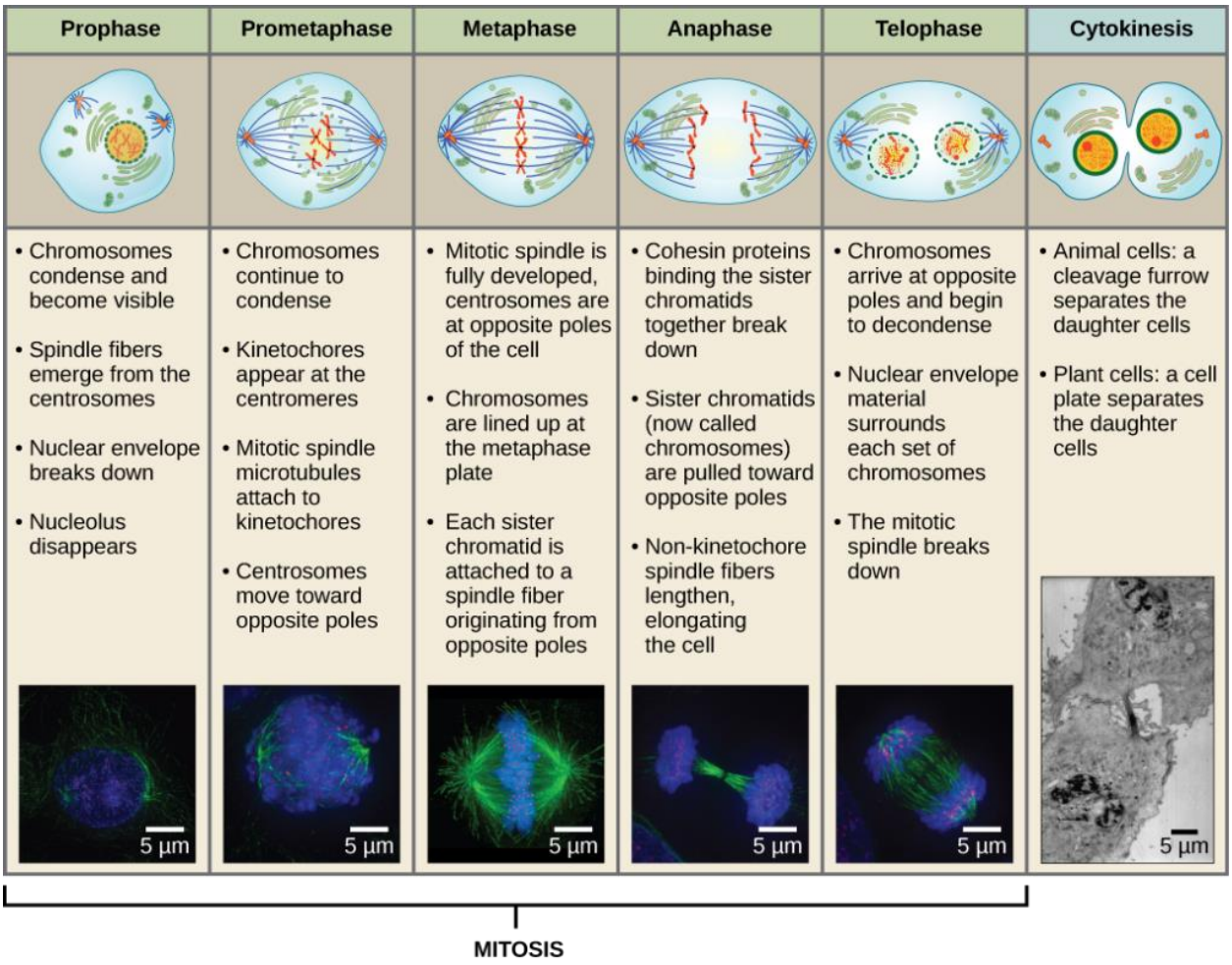


Figure 14- Karyokinesis (or mitosis) is divided into five stages—prophase, prometaphase, metaphase, anaphase, and telophase. (Source: courses.lumenlearning.com)

Metaphase

The complete disintegration of the nuclear envelope marks the start of the second phase of mitosis; hence the chromosomes are spread through the cytoplasm of the cell. By this stage, condensation of chromosomes is completed and they can be observed clearly under the microscope. This then, is the stage at which morphology of chromosomes is most easily studied. At this stage, metaphase chromosome is made up of two sister chromatids, which are held together by the centromere.

The key features of metaphase are:

- Spindle fibres attach to kinetochores of chromosomes.
- Chromosomes are moved to spindle equator and get aligned along metaphase plate through spindle fibres to both poles.

Anaphase

At the onset of anaphase, each chromosome arranged at the metaphase plate is split imultaneously and the two daughter chromatids, now referred to as daughter chromosomes of the future daughter nuclei, begin their migration towards the two opposite poles. As each chromosome moves away from the equatorial plate, the centromere of each chromosome remains directed towards the pole and hence at the leading edge, with the arms of the chromosome trailing behind (Figure 14).

Thus, anaphase stage is characterised by the following key events:

- Centromeres split and chromatids separate.
- Chromatids move to opposite poles.

Cytokinesis

Mitosis accomplishes not only the segregation of duplicated chromosomes into daughter nuclei (karyokinesis), but the cell itself is divided into two daughter cells by the separation of cytoplasm called cytokinesis at the end of which cell division gets completed (Figure 14). In an animal cell, this is achieved by the appearance of a furrow in the plasma membrane. The furrow gradually deepens and ultimately joins in the centre dividing the cell cytoplasm into two. Plant cells however, are enclosed by a relatively inextensible cell wall, therefore they undergo cytokinesis by a different mechanism. Plant cells have walls, so cytokinesis cannot proceed with a cleavage furrow. Instead, during telophase a cell plate forms across the cell in the location of the old metaphase plate.

Significance of Mitosis

Mitosis or the equational division is usually restricted to the diploid cells only. However, in some lower plants and in some social insects haploid cells also divide by mitosis. It is very essential to understand the significance of this division in the life of an organism. Are you aware of some examples where you have studied about haploid and diploid insects? Mitosis usually results in the production of diploid daughter cells with identical genetic complement. The growth of multicellular organisms is due to mitosis. Cell growth results in disturbing the ratio between the nucleus and the cytoplasm. It therefore becomes essential for the cell to divide to restore the nucleo-cytoplasmic ratio. A very significant contribution of mitosis is cell repair. The cells of the upper layer of the epidermis, cells of the lining of the gut, and blood cells are being constantly replaced. Mitotic divisions in the meristematic tissues – the apical and the lateral cambium, result in a continuous growth of plants throughout their life.

Meiosis

The production of offspring by sexual reproduction includes the fusion of two gametes, each with a complete haploid set of chromosomes. Gametes are formed from specialised diploid cells. This specialised kind of cell division that reduces the chromosome number by half results in the
production of haploid daughter cells. This kind of division is called meiosis. Meiosis ensures the production of haploid phase in the life cycle of sexually reproducing organisms whereas fertilisation restores the diploid phase. We come across meiosis during gametogenesis in plants and animals. This leads to the formation of haploid gametes. The key features of meiosis are as follows:

- Meiosis involves two sequential cycles of nuclear and cell division called meiosis I and meiosis II but only a single cycle of DNA replication.
- Meiosis I is initiated after the parental chromosomes have replicated to produce identical sister chromatids at the S phase.
- Meiosis involves pairing of homologous chromosomes and recombination between non-sister chromatids of homologous chromosomes.
- Four haploid cells are formed at the end of meiosis II.

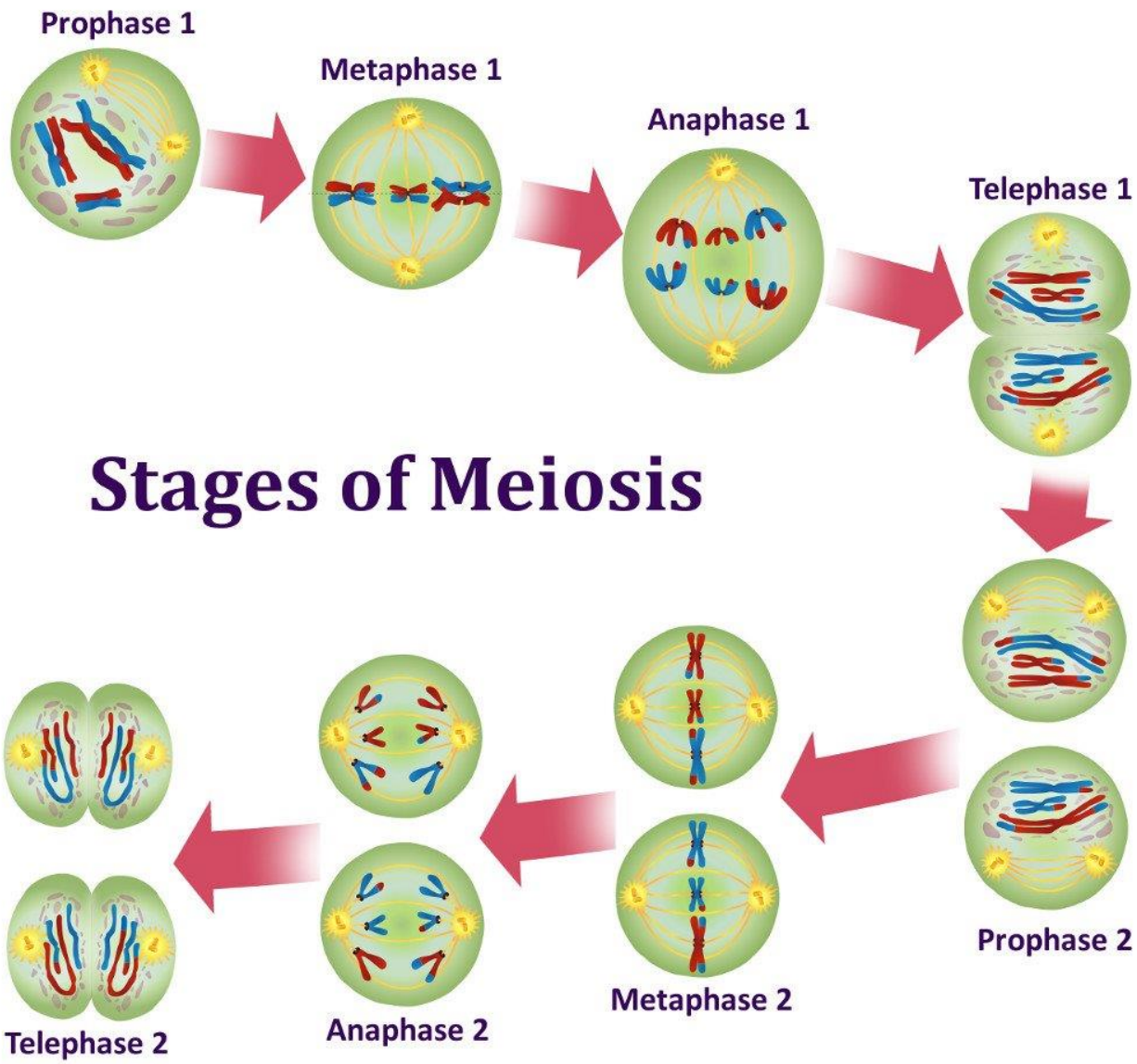


Figure 15- Stages of Meiosis (Photo Credit: Ali Zifan/ Wikimedia commons)

Meiosis I

Prophase I: Prophase of the first meiotic division is typically longer and more complex when compared to prophase of mitosis. It has been further subdivided into the following five phases based on chromosomal behaviour, i.e., Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.

leptotene stage- leptotene stage the chromosomes become gradually visible under the light microscope. The compaction of chromosomes continues throughout leptotene.

zygotene stage- leptotene stage followed by the second stage of prophase I called zygotene. During this stage chromosomes start pairing together and this process of association is called synapsis. Such paired chromosomes are called homologous chromosomes. Electron micrographs of this stage indicate that chromosome synapsis is accompanied by the formation of complex structure called synaptonemal complex. The complex formed by a pair of synapsed homologous chromosomes is called a bivalent or a tetrad. However, these are more clearly visible at the next stage.

Pachytene stage- During this stage, the four chromatids of each bivalentchromosomes becomes distinct and clearly appears as tetrads. This stage is characterised by the appearance of recombination nodules. Crossing over is the exchange of genetic material between two homologous chromosomes. Crossing over is also an enzyme-mediated process and the enzyme involved is called recombinase. Crossing over leads to recombination of genetic material on the two chromosomes. Recombination between homologous chromosomes is completed by the end of pachytene, leaving the chromosomes linked at the sites of crossing over.

Diplotene stage- The beginning of diplotene is recognised by by the dissolution of the synaptonemal complex and the tendency of the recombined homologous chromosomes of the bivalents to separate from each other except at the sites of crossovers. These X-shaped structures, are called chiasmata. In oocytes of some vertebrates, diplotene can last for months or years.

Diakinesis Stage- The final stage of meiotic prophase I is diakinesis. This is marked by terminalisation of chiasmata. During this phase the chromosomes are fully condensed and the meiotic spindle is assembled to prepare the homologous chromosomes for separation By the end of diakinesis, the nucleolus disappears and the nuclear envelope also breaks down. Diakinesis represents transition to metaphase.

Metaphase I: The bivalent chromosomes align on the equatorial plate (Figure 15). The microtubules from the opposite poles of the spindle attach to the kinetochore of homologous chromosomes.

Anaphase I: The homologous chromosomes separate, while sister chromatids remain associated at their centromeres (Figure 15). Telophase I: The nuclear membrane and nucleolus reappear, cytokinesis follows and this is called as dyad of cells (Figure 15). Although in many cases the chromosomes do undergo some dispersion, they do not reach the extremely extended state of the interphase nucleus. The stage between the two meiotic divisions is called interkinesis and is generally short lived.
There is no replication of DNA during interkinesis. Interkinesis is followed by prophase II, a much simpler prophase than prophase I.

Meiosis II
Prophase II: Meiosis II is initiated immediately after cytokinesis, usually before the chromosomes have fully elongated. In contrast to meiosis I, meiosis II resembles a normal mitosis. The nuclear membrane disappears by the end of prophase II (Figure 15). The chromosomes again become compact. Metaphase II: At this stage the chromosomes align at the equator and the microtubules from opposite poles of the spindle get attached to the kinetochores (Figure 15) of sister chromatids.
Anaphase II: It begins with the simultaneous splitting of the centromere of each chromosome (which was holding the sister chromatids together), allowing them to move toward opposite poles of the cell (Figure 15) by shortening of microtubules attached to kinetochores. Telophase II: Meiosis ends with telophase II, in which the two groups of chromosomes once again get enclosed by a nuclear envelope; cytokinesis follows resulting in the formation of tetrad of cells i.e., four haploid daughter cells (Figure 15).

Significance of Meiosis
Meiosis is the mechanism, by which conservation of specific chromosome number of each species is achieved across generations in sexually reproducing organisms, even though the process, per se, paradoxically, results in reduction of chromosome number by half. It also increases the genetic variability in the population of organisms from one generation to the next. Variations are very important for the process of evolution.

Molecular genetics
Molecular genetics is the study of the processes whereby biological information is stored, copied, repaired and decoded to create protein and other molecules within cells and tissues.

DNA replication

DNA replication is semi-conservative. This means that each of the two strands in double-stranded DNA acts as a template to produce two new strands.

Replication relies on complementary **base pairing** that is the principle explained by Chargaff's rules: adenine (A) always bonds with thymine (T) and cytosine (C) always bonds with guanine (G).The replication process

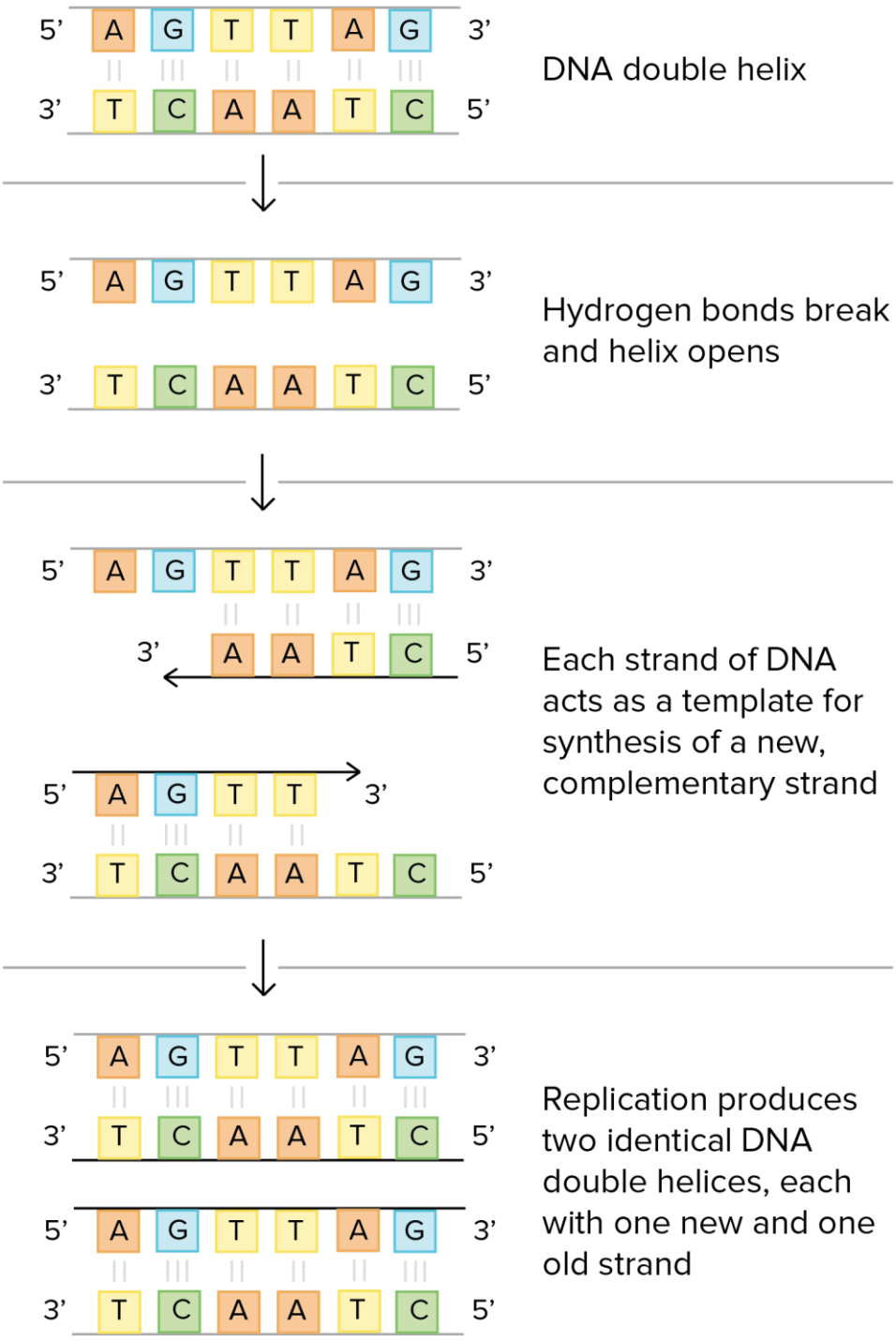


Figure 16

DNA replication occurs through the help of several enzymes. These enzymes "unzip" DNA molecules by breaking the hydrogen bonds that hold the two strands together.

Each strand then serves as a template for a new *complementary strand* to be created. Complementary bases attach to one another (A-T and C-G).

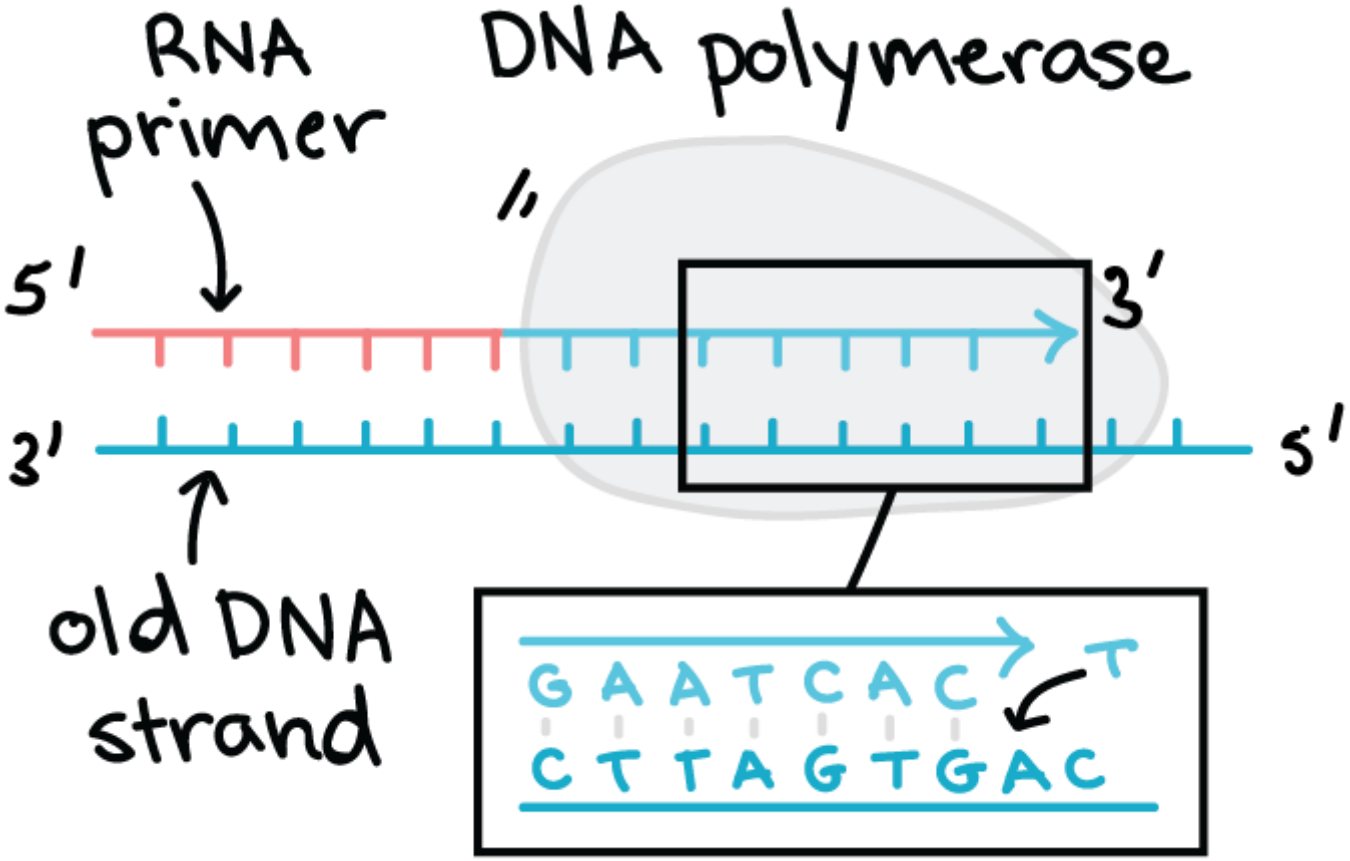


Figure17- DNA template strand and the creation of its complementary strand. (Source: www.Khanacademy.com)

The primary enzyme involved in this is *DNA polymerase* which joins nucleotides to synthesize the new complementary strand. DNA polymerase also proofreads each new DNA strand to make sure that there are no errors.

Leading and lagging strands

DNA is made differently on the two strands at a replication fork.One new strand, the *leading strand*, runs 5' to 3' towards the fork and is made continuously. The other, the *lagging strand*, runs 5' to 3' away from the fork and is made in small pieces called *Okazaki fragments (Figure-!8)*.

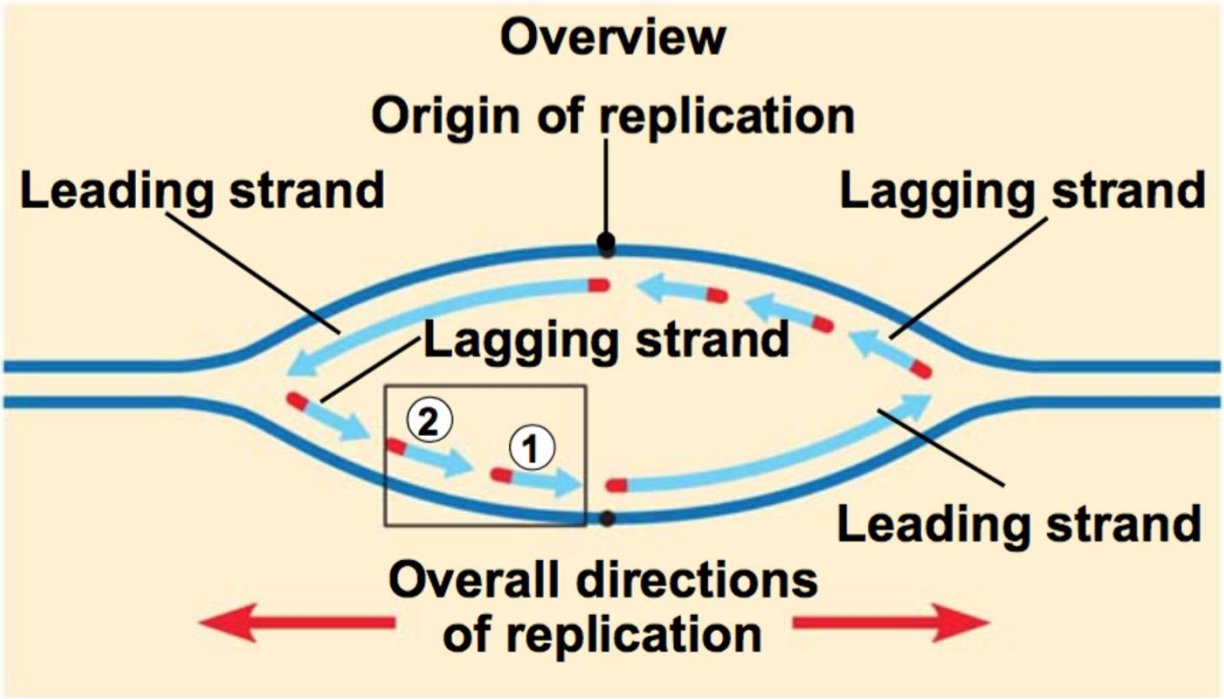


Figure 18- Overview of Replication Procedure

Genes provide information for building proteins. They don't however directly create proteins. The production of proteins is completed through two processes: transcription and translation.
Transcription and translation take the information in DNA and use it to produce proteins. Transcription uses a strand of DNA as a template to build a molecule called RNA.

The RNA molecule is the link between DNA and the production of proteins. During translation, the RNA molecule created in the transcription process delivers information from the DNA to the protein building machines.

DNA → RNA → Protein

DNA and RNA are similar molecules and are both built from smaller molecules called nucleotides. Proteins are made from a sequence of amino acids rather than nucleotides. Transcription and translation are the two processes that convert a sequence of nucleotides from DNA into a sequence of amino acids to build the desired protein. These two processes are essential for life. They are found in all organisms – eukaryotic and prokaryotic. Converting genetic information into proteins has kept life in existence for billions of years.

Transcription= DNA re-written in to RNA

DNA is "transcribed" or re-written into **RNA** in a very complicated process called transcrption. Simply stated, during transcription, one gene (**DNA**) is 're-written' into an **RNA** in the nucleus:

- A team of **enzymes and proteins** binds to the **promoter, or starting region**, of a gene.
- These **enzymes and proteins** unzip the DNA double helix just at the region of the gene.
- The enzyme **RNA polymerase** uses one of the DNA strands to make an RNA **copy of that one gene**.
- This copy, which contains the instructions to make **1 protein**, is called an **mRNA** or **messenger RNA**.
- After the **mRNA** is made, it is trimmed down to a final size, and shipped out of the nucleus!
- When the **mRNA** gets into the cytoplasm, it is made into protein.

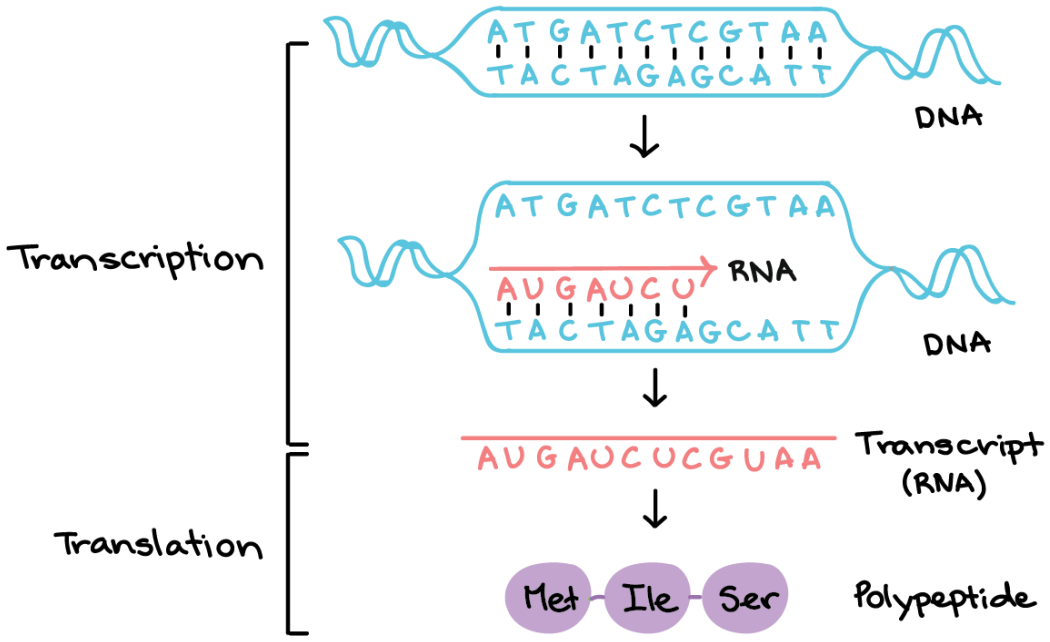


Figure 19- Transcription and Translation (Source: www. khanacademy.org)

Translation = De-coding RNA into protein

- During **translation**, the **mRNA** transported to the cytoplasm is "de-coded" or "translated" to produce the correct order of amino acids in a protein.. Translation requires numerous **enzymes.** To know the full story, we need to look at two other RNA "Key Players" - **rRNA** and **tRNA**

- **rRNA** = ribosomal RNA; these RNA molecules associate with other proteins to form the **ribosomes**. Each ribosome can accept two **tRNAs** at a time (carrying amino acids) and one **mRNA.**
- **tRNA** = transfer RNA; small RNA molecules that carry a **specific amino acid** at one end and an **anticodon** region that recognizes and binds **mRNA** at the other end. The **tRNA** that binds to that mRNA codon determines what amino acid is added to a protein chain.
- The Three RNAs (**mRNA**, **tRNA**, and **rRNA**) all work together to turn the information in DNA into a beautiful, 3-dimestional protein!!!

