COURSE CODE:	EMT 513
COURSE TITLE:	Environmental Biotechnology
NUMBER OF UNITS:	2 Units
COURSE DURATION:	2 hours per week

COURSE DETAILS:

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Office Location: EMT Dept COLERM

Other Lecturers:

COURSE CONTENT:

Definition and historical development of biotechnology, Genes and genetic engineering. Tools and practice of genetic engineering, Applications of bio-technology (bio-remediation, bio-control, bio-fertilizers, bio-gas, bio-informatics etc.), Socio-economic implication of bio-technology

COURSE REQUIREMENTS:

This is a compulsory course for all 500 level students in the EMT Department. It is compulsory that students should participate in all the course activities and have minimum of 75% attendance in order to be qualify to write the final examination.

READING LIST:

1. A Text book of Environmental Biotechnology by R.C. Dubey, Department of Botany & Microbiology, Guruku Kangri University, Hardiocs 2494OU (Uttranchas)
2. Baxeeranis, A.D. & Quellette, B.F.F. 2001. Bio- Infomatics: A Guide to the Analysis of Genes & Protein. John Wiley & Son. Incv.
3. Bernard, H.U. & Helinski, D.R. (1980). In Genetic Engineering: princ Plus & Methods, vol. II (eds. Setlow, J. & Hollaendear) pp. 133-168 Plenum Press: New York
4. Glover, D.M (1984). Gene cloning – The Mechanising of DNA Manipullation, Chapman & Hall: London, P.222.
5. Subba Rao, W.S (1982). Biofertizers, In Advances in Agricultural Microbiology (ed, Subba Rao N.C) pp 219-242, Oxford & IBH Pub.Co. Mew Delhi

LECTURE NOTES

EMT 513 ENVIRONMENTAL BIOTECHNOLOGY.

Biotechnology is defined as the development and utilization of biological processes, forms and systems for obtaining maximum benefits to man and other forms of life. Biotechnology is the science of applied biological process.

Biotechnology is the application of scientific and engineering principles to processing of materials by biological agents to provide goods and service.

It is the application of biochemistry, biology, microbiology and engineering sciences in order to achieve technological application of capabilities of microorganisms, cultured tissue, cells, and parts their of.

It is also the application of biochemistry, biology, microbiology agents such as microorganisms or cellular components for beneficial use.

HISTORY OF BIOTECHNOLOGY

Biotechnology occurs in two phases: (i)the traditional (old) biotechnology, and (ii)the new (modern) biotechnology

Traditional Biotechnology

The traditional biotechnology is really the kitchen technology developed by our ancestors using the fermenting bacteria. Kitchen technology is as old as human civilization. During vedic period (5000-7000 BC), Aryans had been performing daily Agnihotra or Yajna. One of the materials used in Yajna is animal fat which is a fermented product of milk. The traditional biotechnology refers to the conventional technology which have been for many centuries. Beer, wine, cheese and many foods have been produced using traditional biotechnology. Thus, the traditional biotechnology includes the process that is based on natural capabilities of microorganisms. The traditional biotechnology has established a huge and expanding world market. The Egyptians (about 2000BC) used to prepare vinegar from crushed dates by keeping longer time. But the crushed dates produce intoxicants at first. In Egypt, Mesopotamia and Palestine (about 1500BC) the art of production of wine from crushed grapes, and beer from germinated cereals (malt) using a bread leaven (a mass of yeast) was established.

Role of Microorganisms in Fermentation: A French man, Nichola Appert (1810) described the method of food preservation. In the same year, Peter Durand also gave the use of tin container for food preservation. It was done by putting an air-tight vessel containing food material in the boiling water. It increased the importance of canning industry. Lack of oxygen in such a closed and heated vessel was reported by Ga y Lessac. He concluded that oxygen was required for initiation of alcoholic fermentation, but not for further progress of fermentation. After 1830, Charles B. Astier gave the concept that here is the career of all kinds of germs.

In 1837, Theodore Schwann after a series of experimentation demonstrated that the development of the fungus (sugar fungus) on fruit juice causes fermentation. He was the first to observe and describe the yeast in growing process. Charles Cagniard-Latour (1838) observed yeast budding using a microscope allowing 300-400 power magnification.