The steps of translation:

1. Initiation: **mRNA** enters the cytoplasm and becomes associated with **ribosomes** (**rRNA + proteins**). **tRNAs**, each carrying a specific amino acid, pair up with the mRNA codons inside the ribosomes. Base pairing (A-U, G-C) between **mRNAcodons** and **tRNA anticodons** determines the order of amino acids in a protein.

2. Elongation: addition of amino acids one-by-one: As the **ribosome** moves along the **mRNA**, the **tRNA** transfers its amino acid to the growing protein chain, producing the protein - codon by codon!

3. Termination: when the **ribosomes** hits a stop codon - UAA, UGA, or UAG - the ribosome falls apart!

The same **mRNA** may be used hundreds of times during translation by many **ribosomes** before it is degraded (broken down) by the cell.

Control of gene expression at transcription and translation level

In eukaryotic cells like photoreceptors, gene expression is often controlled primarily at the level of transcription. However, that doesn't mean transcription is the last chance for regulation. Later stages of gene expression can also be regulated, including:

- RNA processing, such as splicing, capping, and poly-A tail addition
- Messenger RNA (mRNA) translation and lifetime in the cytosol
- Protein modifications, such as addition of chemical groups

Recombinant DNA technology

This is joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture, and industry. Since the focus of

all genetics is the gene, the fundamental goal of laboratory geneticists is to isolate, characterize, and manipulate genes. So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology.

History: The possibility for recombinant DNA technology emerged with the discovery of restriction enzymes in 1968 by Swiss microbiologist Werner Arber. The following year American microbiologist Hamilton O. Smith purified so-called type II restriction enzymes, which were found to be essential to genetic engineering for their ability to cleave at a specific site within the DNA (as opposed to type I restriction enzymes, which cleave DNA at random sites). Drawing on Smith's work, American molecular biologist Daniel Nathans helped advance the technique of DNA recombination in 1970–71 and demonstrated that type II enzymes could be useful in genetic studies. About the same time, American biochemist Paul Berg developed methods for splitting DNA molecules at selected sites and attaching segments of the molecule to the DNA of a virus or plasmid, which could then enter bacterial or animal cells. In 1973 American biochemists Stanley N. Cohen and Herbert W. Boyer became the first to insert recombined genes into bacterial cells, which then reproduced.

Restriction Enzymes

Steward Linn and Werner Arber (1963) isolated two enzymes which restricted the growth of bacteriophage in bacterium E. coli. One of these enzymes added methyl groups to DNA and second one cut DNA. The second enzyme was named as "restriction endonuclease." H.O. Smith, K.W. Wilcox and T.J. Kelley (1968) isolated restriction endonuclease whose working depended on a particular nucleotide sequence. They isolated this enzyme from bacteria Haemophilus influenzae and called is as Hind II. It was observed that Hind II always cut DNA molecules at specific place by identifying a particular sequence of six base pairs. Restriction enzymes belong to a larger class of enzymes called nucleases.

They are of two types:

Exonucleases: They remove nucleotides from the ends of DNA.

Endonucleases:They make cuts at specific positions within DNA.

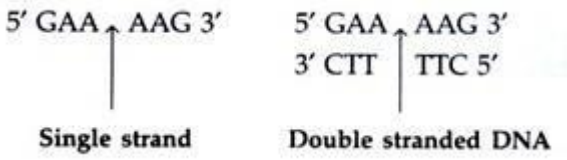
Thus, a restriction enzyme (or restriction endonuclease) recognizes a specific base pair sequence in DNA called a restriction site and cleaves the DNA (hydrolyzes the phosphodiester back bones) within the sequence. Restriction enzymes are widely found in prokaryotes and provide protection to host cell by destroying foreign DNA that makes entry into it.

Types of Restriction Endonucleases:

Three main types of restriction endonucleases i.e., Type I, Type II and Type III are known with slightly different mode of action. Type II restriction enzymes are used in rDNA technology because they can be used in vitro to identify and cleave within specific DNA sequences usually having 4-8 nucleotides.

More than 350 different type II endonucleases with 100 different recognition sequences are known. They need Mg^{2+} ions for cleavage. The first type II enzyme isolated was Hind II in 1970.

The recognition sequences for Type II restriction enzymes form pallindromes with rotational symmetry. In a pallindrome, base sequence of second half in DNA strand represents the mirror image of the base sequence of first half. Due to this in DNA double helix, complementary strand also represents the same mirror image.



(Pallindromes are groups of letters that form the same words when read both forward and backward e.g., 'MALAYALAM'. As against a pallindrome when same word is read in both the directions, pallindrome in DNA is a sequence of base pairs that reads same on the two strands when orientation of reading is kept the same.)

However in pallindromes with rotational symmetry, second half of complementary strand in DNA double helix is the mirror image of base sequence in the first half of another strand. In such cases, base sequences in both the strands of DNA helix represents the same when read from same and i.e., either 5′ or 3′ of both strands in DNA duplex, e.g.,

An example of pallindrome with rotational symmetry.

Nomenclature:

Nomenclature of restriction enzymes is usually done by following technique:

(i) The first letter of the genus is taken in which said enzyme was discovered. This letter is written in capital.

(ii) Then, first two letters of species of that organism are written.

(iii) All the above three letters should be written in italics.

Examples:

Eco from Escherichia coli, Hin from Haemophilus inflenzae and Hpa from Haemophilus parainfluenzae.

(iv) This followed by strain or type identification e.g., Eco K.

(v) When the enzyme is encoded by plasmid, the name of plasmid is written e.g. Eco RI i.e., Eco RI comes from Escherichia coli RY13. Here 'R' is derived from the name of strain. Roman numbers following the names indicate the order in which enzymes were isolated from the strain of bacteria.
(vi) If an organism forms many enzymes, they are identified by sequential Roman numerals.

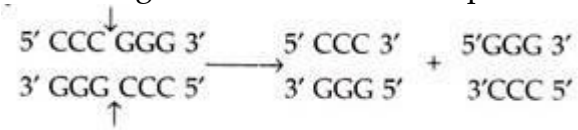
Example:

Enzymes formed by H. influenzae strain RD have been named as Hin II, Hin III etc. Discovery of Enzyme Eco RI led to award of Nobel Prizes to W. Arber, H. Smith and D. Nathans in 1978.

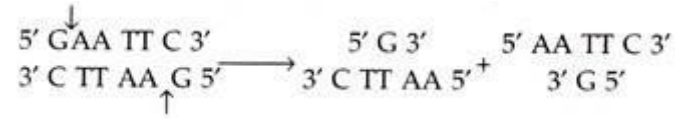
Types of Cleavage Produced By Restriction Enzymes:

Many restriction enzymes like SmaI isolated from Serratia marcescens cleave both the strands of DNA at exactly same nucleotide position almost in centre of recognition site resulting in blunt or flush end.

SmaI recognizes the 6 nucleotide palindromic sequence and cleave at both the ends.



Still some other restriction enzymes cleave the recognition sequence asymmetrically. Thus due to cleavage, they produce short, single stranded hanging structures. Such ends are called sticky or cohesive ends because base pairing between them can stick the DNA molecule again. A 6 nucleotides palindromic nucleotide sequence recognised by Eco RI cleave both strands at different points.



b. Cloning Vehicles (Vectors)-A vector is a DNA molecule which has the ability to replicate in an host cell and into which the DNA fragment to be cloned known as DNA insert is integrated for cloning. The cloning of a foreign fragment of DNA in bacteria is made possible due to the ability of cloning Vectors' or **"carriers"** to continue their lifestyle after additional sequences of DNA have been inserted into their genome. The insertion results in a "hybrid" or "chimeric" or "recombinant" vector which consists in part of the additional **"foreign"** fragment of DNA.

To act as vector, DNA molecule should bear following characteristics:

(i) Origin of Replication (Ori):

It represents the sequence from where replication initiates and any fragment of DNA when integrated to sequence can be made to replicate with in host cells. This sequence also controls the copy number of linked DNA. For getting several copies of target DNA, it is desirable that cloning should be carried out in a vector where origin facilitates high copy number.

It should bear origin of replication (ori) due to which it is able to multiply within the host cell i.e., it should be able to replicate autonomously. Due to this any foreign DNA introduced into vector will also replicate during this process.

(ii) Selectable Marker:
It should incorporate a selectable marker gene. This gene permits to select those host cells which bear the vector from amongst those which donot. Selectable marker helps in eliminating non- transformants and selectively permitting the growth of the transformants. In transformation DNA is introduced into host bacterium.

Examples of few selectable markers are:
(a) Genes which code for antibiotic resistance e.g., ampicillin, chloramphenicol, tetracycline or terramycin.
(b) Genes which encode enzymes like β-galactosidase (product of lac Z gene) which can be identified by colour reaction.

(iii) It should be easy to isolate and purify. Cloning vector should be relatively smaller in size. Large molecules can breakdown during purification and difficult to manipulate.
(iv) Vector should definitely bear atleast one restriction endonuclease recognition site. It will allow foreign DNA to be inserted into vector during the generation of recombinant DNA molecule.
Types of vectors used in **Recombinant DNA technology**

(A) Plasmids:

Plasmids are the most widely used cloning vectors in the technique of gene-manipulation in bacteria. They are circular, double-stranded DNA molecules occuring in extrachromosomal state and self-replicating. Some plasmids may have one or two copies per cell. Plasmids may be present in greater amounts, typically about 15- 100 per cell.
The plasmid vector is isolated from the bacterial cell and cleaved at one site by restriction endonuclease. The cleavage converts the circular plasmids into a linear molecule. Now the two ends of linear plasmid are joined to the ends of the foreign DNA (the gene) to be inserted with the help of enzyme DNA ligase. This regenerates a recombinant plasmid or circular hybrid or chimeric plasmid (Figure 20). The chimeric plasmid is transferred of a bacterium wherein it replicates and perpetuates indefinitely.
One of the earliest plasmid vectors to be constructed was pBR 322. This plasmid bears two different antibiotic resistance genes and recognition sites for several restriction enzymes.

i) Plasmid vectors of pUC family. Such vectors bear a site of the lac Z gene which codes for enzyme B-glactosidase. This site also bears a polylinker and thus, introduction of any foreign DNA into any of restriction enzyme sites will lead to a non-functional enzyme.Plasmid vectors discussed able can replicate only in E.coli.
(ii) In eukaryotic cells, many vectors have been constructed in such that can exist in both eukaryotic cells and E.coli. Such vectors (shuttle vectors) bear two types of origin of replication and selectable marker genes. One of them acts in eukaryotic cells and other in E.coli For example, shuttle vector of yeast episosmal plasmid YEp In plants a naturally occurring plasmid of bacterium Agrobacterium tumefaciens called Ti plasmid has been suitable formed to act as vectors.

An ideal cloning plasmid vector has three properties:

(i) Low molecular weight,
(ii) Ability to confer readily with selectable phenotypic traits on host cells, and
(iii) Several sites for large number restriction enzymes.

The advantages of a low molecular weight are several. First the plasmid much easier to handle. Second, low molecular weight plasmids are usually present as multiple copies. Finally, with a low molecular weight there is less chance that the plasmid will have restriction enzyme.

(B) Phages as Vectors:

Bacteriophages are viruses that infect bacterial cells by injecting their DNA into these cells. Two phages which have been extensively modified for development of cloning vectors are lambda (λ) and M13.

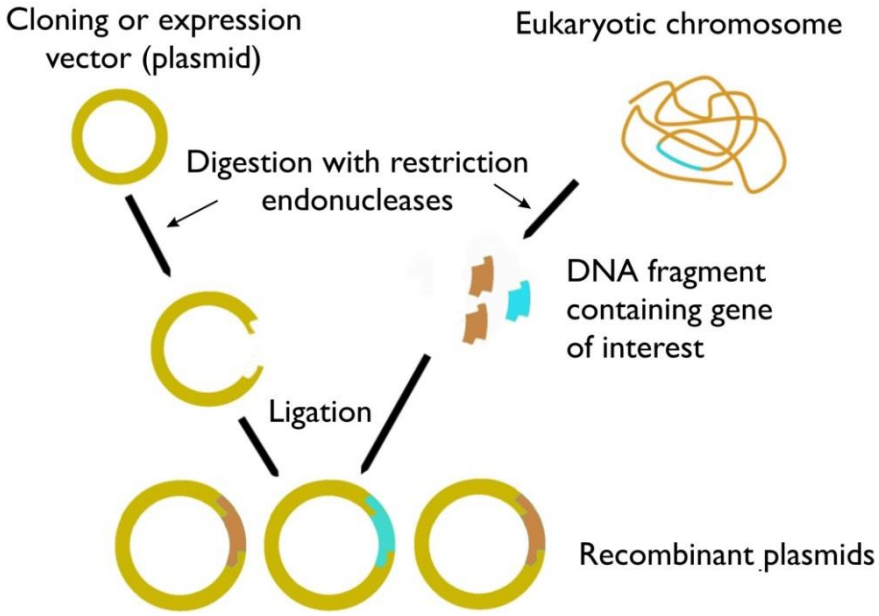


Figure 20 Incorporation of the insert into a plasmid vector

Lambda (λ) phages provide another type of useful vector system for cloning in bacteria. Usually the DNA of phage, λ, in the form in which it is isolated from the phage particle, is a linear double-stranded molecule of about 48.5 kb paris.

Foreign DNA can be introduced into genome of M13 without disrupting any of the essential genes. When M13 phage DNA enters into E. coli host replicative form (RF) a double stranded form is constituted. It replicates until 100 copies are formed. Now the DNA replication becomes a symmetric and it starts producing single stranded copies of genome and extruded from cell as M13 particles.

(C) Cosmids:

Cosmids have been constructed by combining certain features of plasmid and the 'cos' sites of phage lambda. They have been constructed to add some of the advantages of phage vectors to the plasmid vectors' the cos sites endeavour in vitro packaging system to the plasmid vector. The cosmid vectors, however, provide an efficient means of cloning large fragments of foreign DNA (32-48 kb of foreign DNA)—much more than a λ phage vector can accommodate.

When injected into a bacterium, the recombinant-DNA of a cosmid circularizes like phage DNA but replicates as a normal plasmid without the expression of any phage functions. Cosmid vectors are particularly attractive for constructing libraries of DNA fragments of eukaryotes because of their capacity to accommodate large fragments of DNA.

(D) Phasmids:

Phasmids are also a type of plasmid vectors containing a fragment of phage DNA including its att site. Like cosmids, they have been constructed to exploit the advantages of both-plasmid vector and λ phage vector. The phasmid may insert into a phage DNA in the same way by which phage DNA inserts into the bacterial chromosome during lysogenic phase of life cycle.

(E) YAC Vectors:

YACs or Yeast artificial chromosomes (Fig. 11.3) are being used as vectors to clone DNA fragments of more than 2500 Mb in size. They are being highly used in mapping larger genomes like Human Genome Project.

(F) BAC Vectors:

BACs or Bacterial artificial chromosomes (Fig. 11.14) are used as vectors which are based on natural extra- chromosomal plasmid of E.coli the fertility or F-plasmid IHs vector bears genes for replication and maintenance of F-factor, a selectable marker and cloning sites.

(G) Shuttle Vectors:

They are the plasmids capable of shuttling genes between two organisms. One of the organisms is prokaryote like E. coli and other is a eukaryote like yeast. Such vectors should bear unique origins of replication for every cell type. They should have separate markers for transformed host cells harbouring the vector.

Vectors for Cloning Genes in Plants

Genes are transferred from bacterium or virus to eukaryotic cells and these genes force the transformed cell to work at their will e.g., Agrobacterium mediated gene transfer (Figure 21). Agrobacterium tumefaciens is a soil bacterium which causes crown gall tumors in dicotyledons.

These tumors are formed due to insertion of T_i-plasmids into nuclear genome of the infected plant. T_i-plasmid contains T-DNA within it. This bacterium is able to transfer T-DNA to transform normal plant cells into tumour and direct these tumour cells to produce the chemicals required by the pathogen.

The T-DNA causes hormonal disturbances in the transformed plant cell. Most notable of these are increased level of growth hormones i.e., auxins and cytokinins. The tumour inducing (T_i) plasmid of Agrobacterium has been modified as cloning vector. It is not now disease causing to plants but capable of utilising the system to insert desirable genes into many plants.

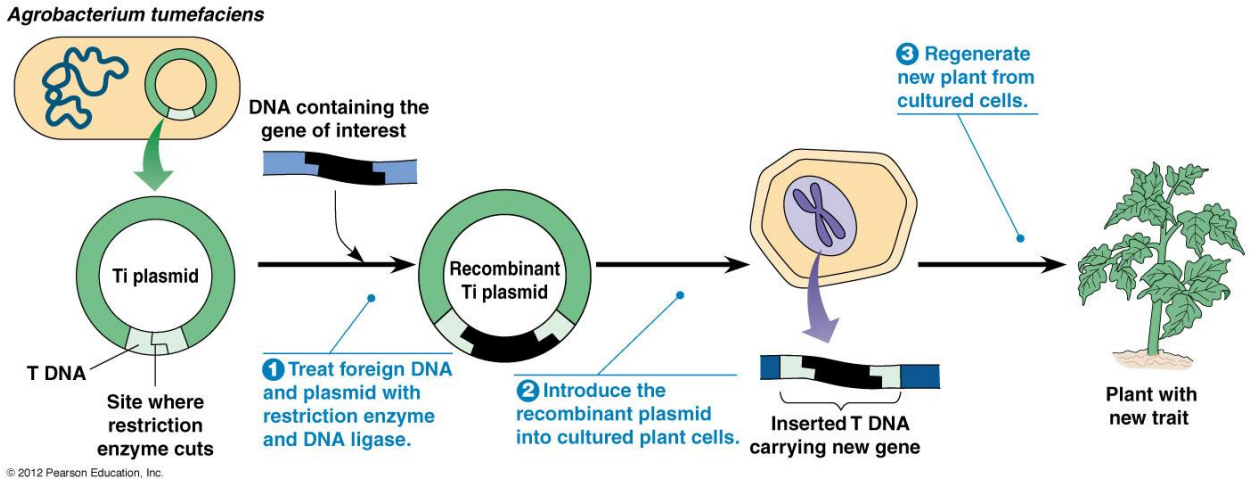


Figure 21 Genetic engineering of plant crops

Gene transfer in higher plants through T_i– plasmid can be achieved by tumour formation on intact plants or plant parts with A. tumefaciens carrying a T_i-plasmid. This can also be achieved by co-culturing protoplasts of A. tumefaciens carrying T_i-plasmid or fusion of protoplasts with spheroplasts of A. tumefaciens.

C. Ligases- The ligases used in DNA cloning do basically the same thing. If two pieces of DNA have matching ends, DNA ligase can join them together to make an unbroken molecule. How does DNA ligase do this? Using ATP as an energy source, ligase catalyzes a reaction in which the phosphate group sticking off the 5′

end of one DNA strand is linked to the hydroxyl group sticking off the 3′ end of the other. This reaction produces an intact sugar-phosphate backbone.

E. coli

The E. coli DNA ligase is encoded by the lig gene. DNA ligase in E. coli, as well as most prokaryotes, uses energy gained by cleaving nicotinamide adenine dinucleotide (NAD) to create the phosphodiester bond.[3] It does not ligate blunt-ended DNA except under conditions of molecular crowding with polyethylene glycol, and cannot join RNA to DNA efficiently.The activity of E. coli DNA ligase can be enhanced by DNA polymerase at the right concentrations. Enhancement only works when the concentrations of the DNA polymerase 1 are much lower than the DNA fragments to be ligated. When the concentrations of Pol I DNA polymerases are higher, it has an adverse effect on E. coli DNA ligase[4]

T4

The DNA ligase from bacteriophage T4(Enterobacteria phage T4 is a bacteriophage that infects Escherichia coli bacteria. The T4 phage is a member of the T-even phages, a group including enterobacteriophages T2 and T6. T4 is capable of undergoing only a lytic lifecycle and not the lysogenic lifecycle.) is the ligase most-commonly used in laboratory research.[5] It can ligate either cohesive or blunt ends of DNA, oligonucleotides, as well as RNA and RNA-DNA hybrids, but not single-stranded nucleic acids. It can also ligate blunt-ended DNA with much greater efficiency than E. coli DNA ligase.

D. Competent Host (For Transformation with Recombinant DNA):
For propagation of DNA molecules host cells are required. Host cells like E. coli, yeast and plant and animal cells are being used.

Most popular and extensively used bacterium is E. coli due to following reasons:
(i) E. coli a gram negative bacterium is easy to handle and grow.
(ii) It can accept a variety of vectors.
(iii) Under optimal conditions, bacteria double their numbers every 20 minutes. When bacteria reproduce recombinant DNA also reproduces. Eukaryotic cells are also being used as host cells for expression of eukaryoticin. This leads to proper folding of polypeptide Cham into exact 3-dimensional form. Simple eukaryotic organisms like yeast are being widely used being single called, easy to grow and manipulate and genetically well characterized.
A cell membrane does not permit DNA to pass through being hydrophilic in nature. Bacterial host cells are made competent to take up plasmid. For this bacteria are treated with divalent cation like calcium. This enhances the efficiency of entry of DNA into bacterium through pores on cell wall.

Recombinant DNA is forced into such cells by:
(i) Incubation of cells with recombinant DNA on ice.
(ii) It is followed by giving them a heat shock (42°C) and again putting back on ice.

Processes of Recombinant DNA Technology

Recombinant DNA (rDNA) technology refers to the process of joining DNA molecules from two different sources and inserting them into a host organism, to generate products for human use. Can you put the DNA molecules in the host organism first and then cut and join them? No! This process involves multiple steps that have to proceed in a specific sequence to generate the desired product. Let's understand each step in detail.

1. Isolation of Genetic Material
We already know that the genetic material of all living organisms is 'nucleic acid'. In most organisms, it is DNA, whereas in some it is RNA. The first step in rDNA technology is to isolate the desired DNA in its pure form i.e. free from other macromolecules (Figure 21).
However, in a normal cell, the DNA not only exists within the cell membrane, but is also present along with other macromolecules such as RNA, polysaccharides, proteins, and lipids. So, how do we break open the cell and obtain DNA that is free from other macromolecules? We can use the following enzymes for specific purposes:
Lysozyme – to break bacterial cell wall.
Cellulase – to break plant cell wall.
Chitinase – to break fungal cell wall.
Ribonuclease – removes RNA.
Protease – removes proteins (such as histones that are associated with DNA).
Other macromolecules are removable with other enzymes or treatments. Ultimately, the addition of ethanol causes the DNA to precipitate out as fine threads. This is then spooled out to give purified DNA.

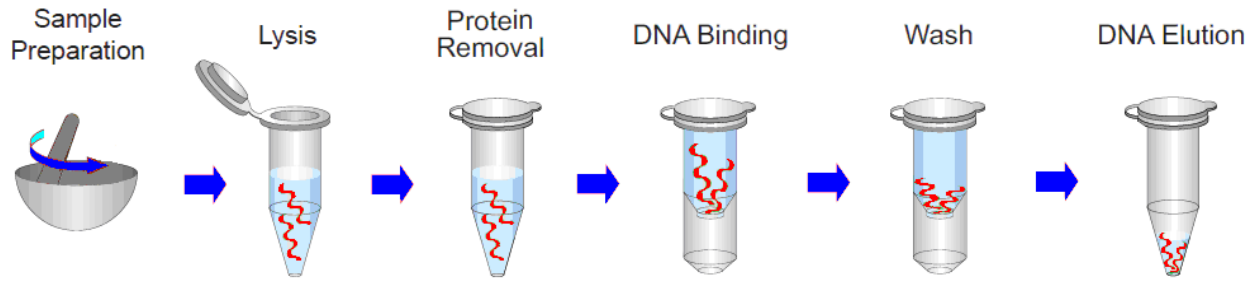


Figure 21- Steps in DNA isolation

2. Cutting of DNA at Specific location

Restriction enzymes act as molecular scissors that cut DNA at specific locations. These reactions are called 'restriction enzyme digestions'. They involve the incubation of the purified DNA with the selected restriction enzyme, at conditions optimal for that specific enzyme.

The technique – 'Agarose Gel Electrophoresis' reveals the progress of the restriction enzyme digestion. This technique involves running out the DNA on an agarose gel. On the application of current, the negatively charged DNA travels to the positive electrode and is separated out based on size. This allows us to separate and cut out the digested DNA fragments. The vector DNA is also processed using the same procedure (Figure 22).

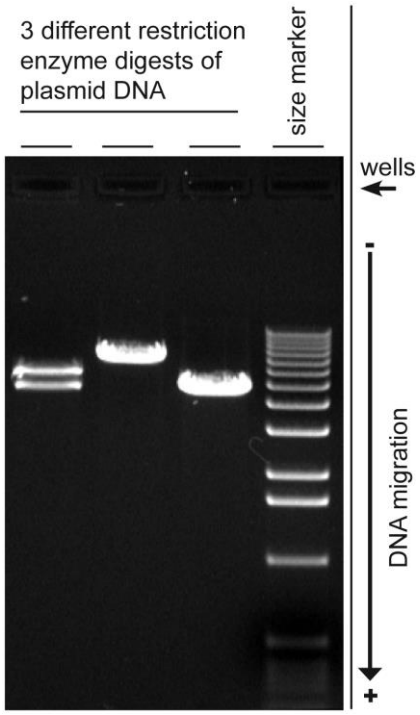


Figure 22 *Image of digested DNA after agarose gel electrophoresis*

3. Amplification Using PCR

Polymerase Chain Reaction or PCR is a method of making multiple copies of a DNA sequence using the enzyme – DNA polymerase. It helps to amplify a single copy or a few copies of DNA into thousands to millions of copies (Figure 23). PCR reactions are run on 'thermal cyclers' using the following components:

Template – DNA to be amplified

Primers – small, chemically synthesized oligonucleotides that are complementary to a region of the DNA.

Enzyme – DNA polymerase

Nucleotides – needed to extend the primers by the enzyme.

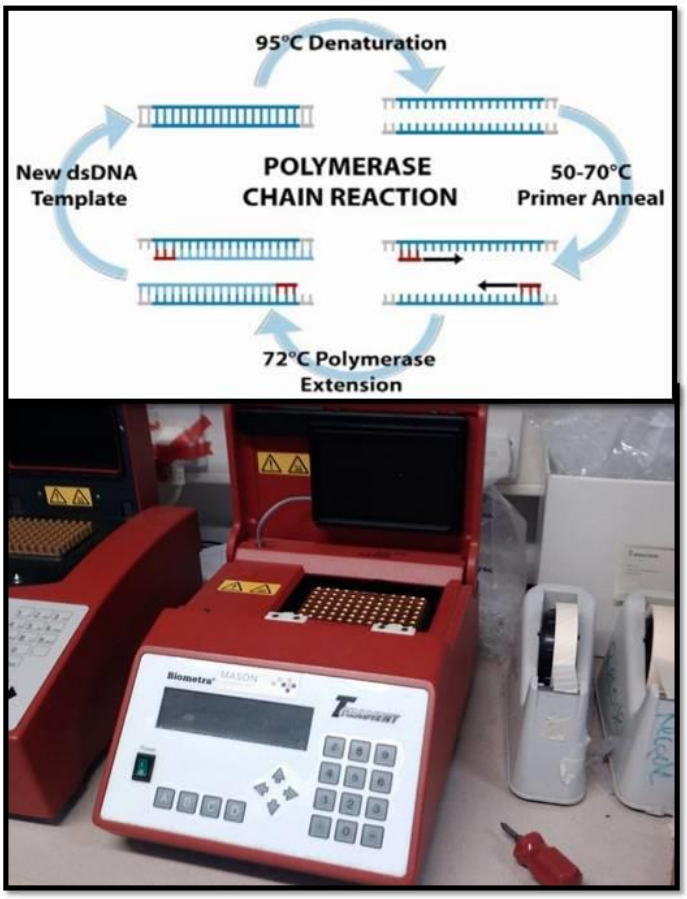


Figure 23: Polymerase Chain Reaction and Thermal Cycler

The cut fragments of DNA can be amplified using PCR and then ligated with the cut vector as explained below.

4. Ligation of DNA Molecules

The purified DNA and the vector of interest are cut with the same restriction enzyme. This gives us the cut fragment of DNA and the cut vector, that is now open. The process of joining these two pieces together using the enzyme 'DNA ligase' is 'ligation'. The resulting DNA is '**recombinant DNA**' (Figure 20).

5. Insertion of Recombinant DNA Into Host

In this step, the recombinant DNA is introduced into a recipient host cell. This process is **'Transformation'.** Bacterial cells do not accept foreign DNA easily. Therefore, they are treated to make them **'competent'** to accept new DNA. (The topic – Tools of Biotechnology explains a few ways to make cells competent).

During transformation, if a recombinant DNA bearing a gene for ampicillin resistance is transferred into recipient *E. coli* cells, then the *E. coli* cells also become ampicillin-resistant. This aspect is useful in differentiating transformed cells from non-transformed cells.

For example, if we spread the transformed cells on agar plates containing ampicillin, only the transformed, ampicillin-resistant cells will grow while the untransformed cells will die. Therefore, in this case, the ampicillin resistance gene acts as the 'selectable marker'.

6. Obtaining Foreign Gene Product

The recombinant DNA multiplies in the host and is expressed as a protein, under optimal conditions. This is now a **recombinant protein**. Small volumes of cell cultures will not yield a large amount of recombinant protein. Therefore, large-scale production is necessary to generate products that benefit humans. For this purpose, vessels called **bioreactors** are used.

Bioreactors are large containers with a continuous culture system, where the fresh medium is added from one side and used medium is taken out from another side. Bioreactors can process about 100-1000 litres of cell cultures. A bioreactor provides optimum conditions (temperature, oxygen, pH, vitamins etc.) to biologically convert raw materials into specific proteins, enzymes etc.