Modern Biotechnology

The two major features of technology differentiate the modern biotechnology from the classical biotechnology: 1) capability of science to change the genetic material for getting new products for specific requirements through recombinant DNA technology and 2) ownership of technology and its socio-political impact. Now the conventional industries, pharmaceutical industries, agro-industries, etc.are focusing their attention to produce biotechnology-based products.

Emergence of modern biotechnology: the new or modern biotechnology embraces all methods of genetic modification by recombinant DNA and cell fusion technology. It also includes the modern development of traditional biotechnological processes. The new aspects of biotechnology founded in recent advancement of modern biology, genetic engineering and fermentation process technology are now increasingly finding wide industrial application. But the rate of application will depend on: 1)

Adequate investment by the industries, 2)improved system of biological patenting, 3) marketing skills, 4) economics of the new methods and 5) public perception about the biotechnology products.

With the end of 19th century, the traditional biotechnology associated with fermentation was gradually industrialized. This resulted in gradual growth of industries producing beer, whisky, wine, rum, canned food, etc.

In 20th century, biotechnology brought industries and agriculture together. During World war I fermentation processes were developed which produced the acetone from starch and paint solvent from automobile industries. During World war ll the antibiotic penicillin was discovered. Manufacture of penicillin studied the biotechnological focus towards pharmaceuticals. Linking the fermentation with biochemistry, bioprocess, chemical engineering and instrument designing helped substantially in the progress of industries. During Gulf (1991) the work on microorganisms dominated for the preparation of biological warfare led to US attack on Iraq in 2003.

After the discovery of double helix DNA by Watson and Crick (1953), Werner Arber (1971) discovered a special enzyme in bacteria which he called the restriction enzymes. These enzymes can cut the DNA strand and generate fragments. The cut ends of two fragments are single stranded sticky ends because the single stranded ends having identical base pairs can re-join. In 1973, S. Cohen and H. Boyer removed specific gene from bacterium and inserted into another bacterium using restriction enzymes. This discovery marked the start of recombinant DNA technology or genetic engineering. In 1976, Baltimore successfully transferred human growth hormone gene into a rabbit. In 1978, a US. Company Genetech used genetic engineering technique to produce human insulin in E. coli. In 1980, trials of new hormone was conducted in the U. S. A., France, Japan, and the United Kingdom. In 1982, the US Food and Drug Administration gave marketing approval to Humulin i.e. human insulin made by Eli Lilly (USA) by the end of 1982. Another hormone somatotropin was produced in industrial scale.

In May 2005, scientists in south Korea have used a method called therapeufic cloning to produce stem cell lines. These are genetic matches to patients. Such stem cell lines could be used for disease research. The USA condemned this approach. In this method human embryos were produced through eloning and stems cells were obtained from blastocyst. The excised stem cells could be grown in vitro and used further. Many countries have developed collaborative networks on several aspects such as Regional Microbiology Network for South-East Asia (supported by Japan UNESCO). Microbiological Resource Centres (MICRENS)(supported by UNESCO), United Nations Environmental Programmes (UNEP). Gene Concept

Although the role of hereditary units(factors) in transfer of genetic characters over several generations in organisms was advocated by Gregor John Mendel, yet the mystery of the ‘ hereditary units’ was unraveled during early 1900s. In 1909, W. Johanson coined the term ‘gene’ that acts as hereditary units. However, early work done by several workers proposes various hypotheses to explain the exact nature of genes. In 1906,W. Bateson and R.C. Punnet reported the first case of linkage in sweet pea and proposed the presence or absence theory. According to them, the dominant character has a determiner, and the recessive character lacks determiner. In 1926, T.H. Morgan discarded all the previous existing theories and put forth the *particulate gene theory.* He thought that genes are arranged in a linear order on the chromosome and look like beads on a string. In 1928, Belling proposed that the chromosome that appeared as granules would be the gene. This theory of gene was well accepted by the cytologists. In 1933, Morgan was awarded Nobel Price for advocating the theory of genes. After the discovery of DNA as carrier of genetic informations, the Morgan’s theory was discarded. Therefore, it is necessary to understand both, the classical and the modern concepts of gene.

According to the classical concepts a gene is a unit of 1) physiological functions, 2) transmission or segregation of characters and 3) mutation. In 1969, Shapiro and co-workers published the first picture of isolated genes. They purified the lac operon of DNA and took photographs through electron microscope.

Units of a Gene

After much extensive work done by the molecular biologists, the nature of gene became clear. A gene can be defined as a polynucleotide chain that consist of segments each controlling a particular

trait. Now, genes are considered as a unit of function (cistron), a unit of recombination (recon) and a unit of mutation (mutan).