'Stirred-tank bioreactor' is the most common type of bioreactor. It is usually cylindrical and has the following parts:

Agitator system – to stir the contents evenly.

Oxygen delivery system – to introduce air into the system.

Foam control system

Temperature control system

pH control system

Sampling ports – to take out small amounts of culture.

7. Downstream Processing

Before the protein is marketed as a final product, it is subjected to downstream processing which includes:

Separation and purification.

Formulation with suitable preservatives.

Clinical trials to test the efficacy and safety of the product.

Quality control tests.

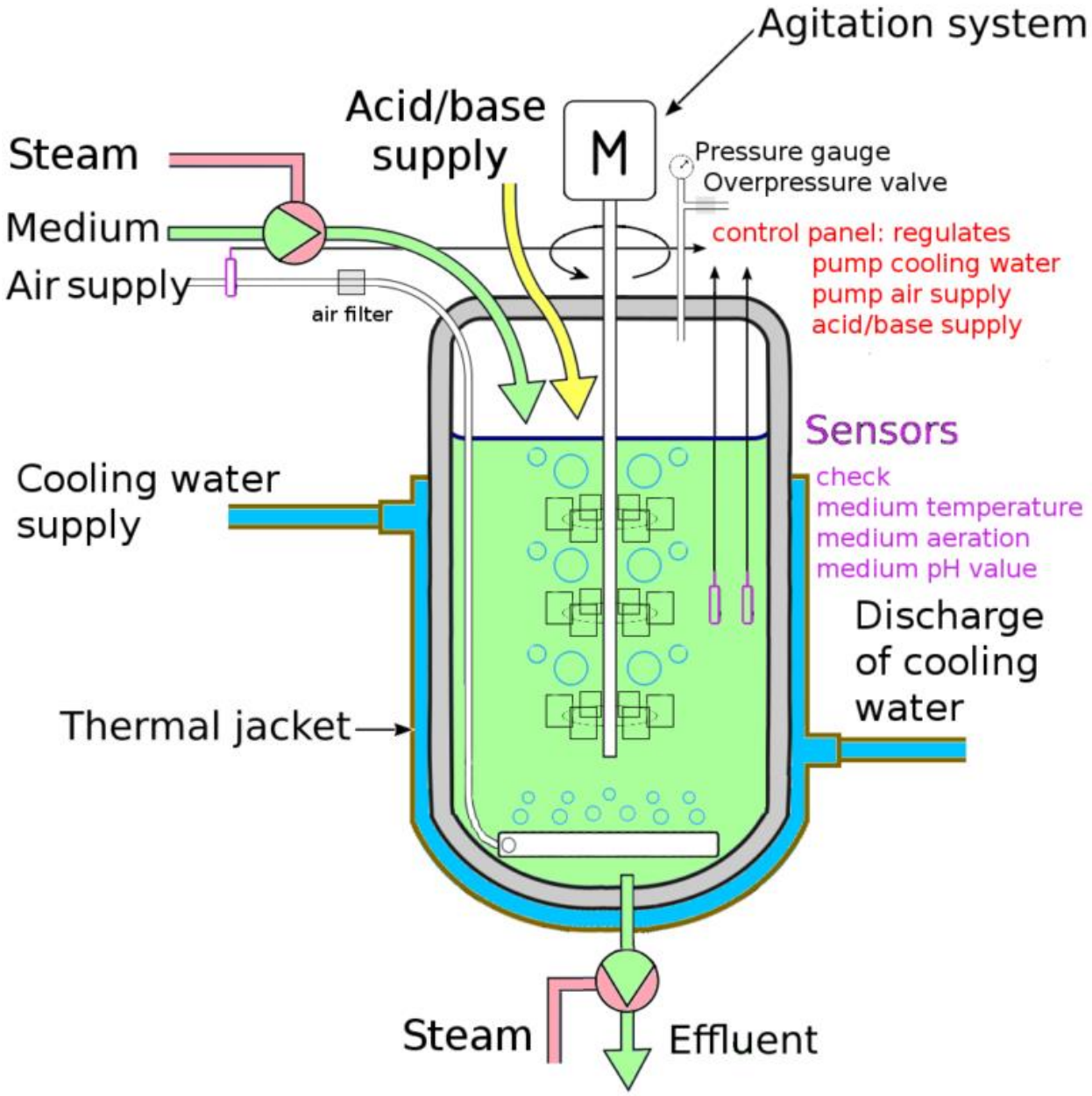


Figure 24 A Typical design of Bioreactor

Applications of Biotechnology to Control Environmental Pollution

Environmental Biotechnology is a branch of biotechnology which deals with the application of new techniques of modern biotechnology along with the approaches of traditional biotechnology for the detection of contaminants present in the

environment as well of remediation of pollution caused by the contaminants. Biotechnology provides us enabling techniques for bringing man-made changes in deoxyribonucleic acid (DNA) or the genetic material in plants, animals and microbial systems leading to development of new products and techniques. It is based on the molecular basis of biological cell function and the ability of mankind in bringing changes by altering the cell function to produce suitable produced as needed in the society. The application of new techniques in the field of biotechnology holds potential for development of products in sectors of agriculture, horticulture, forestry, animal husbandry, health care, energy generation and environment protection. Since microbes are one of the oldest inhabitants of earth with versatility and adaptability to changing environment, therefore they acts as an effective medium for bringing sustainability to nature. The consequences faced by physical and chemical methods in controlling the environment can be overcome by utilisation of micro-organisms. As human activity generates huge amount of wastes thereby creating a burden to society so, the application of biotechnology will ensure the protection in terms of environmental monitoring, waste degradation, and replacement of non-renewable resource with renewable resources by developing eco-friendly products and methods. Altogether environmental biotechnology is a path for creating sustainable environment by utilising various processes and techniques in the following ways:

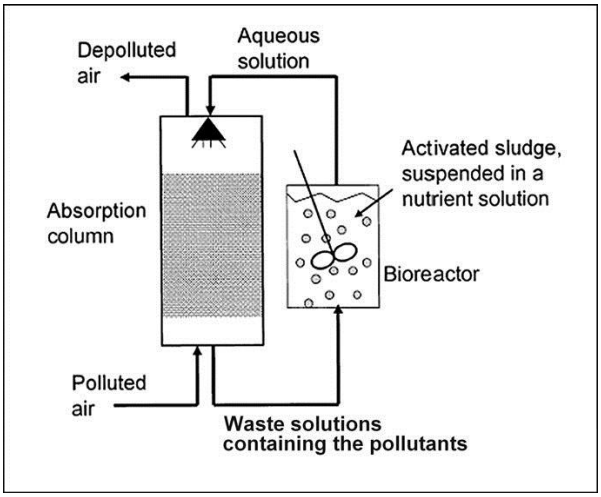
- Abatement of pollution through biodegradation, biotransformation, bioaccumulation of toxic substances like organics, metals, oil and hydrocarbon, dyes and detergents
- Energy management by developing nonconventional non-polluting energy like biodiesel, methanol, biogas, bio hydrogen etc.
- Safeguarding the pollution in agricultural sector via application of bio fertilizer, bio pesticides and bio organics.
- Resource recovery from hazardous waste
- Development of biosensor for pollution monitoring and allied issues.

Application to control of air pollution

The presence of pollutants in the atmosphere namely sulphur dioxide, nitrogen oxides, volatile organic compounds and suspended particulates are the major components of air pollution besides acting as an important determinant of local air quality are responsible for producing environment hazards. Awareness related to air quality has increased in recent years rising rate of health issues. Waste gases generated from production unit, open wastewater treatment plants and garbage dumping sites not only degrades the air quality but also creates an impact on health. Types of biological waste gas purification systems includes: bioscrubbers, biofilters and biotrickling filters.

Bioscrubber

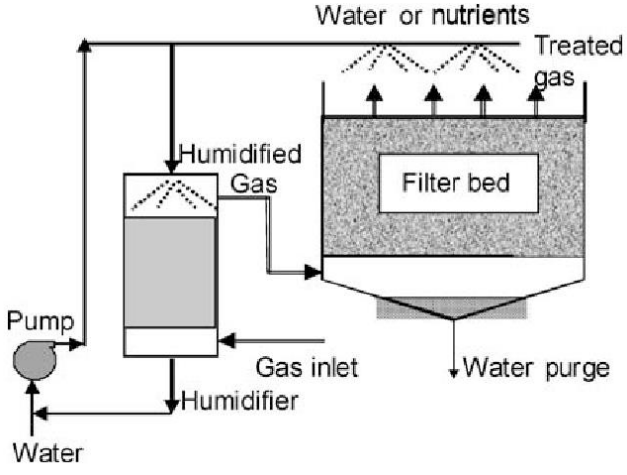
The mechanism of bioscrubber involves an absorption column attached with section of one or more bioreactor. Biological oxidation takes place within the bioreactor while the reaction tank are aerated and supplied with a nutrient solution. Microbial mass remains inside the circulating liquor and passes through the absorption column. The development of biofilm in the absorption column depends upon the circulation whereas the removal rate varies according to time.



Schematic diagram of Bioscrubber system

Biofilters

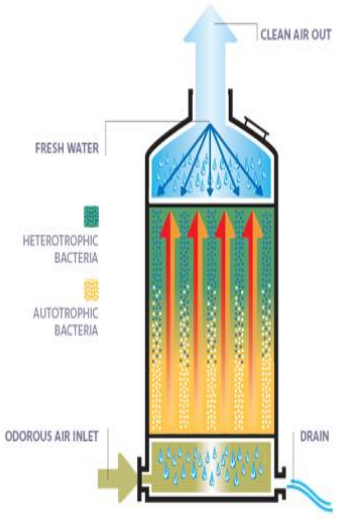
Its mechanism depends on role of microorganism oxidising the volatile organic compounds. The structure of biobed consists of combination of soil, peat, compost, heather, bark etc whereas the proper treatment of gas depends on the uniformity and permeability of biobeds. They require a huge space for gas treatment and can turned 2-3 times in a year besides having a proper drainage facility at the bottom. The flow rate of gas is maintained at 130 m^3/hm^2 and the height of packing of bed is 1m. Mesophilic microbes are used in biofilters with temperature is kept at 15-40°C, moisture 40-60% and gas contact time at 10-30 seconds are properly inside the chamber unit.



Schematic diagram of Biofilter unit

Biotrickling filters

It is based on the mechanism of biosorption of polluted gas to generate clean and purified air. The packing system of such filters consists of single unit through which gasses are passed and water trickles down dissolving the gas while the pure air comes out. Many different types of microbes had been identified for degrading specific air pollutants. For example, some Pseudomonas species and genetically engineered E. coli have been found to be efficient and capable of degrading trichloroethylene (TCE). VOCs are degraded to form end products of carbon dioxide, water, biomass and inorganic salts. For treatment of sulphur containing gases like hydrogen sulphide and sulphur dioxide, some sulphur degrading bacteria like Chiobacillus ferroxidans are utilized for degradation besides producing solid sulphur as end product. The advantage of using biotrickling filter is they are cheaper than thermal and catalytic oxidation.



Schematic diagram of Biotrickling filter unit

Application to control of water pollution

Wastewater is the part of water supplied to community or industries which has been utilized for different purposes and results in mixing with solids either suspended or dissolved. The undesirable waste characteristics of waste present in polluted water includes: (1) suspended solids and soluble organic compounds undergoing progressive decomposition leading to depletion of oxygen level and generating harmful noxious gases, (2) heavy metals, cyanides and toxic organics causing danger to aquatic species, (3) unwanted levels of nitrogen and phosphorous causing eutrophication in water bodies leading to deprivation of dissolved oxygen besides stimulating algal bloom, (4) Non-biodegradable chemicals and volatile materials like hydrogen sulphide and sulphur dioxide. Primary aim of biological treatment of wastewater is to coagulate and to remove non-settable colloidal solids while stabilizing the organic matter. Besides removing odour, they also assist in removal of carbonaceous content of organic matter in waste water measured as BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand) and TOC (total Organic Carbon). Nitrification, denitrification and stabilization are also include under the process of biological treatment.

Biological treatment is carried out when the effluents are rich in unstable organic matter and role of microbes is to break-up these unstable organic pollutants into stable products of carbon dioxide, carbon monoxide, ammonia, methane and hydrogen sulphide. Many industrial effluents vary in concentration, load of pollutants, toxic substances and biodegradable or non-biodegradable pollutants or both. Therefore, biological is one of the efficient measures to treat the waste water. The treatment process of effluents broadly classified into two categories: aerobic and anaerobic

Aerobic biological treatment

They are based on mechanism of treating the sewage by utilising oxygen for degradation of biodegradable organic matter. For treating the industrial effluents focussed must be made based on design and working parameters. The basic reaction involves in aerobic treatment plant is:

$$\text{Organic material} + O_2 \xrightarrow[\text{Other Nutrients}]{\text{Cells}} CO_2 + H_2O + \text{new cells}$$

Microbes undergoing auto-oxidation of cell mass:

$$\text{Cells} + O_2 \longrightarrow CO_2 + H_2O + NH_3$$

The different aspects of biological treatment process are broadly explained below:

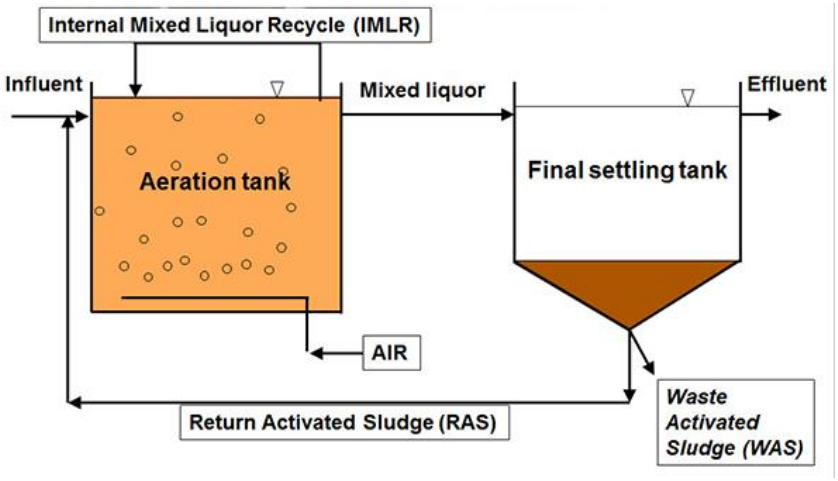
Activated sludge process

Its mechanism is based on homogenous continuous culture in which the biosorption and flocculation removes the organic content rapidly which oxidation and biosynthesis are operated at lower rate. It consists of two chambers: aeration tank and sedimentation tank. The flocs gets settled into secondary sedimentation tank while a portion of this floc are returned as an inoculum. BOD and suspended solids are reduced by 85-95% and the organisms in activated sludge are similar to percolating filters. In terms of reaction vessel, the contents are referred to as Mixed Liquor Suspended Solids (MLSS) or Mixed Liquor volatile Suspended Solids (MLVSS) and consist of microorganisms and inert and non-biodegradable suspended matter. Various modifications of activated sludge process are:

- Tapered aeration: Aeration capacity depends on the demand and less at the outlet than at the inlet.
- Step aeration: In this type of chamber unit both feeding and aeration is done at steps throughout the length of the tank
- Contact stabilization: In such unit, the returned sludge is aerated to encourage organisms to utilize the stored nutrients. Waste gets digested while the sludge

volume gets reduced through aerobic digester chamber. It is based on principle of extended aeration treatment in which both aeration and mixing of sludge along with effluents are done in the same unit.

Advanced activated sludge process: Such type of system operates with pure oxygen therefore can operate at a higher biomass concentration. The residence time is less while bulking i.e. the extensive growth of filamentous bacteria and fungi which can inhibit sludge setting is restricted.



Schematic process diagram of activated sludge treatment unit

Biological filters-Fixed film systems

In such type system, microbes are attached to inert supporting medium packed into tower or tank. It is based on mechanism of aerobic systems in which fixed film layer of microorganisms are grown with even distribution of effluent is done in the reactor while air is introduced from the bottom vents and passes across media bed as effluent percolates and distributes. Microbial slime layer develops on supporting media by utilising organic matter and oxygen. When the thickness of slime increases, extra biomass sloughs off and sludge is collected by gravity in sedimentation tank.

The first trickling filter was operated in 1893 and the filter media normally used consists of rocks ranging in size from 25-100 mm in diameter. The depth of rock depends on design and ranges from 3-8 ft. Rock filter bed are circular while the rotating arm distributing the effluent liquid at the top of bed. Filters have under drainage system to collect sludge as well as treated effluents. Organic matters decomposed by microbes are grown as film on inert support. Development of slime takes place when metabolic activities of microbes in slime increases in thickness of slime whereas the detachment of slime happens when microorganisms near to medium reaches endogenous phase i.e. lose the ability to cling thereby resulting in formation of new slime occurring in a cyclic manner. The group of microbes mostly used are facultative bacteria like Achromobacter, Flavobacterium, Pseudomonas,

Adcaligenes, filamentous form like Sphaerotilus natans, Baggiatoa while at lower levels beds of nitrifying bacteria like Nitromonas, Nitriobacter are mostly common. Apart from these few fungi, algae and protozoa are also present.

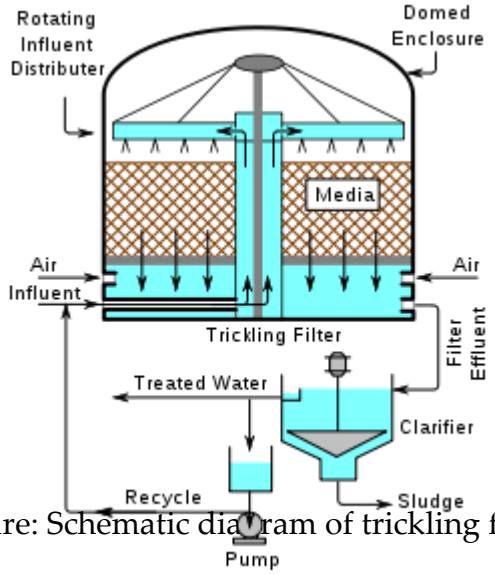


Figure: Schematic diagram of trickling filter unit

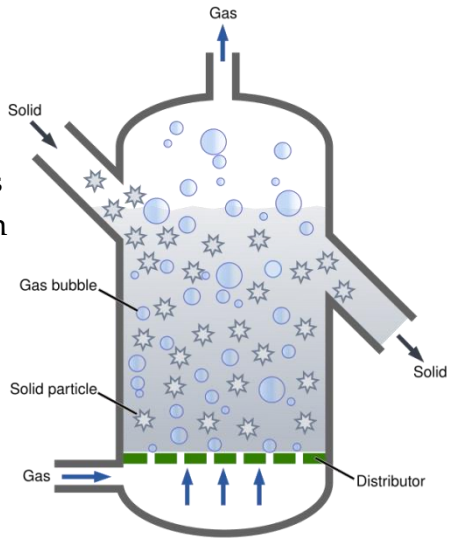
Rotating Biological Contactors

Based on the principle of fixed film-moving medium systems acting as a digester in which the biological growth is established on disc surface made of polystyrene, polyethylene, polypropylene, stainless steel, cement, aluminium, glass, PVC, rubber, Teflon, wood and wire screens. Discs are normally 2-3 meters in diameter with 10-20 mm wide and mounted on horizontal shaft. The distance between horizontal discs is 20 mm and are partly submerged (40% of the area in medium) while rotation takes place at 1-7 revolutions per minute. Air is continuously sparged to reduce effect of anaerobiosis in multiple unit systems. Retention time varies with percolation time whereas the biomass formation or the biological growth on the discs is 200 gm dry weight per square meter of disc surface with thickness of 2.4mm. The advantages of such system are easy in operation with low maintenance cost, free from channeling problem in percolators, reduces power costs (no sludge recycling), requires less space than activated sludge plant, efficient mixing with little sloughing off of biomass, foaming, aerosol and air striping is reduced along problems of clogging, ponding and filter flies are eliminated.

Figure: Rotating Biological Contactor

Fluidised Bed Reactors (FBR)

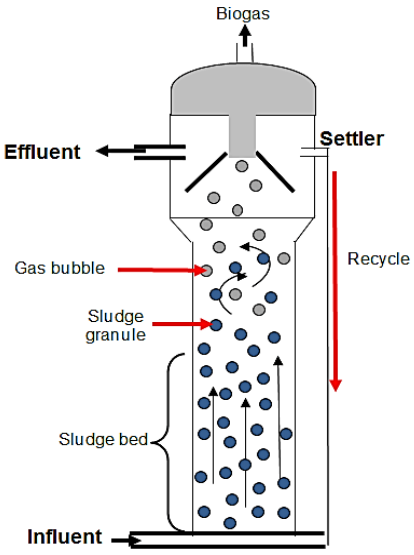
It is based on the mechanism of combination of attached growth (percolating filter) and suspended growth (activated sludge) in which the biological slime film is developed and maintained on a solid support medium consisting of particles less enough for maintaining in suspension by the upward flow of liquid utilized for treatment. The particles in the support medium neither sink nor outflow and the reactors are generally cylindrical in nature attached with perforated distribution plates. Fluidization of support particles are allowed but clumping is prevented.



Schematic diagram of fluidized bed reactor

Expanded Bed Reactor (EBR)

Such reactors have mechanism similar to packed filters and fluidized bed processes. The expanded bed system utilizes the operating mode of fluidized bed reactor. The process of expanded bed is different in certain aspects from fluidised bed for biological applications and differ in terms of: (a)velocities or maintaining the delicate attached living film, (b) separation, retardation and bioaccumulation of fine suspended solids and (c) achievement of maximum biomass concentration.



Schematic diagram of expanded bed reactor

Anaerobic biological treatment

Anaerobic digestion is the process in which decomposition of biodegradable material is taken place by micro-organisms in the absence of oxygen. Such type of digestion process is often used for industrial or domestic purposes to manage waste streams. Three principal products are produced through the process of anaerobic digestion. First, the process produces a biogas, consisting mainly of CH4 and CO2, which can be used for energy production in a combined heat and power plant. Second, the process results in a nutrient-rich digestate which is similar to compost. Finally, the process results in liquid liquor that can be utilized as a fertilizer. In terms of types and variations of anaerobic digester; there a are two basic types: batch and continuous. Batch-type of digesters is the simplest to build since their operation consists of loading the digester unit with organic materials and allowing it to digest while the retention time depends on temperature and other factors. Once the digestion is complete, the effluent is removed and the process is repeated. In case of continuous digester, organic material is constantly or regularly fed into the digester. The material moves through the digester either mechanically or by the force of the new feed pushing out digested material. Unlike batch-type digesters, continuous digesters produce biogas without any interruption of loading material and unloading effluent. They may be better suited for large-scale operations while proper designing, operation, and maintenance of such digesters produces a steady and predictable supply of usable biogas.

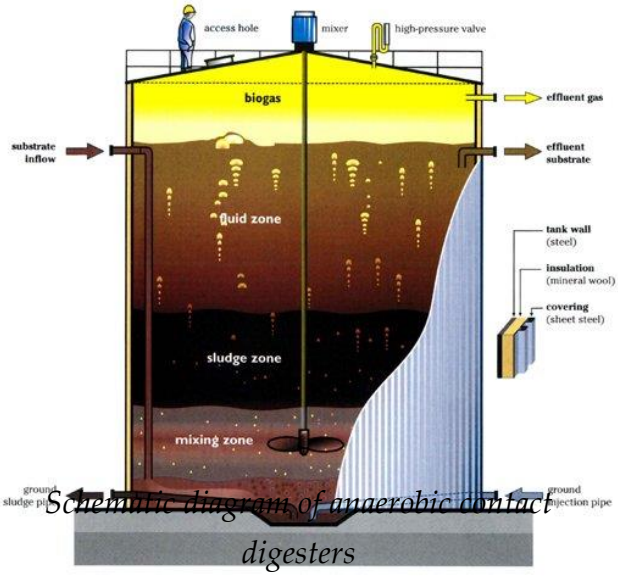
There are four key stages involving in anaerobic digestion: hydrolysis, acidogenesis, acetogenesis and methanogenisis. The overall process can be described by the chemical reaction, where organic material such as glucose is biochemically digested into carbon dioxide (CO_2) and methane (CH_4) by the anaerobic microorganisms.

$$C_6H_{12}O_6 \longrightarrow 3CO_2 + 3CH_4$$

The different aspects of anaerobic treatment process are explained below:

Anaerobic contact digesters

Such reactors are equivalent to aerobic activated sludge process. It consists of a stirred tank along with a tank consisting of anaerobic conditions. The output of the

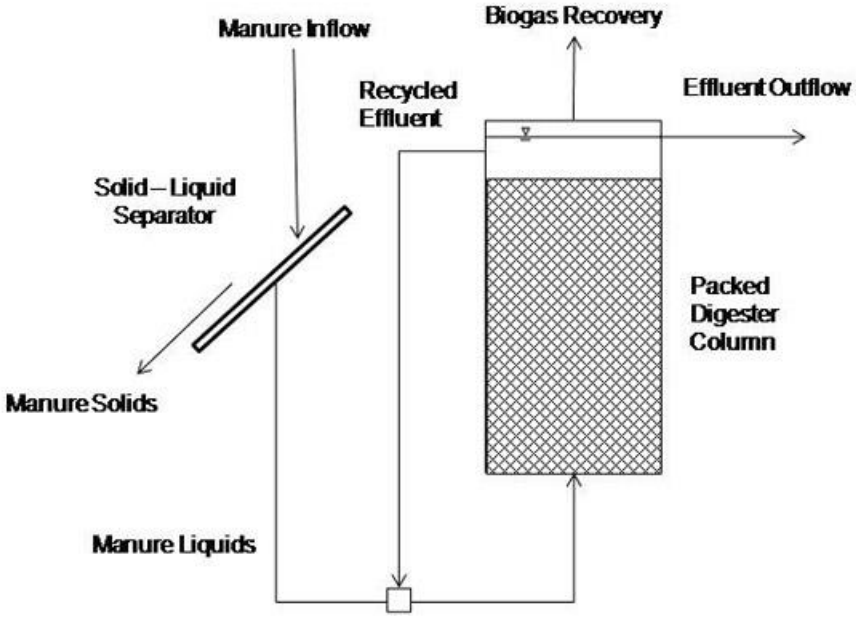


Schematic diagram of anaerobic contact digesters

stirred tank (digester) is settling under anaerobic conditions while a part of settled sludge is returned to the digester unit which results in concentration of sludge with longer retention time. Under such conditions, growth of methanogenic microbes takes place over wide range of loading. Bacterial load separation is hindered by gassing of the effluents, whereas the effluent is normally degassed before settling of biomass. They have wide range of application which includes treatment of effluents from sugar processing, distilleries, citric acid and yeast production, industries producing canned vegetables, pectin, starch and meat products as well as farm slurries.

Anaerobic Packed Bed Reactors (Packed Column Reactors)

In such type of reactors the organisms are mixed within the packing medium in an enclose chamber while liquid wastes passes upwards. Formation of slime does not occur due to organisms on packing while regular backwashing prevents from clogging. Removal rate depends on the surface area of packing. For packing material usually polystyrene spheres or Neptune-Microfloc filter containing sand, silica or a



Schematic diagram of anaerobic packed bed column

Upflow anaerobic sludge blanket reactor

Such type of reactor requires active bacteria in the form of high density granular sludge collected within the digester tank despite gassing and upflow velocity of effluents. Sludge granulation formation is a complex process but initially 10-15%

inoculation of granular sludge is needed. Hydrodynamics formation within the digester created by feed distribution and shape of the digester will determine the retention of correct granular form while well adapted sludge may be sufficient in 1% volume only. The feed substrate mainly consisting of particles or granular elements of calcium, phosphorous, magnesium, ammonia, aluminum and silicon. For undergoing complete process inside the digester, a large population of filamentous microorganisms is very essential. Development of granular sludge takes place with the dissolved wastes while the baffles within the digester promote gas solid separation and upward liquid velocity. Apart from these, baffles also provide larger surface area which assists in formation of biomass flocculation. The nature of baffles must corrosion-resistant whereas long chain fatty acids had be proved to be toxic to methanogenic bacteria but the addition of calcium chloride reduces such toxicity enables better granulation.

Schematic diagram of upflow anaerobic sludge blanket column

Membrane bioreactors

Such type of reactor is based on membrane separation technology useful in removing the inhibitory effects of biodegradable pollutants. Mainly applied for removal of volatile organic compounds like Dichloroethylene (DCE), the whole unit consists of effluent inlet chamber allowing the incoming wastewater containing pollutants to cross through the membrane and reach over biofilm. Biodegradation occurs within the biofilm unit while the aerating gas as well as the wastewa

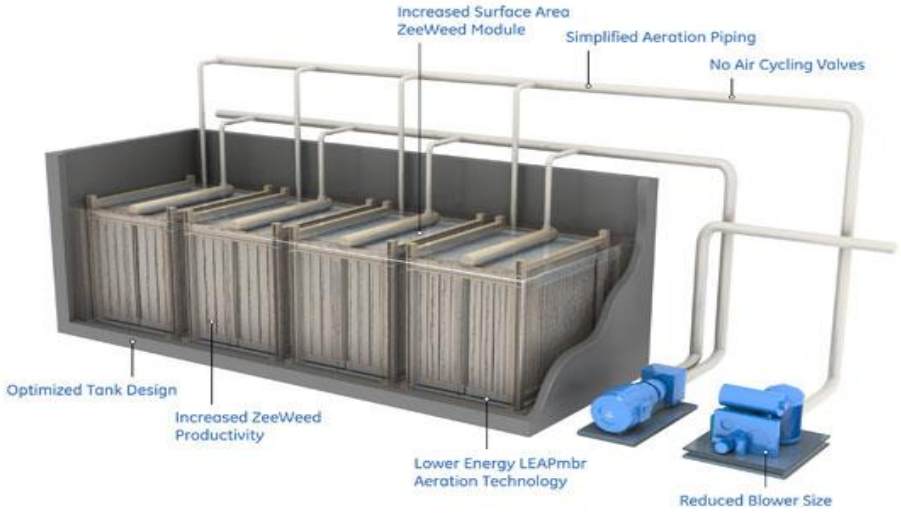


Figure: Membrane bioreactor

Utilisation of immobilized enzymes or microbial cells for effluent treatment

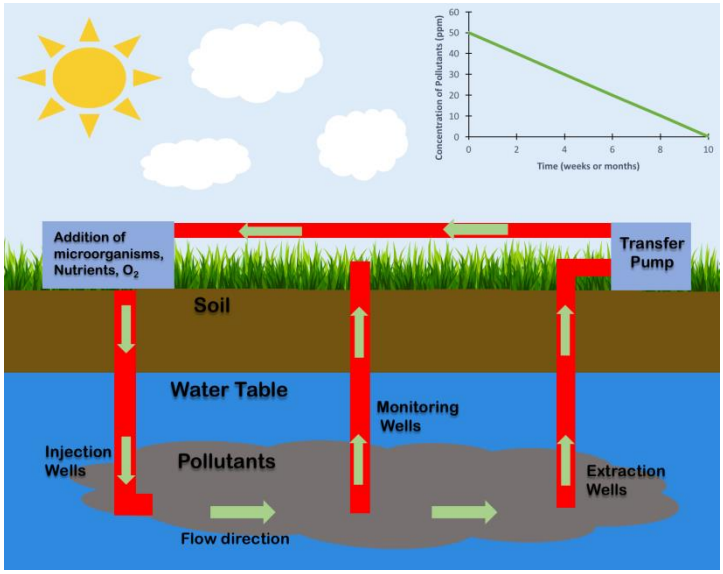
For treatment of effluents fixed film system and suspended growth are the two most common types. Since both types of processes are based on microbial growth, metabolic activities of microorganisms, eventual death of organisms sludge production and bioconversions taking place during the entire pathway therefore, immobilization of biocatalyst cells or enzymes can provide an added advantage to the treatment system. An immobilized biocatalyst can be termed as biocatalyst in which the movement of cells or enzymes are completely fixed or severely restricted to develop a distinct phase within the bulk phase where the substrate, effector, inhibitor molecules are dispersed and exchange is made.

Bioremediation

Bioremediation is a treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances. At sites filled with waste organic material, bacteria, fungi, protists and other microorganisms keep on breaking down organic matter to decompose the waste. If such environment is filled with oil spill, some organisms would die while some would survive. Bioremediation works by providing these organisms with different materials like fertilizer, oxygen and other conditions to survive while helping the organic pollutant to break at a faster rate. In other words, it can help to clean up oil spill. Bioremediation is important as it uses no chemicals which are one of the key issues since usage of man-made chemicals in the treatment and removal of contamination is that the chemicals eventually make it into the water supply. There were many chemicals used at the beginning of the waste management era which are known to be very harmful to plant, animal and human life once they reached the water supply. On the other hand bioremediation allows waste to be recycled. When chemical remediation types are used, the waste is still contaminated just with a less toxic substance and in general, cannot then enter into the recycle process therefore bioremediation allows for more waste to be recycled while chemical methods still create waste that cannot be used and has to be stored somewhere.

There are two classes of bioremediation based on the general application of the organisms. The two classes are:

- ***In-situ*** –refers to when contaminated waste is treated right at its point of origin. For example, there may be soil that is contaminated. Rather than remove the soil from its point of origin, it is treated right where it is. The benefit to in situ treatment is that it prevents the spread of contamination during the displacement and transport of the contaminated mater.
- ***Ex-situ*** –refers to treatment that occurs after the contaminated waste has been removed to a treatment area. To use soil as the example again, the soil may be removed and transported to an area where the bioremediation may be applied. The main advantage to this is it helps to contain and control the bioremediation products, as well as making the area that was contaminated available for use.



Schematic flow of bioremediation process

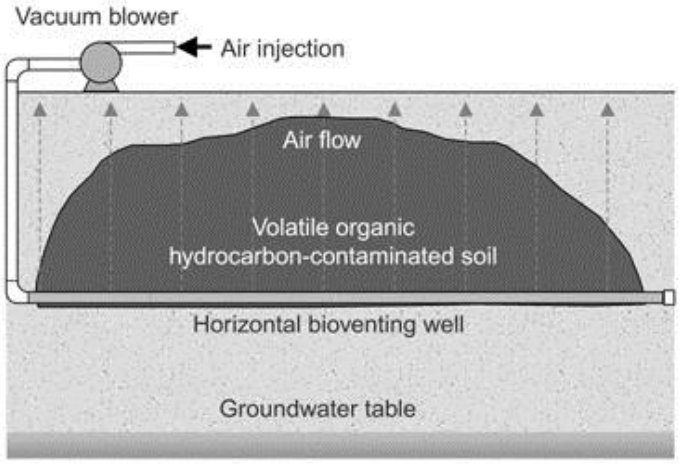
The major advantage of bioremediation method is that it allows the contamination to be treated, neutralized or removed and then produces a waste product which itself more easily disposed of while in some cases, there is no need for disposal at all. In case of plants, they utilize phytoremediation and rhizofiltration method through process called bioaccumulation in removing the contaminants. This means is holds onto the contaminant. As the plant is still growing, there is no need to remove and destroy it. In case of contaminated waste, it is the plant that keeps growing to allow for more storage of waste which is uniquely a cost effective solution for contaminated waste. The factors responsible for microbial bioremediation to be effective include:

- **Microbial Population:** Suitable kinds of organisms that can biodegrade all of the contaminants

- **Oxygen:** Enough to support aerobic biodegradation (about 2% oxygen in the gas phase or 0.4 mg/L in the soil water)
- **Water:** Soil moisture should be from 50–70% of water holding capacity of the soil
- **Nutrients:** Nitrogen, phosphorus, sulfur, and other nutrients to support good microbial growth
- **Temperature:** Appropriate temperatures for microbial growth should be between 0–40˚C
- **pH:** Must be ranged from 6.5 to 7.5

Bioventing

Bioventing is an *in situ* remediation technology that uses microorganisms to biodegrade organic constituents adsorbed on soils in the unsaturated zone. Bioventing enhances the activity of indigenous bacteria and simulates the natural in situ biodegradation of hydrocarbons in soil by inducing air or oxygen flow into the unsaturated zone and, if necessary, by adding nutrients. During bioventing, oxygen may be supplied through direct air injection into residual contamination in soil. Bioventing primarily assists in the degradation of adsorbed fuel residuals, but also assists in the degradation of volatile organic compounds (VOCs) as vapors move slowly through biologically active soil. The rate of natural degradation is generally limited by the lack of oxygen and other electron acceptor (i.e., a compound that gains electrons during biodegradation) rather than by the lack of nutrients (i.e., electron donors). In conventional bioventing systems, oxygen is delivered by an electric blower to subsurface wells. In contrast to soil vapor vacuum extraction, bioventing uses low airflow rates to provide only enough oxygen to sustain microbial activity. Passive bioventing systems use natural air exchange to deliver oxygen to the subsurface via bioventing wells. A one-way valve, installed on a vent well, allows air to enter the well when the pressure inside the well is lower than atmospheric pressure. When atmospheric pressure drops (due to a change in barometric pressure) below the subsurface pressure, the valve closes, trapping the air in the well and increasing oxygen to the soil surrounding the well. The major application of bioventing includes treatment of VOCs, petroleum hydrocarbons, non-chlorinated solvents and some pesticides & wood preservatives



Schematic diagram of Bioventing process

Bioremediation of Xenobiotics

'Xenobiotic' means foreign to the biological community i.e., substances which are not or negligibly present in nature but are introduced to the environment through anthropogenic activities. Organisms are not exposed to these chemicals and hence their biological mechanisms do not have a pathway for metabolising such compounds. As a result, a large number such chemical are not easily degraded by indigenous microflora and fauna. The chemicals used for various purposes like pesticides, paints and dyes can be categorized into biodegradable and non-biodegradable (recalcitrant). A biodegradable chemical can be converted by microbial action into non-toxic compound within few months and hence may be considered too hazardous. However, a recalcitrant chemical can persist in the environment for several years in toxic form. Such chemicals are introduced into environment mainly by untamed human activities although some natural processes such as erosion, oil seepage and volcanic eruptions are also known to be contributors. Many of these recalcitrant compounds are highly persistent in nature and lead to removal of elements from biogeochemical cycles. With increase in environmental awareness, degradation of xenobiotics has become an intense area of research.

Xenobiotics substances include a family of semi-volatile organic pollutants of polycyclic aromatic hydrocarbons (PAHs) which include anthracene, pyrene, benzo(a)pyrene, benzo(b)flouranthene, chlorobenzenes, dioxins, bisphenol A and chlorophenols. There are typically two sources of PAHs in which one of them is spilled or released petroleum products and the other is the chemicals used for various materials such as pesticides, paints, dyes and etc.

Many xenobiotic compounds are toxic in nature and adding to the toxicity is their bioconcentration in organisms and biomagnification along with food chains, which finally affects human beings. Their concentration in the environment when diluted varies from ppm (parts per million) to ppb (parts per billion) levels and at lower levels they may not effect at all. Further many physical, chemical and biological processes are applied for removal of xenobiotics. Biological approaches involve extensive biodegrading capabilities of certain microbes that have been extremely successful in many cases even for in-situ remediation. However, for effective bioremediation, the pollutant must be amenable to metabolic transformation in which product must be safe with having environmental conditions favourable for microbial activity besides having cost-effective and eco-friendly process. To achieve harmony among these varying factors is difficult task since environmental conditions are hardly conducive for microbial growth. However, after optimization of different environmental parameters with suitable modifications, biodegrading microbes can be made to grow optimally.

Bioremediation of Heavy Metals

Degradation of heavy metals is a major concern since they mainly originate from municipal waste incinerators, car exhausts, as residues from metalliferous mining industry, smelting industry, urban compost, pesticides, fertilizers, sludge and sewage. Due to rapid growth in industrialization in residential areas is one of the major causes for contaminants being discharged into sewage thereby creating environmental pollution. Since urban sewage releases effluents containing large amounts of heavy metals such as Ni, Cr, Pb, Cd and Co with high salt load capable of causing alkalinity and salinity hazards which in turn reduces soil microbial activity, soil fertility and yield loses.

The heavy metals present in the soil can be either removed by conventional chemical methods or by bioremediation. Conventional method employs technology having stringent physio-chemical agents which drastically inhibits soil fertility and damage the ecosystem. Numerous methods such as chlorination, use of chelating agents and acid treatment at high temperature have been utilized to remove heavy metals from sewage sludge. However, such methods are difficult to apply due to high operational cost and difficulty as well as low metal leaching efficiency. Therefore utilization of microbes and plants for decontamination of heavy metals has emerged as growing attention because of several drawbacks associated with removal of pollutant using conventional practices. Bimineralization is a bioremediation method involving microbial leaching in which hazardous wastes are removed using microbes or plants from the soil. It can play a major role in several sectors ranging from bioremediation of contaminated groundwater to bioleaching of metals present in trace amounts. Many fungi can be effective for biomineralization, utilizing an integral approach to increase the soil fertility along with decontamination of sites facing problem of heavy toxicity.

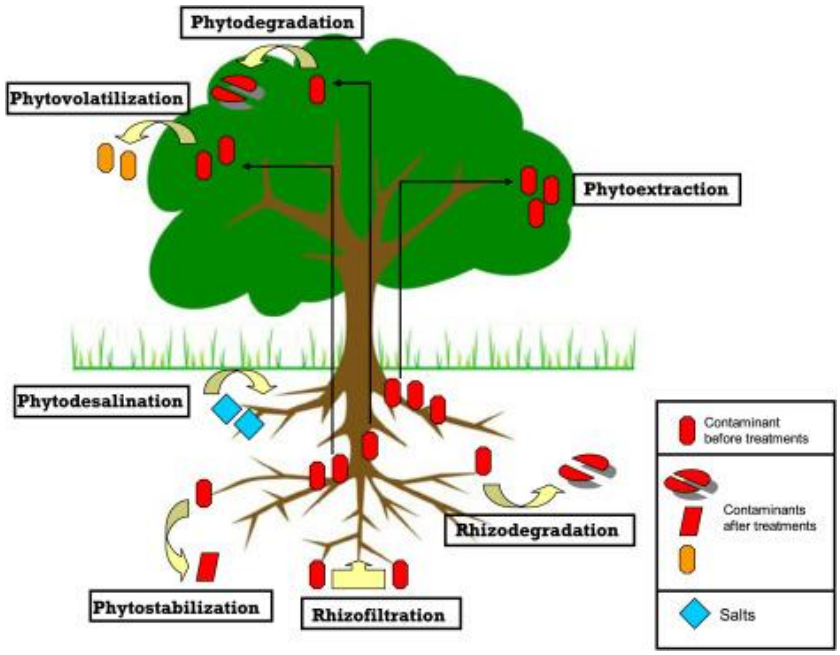
Phytoremediation

Phytoremediation ('phyto' means plant) is a generic term for the group of technologies that use plants for remediating soils, sludges, sediments and water contaminated with organic and inorganic contaminants. Phytoremediation can be defined as "the efficient use of plants to remove, detoxify or immobilise environmental contaminants in a growth matrix (soil, water or sediments) through the natural biological, chemical or physical activities and processes of the plants". Plants are unique organisms equipped with remarkable metabolic and absorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the growth matrix, soil or water. Phytoremediation involves growing plants in a contaminated matrix, for a required growth period, to remove contaminants from the matrix, or facilitate immobilisation (binding/containment) or degradation (detoxification) of the pollutants. The plants can be subsequently harvested, processed and disposed.

Plants have evolved a great diversity of genetic adaptations to handle the accumulated pollutants that occur in the environment. Growing and, in some cases, harvesting plants on a contaminated site as a remediation method is a passive technique that can be used to clean up sites with shallow, low to moderate levels of contamination. Phytoremediation can be used to clean up metals, pesticides, solvents, explosives, crude oil, polyaromatic hydrocarbons, and landfill leachates

How does phytoremediation works?

There are several ways in which plants are used to clean up, or remediate, contaminated sites. To remove pollutants from soil, sediment and/or water, plants can break down, or degrade organic pollutants or contain and stabilise metal contaminants by acting as filters or traps.



Different process involving in phytoremediation

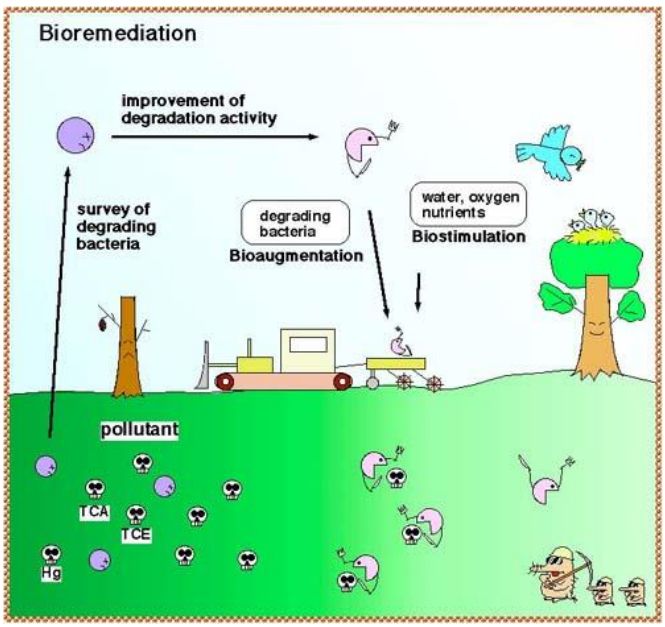
The uptake of contaminants in plants occurs primarily through the root system, in which the principal mechanisms for preventing contaminant toxicity are found. The root system provides an enormous surface area that absorbs and accumulates the water and nutrients essential for growth, as well as other non-essential contaminants. Researchers are finding that the use of trees (rather than smaller plants) is effective in treating deeper contamination because tree roots penetrate more deeply into the ground. In addition, deep-lying contaminated ground water can be treated by pumping the water out of the ground and using plants to treat the contamination.

Plant roots also cause changes at the soil-root interface as they release inorganic and organic compounds (root exudates) in the rhizosphere. These root exudates affect the number and activity of the microorganisms, the aggregation and stability of the soil particles around the root, and the availability of the contaminants. Root exudates, by themselves can increase (mobilise) or decrease (immobilise) directly or indirectly the availability of the contaminants in the root zone (rhizosphere) of the plant through changes in soil characteristics, release of organic substances, changes in chemical composition, and/or increase in plant-assisted microbial activity.

Phytoremediation is an alternative or complimentary technology that can be used along with or, in some cases in place of mechanical conventional clean-up technologies that often require high capital inputs and are labour and energy intensive. Phytoremediation is an in situ remediation technology that utilises the inherent abilities of living plants. It is also an ecologically friendly, solar-energy driven clean-up technology, based on the concept of using nature to cleanse nature.

Bioaugmentation

Bioaugmentation is the practice of adding cultured microorganisms into the subsurface for the purpose of biodegrading specific soil and groundwater contaminants. Since the 1970s, bioaugmentation, or the addition of oil-degrading microorganisms to supplement the indigenous populations, has been proposed as an alternate strategy for the bioremediation of oil contaminated environments. The rationale for this approach is that indigenous microbial populations may not be capable of degrading the wide range of potential substrates present in complex mixtures such as petroleum or that they may be in a stressed state as a result of the recent exposure to the spill. Other conditions under which bioaugmentation may be considered are when the indigenous hydrocarbon-degrading population is low, the speed of decontamination is the primary factor, and when seeding may reduce the lag period to start the bioremediation process For this approach to be successful in the field, the seed microorganisms must be able to degrade most petroleum components, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and move through the pores of the sediment to the contaminants. Different microbial species have different enzymatic abilities and preferences for the degradation of oil compounds. Some

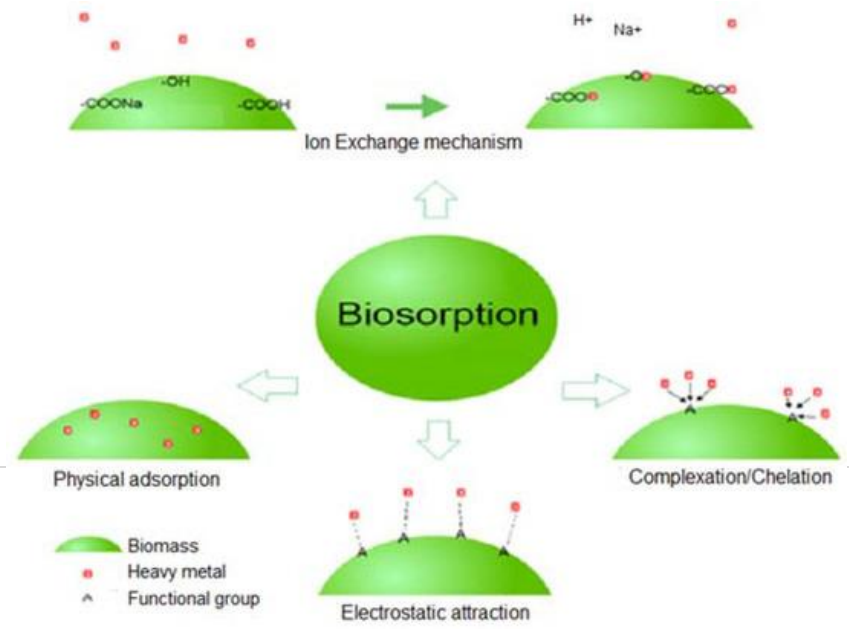


microorganisms degrade linear, branched, or cyclic alkanes. Others prefer mono- or polynuclear aromatics, and others jointly degrade both alkanes and aromatics. The study of microbes in bioremediation systems makes possible the selection of microorganisms with potential for the degradation and production of compounds with biotechnological applications in the oil and petrochemical industry.

Successful bioaugmentation treatments depend on the use of inocula consisting of microbial strains or microbial consortia that have been well adapted to the site to be decontaminated. Foreign microorganisms (those in inocula) have been applied successfully but their efficiency depends on ability to compete with indigenous microorganisms, predators and various abiotic factors. Factors affecting proliferation of microorganisms used for bioaugmentation including the chemical structure and concentration of pollutants, the availability of the contaminant to the microorganisms, the size and nature of the microbial population and the physical environment should be taken into consideration when screening for microorganisms to be applied. Bioaugmentation involves the introduction of microorganisms isolated from the contaminated site, from a historical site or carefully selected and genetically modified to support the remediation of petroleum hydrocarbon contaminated sites based on the assumption and/or confirmation that indigenous organisms within the impacted site cannot biodegrade petroleum hydrocarbon.

Biosorption

Biosorption involves uptake or binding of heavy metals or radionuclides to cellular components. Plant, algae and microbial biomass can be actively utilized for metal recovery by enhancing their metal-binding ability i.e., capable to remove metal species from aqueous solutions. Presently bacteria and biopolymers are being used for biosorption. Biosorbents may be described as natural ion-exchange materials that primarily contain weakly acidic and basic groups, where the chelation process is unspecific. Metals can be stripped from the matrix after loading by H_2SO_4, HCl, NaOH or complexing agents where dead biomass or live bacteria are used. The system may further be regenerated by employing alkali treatment for further application in sorption-desorption cycles.



Biosorption process of heavy metal

Addressing Pesticide Pollution through Bioremediation Technology

According to world health organisation (WHO) pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease such as mosquitos, and in agriculture to kill pests, that damage crops. By their nature pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly.

The UN Food and Agriculture Organisation estimated that in developing countries, pests, weeds and disease destroy about 40 per cent of crops while they are still in the fields and 6 to 7 per cent of them after the harvest. In Africa and Asia, the pre-harvest losses are estimated at 50 per cent. Many researchers (Cramer,1967; FAO, 1975; Pimentel, 1992; Oerke et al.,1995) have put global crop losses due to pests between one-third and one-half of the attainable crop production, with crop losses in developing countries at the higher side. The crop damage is caused highest by insects, followed by pathogens and weeds. Consequently, the use of chemical pesticides in agriculture has been an integral part of crop production in many regions, often at very high levels and unscientific pattern of application (Atreya, 2007; Devi, 2010; Shetty et al., 2010). The role of pesticides in augmenting agricultural output has been well perceived and these have been considered as essential inputs in agricultural production.

History of pesticide development

The history of pesticide development and use is the key to understanding how and why pesticides have been an environmental threat to environmental systems, and why this threat is diminishing in developed countries and remains a problem in many developing countries. Stephenson and Solomon (1993) outlined the chronology presented in Table 1. Agricultural use of pesticides is a subset of the larger spectrum of industrial chemicals used in modern society. The American Chemical Society database indicates that there were some 13 million chemicals identified in 1993 with some 500 000 new compounds being added annually. Dominant organochlorine group of pesticide including hexachlorocyclohexane (HCH) has been extensively used since 1950 IN India for pest control in agricultural production, food and seed storage, and forestry. HCH has mainly been applied in two formulations: i) pure grade gamma-HCH (lindane) and ii) technical grade mixture of HCH isomers (alpha-beta-gamma-delta). Due to their toxicity, persistence, and serious environmental problems gamma-HCH and technical grade HCH gradually been banned and their use is now restricted in most countries. Unfortunately, HCH residues still remain present in many agricultural soils where they had been applied previously. Due to long persistence and toxicity of

organochlorine pesticide, the use of organophosphorus (OP) pesticide has been growing as they were considered environmentally less problematic (due to their biodegradability) and biologically more efficient. At present, OP pesticides constitute the largest group of pesticides used globally (34%).

Pesticide manufacturing scenario in India

The manufacturing and use of pesticides has been rising extensively in India and generation of waste by pesticide industry has become an environmental threat due to available insufficient waste management technology. The available information and reports suggest that pesticide residues present in surface soil leading to toxicity in soil. In India, the production of pesticide started at Rishra, very close to Calcutta during the year of 1952 with production of benzene hexachloride (BHC) at larger scale. Subsequently, Hindustan Insecticides Limited started production of {1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane} (DDT) on 1954 with the objective of supplying DDT for National Malaria Eradication Programme launched by Government of India. Since 1972, India Pesticides Limited started production of DDT in a large scale at Deva Road of Barabanki District near Lucknow. During course of time many more pesticides industries came up and started their production in several pockets in India. Now India is the second largest manufacturer of pesticides in Asia after China and ranks twelfth globally (CLI, 2002). The growth in production of technical grade pesticides from 5,000 metric tonnes in 1958 to 102,240 metric tonnes in 1998 was reported to be very sharp and steady (Geetha and Fulekar, 2008). However, very recent data shows that, the production capacity of pesticide in India is now increased to 132,000 metric tonnes (Indira Devi et al., 2017). Table 2 shows the production, import, export and consumption of pesticides in India.

Major group of pesticide uses in India

Organochlorine group of pesticide: HCH is synthesized by photochemical chlorination of benzene. During this process several isomers are formed of which the most abundant ones are designated as alpha, beta gamma and delta HCH. The isomers are formed in the approximate proportion of alpha HCH (60-70%); beta HCH (5-12%), gamma HCH (10-15%) and delta HCH (6-10%) (Walker et al., 1999). Off all the isomers only gamma HCH has insecticidal activity. During gamma HCH purification, the other non-active isomers were buried or dumped without further treatment which resulted as creating highly contaminated areas. Conversely, the OP pesticides are esters or thiols derived from phosphoric, phosphonic or phosphoramidic acid (Sogorb and Vilanova, 2002). The general chemical structure of OP is shown in Figure 1. R1 and R2 are generally aryl or alkyl groups that are bonded to P atom either directly (phosphinates) or through oxygen (phosphates) or sulphur (phosphothioates) atom. In phosphonates, R1 is directly bonded to the P

atom and R2 is linked either to an oxygen or sulphur atom (phosphonothioates). At least one of the R groups is linked to –NH2 in phosphoramidates. Finally, the X group which is also called the 'leaving group' (because this group is released upon hydrolysis of OP compounds) may be a halogen, aliphatic, aromatic or hetrocyclic group.

Fate and effects of pesticides

The mode of action of OPs involves inhibition of acetylcholine breakdown. Acetylcholine plays an important role in transmitting nerve impulses in the brain and skeletal and muscular systems. However, after the transmission, acetylcholine must be hydrolysed to avoid over-stimulation of the nervous systems. This hydrolysis is brought about by an esterase called acetylcholine esterase (AChE) which results in formation of choline and acetyl CoA. OP compounds bind to the active site of the AChE and then the leaving group breaks off from it resulting into phosphorylated AChE. The hydrolysis of phosphorylated AChE is extremely slow and results in the over-stimulation of the nervous system which in turn cause agitation, hypersalivation, confusion, convulsion, respiratory failure and ultimately death to insects and mammals.

The policy towards use of chemical pesticides during green revolution era has been promotive in nature. The policy support in favour of high yielding variety's (HYVs) naturally necessitated the application of chemical pesticides. Later on, these chemicals were used in a highly unscientific way, starting from the choice of chemical to the application practices, timing and even necessity (Devi, 2009). The pesticide application has often been adopted as a risk avoidance strategy, where the chances of pest incidence/critical pest population are often wrongly perceived to be on the higher side. The professionalism in the choice of chemical and its application has slowly been replaced by the private operators who handle the retail sales, mostly without any formal training or information on these aspects. Consequently, the concerns on the negative externalities of chemical pesticides across the world have resulted in increasing awareness on the pesticide use, especially in the socioeconomically advanced societies (Kerala, for example). Despite this, the supply side of pesticide management is largely a neglected area, where the operators lack proper awareness, training, and education (Devi, 2015).The situation is further polluted by the poor monitoring and regulatory mechanism (Devi, 2010). The WHO reports that the negative externalities due to pesticide exposure as more prominent in the developing economies despite their lower consumption. It indicates adoption of unscientific use practices that exist in these regions.

Mitigation measure of pesticide contamination

One of the options to mitigate HCH risks would be to mineralize it to CO2 and H2O and chloride, which might be achievable by targeted bioremediation. Bioremediation or bio-augmentation of contaminated soils through exploitation of microbial catalytic activities have been increasingly projected as an alternative to more costly physical and chemical methods such as incineration (Table. 3). However, OP compounds are metabolised by four major reactions: hydrolysis, oxidation, alkylation and dealkylation. Hydrolysis of the phosphoesteric P-O-C or phosphothiesteric P-S-C bonds present in the OP molecules is considered the initial step in their metabolism. The major intermediate products are diethyl thiophosphate (DETP) and 3,5,6-Trichloro 2-methoxypyrimidine (TCP) and finally mineralised to CO2. These approached convert compounds from toxic to less toxic which in some cases can accumulate in the environment and still be toxic to a range of organisms. Bioremediation provides a suitable way to remove contaminants from the environment as in most of cases, OP compounds are totally mineralised by the microorganisms. Most organochlorine (OC) and organophosphorus (OP) compounds are degraded by microorganisms in the environment as a source of carbon, energy or both. Several soil bacteria have been isolated and characterised which can degrade OP compounds in laboratory cultures and in the field. Several potential bacteria and consortium of bacteria have been developed by different group of researchers. Few of them are tested at filed level for bio-augmentation. Furthermore, several genes/enzymes which provide microorganisms the ability to degrade OC and OP pesticide compounds have been identified and characterized. Some of these genes and enzymes have been engineered for better efficacy. Bacteria capable of complete mineralization are constructed by transferring the complete degradation pathway for specific compounds in one bacterium.

Solid Waste management

The term 'solid waste' refers to different kinds of wastes that are normally solid in nature which are produced due to various activities of humans and animals, and are discarded as useless or unwanted substance. Solid waste are also defined as heterogeneous mass refuse by urban dwellers along with accumulation of agricultural, industrial and mining wastes. Solid waste management encompasses all the activities related to control of production, storage, collection, transportation, processing and disposal of solid wastes in a way that is in accord with best principles of public health, economics, technology and environmental factors. The scope of solid waste management includes administrative, planning, financial and engineering functions that are involved in providing solutions to problems related to disposal of solid wastes.

Biological conversion process for treatment of wastes

In recent years, for treating solid wastes and in particular Municipal Solid Waste (MSW), biological conversion is the most preferred and economical process of waste treatment. Using such type of method both volume and weight of solid wastes can be reduced, producing a humus-like material and compost acting as a soil conditioner. Apart from these, methane gas is also generated depending upon the process. The main type of organisms involved in biological transformation of organic wastes includes bacteria, fungi, yeasts and actinomycetes. The operational process is either performed in the presence of oxygen known as aerobic composting or absence of oxygen known as anaerobic digestion. Depending upon the process the nature of end products also differs since conversion reactions of aerobic and anaerobic processes are differently accomplished.