1. **Cistron**

   One-gene-one enzyme hypothesis of Beadle and Tatum was redefined by several workers in coming years. A single mRNA is transcribed by a single gene. Therefore, one-gene-one mRNA hypothesis was put forth. Exceptionally, a single mRNA is also transcribed by more than one gene and it is said to be polycistronic. Therefore, the concept has been given as one-gene-one protein hypothesis. The proteins are the polypeptide chain of amino acids translated by mRNA. Therefore, it has been correctly used as one-gene-one polypeptide hypothesis.

TOOLS OF GENERIC ENGINEERING.

Generic engineering (gene cloning, rDNA technology) can be defined as "changing of genes by using in vitro processes". A gene of known function can be transferred from its normal location into a cell that does not exist via a suitable vector. The transferred gene replicates normally and is handled over to the next progeny. On confirmation for its presence through biochemical procedures replica of the same cell (clones) can be produced. The derivation of procedures for the reintroduction of the foreign DNA fragment into a bacterium have led to evolution of new technology i.e the recombinant DNA technology, gene cloning , gene manipulation or genetic engineering.

The macromolecules such as DNA, RNA, proteins, etc. are synthesized inside the living cells which vary with each other in respect of molecular weight (size), solubility, presence of charges, absorbance of light wavelength (spectrum), etc. There are many techniques that are used to isolate and characterize the macromolecules on the basis of differentiating features.

Size of different types of molecules varies and therefore, their molecule weight also varies. For example, a macromolecule of small size shows less molecular weight and vice versa. Techniques used on the basis of molecular weight are: gel permeation, osmosis pressure, polarity of charges.

Gel Permeation or Gel Filtration: In this technique, polymeric organic compound is used to prepare a porous medium. The polymers form a three-dimensional network of pores. The pore size is determined by degree of cross-linking of polymeric chains. Solutes present in the mixture are separated on the basis of their size and shape when they pass through a column consisting of packed gel particles.

Separation of macromolecules by using gel permeation offers many advantages such as : 1) separation of labile molecules, 2) recovery of solutes in maximum quantity, 3) short time and expensive equipment , and 4) high reproducibility.

Characteristics Desired for Gel Filtration Media

The characteristics desired of gel filtration media are: inertness of gel matrix, presence of low amount of inorganic groups, uniform pore size, wide choice of gel particles and pore sizes and high mechanical rigidity.

Application of Gel Permeation

It is used for several purposes as given below:

i. Desalting or group separation: In many experiment (e.g. protein separation) inorganic salts are used. Salts should be removed during final preparation by passing through a column of Sephadex G-10.
ii. Fractionation or purification: Viral particles and low molecular weight compounds like sugars, proteins and nucleic acids of varying molecular weight can be separated.
iii. Determination of molecular weight: The macromolecules are separated on the basis of their relative size.

   If molecular weight of a compound is large, it will have lesser elution volume. The method has been used to determine the molecular weight of protein from their evolution characteristics.

Ion-Exchange Chromatography

When molecules are dissolved in solvent, they dissociate into charged ions. Therefore, they develops polarity. On the basis of polarity i.e. presence of charges they can be separated.

On the basis of counter ions, the ion exchanger has been grouped into two types: the cation exchangers and anion exchangers.

Cation exchangers: These are negatively charged containing positively charged counter ions (cation). On the basis of strength of charged functional groups, cation exchanges are divided into three types: strongly acidic, intermediate acidic and weekly acidic cation exchangers.

Anionic Exchangers: These are positively charged exchangers containing negatively charged counter ions (anions). On the basis of strength of charged functional groups anions, exchangers are also divided into strongly basic, intermediate basic, and weakly basic anion exchangers.

Matrix quality: Inorganic compounds, synthetic resin or polysaccharide based matrix are used in ion exchangers. The chromatographic properties of exchangers are governed by the matrix. The matrix also influences the biological activity of molecules. The basic three group of materials used in construction of matrix for ion exchangers are cellulose, resins and polymers or dextran or acrylamide.

Separation of solutes in ion-exchange chromatography: The steps for separation in ion-exchange chromatography are given as below:

1) The ion-exchanger is first treated with alkali, then acid to neutralize it. Finally it is washed with water. In contrast, the cation exchanger is first treated with acid, neutralized with alkali and finally washed with water.
2) The ion-exchanger is packed into a column and equilibrated with counter ions passing buffer of required pH.

Electrophoresis

It is a method of separation of charged molecules applying an electric field. When the charged molecules are placed in an electric field, they migrate depending on their net charges, size, shape and applied current. The velocity of movement of molecules can be represented by the following formula.

$$V=\frac{E.q}{F}$$

V= velocity of migration of molecules

E=electric field in volts/cm

q= net fractional co-efficient which is a function of the mass and shape of molecules.

Electrophoresis is applied for separation of RNA, DNA and proteins. DNA molecules have negative charges. Therefore, based on their size,DNA molecules migrate to anode i.e. small molecules move faster through the pores of matrix than the larger molecules. Similarly, Protein macromolecules are made up of positively and negatively charged amino acids at specific pH. The relative proportion of these two charges governed the net charges of protein macromolecules. If two proteins are of similar size and have identical charges, there shall be a little or no separation of such proteins due to similar charge: Mass ratio. Because the electrophoretic mobility depends on the charge density(charge/mass ratio).

On the basis of types of support medium, electrophoresis is of different types such as:1) paper electrophoresis (a strip of Whatman filter paper or cellulose acetate paper is used as support to separate macromolecules on the basis of their varying sizes. Later on, the spots developed on filter paper are cut and dissolved in solvent for separation and further work). 2) Starch gel electrophoresis (starch is partially hydrolysed in buffer to prepare a solution. Then it is heated and cooled to get starch gel. The gel acts as molecular sieve to separate molecules. Molecules of varying sizes migrate in gel and separated), 3)Immuno-electo focusing (it works like agar or agarose gel electrophoresis. It is used for

separation of proteins based on charge:mass ratio and their antigenicity. First proteins are separated, then allowed to react with antigens through diffusion via gel. Therefore, it is called immune electrophoresis), agarose gel electrophoresis, pulsed field the gel electrophoresis, polyacrylamide gel electrophoresis, sodium dodecyl polyacrylamide gel electrophoresis, two dimensional gel electrophoresis.

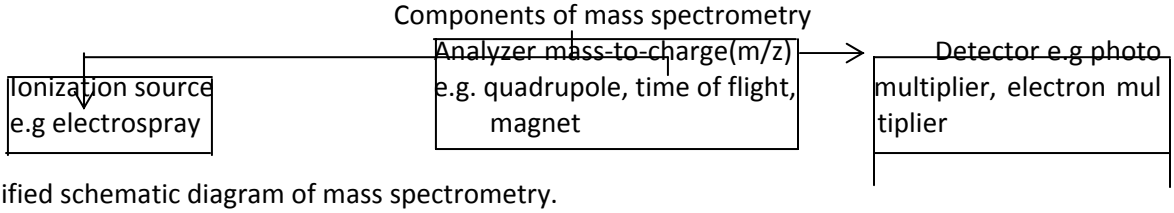
MASS SPECTROMETRY

Mass spectrometer employed fixed magnetic and electric fields to separate ions of different mass and energy. Two-dimensional electrophoresis is more powerful when coupled with MS. The unknown protein spot is cut from the gel and cleaved by trypsin digestion into fragments which are then analyzed by mass spectrometer and the mass of the fragment is plotted. This mass finger print can be used to estimate the probable amino acid composition of each fragment and tentatively identify the protein. The proteome and its changes can be studied very effectively by employing the two techniques together. The MS can also provide valuable information about covalent modification of proteins which can affect their activity. Mass Spectrometry is very useful technique. It is used in identification of unknown compounds, quantification of known compounds and determination of structural and chemical properties of compounds when present in small amounts. In recent years, mass spectrometry has become essential tool for analysis of genome and proteome in its many forms. This technique involves (i)the production of ions of materials in sample, (ii)their separation on the basis of their mass: charge (m: e) ratio, and (iii)determination of relative abundance of each ion. Therefore a mass spectrometer consists of three components:

(i)Source of ignition: It involves the gaseous ionization of analyte (the molecule to be analyzed)to be examined. When the molecules gain or lose a charge (through electron ejection, protonation), molecular ions are generated.