Composting is a biological method majorly used by municipalities to manage the organic solid waste. Biodegradation is bio-technique suitable for xenobiotic compounds. Bioremediation is a natural process which uses microorganisms to treat the pollutants from water or soil.

Aerobic Composting of Solid Wastes

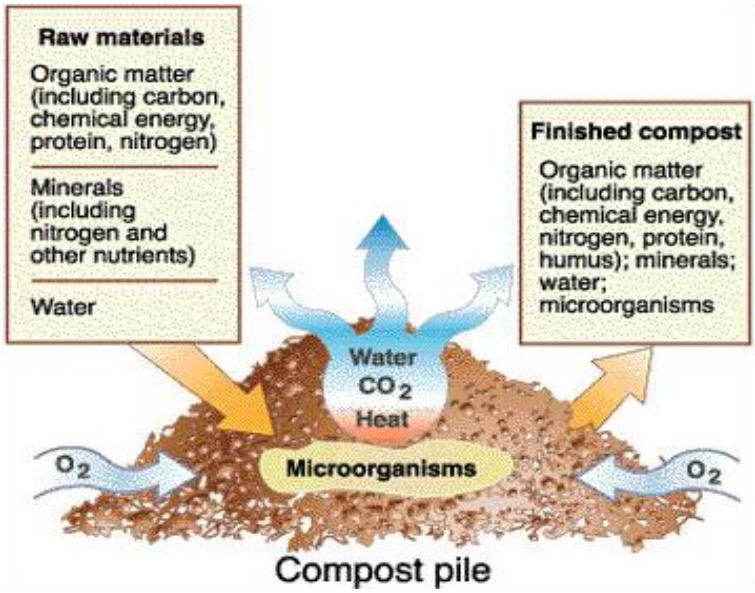
Among the available technologies, composting is one of fundamental method that suits various scales of application, from individual level to community level. The process involves decomposition of waste and treatment of waste water in controlled conditions with the help of micro-organism and Flea present in the nature. It is small scale decomposition process, in which the controlled parameters such as moisture, pH, and temperature and C/N ratio are considered.

The common materials which are composted easily are leaves, grass, animal food, old herbs & spices, newspapers, vegetable, bread, cereals & organic waste etc. The materials that are avoid for composting are plastic, bones, meat, synthetic

chemicals, diseased plants and animals etc. Additional materials such as saw dust and fibre powder can be applied for quicker breaking of organic compounds.

This process involves the following easy steps to complete its process:

- Preparation of site for the compacting
- Selection of type of composting
- Separation of the waste (food and vegetable waste)
- Collection of dry organic matter (saw dust, sludge)
- Addition of of waste layer by layer
- Covering the container with plastic cover to maintain required moisture content and temperature.



Aerobic composting process

In this process the organic matter is converted to humus like substance, in which the final product looks like soil, acting as a good carbon and nitrogen source thus, providing as optimum medium for growth of plants. At the end of composting process, the final product produced increases the soil sensibility to hold water and makes soil easier to cultivate. However, the entire decomposition process is controlled by various factors such as availability of oxygen, nutrients, moisture content and nature of waste. Under control conditions, within a short period of 4 to 6 weeks of time, the yard waste as well as the organic fraction of solid waste is converted into stable organic residue known as compost.

The transformation reaction taking place during the aerobic composting of Solid Waste can be represented as:

microorganism

Organic matter + nutrients + O_2 ⟶ new cell mass + residual organic matter + CO_2 +H_2O +NH_3 +SO_4^{2-} + heat

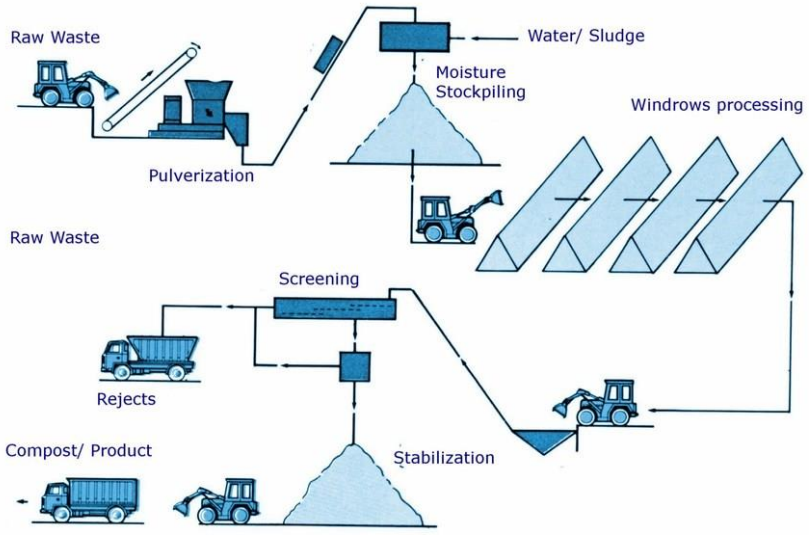
The residual organic matter is the compost consisting mainly of cellulose, lignin, and inorganic substances.

Composting systems

Pre-processing of MSW for composting includes receiving, removal of inorganic or non-biodegradable materials, size reductions, adjustments of C/N ratio, addition of moisture along with nutrients as per requirement.

Windrow composting

It involves the pre-processed MSW or industrial solid waste placed in a windrow in an open field. Windrows are made by dumping the solid waste into small heaps having height 2.5 to 3 m and 6 to 7.5m wide at the base. Smaller heap size will ensure better control whereas the composting period would normally last for 4 to 5 weeks, during which the windrows are needed to be turned once or twice per week in between to ensure better power supply and maintaining proper operating temperature around 55°C as well. Various consortiums of microbes occurring naturally inside the surrounding environment would assist decomposition of organic matter whereas completion of decomposition is indicated by drop in temperature. In order to ensure complete stabilization of solid waste, composted material should be cured for another 2 to 4 weeks in open windrows.



Stack pile system

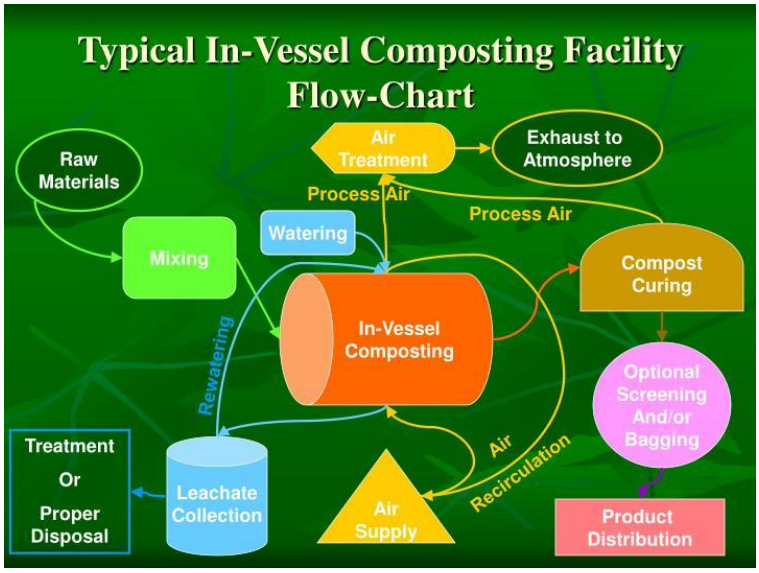
It involves grid based exhaust perforated piping system over which dumping of pre-processed organic material is made. The pile heights are about 2 to 2.5m and in order to control odours emitted during the initial stages and also to provide proper insulation, a layer of screened compost shall be placed on top of each newly formed

pile. To facilitate proper aeration control, each pile should be provided with individual blower while air is introduced through disposable plastic drainage pipes which provide oxygen needed for biological conversion and also help to control temperature within pile. The entire process takes about 3 to 4 weeks for composting the organic material, whereas curing period takes another 4 weeks.

Schematic process of stack pile composting

In vessel composting

In such type of composting system, the entire process is comprised of vessel or enclosed container of any size, shape including vertical towers, horizontal circular and rectangular tanks. It can either be of plug type or agitated bed type. The plug flow system operates in form of first-in and first-out basis whereas in agitated bed system, the material is mixed mechanically during processing.



Schematic process flow of In vessel composting

Design aspects of aerobic composting

For designing an efficient composting system, the most desirable particle size must be less than 5cm, since smaller size increases the biochemical reaction rate of composting. The ideal carbon to nitrogen (C/N) ratio for entire process lies between 25 and 30. In some cases, the ratio either becomes very low or very high therefore; it is prerequisite to blend carbon and nitrogen in proper proportions to bring the ratio to 30 whereas to maintain desired moisture content of 50-60% dry material must be blended with wet material but moisture <40% is undesirable for composting process. Seeding or inoculation of microbial culture depends on the volume as well as the fraction of decomposition of organic fraction of solid waste. The duration of mixing or turning of organic wastes during composting with 55-60% moisture content takes about a period of 15 days, in which the first turning can be achieved on the third day and thereafter on every alternate day. Since aerobic composting is based upon microbial activity therefore the type of organisms involved are either mesophilic or thermophilic. Composting beds is maintained either at 30-38°C or 55-60°C for mesophilic and thermophilic microbes respectively but however the entire process is exothermic in which bed temperature rises automatically. Apart from temperature microbial waste stabilization is also dependant on pH. The pH value of composting bed varies from time to time as the process continues. The initial pH of organic fraction of solid waste or MSW is normally between 5 and 7 while at the beginning of composting system pH reduces to 5 or less and population of mesophilic microbes increases rapidly as a result temperature also rises rapidly from ambient conditions. In about 3 days' time the temperature reaches the thermophilic stage whereas the pH begins to rise to 8 or 8.5 and this continuous for remainder of aerobic process. At end of final stage when temperature starts to decrease and the compost becomes matured, the pH value settles between 7 and 8.

Anaerobic composting of solid waste

Anaerobic composting of solid waste is a process for treating solid waste biologically in the absence of oxygen. It can be also termed as anaerobic fermentation or anaerobic digestion in which methane gas is produced along with compost during the process. Three groups of microorganisms are involved in anaerobic composting process. One group of microbes is responsible for hydrolysing organic polymers and lipids to fatty acids, monosaccharides, amino acids and others while a second group of anaerobic bacteria ferments the chemical produced by first group to simple organic acids such as acetic acid. The second group of organisms consists of facultative or obligate anaerobes identified as acetogens whereas the third group of microbes which are strictly anaerobes convert

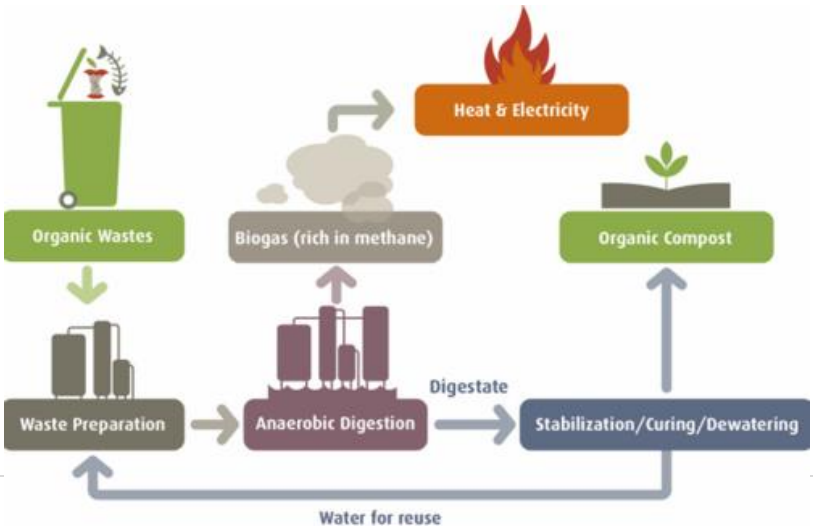
the hydrogen and acetic acid formed by acetogens into biogas (methane and CO_2). The microbes of third group are known as methanogens.

To achieve efficient performance in anaerobic composting system, both methanogens and non-methanogens bacteria must be kept in state of dynamic equilibrium as well as keeping the reactor content free from dissolved oxygen and excess ammonia. The pH should be between 6.5 and 7.5 since the digestion mixture needs to be alkaline in nature. Alkalinity range must be varied from 1000 to 5000mg/L while the volatile fatty acid concentration should be below 250 mg/L. To establish proper growth of biological community, sufficient amount of nutrients such as nitrogen and phosphorous must also be present. The transformation reaction taking place during the aerobic composting of Solid Waste can be represented as:

$$\text{Organic matter of solid waste} + H_2O \longrightarrow \text{new cells} + \text{residual organic matter} + CO_2 + CH_4 + NH_3 + H_2S + \text{heat}$$

Types of anaerobic digestion

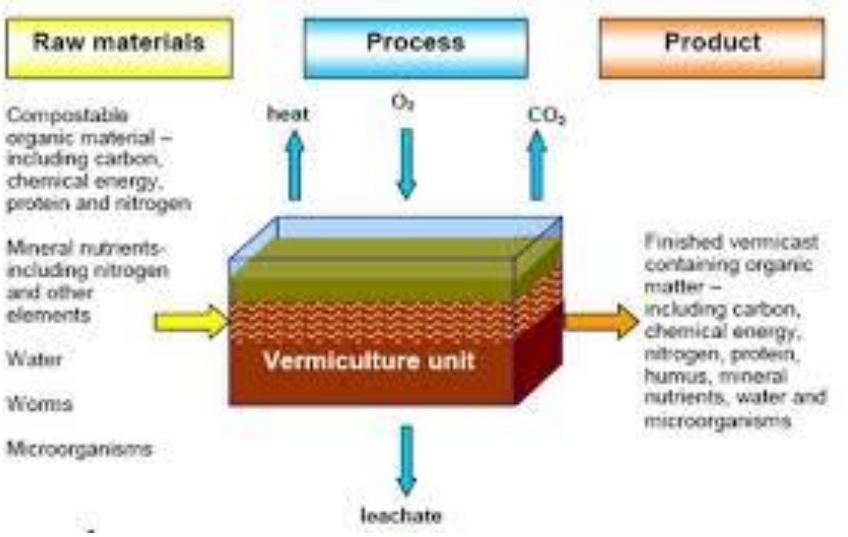
Anaerobic digestion can be either of low solids or high solids type. Both types differ in concentration of solids in the digester. In case of low solids, the solid concentration equals to or less than 4-8% whereas the solid concentration for high solid anaerobic digestion process is 22% or more. In production of methane from low-solids organic fraction of solid wastes involves three basis steps which involve preparation of organic fraction of solid waste such as receiving, sorting, separation and size reduction. The second step includes addition of moisture and nutrients, blending, pH adjustment to 6.8 as well as heating of slurry if digestion is to take place in thermophilic range of 55-60°C whereas the final step comprises separation of gas, dewatering and disposal of digested sludge. Similarly, high solids anaerobic digestion also involve three steps however, the major difference between the two is that for high solid process, less effort is required to dewater and dispose of digested sludge.



Process flow of anaerobic composting system

Vermicomposting

Vermicomposting is a type of composting in which certain species of earthworms are used to enhance the process of organic waste conversion and produce a better end-product. It is a mesophilic process utilizing microorganisms and earthworms. Earthworms feeds the organic waste materials and passes it through their digestive system and gives out in a granular form (cocoons) which is known as vermicompost. It is earthworm excrement, called castings, which can improve biological, chemical, and physical properties of the soil. The chemical secretions in the earthworm's digestive tract help break down soil and organic matter, so the castings contain more nutrients that are immediately available to plants.



Process flow of vermicomposting

How is vermicompost produced?

A wide range of organic residues, such as straw, husk, leaves, stalks, weeds etc can be converted into vermicompost. Other potential feedstock for vermicompost production are livestock wastes, poultry litter, dairy wastes, food processing wastes, organic fraction of MSW, bagasse, digestate from biogas plants etc. Earthworms consume organic wastes and reduce the volume by 40–60%. Each earthworm weighs about 0.5 to 0.6 gram, eats waste equivalent to its body weight and produces cast equivalent to about 50% of the waste it consumes in a day. The moisture content of castings ranges between 32 and 66% and the pH is around 7. The level of nutrients in compost depends upon the source of raw material and species of earthworm applied while the types of vermicomposting depend upon the amount of production and composting structures. Small-scale vermicomposting is done to meet personal requirements and farmers/gardeners can harvest 5-10 tons of vermicompost annually whereas large-scale vermicomposting is done at

commercial scale by recycling large quantities of organic waste in modern facilities with the production of more than hundreds of tons annually.

There are nearly 3600 types of earthworms which are divided into burrowing and non-burrowing types. The most commonly earthworms utilized for vermicomposting includes pontoscolex corethrurus, Megascolex konkanensis, Lampito mauritii, Drawida willsi, Lumbricus rebellus, Octochaetona surensis, Amynthas cortices and Metaphire houlleti however, red earthworm species, like *Eisenia foetida,* and are most efficient in compost making. The non-burrowing earthworms eat 10% soil and 90% organic waste materials; these convert the organic waste into vermicompost faster than the burrowing earthworms. They can tolerate temperatures ranging from 0 to 40°C but the regeneration capacity is more at 25 to 30°C and 40–45% moisture level in the pile. The burrowing types of earthworms come onto the soil surface only at night making holes in the soil up to a depth of 3.5 m and produce 5.6 kg casts by ingesting 90% soil and 10% organic waste. The types of vermicomposting depend upon the amount of producton and composting structures. Small-scale vermicomposting is done to meet personal requirements and farmers/gardeners can harvest 5-10 tons of vermicompost annually whereas large-scale vermicomposting is done at commercial scale by recycling large quantities of organic waste in modern facilities with the production of more than hundreds of tons annually.

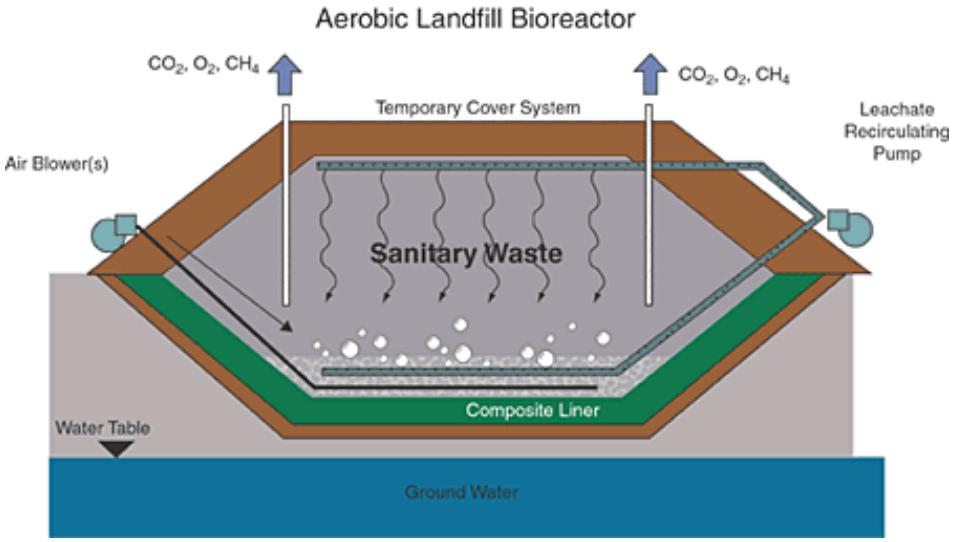
Advantages of vermicomposting

- It reduces household garbage as well as agro residues and yard waste disposal costs
- Produces fewer odours and attracts fewer pests
- The method saves water and electricity consumed by kitchen sink garbage disposal units
- It produces free, high quality soil amendment (compost) having lower C/N ratio than traditional backyard compost.
- The compost produced requires only little space, labour and maintenance
- Process is much faster

Landfill Bioreactor for solid waste treatment

A bioreactor landfill changes the goal of landfilling from the storage of waste to the treatment of waste. A bioreactor landfill is a system that is isolated from the environment and that enhances the degradation of refuse by micro-organisms. The bioreactor landfill technology is based on the acceleration of the anaerobic degradation of the biodegradable fraction of the wastes leading to faster mineralization and hence stabilization. The most widely used and understood method of creating a landfill bioreactor is the recirculation of leachate, because the element that usually limits microbial activity in a landfill is water. By recirculating leachate and/or adding additional liquid wastes, bioreactor landfills operate to rapidly degrade and transform organic wastes. The intensity and duration of waste

stabilization are primarily reflected by characteristic changes in leachate and gas quantity and quality. In the absence of inhibition, these microbially mediated changes are temporally and spatially predictable and are influenced by the availability and sufficiency of moisture, nutrients, and



substrates. Accordingly, moisture accumulates and becomes more uniformly distributed with leachate recirculation, waste stabilization in each compartment of a landfill bioreactor progresses through the initial, transition, acid formation, methane formation, and final maturation phases. During these five phases, biodegradable waste constituents are converted into intermediates and end products, primarily by initial hydrolysis to intermediate substrates supporting acidogenesis and subsequent utilization of the products of acidogenesis as precursors to gas formation during methanogenesis. The nature of the ecological milieu established during the phases of landfill stabilization is a reflection of the transformation processes occurring within the multiple reaction zones of the bioreactor landfill system. Many of these processes can be described in terms of established reaction mechanisms as the overall system progresses towards an equilibrium or steady-state condition. Therefore, a landfill compartment operated with leachate recirculation is essentially analogous to an attached growth anaerobic process with internally generated and diminishing organic substrate loadings, unless leachate from other landfill compartments is applied. This sequencing batch-type configuration features retention times regulated by leachate recirculation intervals. Moreover, in the absence of inhibition, a natural progression of microbial populations develops and proliferates until substrates and/or environmental conditions become limiting. The thermodynamic favorability of reactions recognized as potentially operative during landfill stabilization. Approximately in all cases, the role of hydrogen (H_2) is apparent and has led not only to the suggestion that H_2 will regulate reaction opportunity and pathway, but the relative predominance of process intermediates as well. In comparison to a "dry-tomb" landfill, bioreactor landfill technology has shown an early stability, reduced

leachate disposal costs, increased capacity, and increased LFG recovery. In addition to elevated moisture content, elevated temperatures are typical in a bioreactor landfill. Typical temperature ranges are from 45 to 60°C. The recirculation of leachate increases the moisture content of the refuse in the landfill and, therefore, promotes microbial degradation. If leachate recirculation alone cannot raise the moisture content to levels at which microbial growth is enhanced (40% by weight, minimum), water may need to be added to the waste. Methanogenesis is the ultimate process reached by a stabilizing MSW. Optimum moisture contents of 60% and above wet mass hasten the degradation towards methanogenesis. Anaerobic conditions are essential indicating an optimum redox potential below −100 mV. Alkaline environments with pH ranging from 6.0 to 8.0 and an alkalinity of around 2000 mg/L are favourable for methanogens. Temperatures anywhere between 35 and 45°C can support methanogenic population and useful nutrients to maintain biomass are usually adequately available in MSW.

Cutting edge technologies for the sustainable management of wastewater

Global water crisis is one of the burning problems in the 21st century mainly caused by exponentially increasing incessant population expansion, climate change, urbanization, industrialization, rising livelihood standards etc. Water has a direct impact onorganic and inorganic components of aquatic systems and contributes in maintaining the integrity of our life. Wastewater is any water which has been adversely affected by various anthropogenic activities. Hence, proper disposal and management of wastewater is a major concern for the researchers worldwide. From the beginning of humankind, adapting to the accessibility or in accessibility of water assets has been a basic component of individuals. The quality of water determines the growth of a civilization. There are numerous meanings of sustainability supporting different principals and ideas. Basically, sustainability can be portrayed as 1) an arrangement of objectives; and 2) practices and conduct that support such objectives. Sustainability is vital on the grounds, that the decisions and activities of today, influences everything later on. Conventional sustainable waste water resource management mainly includes small and copious tools like rain water harvesting, filtration and reuse of water which does not cover broad management of waste water. Hence in this scenario we need some cutting edge technologies which not only provide management issues rather provide an insight towards recovery of the essentials from the waste water. Developing a cutting edge technologies consist of great ideas and broad applications. Among the broad spectrum of great ideas our focus lies on, development of water saving sensors, Geo-informatics based application and management by utilizing metal nanoparticle. These three procedures can be explored and managed in various references of time and space and within contexts of environmental, social and economic order. (Corcoran et al, 2010).

Global and Indian scenario of water crisis

Various factors like culture, climate, work and working conditions, food habits, and physiology of common people decides the requirement of water. According to the Bureau of Indian Standards, IS:1172-1993, a base water supply of 200 liters per capita per day (lpcd) should be accommodated for domestic consumption utilized in urban communities with full flushing frameworks (Shaban and Sharma, 2007). IS:1172-1993 additionally specifies that the measure of water supply might be decreased to 135 lpcd for the LIG (low income group) and the monetarily weaker sections (MWS) of society in small towns(Shaban and Sharma,

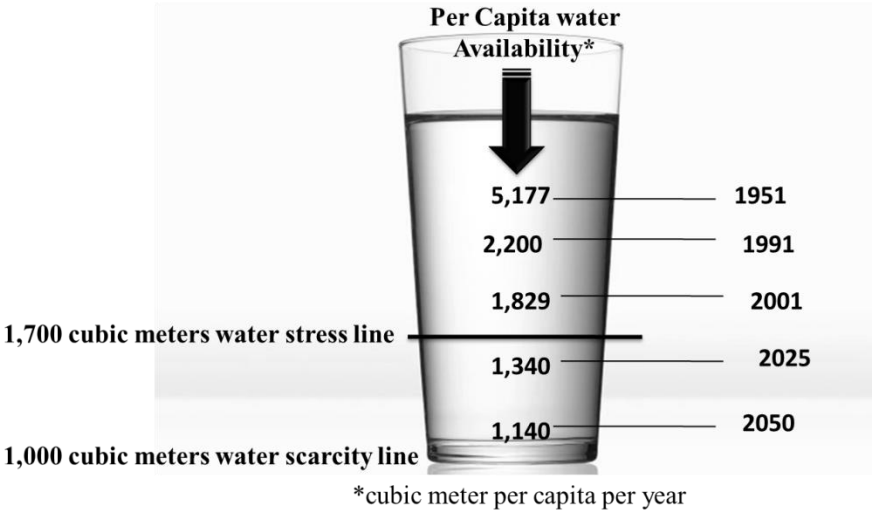
2007). According to the 10th plan (2002-2007), the cities based on the planned sewerage are classified in two different sectors based on the population statistics, metropolitan and non-metropolitan areas. In metropolitan areas water supply level is 150 lpcd and in non-metro Politian areas it is 135 lpcd. According to National commission on Urbanization per capita water supply should be 0-100 liters per day for hygienic life style to all citizens (Shaban and Sharma, 2007). The World Health Organization (WHO) has categorized supply and access to water in 4 categories. These are:

(1) No access (Water availability below 5 lpcd)
(2) Basic access (Water availability approximately 20 lpcd)
(3) Intermediate access (Water availability approximately 50 lpcd)
(4) Optimal Access (Water availability average of 100-200 lpcd)

Population growth is one of the main drivers of freshwater demand. Most projections estimate that the world population will stabilize at between 8 and 9.5 billion people by around 2050 and most of this growth will take place in the developing world (UNDP, 2004). Urbanization is another demographic factor whose trend results in greater water use. Theproportion of the world's population residing in cities was only 14% in the early 1900s and still only 29% in the 1950s. Since then, the urban population has grown rapidly, and more than 60% of people are expected to live in cities by 2030 (UNDP, 2004). Prices, subsidies and taxes often inadvertently, discourage efficient water use. Governments in developing countries give out 75% of the world's water subsidies, amounting to 45 billion USD annually (UNDP, 2004).

Water consumed in most of the Indian cities is not determined by the demand but the supply. People attempt to adjust to the quantity (as well as quality) of water supplied. The 54th round of National Sample Survey Organisation (NSSO) data reveal that 80 per cent of the households in urban India, across different segments, consider that they have sufficient water supply (Bajpai and Bhandari, 2001), while the study provided by Abdul Shaban and R. N. Sharma in their article "Water Consumption Patterns in Domestic Households in Major Cities", finds that about 71 per cent of the households in Delhi, greater Mumbai, Kolkata, Hyderabad, Kanpur, Ahmedabad and Madurai consider the water supply adequate. But the city-wise figures are 73 per cent in Delhi, 77 per cent each in Mumbai and Kolkata, 49 per cent in Hyderabad, 75 per cent in Kanpur, 63 per cent in Ahmedabad and 82 per cent in Madurai. In reality, this shows nothing but an adjustment made by people to the supply

such that they do not feel that more water is needed. This, in turn, creates hygiene and sanitation problems resulting in several health consequences (Shaban and Sharma, 2007). Water consumption for India was 987 billion m^3/yr for the time period 1997-2001 (Hoekstra and Chapagain, 2008), which means 980 m^3/yr per capita which lies below the global average of 1,243 m^3/yr. As provided by a seminar water day 2010 organized by CII New Delhi in 2010 which is depicted in Fig 1 clearly shows constantly declining per capita water availability by 2050.



Per capita availability of water in India

Concept of sustainability in waste water management

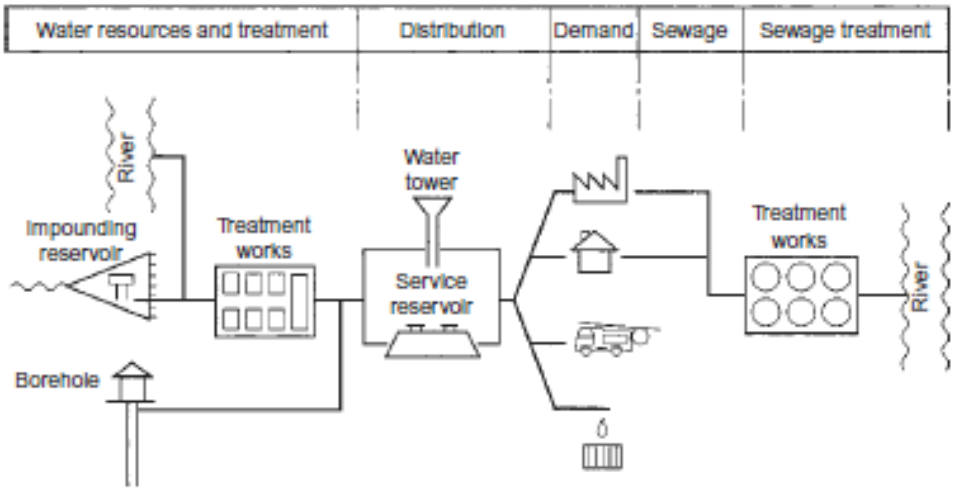
According to the Bruntdland Commission, sustainability is defined as **"Development that meets the needs of the present without compromising the ability of future generations to meet their own needs"** (Sustainable development, 2015).As depicted in Fig 2 Sustainability not only defined by the natural resources, rather it has two other components like social and economic resources (Corcoran et al, 2010). The goals of the sustainability is to have a bearable, and viable load put on the environmental resources by social resources and economic resources, in such a way that equitability must be maintained between social and economic resources.

Recycling and social issues are very much crucial regarding the evaluation of sustainability in wastewater treatment. Therefore selection of adequate system for certain condition is a crucial parameter while evaluating the sustainability. This whole process requires multidisciplinary method where engineers cooperate with social scientists, economists,

biologists, health officers and the general public to develop cutting edge technologies which can enlighten the effectiveness of waste water treatment both in developed, developing and under developed countries.

Sustainability approach in planned management of water service cycle

In different counties hydrological cycle is managed in such a way that sufficient water can be made available to the agriculture, industries and domestic use. This whole process requires proper management of ground water and surface water resources, the supply and treatment of water and its successive collection, purification and return to the cycle.(Fig 3). Water service cycle helps in counteracting the demand of fresh hygienic water; it has the potentiality to purify waste water available from either industries or swage and directs the water to different sectors involving in agriculture, industries or domestic purposes. The main components for water service cycle are treatment and distribution of water which helps in controlling the demand of fresh water.(Gray, 2010)



Management plan of water service cycle (Gray, 2010)

Integrated resource recovery (IRR)

Integrated resource recovery (IRR) is another concept within the waste water management which views waste as a resourceandplans for developed infrastructure which maximizes the recovery of the valued substances. As depicted in Fig 4IRR works on the principle where every source is seen as a resource and its recovery is designed with respect to nature and has a well architected land use and infrastructure decision.

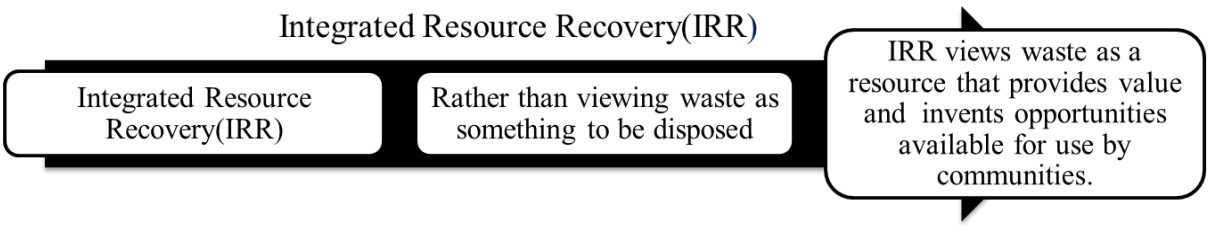


Fig4. *Flow Diagram for the Principle of IRR*

Its main focus is to recycle rather than avoid, reuse rather than reduce and recovery of certain things rather than disposing it. It visualizes waste as a wealth. As produced in Fig 5 the key objective of IRR is conversion of waste to wealth considering the parameters of Sustainability.

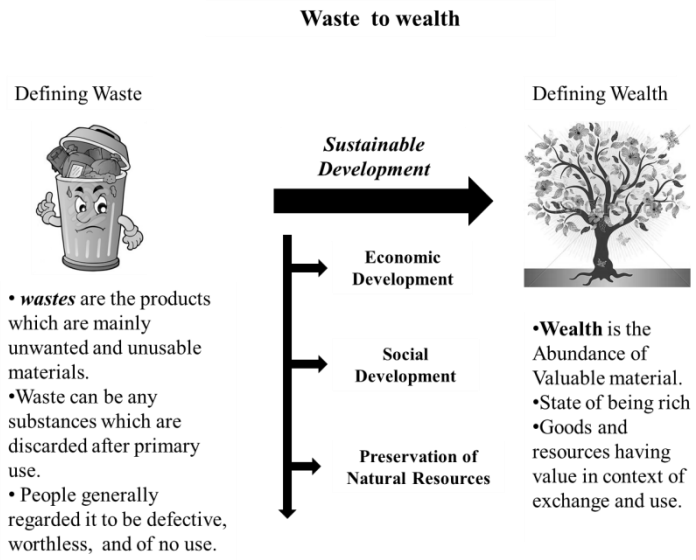
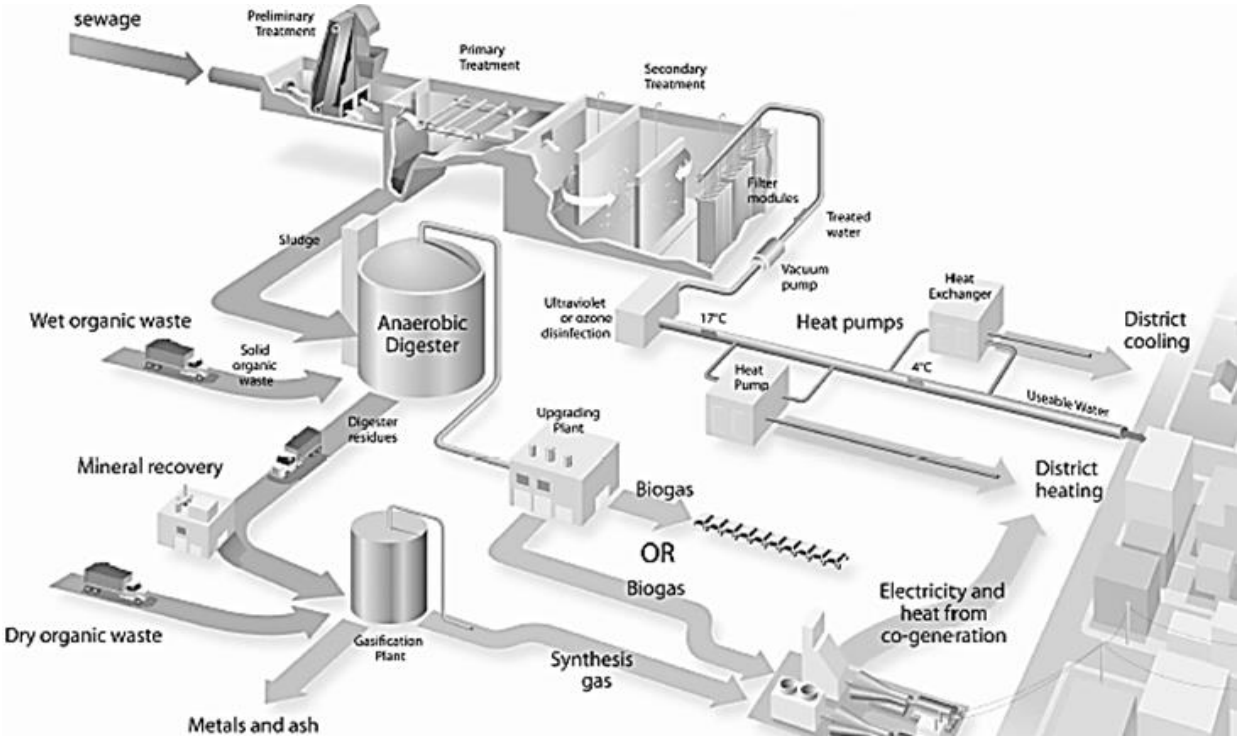


Fig5*. Key objective of IRR*

Utilization of Integrated resource recovery (IRR) to produce green energy from waste water



Green energy by utilizing IRR tools (Stephen,2009)

As discussed by Stephen (2009) which is shown in Fig 6 IRR consist of various tools, methods and techniques which can be utilized to produce green energy thus concreting the fact of using waste to build energy. Among several points, our focus lies on following five perspectives which are key point in recovery from green energy:

1. Energy system for heating and cooling
2. Reclaiming heat and cold from waste water using heat pumps
3. Reclaiming wastewater
4. Cogeneration of electricity and heat&
5. Other recovery processes

Table below clearly brings a comparison between resource consumption and resource production during green energy production and relating it to the benefits related to the components recovered from green energy

Table1.Comparison of resource consumption and resource production during green energy and relating it to both socio-economic benefits.

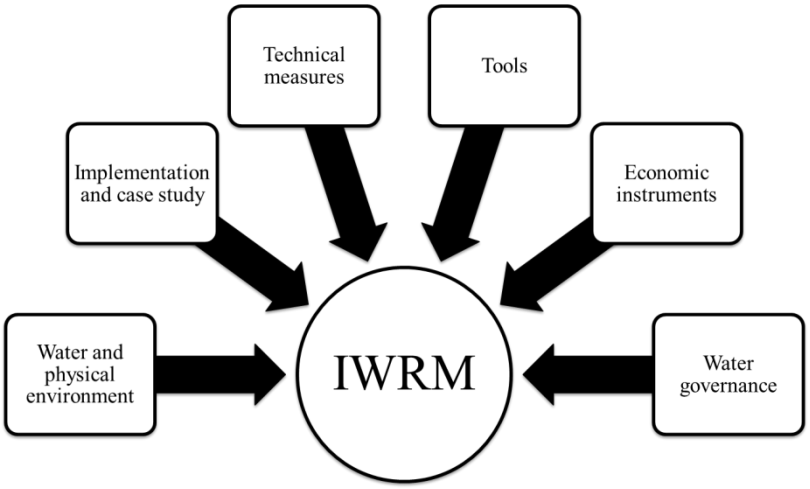
(Stephen, 2009)

Sl. no.	components recovered from green energy	Resource consumption	Resource production	Benefits
1	Energy system for heating and cooling	• Waste heat from cogeneration • Heat recovered from wastewater through heat pumps and • Electricity required to pump hot or cold water through district energy pipes.	• Heating for space heating and domestic hot water; and • Cooling for refrigeration and air conditioning	• Local sustainable employment; and • Increased energy independence.
2	Reclaiming heat and cold from waste water using heat pumps	• Electricity to operate heat pumps	• Heat for space heating and domestic hot water; and • Cooling for refrigerator and air conditioner	• Local sustainable employment • Increase energy independence with re fossil fuels.
3	Reclaiming wastewater	• Energy to treat wastewater and electricity to operate the pumps to move the water to users	• Reclaimed water for non-portable purposes.	• Environmental benefits of recharging and ground water; • Social benefits of amenity and beauti of water features which could not oth have been developed and • Economic benefits of using lower-cos reclaimed water for non-portable pur and savings in portable water treatme infrastructure.
4	Cogeneration ofelectricity and heat	• Cleaned gas from gasification	• Electricity and heat from greenhouse gas	• Local sustainable employment
5	Other recovery		• Biodiesel, liquid fuel,	

processes	cellulosic ethanol, hydrogen

Integrated resource recovery (IRR) in context to integrated water resourcemanagement (IRWM)

The integrated water resources management (IWRM) helps to control and expand water resources in a sustainable and reasonable way, taking in account of social, economic and environmental interests. It recognizes many different and challenging interesting groups, the sector that use and abuse water, and the needs of the environment. The integrated water resource management co-ordinates water resources management across various sectors and significant groups, and at different scales, from local to international (Stephen, 2009). It emphasizes on the involvement of national policy and law making processes, establishing effective governance and thereby creating effective institutional and regulatory arrangements as routes to more fair and sustainable decisions. A range of tools, such as communal and environmental assessment, financial instruments, and information and monitoring systems, must be developed to support this process (Fig 7).

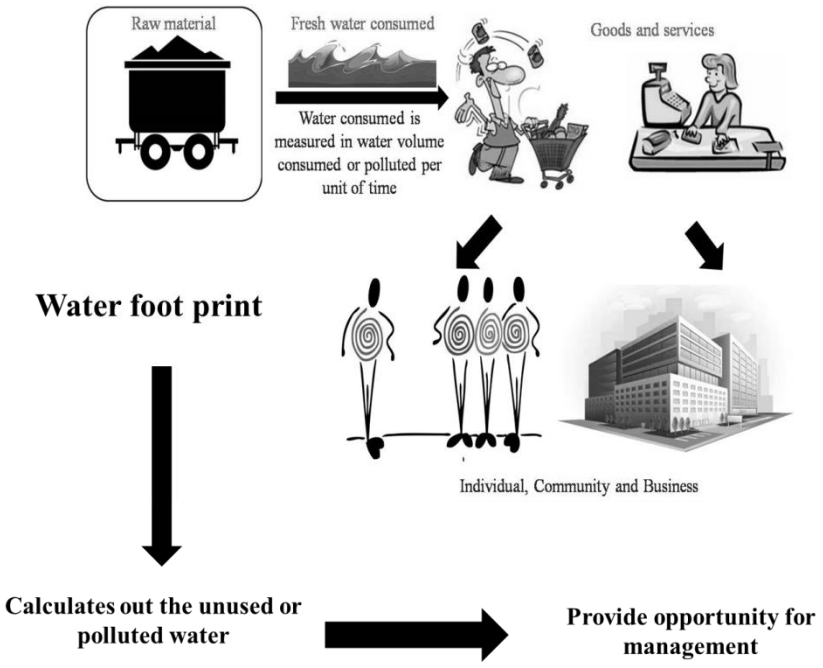


Components of IWRM)

Concept of water foot print

Water foot print provides a basal platform for the IRR to function. It provides basal pattern for the water utilized and gives accountable information regarding the water scenario in certain place at a point of time. As depicted in Fig 8Water footprintfor an individual, community and business is defined in terms of the entire volume of freshwater which is being consumed to produce the services and goods utilized by an individual, community, or produced by the

business. Water consumed is measured in water volume consumed or polluted per unit of time (Ercin and Hoekstra, 2014).Water foot print revels the water use pattern from individual level to the national level; it provides clear cut information regarding the uses of water in production of goods and services. Water footprint gives an account for the contamination of water due to industries and thus calculates out the unused or polluted water from the system (Ercin and Hoekstra, 2014).



Concept of Water Footprint

Water footprint consists of three components

Blue water Footprint: It is the amount of water and ground water necessary to make a product; the required water may be evaporated or directly used.

Green Water Footprint: it is the amount of rain water necessary to make a product. The required water may be evaporated or directly used.

Grey Water Footprint: It is the amount of water from the freshwater source required to mix or dilute pollutant to such context such that the water quality standards maintains.

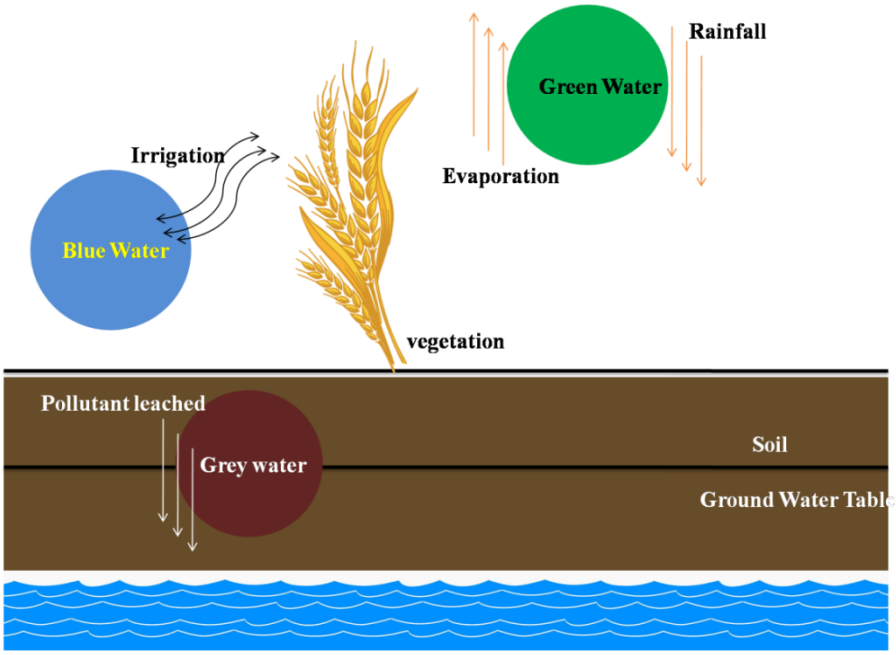


***Fig9**. Inter relationship of different types of water foot print (Blue, Green, Grey Water footprint)*

Application of Cutting edge technologies for sustainable wastewater management

In placing ourselves parallel to the above memorandum of concepts cutting edge technology not only represent itself as a technology, but also revolves around development of great ideas as put forwarded in Fig 10 following the principles of sustainability and IRR

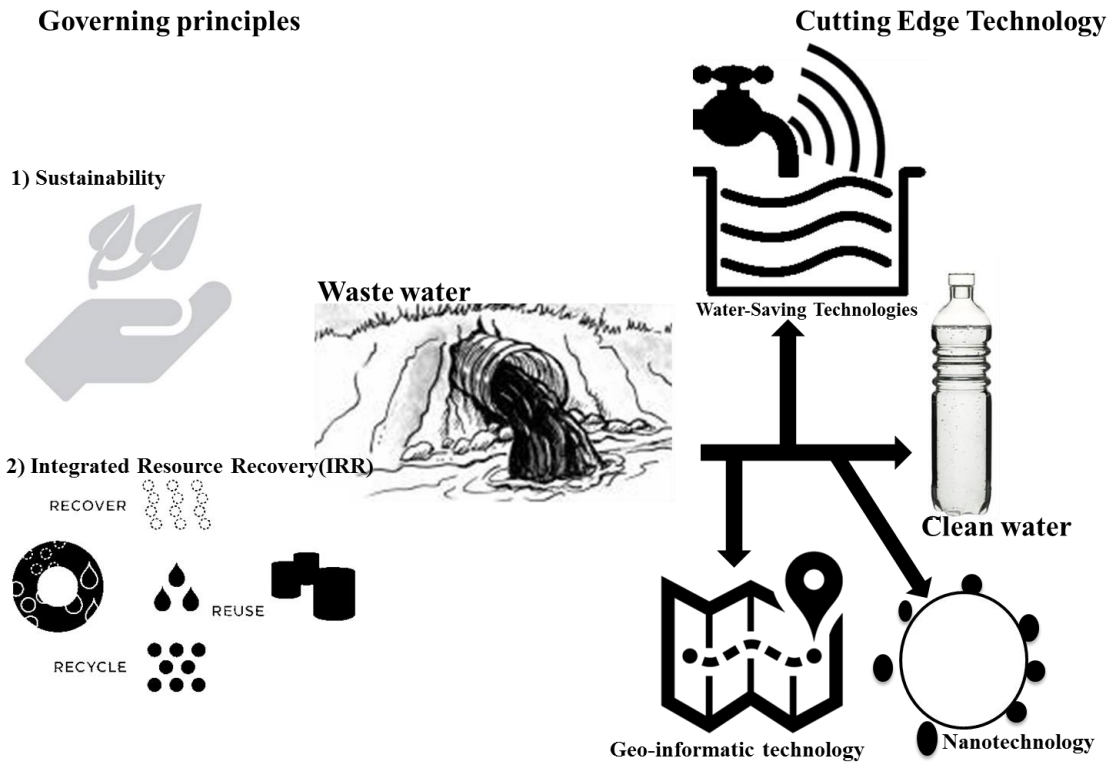


Fig10 *Application of the Technologies for sustainable wastewater management*

Among the ocean of so much vivid verified tools our focus relies on the three effective ideas which has the potentially to mark itself as an effective resource in waste water management. Those are:

- Development of water-savingtechnology
- Geo Informatics and its application in waste water treatment. And
- Use of nanoparticle for waste water treatment.

Development of water saving technology by utilizingwater saving sensors

A water savingsensorsaresome objects, whose purpose is to detect events or changes in its environment and sends the information to the artificial unit which then tells the output devices to provide the corresponding output.

Two types of water saving sensors can beutilized

1) Mechanical Sensor
2) Bio sensor

Mechanical Sensor

Various mechanical sensors can be utilized for saving the water from getting wasted. Few of them are listed below:-

The fill cycle diverter is a simple plastic device that can be used for older toilets that use 3.5 or more gallons of water per flush.

Watering gauges are small cup-like devices that can be used for the measurement of the amount of water utilized by a sprinkler over a given time.

An automatic faucet is equipped with asensoropens its valveto allow water to flow in, in which controls the flow of water through faucet by mixing air with the water as it flows through faucet. Flowalert is a depth-based alarm system offering an economical approach to wastewater collection system monitoring. This new wireless alarming system features a combination float switch and depth sensor device designed for deployment in networks requiring rapid notification and response to abnormal flow conditions

Biosensors

The development of biosensors has opened up great perspectives and, simplified cost-effective monitoring of water quality. In a biosensor, a biological recognition element combines with a physical transducer which converts the biological response into a signal depending on the analytic concentration(Chouler& Lorenzo, 2015).

Most of the biosensors are enzymatic, and operates via electrochemical means. Enzymatic biosensors have high selectivity towards the target analyte. The use of bacteria offers a great simplicity associated, with biocatalyst preparation. Microbial biosensors are versatile and sensitive, to a large variety of analytes. Microbial biosensors are mainly utilized as water quality monitoring devices (Chouler and Lorenzo, 2015).The use of various microbes that survive under highly adverse condition opens up attractive perspectives on water monitoring for industrial process. The full utilization of microbial biosensors is however faced with various difficult challenges (Chouler and Lorenzo, 2015). These include low detection limits, low selectivity, and risk of contamination with other microorganisms.

Geo-informatics and its application in waste water treatment

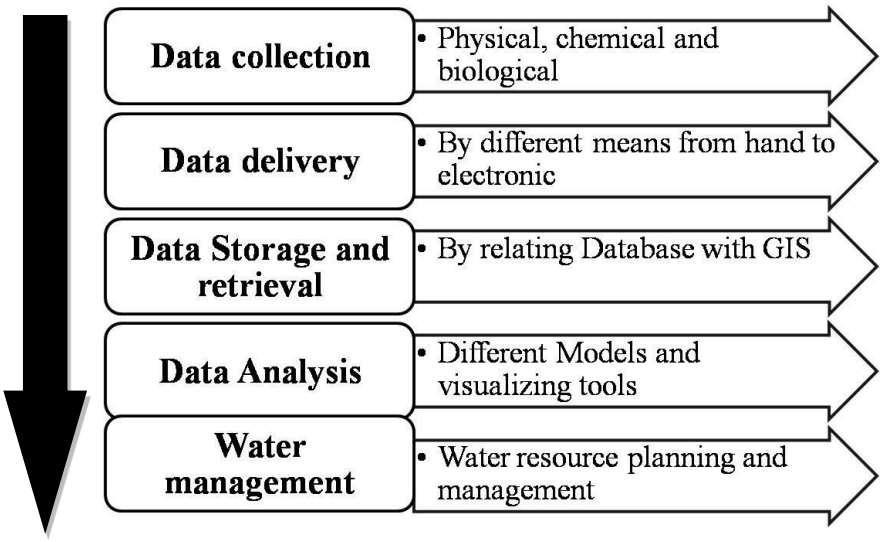
Geo-informatics is a data framework which is produced or utilized as a part of a worldwide setting. Within Geo-informatics lies Global information system (GIS), this is any data framework whose actions are to convey the totality of quantifiable information inside a characterized setting.

Application of Geo-informatics in waste water treatment

Water management requires a decent comprehension of the topographical space and related spatial data, for example, water sources, landscape surface, watershed, precipitation, temperature, moistness, soil condition and, topography, conditions on the climate, human exercises, natural information, and so forth. The exact learning of the landscape surface comprehends most hydrological procedures. There are numerous hydrological models (Maidment, 1993) for surface and subsurface water hydrology and GIS is the way that numerous scientists are attempting to use to deal with the data. The landscape surface is the medium on which numerous exercises of the water happen, for example, rainfall, water transport over the surface or through the surface, water system, flooding, plant evapo-traspiration, and this work is centeredon the territory surface.

Geo-informatics has huge applications inwastewater management because of the spatialcomponent of the system. In this way analysis, monitoring, and display of various components and waterish enabled. There are various software packages based on GIS and they are designed formanagement of wastewater systems. Detailed analysis consisting of a large database and its management, display by transverse and longitudinal profiles, monitoring, etc. isenabled by these software packages. Because of its unique characteristics, GIS can be used for location selection for wastewater treatment plant, which emphasize sits use in combination with multi-criteria analysis.

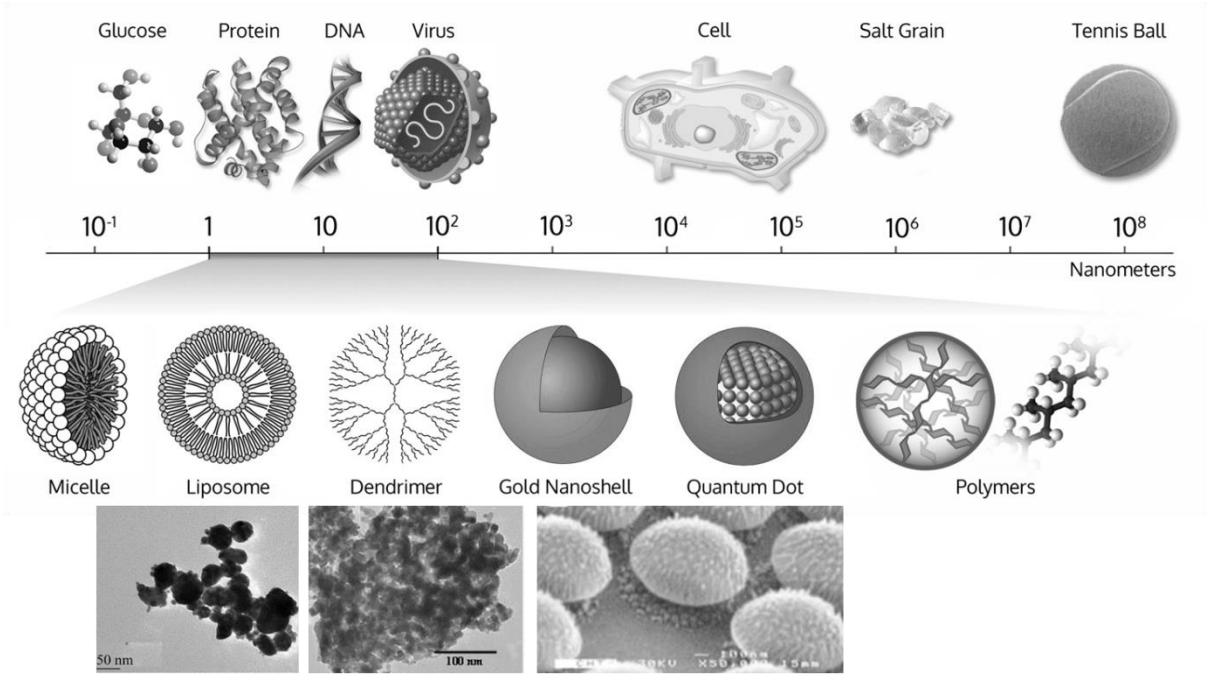
In a nut shell as shown in Fig 11 waste management by Geo-informatics module consist of data collection which can done by physical chemical and biological parameter, thus delivering data to different means or electronic sets and relating it toa main data base with GIS. These data's get analyzed in different software modules and which results into production of effective resource planning and management.



Flow chart for Water Management by Geo-informatics

Use of nanoparticle for waste water treatment

Nanotechnology, in Greek the word *"nanos"* refers a thing of one billionth (10^6 m) in shape and size.Nanoparticles are usually 0.1 to 100nm in each dimension from spatial angle of view as shown in Fig 12 (Sung et.al, 2004).

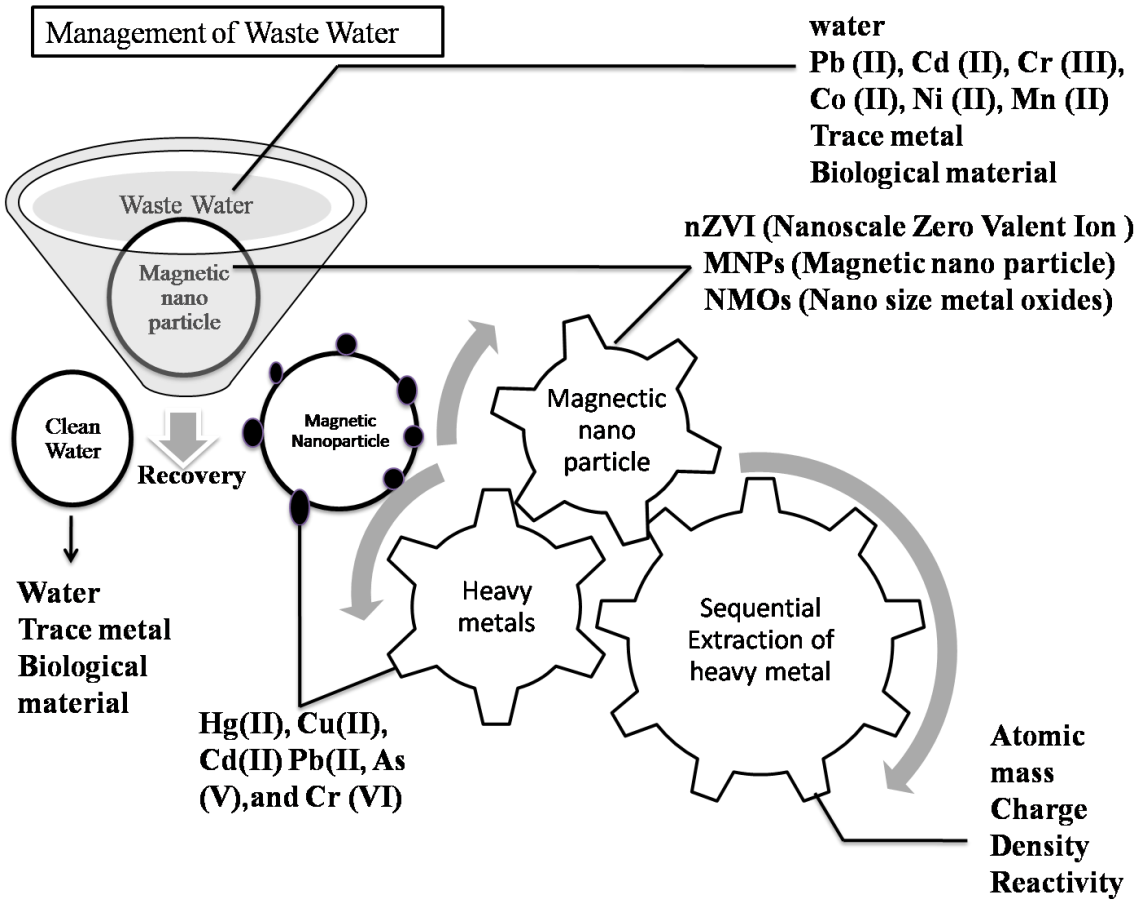


Comparison of Nanoparticles in context to real life and microscopic objects.

(Sung et.al, 2004)

Magnetic nanoparticles (MNPs) (Wassana et. al, 2007) with its name defines its special characteristic of possessing super paramagnetic properties, includes metal oxides like iron oxide $Fe^{3}O^{4,}$ nanoscaleZerovalemt ion (n ZVI) (Simeonidiset.al, 2016) could be able to absorb heavy metals on its surface. Other metal oxide nanoparticles (NMOs) which includes ferrite oxide, aluminum oxides, manganese oxide, magnesium oxide and titanium oxide bears the same properties like MNPs (Agrawal and Sahu, 2006).

From the flow chart depicted in Fig 13, waste water may contains heavy metals like Mn(II), Ni(II), Cd(II) Pb(II) with few trace metal and biological material. This waste water when put inside with solution of nanometals consisting of nZVI, MNPs and NMOs, due to its natural potential of being working as an absorbent it can remove out the heavy metals from water and thereby giving out water which may contains trace metal or biological elements. These heavy metals can be recovered according to their atomic mass, charge, density, reactivity, through sequential extraction procedure and can be utilized in various sectors like anti-microbial agents (Bakshi et.al, 2015), pest control, biosensor, drug delivery and energy generation.



Nanometal in waste water treatment

Hematite (α-Fe_2O_3) and Goethite (α-FeOOH) as shown in Table 2, poses high surface area which makes it an efficient absorbent, Cu^{2+} adsorption from goethite is much effective by pressure pump technique. CU^{2+} absorption on nano-hematite and nano-geolith in terms of, kinetics and dynamics are same. The adsorption process follows the pseudo-second-order kinetics. The equilibrium for the reaction involving the Cr (VI) removal by nano-maghemite (γ-Fe_2O_3),andnano-magnetite (Fe_3O_4) is independent of initial Cr (VI) concentration and the absorption capacity increases when pH of the reaction decreases. Nano-maghemite emerges as the high selectivity component for Cr (VI) from water. (Mandavian and Mirrhimi, 2010).

Superparamagnetic Fe_3O_4(iron oxide) as shown in Table 2, with surface functionalization coating of DMSA (Di-mercapto succinic acid) plays an effective role in adsorption of soft metal like Ag, Hg, Cd, Pb and Ti.(Cumbal and Sengupta, 2005). The stability, capacity, chemical affinities of magnetic, nanoparticle is much more stable then resin based adsorbents,

nano-porussilica(SAMMS) and activated carbon in river water, ground water, sea water and human blood. Fe_3O_4 coated with DMSA has the capacity to remove Hg, 30 times larger than resin based sorbents. Even for the removal Pb its takes just a minute for magnetic particle in comparison to resin based absorbent (Cumbal and Sengupta, 2005).

Superparamagnetic Fe_3O_4(iron oxide) coated with humic acid (HA) is effective in removal of Hg(II), Cu(II), Cd(II) and Pb(II) from water. Fe_3O_4 coated with HA could able to remove 99% of Pb(II) and Hg(II) in comparison to 95% of Cd(II) and Cu(II) from tap and natural water at, optimized pH (Cumbal and Sengupta, 2005).

3D flower like self-assembled nano particle of iron oxide has the potentiality of removal of As (V), Orange II and Cr (VI). Decreasing the diameter of nano-crystle of iron oxide nano particle from 300 nm to 12 nm will increase the efficiency of As (III) and As (V) by several orders (Cumbal and Sengupta, 2005).

Table 2.Shape, size, surface area, and targeted metals for various NMOs/MNPs

NMOs absorbent	**Targeted metal**
Goethite	Cu(II)
Hematite	Cu(II)
Amorphous iron oxide	Pb(II)
Hydrous manganese Dioxide	Pb(II)
MNPs absorbent	**Targeted metal**
Superparamagnetic Fe_3O_4(iron oxide) with surface functionalization by DMSA(di mercapto succinic acid)	Ag, Hg, Cd, Pb and Ti
Superparamagnetic Fe_3O_4(iron oxide) coated with humic acid (HA)	Hg(II), Cu(II), Cd(II) and Pb(II) from water

The New Faces of Diatoms: Source for new age nanotechnology application

As famously told by Netaji Subhas Chandra Bose in his freedom fighting lecture "Give me blood I will give you freedom" where he wanted freedom fighters to raise for our country. Similarly in this present context its time that we must raise ourselves for facing this global situation of water crisis and propagate a definitive management plan. Cutting edge sustainable management technologies of water is utter most necessary for India as immense developing population is putting a serious strain on the majority of the nation's characteristic assets. Most water sources are sullied by sewage and farming overflow. In spite of the fact that entrance to drinking water has enhanced, the World Bank evaluates that 21% of transferable illnesses in India are identified with dangerous water. This study of cutting edge technology for sustainable waste water management consisting of three components i.e. Sensors, Geo-informatics application and use of nanoparticle clearly has the potentiality to recover the useful material from waste water. These three components are not only relying on the technological development but also include development of different socio-economic factors. Biosensors utilized for proper use of resources has the potentiality to create a renaissance for the next gen era. Geo-informatics with its simple tools provide us opportunity to gather knowledge form a potential space and keep ourselves updated in every context of any situation, thus it provides us a three dimensional knowledge about the ongoing situation. And lastly utilization of nanoparticle is favored, due its high absorbance which in practical perspective can purify large volume of waste water.

As famously told by Yeboah, (2014) "The uncertainties in life are so uncertain for us to determine the kind woe we shall be entangled in in the next future. When you stay dormant, your life is at risk; when you dare to take a step, you take a step to take a risk. We have a choice. Yes! a choice to choose to dare to get to our real reasons on earth or to choose to live in mediocrity and conformity, but, we ought to note that, it is riskier to risk nothing when the life we live is always at risk" can be related to this article that with the uncertainties lies the success, and its upon us whether to take a risk to save our planet or not.

The first single-cell organisms started moving around in the oceans started around 3.5 billion years ago. Ever since, every life form has spent their time uniquely adapting to their surroundings. In the case of diatoms, which evolved around 200 million years ago, this adaptation resulted in a tough silica containing exoskeleton capable of protecting the organism from predators. Diatoms are single celled photosynthetic algae that live in both seawater and fresh water, wherever enough light and nutrients

are found. This group of phytoplankton is estimated to comprise more than 200 000 living species, varying in size from 2μm to 2mm. Diatoms are solely responsible for about 20–25% of global oxygen production, i.e., approximately every fourth breath of oxygen we inhale. Diatoms alone account for around 40% of the phytoplankton on Earth which implies 20–25% global net primary production. This is more than the primary production of all. Diatoms are estimated to contribute up to 25% to the world primary production of organic carbon, capturing carbon dioxide (CO_2) and sunlight to sustain themselves, and releasing oxygen in the process.

Evolution of Diatoms

The entry of diatom in the plant kingdom happened late in the evolution process and through an unusual entry. Researchers believe they are secondary endosymbionts, meaning that their precursor was a eukaryote that engulfed another eukaryote, resulting in a quadruple membrane around the chloroplasts the diatom gained from this act of piracy. Diatom chloroplasts are derived by endosymbiosis from those of red algae (Rhodophyta); rather than directly from prokaryotes as in the case of plants. However, recent studies show that nuclear genes in diatoms and few closely-related algal groups are derived from green algae (Chlorophyta). About 16% of the diatom nuclear coding potential genes 2 are similar to green algae. It is hypothesized that they were introduced by a cryptic endosymbiont related to green algae. Thus, it is now assumed that diatoms have recruited genes from both of these major existing algal groups. Several bacterial endosymbionts have also been reported from diatoms. For example, spheroid bodies present inside diatoms are endosymbionts closely related to a cyanobacterium. The ornithine urea cycle (OUC), similar to that in metazoans was recently reported in diatoms. It links them evolutionarily to animals! Although it is involved in nitrogen metabolism in both taxa, it's role is different. The OUC is involved in the removal of fixed nitrogen in metazoans, whereas, it helps in the distribution and repackaging of inorganic carbon and nitrogen in response to nitrogen availability in diatoms. This cycle was earlier thought to have evolved in metazoans. It is absent in green algae and plants. This discovery is significant as it provides evidence that the cycle appeared several hundreds of millions of years before the appearance of metazoans. The evolutionary success story of diatoms only begins some 200 million years ago, but they have spread around the globe and diversified into hundreds of genera and around 100,000 species in this short fraction of the geological timescale. Today, they are present wherever there is liquid water, in the oceans, in freshwater, and even in soil. They have already played a significant role in the global cycles of carbon and nitrogen, and are responsible for large sediments of silica including diatomaceous earth.

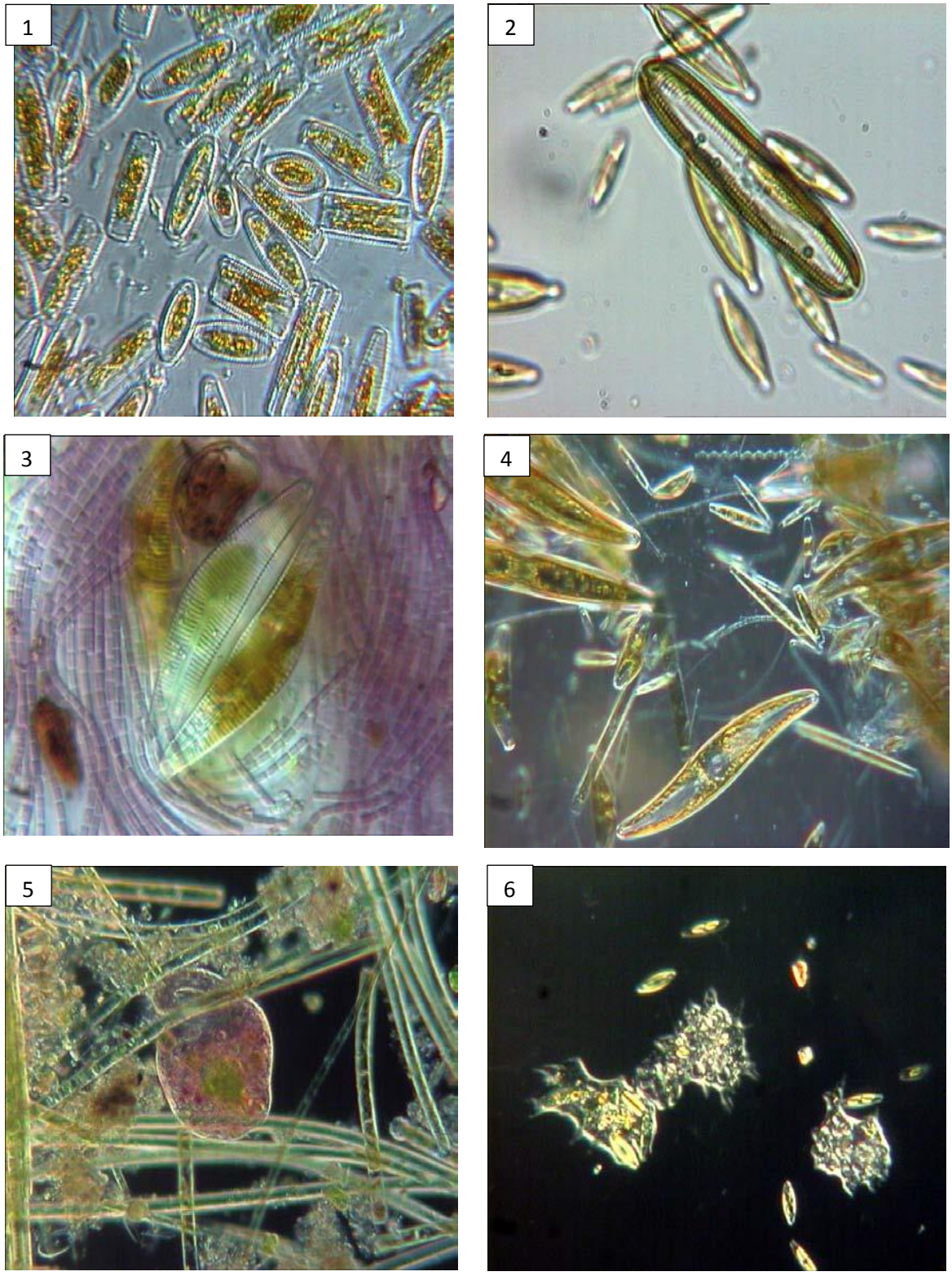


Fig. 1 a diatom community completely dominated by *Diatoma vulgaris* **Fig. 2** a sediment diatom community with *Navicula* spp. and *Pinnularia viridis*. **Fig. 3** mixed diatom community with large cells of *Gyrosigma* sp. **Fig. 4** shows cells of *Cymbella* sp. living in association with the blue-green algae

Oscillatoria. **Fig. 5** shows the filamentous diatom *Aulacosiera granulata* being grazed by a protozoan. **Fig. 6** shows diatoms being grazed by *Amoeba* sp. (adopted fromWRC Report TT 281/07 January 2007)

Diatoms ability to form a rigid outer cell wall, termed a frustule have separated them from other phytoplankton groups is their illustrated schematically in Figure 7, made of amorphous hydrated silica - $SiO_2 * 2 H_2O$.

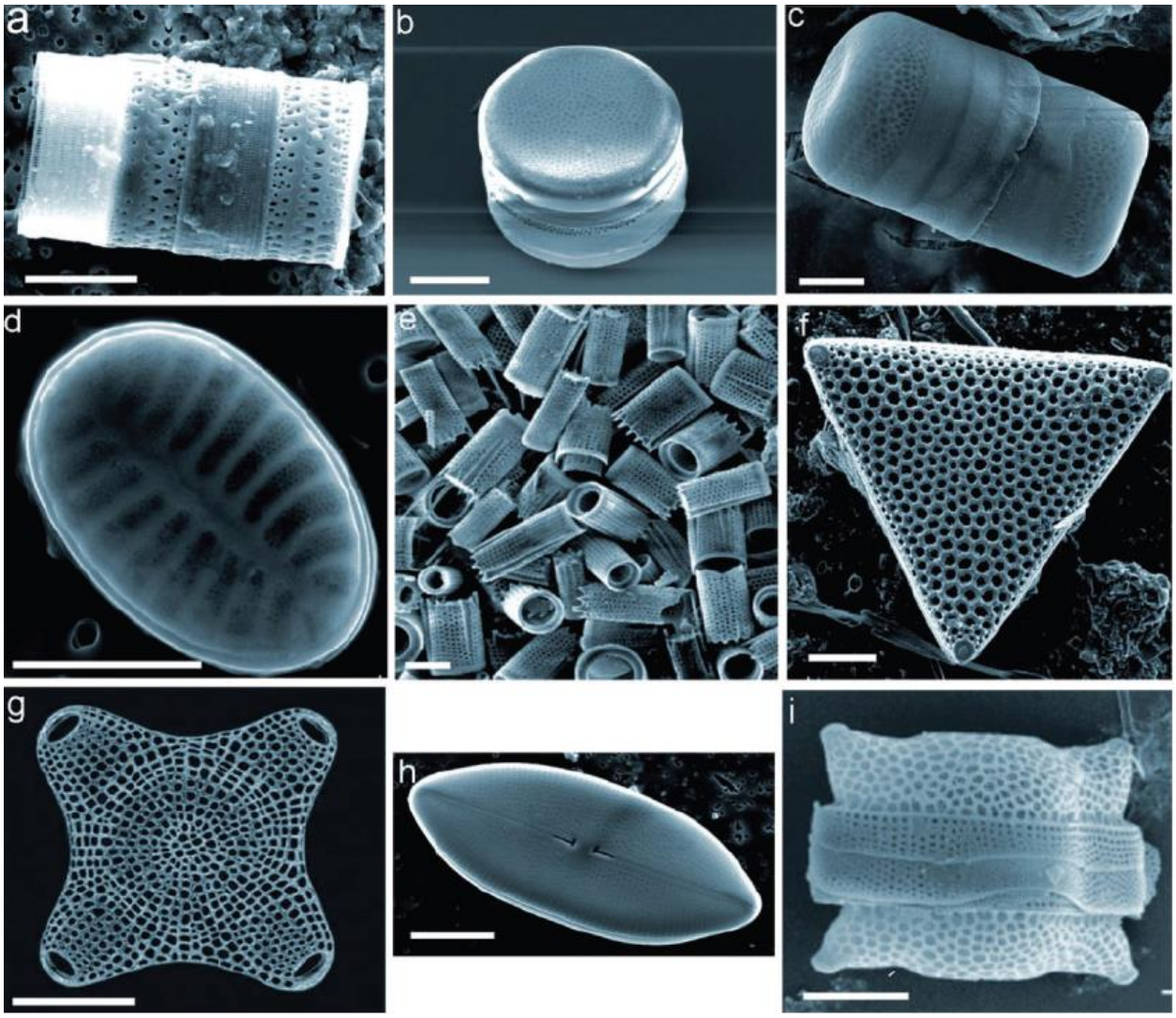


Figure 7: SEM of fossilized diatom biosilica structures from diatomaceous earth (Diatomite mine NSW, Australia). Scale bar: 10 mm. adopted from Losic et.al. (2009). Copyright WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, 2009.

The purpose of this frustule is debated; however, one theory is that the frustule serves as armor to protect the organism from predators. A large variety of organisms feed on diatoms, so any measure that reduces mortality would be an effective first line of defense. Diatoms are divided into two orders based on the shape of the frustule: Centrales and Pennales. These orders comprise round and elongated diatoms,

respectively. Centric diatoms are radially and bilaterally symmetric (Figure 8), whereas pennate diatoms are bilaterally symmetric. This symmetry has given rise to the group name diatoms, from the Greek diatomos, meaning cut in two. Diatoms generally reproduce asexually, and can do so up to 8 times per day. Asexual division, however, reduces the average size of the population over time. After a number of divisions, the cell will resort to sexual reproduction, and a spore is created. This spore will form a fully-grown diatom, and the cycle will begin again.

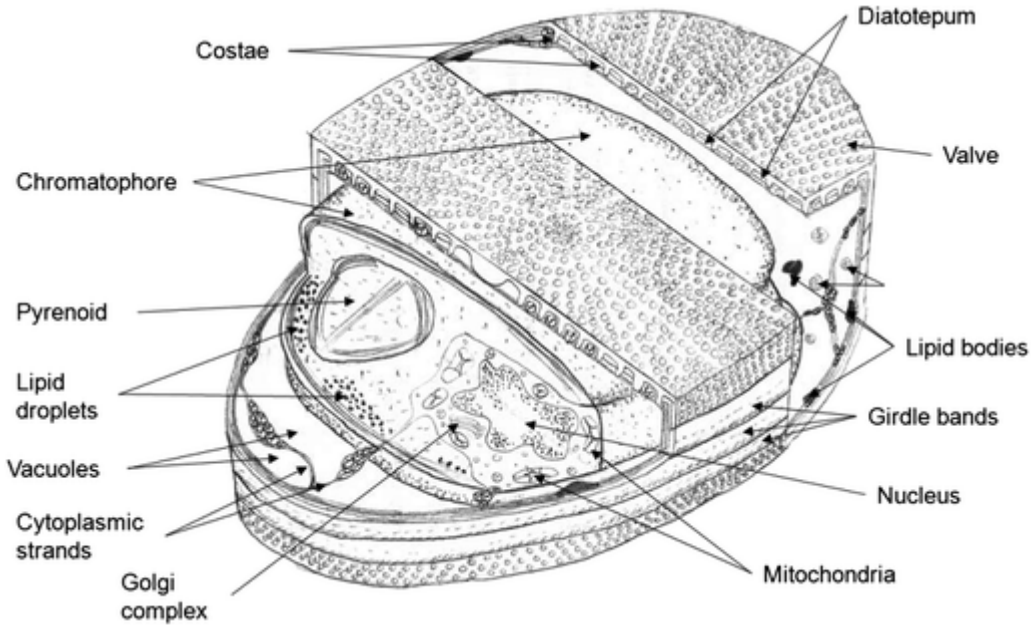


Figure 8: Schematic Representation of Centric diatoms with major components.

Why have diatoms been so successful?

Is it to do with their silica wall, as research from Paul Falkowski at Rutgers University has suggested, silica cell walls are energy efficient to produce and unlike the carbonate biominerals of other species are not sensitive to ocean pH. Fossil traces of diatoms' silica shells (frustules) can be dated back approximately to 185 million years ago, and their rise seems to have been unstoppable since then. Alternatively, results from Christian Wilhelm at Leipzig show that they have a very efficient way to dissipate excess solar energy, known as non photo-chemical quenching. Some experts believe that may be a crucial factor explaining their success

Life cycle

All diatoms exhibit diplontic lifecycle which means that the diploid5 form is dominant over the haploid6 form in the lifecycle. This lifecycle occurs very rarely in algae. Diatoms mostly undergo

vegetative (asexual) reproduction. In vegetative reproduction, daughter cells are formed within the parent cell by mitosis. Each parent valve acts as an epivalve inside

which a hypovalve is synthesized. Thus, each daughter cell contains the valve from the parent as epivalve and a newly-synthesized hypovalve. Interestingly, this means that one daughter cell from the parent epivalve retains the same size; while the other synthesized from hypovalve is slightly smaller than the parent. Therefore, for a given species, the average cell size usually decreases with successive divisions in a population (Figure 9 asexual reproduction). As a consequence of cell size (particularly, length) reduction, the relative cell dimensions change. The entire range of variation in size and shape in a population is termed a 'size diminution series'. Macdonald and Pfitzer documented this phenomenon independently in 1869; hence it is referred as the MacDonald–Pfitzer hypothesis. This unique phenomenon also affects success in undergoing mitotic division. The size diminution occurs only until a minimum viable size, beyond which the cell is unable to survive. Diatoms regain their maximum size by the formation of an auxospore – a specialized cell also called as the perizonium. It is linked to meiosis and sexual reproduction in the diatom lifecycle. The auxospore is formed after the fusion of male and female gametes. The basic shape of each diatom species is formed during the expansion of the auxospore. (Sexual reproduction). An auxospore possesses silica bands (perizonia) rather than a rigid silica cell wall. It allows the cell to expand to its maximumsize and produce a frustule of the basic shape. Diatoms also form resting cells in adverse conditions such as lack of nutrients and very low temperature. These are physiologically different from vegetative cells.

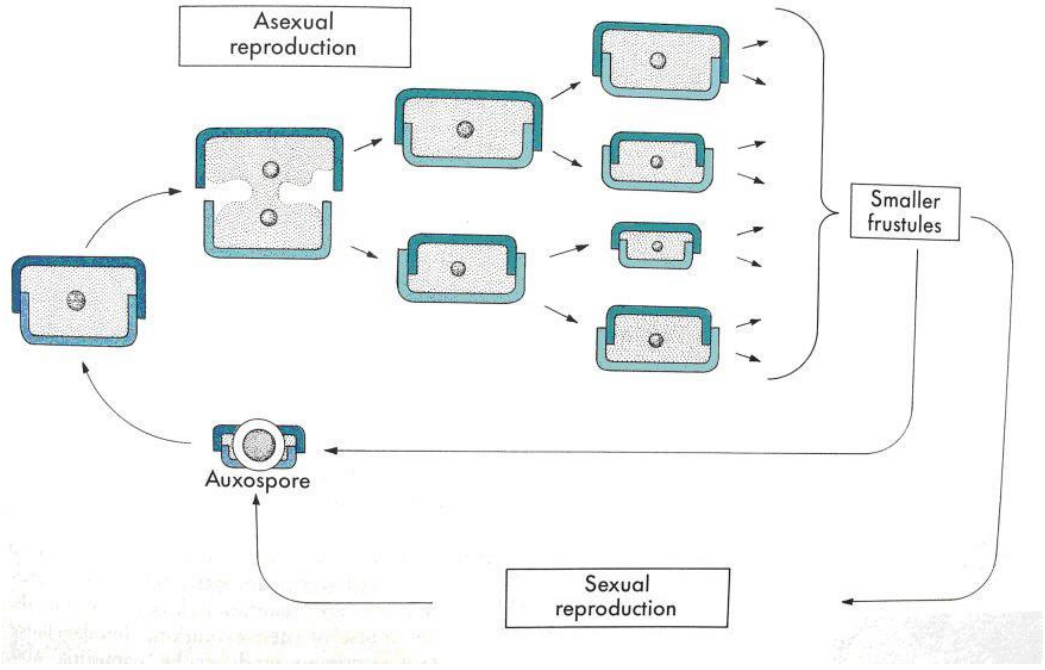


Figure 9: Illustration of Life cycle and reproduction of Diatom.

Frustule Formation in diatom

Diatoms use silicic acid present in the environment for silica formation in frustules. For this, they have specialized structures called silica deposition vesicles (SDVs) which polymerize silicic acid. Two types of SDVs, i.e., valve and girdle band SDVs, work at different stages of the cell cycle. The nucleus of each daughter cell moves nearer to the microtubule centre (cytokinesis). The microtubule centre lies below the plasma membrane where the hypovalve is eventually synthesized. The valve SDV involved in valve formation aligns between the microtubule centre and the plasma membrane forming a region called the pattern centre. The SDV then synthesizes a hypovalve in an organized fashion, using silica and biomolecules, by gradual expansion. The hypovalve then fuses with the plasma membrane giving rise to 2 daughter cells by exocytosis.

Since the siliceous cell walls cannot expand, the daughter cells are smaller than the parent and undergo cell growth. The distance between the rigid valves needs to be increased before mitotic division. Therefore, several succeeding lateral girdle bands are formed in girdle band SDVs and are released (exocytosis) synchronously with cell growth during interphase. As this prevents gap formation in the frustule, cell components never get directly exposed to the environment (Figure 10)

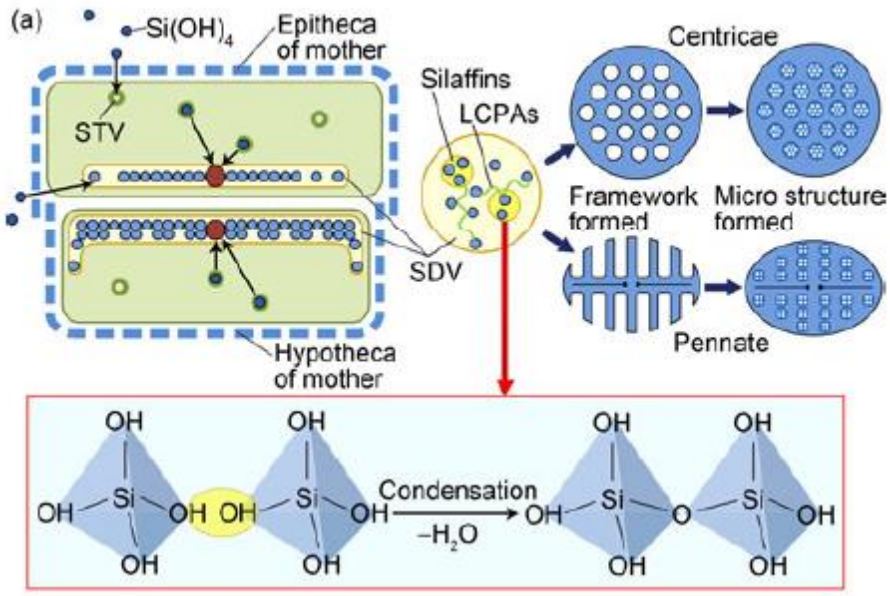


Figure 10: Schematic of Frustule formation in diatoms

Application of Diatom and Diatom bio silica frustule

A variety of potential applications for diatom frustules including optics, photonics, catalysis, nanofabrication, biosensing, drug delivery, filtration, bioencapsulations, and immunoisolations have been proposed and explored. Most of these applications rely on using the unique and ordered porous structures of frustule valves. Porous matter is omnipresent in nature for functional reasons and the material properties of pores provide a large scope for exploitation. Micro- to macroporous membranes are frequently used for separation processes, and there is currently intensive research exploring several novel nanomaterials based on zeolites, metallo-organic composites, inorganic oxides, and polymers.

1) **Riding the global cycles**

Diatoms fix as much carbon dioxide as all the rainforests of the world combined, and they may very well have been the main architects of our current, moderately cool climate situation. The rise of the diatoms began in a much warmer climate than ours, with no ice caps on the poles. The atmospheric carbon dioxide concentration was much higher than today. In the Cretaceous, around 100 million years ago, when diatoms began to become widespread and developed great diversity, the carbon dioxide level was fivefold higher than today, and oxygen was lower. Remarkably, diatoms managed to thrive and expand during a period of extreme climate change, and they also seem to have come through the mass extinction that saw off the dinosaurs without too many problems. The opening of the Drake Passage around 40 million years ago, which created the continent of Antarctica, was particularly beneficial for the diatoms because they began to proliferate dramatically in the cold turbulent waters of the Southern Ocean.

As diatoms evolved thicker and denser cell walls and spread across the oceans, it became more likely that dead diatoms might sink to the ocean floor and thus sequester their carbon. This became a significant cause of carbon dioxide reduction at the planetary level in the next tens of millions of years, until carbon dioxide approached the level that we used to have before the Industrial Revolution. Currently, by burning fossil fuels such as petroleum that were generated in the past by diatoms and other plankton, we are undoing much of the carbon sequestration work that these diatoms did. Recognition of the importance of diatoms for the Earth's carbon cycle has led to the idea of encouraging them to step up their current sequestration activities in the oceans. Iron is a limiting nutrient in large parts of the oceans, so fertilisation with iron could lead to diatom blooms.

2) The "Living Photonic Crystals"

A photonic crystal is defined as a spatial, periodic distribution of refractive index which, properly dimensioned, can inhibit propagation of light in specific wavelength ranges (the so-called photonic bandgaps). The whole mathematical formalism describing the optical behavior of a photonic crystal is the same which describes the electric properties of a semiconductor, where the periodic refractive index plays the role of the periodic electric potential, the photons are the analogous of the electrons, and the photonic bandgaps are the equivalent of the forbidden bandgaps separating valence and conductive bands

Diatom frustules and valves present the most impressive resemblance with artificial photonic crystals, as it can be seen from Figure 11: a single layer of a single valve of C. wailesii can be described, in first approximation, by a photonic crystal slab with hexagonal lattice. Even though it has been numerically demonstrated that, for light propagating out of the plane defined by the slab, photonic bandgaps can be accessible even for refractive index contrasts as small as that of silica and air, they have not been experimentally observed yet; in any case, they can be hardly found for refractive index contrasts similar to that between silica and water/cytoplasm (and 1.34 in the visible range, resp.) and for light propagation in the plane defined by the slab

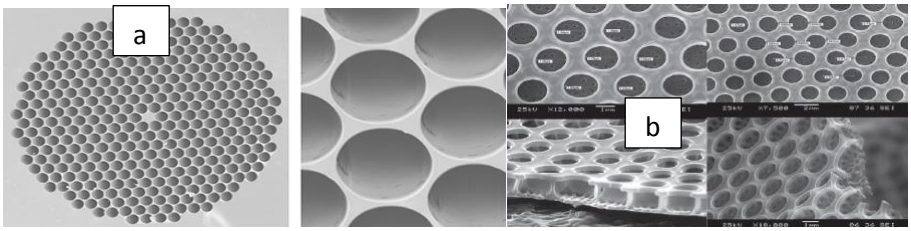


Figure 11: (a) artificial photonic crystal; (b) internal structure of frustule resembling photonic crystal

3) Photoluminescence

A distinctive optical feature, photoluminescence (PL), was also observed with frustules. When porous biosilica was irradiated by UV wavelengths it emitted one or more PL peaks in the visible spectral region. Scientists investigated such features and their potential applications, for example in gas detection Photoluminescence responses of Thalassiosira rotula valves were examined when exposed to several gases and volatiles substances (Table 1). Frustules irradiated by a He-Cd laser (325 nm) showed a multiband PL (between 450 and 690 nm) that was attributed to oxidized silicon nanocrystals (533 nm), porous silicon (609 nm), and hydrogenated amorphous silicon (661 nm). As a result, valves PL were quenched with electrophilic gases while they were enhanced for nucleophilic ones. Furthermore, PL of T. rotula frustules was quenched when exposed to NO_2(g) flux, showing a high sensitivity in the order of sub-ppm level that it is strongly dependent on the structure, porosity, and gas nature. PL

properties of the frustules from other centric and pennate species (T. rotula, Cosinodiscus. Wailesii, and Cocconeis scutellum) were also demonstrated, and high gas detection sensitivity was shown at room temperature. Photoluminescence was also investigated for chemically modified diatoms frustules study on nickel sulfate modified C. wailesii frustules. When irradiated with a He-Cd laser (442 nm), intact frustules exhibit a broad PL peak (500–650 nm) while those growing with $NiSO_4$ quenched the PL. Photoluminescence of chemically modified frustules have been investigated, for medical purposes, by fixing antibodies on the silica shells. Antibodies doped frustules were irradiated by 325 nm laser's wavelength, which show a high sensitivity in the order of 1.2 nm.μm−1 and detection limit around 100 nmol.L−1. Recently, frustules from the pennate diatom Psammodictyon panduriforme were investigated under 325 nm laser excitation. Frustules showed two emission peaks at 417 and 534 nm that were considered as radiative luminescence generated by oxygen vacancy defects in the structure. However, under pulse laser excitation, a single narrow emission peak was observed near 475 nm. This result was interpreted as the consequence of a putative quantum confinement effect due to the mesoporous silica and the quasi-regular pores in frustules structure.

Table 1: **Photoluminescence studies on diatom frustules**

Species	**Modified chemically**	**Main results**
Psammodictyon panduriforme	No	PL emission peaks at 417 and 534 nm.
Coscinodiscus concinnus	Yes	PL of frustules acts as synthetized quantum dots. PL of modified frustules shows high sensitivity toward antibodies.
Coscinodiscus wailesii (modified by nickel sulfate)	Yes	Intact diatoms exhibit a broad PL peak (500–650 nm). Growth with $NiSO_4$ quenches the PL.
T. rotula; C. wailesii and	No	Sensitivity depends on the used bio-silica

Species	Modified chemically	Main results
C. scutellum		(structure and porosity) and gases.
Thalassiosira rotula	No	PL was quenched by NO_2 gas with high sensitivity in the order of the sub-ppm level.
Thalassiosira rotula	No	PL of diatoms was quenched with electrophiles and enhanced with nucleophilic gases.

4) Light trapping for solar applications

The ability of diatom frustules to collect and confine light with high efficiency can be exploited in the development of new bio-based or bio-inspired solar cells if properly chemically modified. Diatom frustules coated with titania nanoparticles (less than 20 nm in diameter) by means of plasma treatment (thus avoiding any linking agent) and used in dye sensitized solar cells (DSSCs) in order to improve their efficiency were reported. In this kind of cells the dye generates, after absorption of light, electrons that are excited into the conduction band of a semiconductor (usually thin films of titania, indeed) which then travel to the working electrode (Figure 12). Titania functionalized frustules allow to obtain three-dimensional titania structures with high specific surface, thus increasing the interaction between titania nanoparticles themselves and dye electrons. After only three cycles of plasma treatment, an increase of DSSC efficiency of about 30% with respect to the use of titania thin films has been achieved.

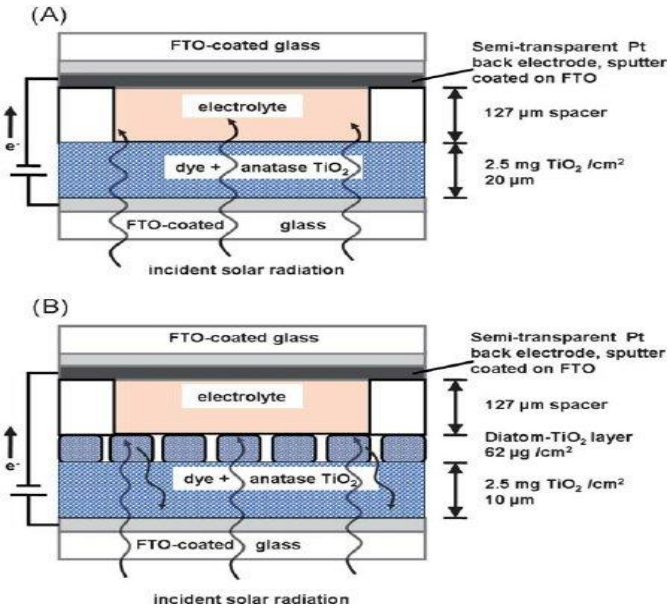


Figure 12: (a) Traditional DSSC, (b) DSSC with enhanced photonic capture resulting from the incorporation of a reflective diatom titanium oxide composite layer

5) Diatoms and bioprospecting

Diatoms produce different lipids, polysaccharides, amino acids, and vitamins. Some diatoms are known to yield pharmacologically- active compounds, which display antibiotic activity, enzyme inhibition, toxicity, etc. The most commercially important among these are lipids. The most-studied molecule in diatoms is eicosapentaenoic acid (EPA), a polyunsaturated fatty acid (PUFA) which also constitutes a major part of cod liver oil. Diatoms are one of the primary producers of PUFA in the aquatic food chain and they can act as the alternative sustainable source for supply of PUFA. Further, studies have shown that the EPA is less expensive and more stable when obtained from a marine diatom *Phaeodactylum tricornutum* Bohlin than from the liver of codfish.

Table 2: Active Compounds and oil content of some microalgea

Phylum	Class	Taxonomy	Oil Content (% d.w.)	High Value Molecules
Chlorophyta	Chlorodendrophyceae	*Tetraselmis suecica*	15–32	Carotenoids, chlorophyll, tocopherol, lipids
Chlorophyta	Chlorophyceae	*Ankistrodesmus* sp.	28–40	Mycosporine-like amino acids,

Phylum	Class	Taxonomy	Oil Content (% d.w.)	High Value Molecules
				polysaccharides Carotenoid, β carotene,
Chlorophyta	Chlorophyceae	*Dunaliella salina*	10	mycosporine-like amino acids, sporopollenin
Chlorophyta	Chlorophyceae	*Dunaliella tertiolecta*	36–42	Carotenoid, β carotene, mycosporine-like amino acids
Chlorophyta	Chlorophyceae	*Neochloris oleoabundans*	35–65	Fatty acids, starch
Chlorophyta	Trebouxiophyceae	*Botryococcus braunii*	29–75	Isobotryococcene, botryococcene, triterpenes
Chlorophyta	Trebouxiophyceae	*Chlorella vulgaris*	58	Neutral lipids
Chlorophyta	Trebouxiophyceae	*Chlorella emersonii*	34	Neutral lipids
Chlorophyta	Trebouxiophyceae	*Chlorella protothecoides*	15–55	Eicosapentaenoic acid (EPA), ascorbic acid
Chlorophyta	Trebouxiophyceae	*Chlorella minutissima*	57	C16- and C18-lipids
Heterokontophyta	Bacillariophyceae	*Nitzschia laevi*	28–69	EPA

Phylum	Class	Taxonomy	Oil Content (% d.w.)	High Value Molecules
Heterokontophyta	Coscinodiscophyceae	*Thalassiosira pseudonana*	21–31	Glycosylglycerides, neutral lipids, TAG
Heterokontophyta	Labrynthulomycetes	*Schizochytrium limacinum*	50–77	Docosahexaenoic acid (DHA)
Myzozoa	Peridinea	*Crypthecodinium cohnii*	20	DHA, Starch
Ochrophyta	Coscinodiscophyceae	*Cyclotella* sp.	42	Neutral lipids
Ochrophyta	Eustigmatophyceae	*Nannochloropsis* sp.	46–68	EPA, TAG, ω-3 LC-PUFA

Algal fuels based on diatoms are beginning to show promise which is reasonable, given their contribution to natural oil deposits. Thus diatoms have the ability to produce large amounts of oil. For example, planktonic diatoms are reported to produce lipids in quantities up to 40% dry cell weight. Cultured marine diatoms had 30%–45% dry weight as lipids. The amount of oil in a single diatom is reported as up to 25% of the algal biomass and could perhaps reach 60% of the nonsilica diatom dry mass. For some diatom species, the amount of oil that could be produced has been projected to reach up to 200 times more oil per hectare than soybeans. Diatoms also have the ability to reproduce quickly and can create very large bio-volumes. Diatoms can reproduce and double in as little as five hours under laboratory conditions, and in as little as two days in the environment. How fast milked, stationary diatom cultures will replace their oil is as yet untested. Diatoms have the ability to produce large amounts of oil. For example, planktonic diatoms are reported to produce lipids in quantities up to 40% dry cell weight. Cultured marine diatoms had 30%–45% dry weight as lipids. The amount of oil in a single diatom is reported as up to 25% of the algal biomass and could perhaps reach 60% of the nonsilica diatom dry mass. For some diatom species, the amount of oil that could be produced has been projected to reach up to 200 times more oil per hectare than soybeans. Diatoms also have the ability

to reproduce quickly and can create very large bio-volumes. Diatoms can reproduce and double in as little as five hours under laboratory conditions, and in as little as two days in the environment. How fast milked, stationary diatom cultures will replace their oil is as yet untested. There is no "puzzling underrepresentation of diatoms in the microalgal biofuels arena, as they have been a focus since 1978. Biodiesel from microalgae or third generation renewable biofuels could meet the demand because they are projected to be capable of generating up to 6–200 times more crude oil per surface area than higher plants. CO2 will be available either from the atmosphere or directly from industries. However, our calculations here indicate that there is not enough industrial CO2 waste to convert to biofuel to replace fossil energies used for transport.

6) New Material Modification of Diatom

Material modification is an important approach for changing the material property or function of frustules. Current research suggests several new materials to add or replace in frustules by chemical, culture or replica method while keeping the original structure of frustules. These methods are summarized in several reviews, including in a review by Losic et al.

(i) Direct processing of frustules. The term Bioclastic and Shape-Preserving Inorganic Conversion (BaSIC) methods was used to describe the method develped for modifying the biosilica frustule into new nanocrystal materials whilst maintaining the original frustule structure by Sandhage et al. Through gas/silica displacement reactions (e.g. magnesio-isothermic reduction), MgO, TiO2 and Si, frustules with fine nanoscale structures were obtained. The technological conditions of fabricating frustules composed of other materials, such as Al_2O_3, CaO, Fe2O3, Li2O, Nb2O5, SrO and Ta2O5 have been established. Subsequent coating or displacement reactions on MgO frustule substrates could produce new products, such as frustules composed of ZrO2 , MgO/BaTiO3, BaTiO3, MgO/BaTiO3 (coated with Eu3+), SrTiO3, C, or Pt/C (the expected properties or potential uses of these frustules are summarized in Figure 13). In addition, both hydrothermal growth and vapor-phase transport can produce zeolites/SiO2 composite frustules, which have many uses such as molecular sieves and water/waste treatment. Culture methods reported by Rorrer et al. for the successful addition of GeO2 and TiO2 to biosilica frustules during the natural growth of a diatom have been described.

(ii) Frustules have been used as templates to obtain cast or composite frustule structures. Several cast structures of frustules were obtained by coating and subsequent removal of the frustules, which were composed of Ag, Au, epoxy, carbon, boron nitride [77] or ZrO_2 [78]. Solution coating and subsequent thermal treatment could coat ZnFe2O4 , Zn2SiO4 (coated with Mn) and Ba-TiO3 onto frustules. Electroless plating has been shown to be effective in coating metal (Au, Ag or Pd) onto frustules. Some composite frustules have new characteristics, for example, a polyaniline coated frustule has a certain degree of electrical conductivity.

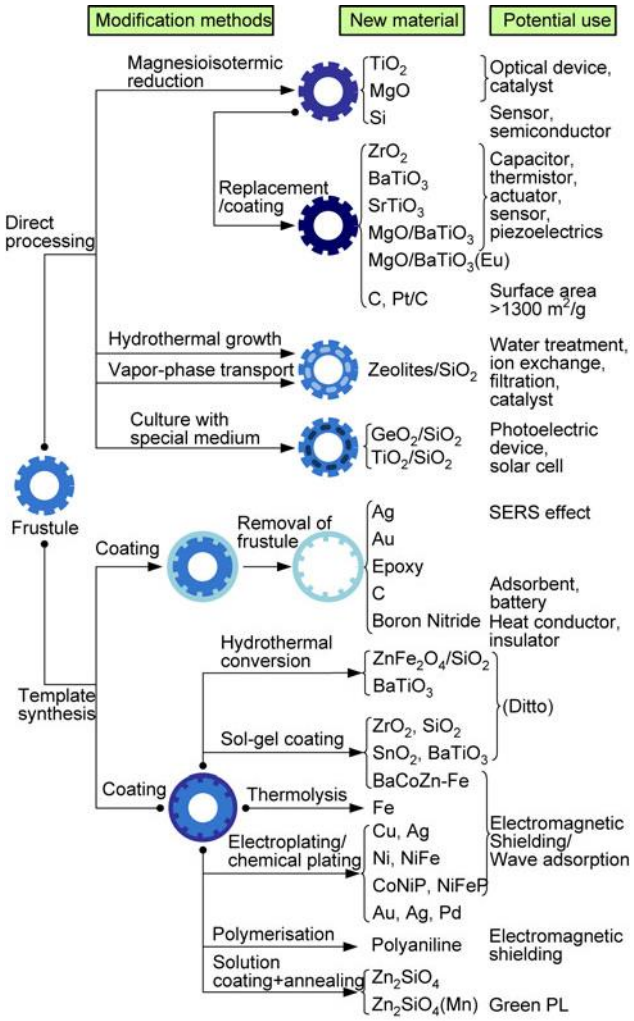


Figure 13: Methods for modification of frustules and its application

Biological materials have been largely used in industrial products due to their fast reproduction rate, green and renewable nature, complex structures, and natural optimized functions. Diatom frustules possess several excellent functions and

potential for micro- and nanoscale manufacturing. Many industrial products utilize the natural functions of diatom frustules. For instance, construction materials made of diatomite can insulate heat and noise, and regulate humidity because of the outstanding pore volumes. Diatomite filters can remove impurities and bacteria from water, and at the same time absorb metallic ions, fluoride ions and dye groups due to their surface electric properties. With high surface area and possibility of material modification diatoms are good candidate for filtration and microfluidics. Currently most of the research anf and applicability process of diatom and diatom based materials are I laboratory stage. The research of these tiny phototrophs are encouraging and it is showing good prospect in creating future devices with varied application possibility due to its versatile form.

Oilzapper and Oilivorous-S

The new technique of using the bacteria to get rid of oil spill has been called "Oil Zapping". Oil Zapping is a bio-remediation technique involving th e use of 'oil zapping' bacteria. It was recently employed to clean up the Mumbai shoreline affected by the oil spill that occurred in August 2010.

The Mumbai Oil Spill happened when a merchant vessel M V Khalijia collided with a cargo ship MSC Chitra 10 km off the coast of Mumbai. MSC Chitra tilted 15 degrees soon after the collision but there was no loss of life. The cargo ship eventually tilted to about 80 degrees dropping around 250 containers into the sea. Before the spill could be plugged 400 tons of oil had leaked into the sea.

The Maharashtra Pollution Control Board used the services of The Energy and Resources Institute (TERI) which has developed the oil zapping bacteria. TERI had developed this technique over a period of seven years. The Oil Zapping project was supported by the Department of Biotechnology (Government of India) and the Ministry of Science and Technology.

How does Oil Zapper Work?

There are five different bacterial strains that are immobilized and mixed with a carrier material such as powdered corncob. bacteria is called Oil Zapper. Oilzapper feeds on hydrocarbon compounds present in crude oil and the hazardous hydrocarbon waste generated by oil refineries, known as Oil Sludge and converts them into harmless CO_2 and water. The Oilzapper is neatly packed into sterile polythene bags and sealed aseptically for safe transport. The shelf life of the product is three months at ambient temperature.

The technique was used successfully and 130,000 tons of oily sludge/ oil contaminated soil was treated as per the leading news sources.

A boon for oil industry

An innovation by TERI has all the makings of a grand Hollywood motion picture. The plot revolves around a mammoth oil tanker that leaks, causing great catastrophe. There are shots of the carnage on television sea birds and fish washed ashore, their bodies sodden with crude oil. Amidst all the chaos, only one man the protagonist, of course knows what to do. He has Oilzapper, a bacterial consortium that degrades crude oil and oily sludge. The rest is a join-thedots game.

A clean innovation

Application of Oilzapper on an oil-spill site in Assam

This is not about a movie script but a real achievement the Oilzapper. Developed by TERI after seven years of research work and partly supported by the DBT (Department of Biotechnology), Ministry of Science and Technology, Government of India, the Oilzapper is essentially a cocktail of five different bacterial strains that are immobilized and mixed with a carrier material (powdered corncob).

Much as its savvy name suggests, the Oilzapper feeds on hydrocarbon compounds present in crude oil and oily sludge (a hazardous hydrocarbon waste generated by oil refineries) and converts them into harmless CO2 and water. The Oilzapper is neatly packed into sterile polythene bags and sealed aseptically for safe transport. The shelf life of the product is three months at ambient temperature.

The scientific term for the abovementioned process is bioremediation. Bio refers to living and remediate means to fix or cure. It is a branch of biotechnology that makes use of living organisms to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals, or hazardous waste. A first-of-its-kind solution, Oilzapper's uniqueness lies in the bio-friendly manner in which it detoxifies oily sludges and cleans up oil slicks.

The story so far

One of the biggest and most frequent threats for any oil refinery is oil sludge. There are 16 refineries in India and all create toxic waste. Estimates suggest that about 20 000 tonnes of petroleum sludge is generated every year. At present, refineries have to construct polymer-lined pits with a special leachate collection system to prevent the dumped sludge from leaking into the earth and groundwater. A pit costs about 10 million rupees and each refinery needs several such pits. With more refineries being set up, space is always a constraint. What's more, a pit gets filled up in three to four years whereas with Oilzapper, one just needs 200 tonnes of environment-friendly bacteria to clean up 20 000 tonnes of oily waste!

When an oil spill occurs at sea, oil floats above water since it is lighter. It therefore catches fire swiftly, endangering the ecosystem for all times. Oil spills on land (due to leakage from pipelines, pilferage, etc.) too cause fire hazards and pollute groundwater and air. With Oilzapper, a contamination of 20% (say, 200 grams per kilogram of oil) can be taken care of in two months. A blessing for the oil exploration and production sites and oil refineries, the Oilzapper has proven particularly relevant in the wake of the everincreasing movement of oil

across land and water, the many oil-transport related accidents in the past and oilwaste management issues at the refineries.

Cleaning up the mess

The good news is that more than 5000 hectares of cropland contaminated with crude oil spills has already been reclaimed in different parts of India and more than 26 000 tonnes of oily sludge successfully treated with Oilzapper. Many oil-slick contaminated lakes in the north-eastern parts of India have also been cleaned up in two years. Table 1 provides details of the amount of oily sludge treated in India.

Pre-bioremediation: a site in Mehsana, Gujarat

Post-bioremediation: the same site after 2 months

Zapping oil - where and how much?

Refinery/ Oil installation site	**Quantity of sludge treated (tonnes)**
Indian Oil Corporation Ltd.	
- Mathura	3750
- Barauni	11400
- Digboi	1000

- Guwahati	80
- Gujarat	1650
- Haldia	3500
- Kanpur	50
Bharat Petroleum Corporation Ltd., Mumba, Hindustan Petroleum Corporation Ltd.	300
Visakhapatnam	2350
Oil India Ltd., Duliajan	700
Reliance Refinery, Jamnagar	20
Indian Petrochemicals Ltd., Nagothane	50
Hindustan Petroleum Corporation Ltd., Panipat	10
Hindustan Petroleum Corporation Ltd., Kandla	100
Indian Oil Corporation Ltd., Rajkot	350
Oil and Natural Gas Corporation Ltd.	
- Jorhat	200
- Mehsana	370
- Nazira	250
- Sanatnagar	20
Total	26150

Beneficiaries

- Oilzapper has elicited tremendous response in India and abroad. The BPCL (Bharat Petroleum Corporation Ltd), the IOCL (Indian Oil Corporation Ltd), the ONGC (Oil and Natural Gas Corporation), the OIL (Oil India Ltd), the HPCL (Hindustan Petroleum Corporation Ltd), and the RIL (Reliance Industries Ltd) are some of its consumers (Table 1).
- The know-how of Oilzapper technology has been shared with BPCL, Mumbai, for their internal use but it is marketed by TERI, New Delhi. Oilivorous-S (Box 1), on the

other hand, was jointly developed by TERI's microbial biotechnology laboratory and the R&D Centre of the IOCL, New Delhi. It is marketed by both the organizations.

Snapping up oil and awards

The name 'Oilivorous-S' suggests an affinity to 'Oilzapper'. Yes, they belong to the same family of oily sludge-degrading consortia of microbes. What makes Oilivorous-S a tad different from Oilzapper is an additional bacterial strain that makes the former more effective against sludge and crude oil with high-sulphur content.

The Oilzapper and Oilivorous-S have not just snapped up wasted oil but also several eminent scientific awards in India and outside like the All India Biotech Association Award 2001, given by the All India Biotech Association, New Delhi; the Biotech Product & Process Development for Commercialization Award 2002 by the DBT, Government of India; the Jawaharlal Nehru Memorial National Gold Medal Award 2002 for excellence in Environmental Biotechnology Research by the International Greenland Society, Hyderabad; and the Best Paper Oral Presentation award in the 5th International Petroleum Conference and Exhibition (PETRO-TECH 2003) organized by the Oil and Natural Gas Corporation Ltd, India; the National Petroleum Management Programme Award 2002/03 for Creativity and Innovation in the R&D category by the Ministry of Petroleum and Natural Gas, Government of India; and the Burhani Foundation-NEERI Award 2002 by the National Environmental Engineering Research Institute, Nagpur. A cheap-and-quick solution to one of the most menacing environmental issues sure deserves these accolades.

Applications/benefits

- Among all the other advantages these technologies offer, two crucial ones stand out. First, both Oilzapper and Oilivorous-S can be used in situ, thereby eliminating the need to transfer large quantities of contaminated waste from the site, a process that poses more threats to the environment. Secondly, the solution is complete in itself contaminants are not merely transferred from one environmental medium to another (from water to air or land) but destroyed.
- Moreover, bioremediation is far more cost-effective than other technologies used to clean up hazardous waste Oilzapper/Oilivorous-S work out 30% cheaper than the conventional physico-chemical treatments.

Biofuels – Importance, Potential and Present Status

Mounting carbon emission-related climate change threat has made the pursuit of renewable energy paramount. Fossil energy reserves are finite anyway and the shift to renewable alternatives is needed sooner than later. Out of the total energy produced in the world for consumption, 25-30% relates to transport fuels. India's transport fuel demand is 112 million tons/year. Biofuels offer a renewable option for such storable and portable energy demand. There are also critical energy security and economic implications to consider. India's crude oil import bill is Rs 5-6 lakh crores per year. This is about one-third of the total import bill of the country and by far the largest expenditure item in the bill.

Biofuels can be broadly classified into first generation, second generation and third generation biofuels. First generation biofuels refer to ethanol from corn and sugarcane and biodiesel from oil crops such soybean and rapeseed. Agricultural lands are utilized for the feedstock development. This led to food vs fuel considerations. As a result, the scope of these fuels is limited in most countries in the world. India has altogether ruled out the development of such fuels from the beginning.

Second generation biofuels refer to fuels developed from not interfering with utilization of agricultural land and not compromising with food production. Representative fuels are ethanol produced from sugarcane molasses by-product, biodiesel from Jatropha grown in marginal lands and fuels developed from agricultural residues – ethanol produced by biochemical treatment of agri-residue feedstock (lignocellulosic ethanol) and pyrolytic fuels produced from thermochemical treatment of such feedstock. Forestry biomass could also be sustainably used for second generation fuel development. Ethanol produced molasses by-product is first utilized by the liquor market and then by the chemical industry dictated by better prices and only the left-over ethanol finds application as transport fuel. Hence this fuel option is unreliable in terms of availability and even in the best case scenario can offer only about 1 mtoe/year (less than 1% of India's transport fuel demand). Jatropha biodiesel suffers from feedstock germplasm development challenge for viable application in marginal lands and has never produced more than 0.1 mtoe/year in the past 15 years.

India has about 200 million tons/year of non-fodder agriculture residue that may be utilized for second-generation fuel production. This has the potential for the production of about 30-40 mtoe/year biofuel production. This relates to 25-35% of transport fuel demand of India. The potential for these fuels is much higher than the first generation biofuels and molasses-based ethanol and Jatropha biodiesel. But the technologies need further development before being widely applicable.

Lignocellulosic ethanol production involves pre-treatment of biomass, enzymatic hydrolysis of the complex cellulose and hemicellulose component to simple sugars and fermentation of sugars to produce ethanol. Pyrolytic fuel production involves thermal decomposition of biomass at high temperatures in the absence of oxygen, followed by deoxygenation and catalytic upgrading. Biochemical process-based lignocellulosic ethanol presently seems to have an edge over its counterpart thermochemical process-based pyrolytic fuels utilizing the same feedstock. However, the thermochemical process-based biofuels have shown less need for high capital investment at smaller-scale than biochemical process based ethanol. The thermochemical process-based fuels are currently trailing lignocellulosic ethanol fuel technologies in terms of development, but the preference may change when both technologies mature. Enzymatic hydrolysis of cellulose remains the main processing challenge in lignocellulosic ethanol technology. Deoxygenation and upgrading to drop-in fuels are the main challenges in pryolytic transport fuel technology. But the methods have one common major hurdle – supply chain and feedstock transportation logistics. Farm fields are spread across over vast areas and with small farmer land holding in a country like India and collecting agricultural residue from such vast areas to a common processing facility is a major challenge. Hence any technology that is being developed may have to be suitable for smaller decentralized processing requirements. One must also be wary of the increasing reach of electric vehicles in the future and the possibility of them replacing light automobiles utilizing petrol-type fuels. Recently Praj Industries Ltd, India has set up a 1 million liter/year second-generation ethanol plant in Maharashtra India. This utilizes corn cobs and stover, rice and wheat straws, cane trash and cotton stalk. India Glycold Ltd has set up second-generation ethanol plant in partnership with the Institute of Chemical Technology (ICT), Mumbai at Kashipur, Uttarakhand. Globally there are about seven other demonstration plants that have been set up so far. But commercialization efforts adopting the demonstration technologies have not yet made progress.

Microalgal biofuels are referred to as third generation biofuels – biofuels based on fast-growing microorganisms without the need for cultivable lands. Microalgae are microscopic unicellular suspensions in water. They are among the fastest growing organisms and they produce lipids as they grow. Their lipid yields are 10-50 times higher than the conventional oil crops. Sea water could be used as water source. Wastelands and marginal lands could be used as land resource. The potential is much higher (could cater to 30-60% of fuel needs), but the technology needs even more development than the second-generation fuels. Translating high yields observed in laboratory conditions to outdoor production, sturdy long-lasting strains that can withstand outdoor contamination, lipid recovery and by-product value chain development are some of the main challenges associated with microalgal biofuels. A

number of research institutes, academic research groups and industrial R&D units all around the world are presently engaged in the development of algal biofuels.

The Government of India initiated mandated biofuel blending programs from 2003 under the National Biofuels Mission (ethanol blending with petrol from 2003 and biodiesel blending with diesel from 2006). These programs specified blending of 5%, 10% and 20% biofuels with fossil fuels in a time bound and phased manner across India. A 'National Policy on Biofuels' was released in 2009 and it focussed mainly on molasses-based ethanol and Jatropha/Pongamia biodiesel with R&D focus on second-generation fuels. The policy specified pricing mechanism guidelines for biofuels such as Minimum Purchase Price for the fuels and Minimum Support Price for oilseeds. However these initiatives achieved only moderate success as described earlier. The Union Cabinet approved a more advanced and detailed National Policy on Biofuels – 2018 earlier this year. This aimed to enable extension of appropriate financial and fiscal incentives for development of advanced biofuels. It expanded the scope of biofuels to a range of new feedstocks/resources and new processing technologies. The policy aims to address many of the aforementioned technological, financial and supply chain challenges.

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