(ii)Analyzer: The ions generated are separated according to their mass-to-charge (m/z)

(iii)Detector: The molecular masses of separated ions are identified from the mass spectra determined after mass spectrometry of analyte . The charged molecules are electrostatically propelled into analyzer. On the basis of m/z ratio analyzer separates the ions. The detector detects the ions and transfer the received signals to computer. The information is stored and processed by the computer.



A simplified schematic diagram of mass spectrometry.

SPECTROMETRY

Spectrometry involves the study of interaction of electromagnetic radiation with matter, Characterisation of chemical compounds on the basis of their spectral properties are done by using spectrometry. In early days, quantitative estimation and identification of biomolecules were done using visible radiation (light) in spectroscopy. But in recent years spectroscopic techniques have been refined. Therefore, its scope has been broadened. Now a days it is used to examine the behavior of chemical substances after electromagnetic irradiation such as gamma rays, ultraviolet (UV) rays, infrared (IR) rays, radio waves and microwaves. Using the techniques, detailed information about inter-molecular bonding types or molecular changes occurring during enzymatic reactions and mitochondrial electron transport chain, etc. can be obtained.. More specifically, qualitative and quantitative measurement of biomolecules even in impure samples can be done rapidly and conviently using spectrophotometric and flavometric methods.

POLYMERASE CHAIN REACTION

The polymerase chain reaction provides a simple and indigenous method for exponentially amplification of specific DNA sequences by in vitro DNA synthesis. The technique has made it possible to synthesize large quantities of a DNA fragment without its coloning. It is ideally suited where the quantity of biological specimen available is very slow such as single fragment of hair or a tiny blood stain left at the site of crime. The PCR requires DNA Template, Primers and Enzyme.

BIOREMEDIATION

Bioremediation is the use of living microorganisms to degrade environmental pollutants or prevent pollution. It is a technology for removing pollutants from the environment, restoring contaminated sites and preventing future pollution. However, it is global, regional and local application. The basis of bioremediation is the enormous natural capacity of microorganisms to degrade organic compounds. Attempts are being made to develop microorganisms that can help reverse desert formation. This work is based on developing biopolymers that retains water reverse desert formation. *Alcaligens luteus* is being used to produce superbioabsorbent, a polysaccharide which is compose of glucose and glucuronic acid. These can absorb and hold more than thousands times of its own weight of water. Bioremediation technology has been used to remove environmentally hazardous chemicals, accumulated in their cells or detoxify them into non-toxic forms. Several members of algae, fungi and bacteria are known to solubilize, transport and deposit the metals, and detoxify dyes and complex chemicals. The toxic materials remain in vapour, liquid or solid phases; therefore bioremediation technology varies accordingly whether the wastes material involved is in its natural setting or is removed and transported into a fermenter (bioreactor). On the basis of removal and transportation of wastes for treatment, basically there are two methods: In situ and ex situ bioremediation.

1. In situ Bioremediation

   In situ bioremediation is the clean up approach which directly involves the contact between microorganisms and the dissolved and sorbed contaminants for biotransformation. Biotransformation in the surface environment is a complex process. Potential advantages of in situ bioremediation methods include minimal site disruption, simultaneous treatment of contaminated soil and ground water, minimal exposure of public and site personnel and low costs. But the disadvantages are (i) seasonal variation of microbial activity resulting from direct exposure to prevailing environmental factors, and lack of control of these factors ii) time consuming method as compared to other remedial methods and (iii) problematic application of treatment additives (nutrients, surfactants and oxygen).

   There are two types in situ bioremediation

(a) Instrinsic Bioremediation: This is the conversion of environmental pollutants into the harmless forms through the innate capabilities of naturally occurring microbial population. The intrinsic i.e. inherent capacity of microorganisms to metabolize the contaminants should be tested at laboratory and field levels before use for intrinsic bioremediation. Through site monitoring programs progress of intrinsic bioremediation should be recorded from time to time. The conditions of the site that favour intrinsic bioremediation are ground water flow throughout the year, carbonate minerals to buffer acidity produced during biodegradation, supply of electron acceptors and nutrients for microbial growth and absence of toxic compounds. The other environmental factors such as pH, concentration, temperature and nutrient availability determine whether or not bioremediation takes place. The ability of surface bacteria to degrade a given mixture of pollutants in ground water is dependent on the type and concentration of compounds, electron acceptor and duration of bacteria exposed to contamination. Therefore, ability of indigenous bacteria degrading contaminants can be determined in the laboratory by plate count and microcosm studies.

(b) Engineered in situ Bioremediation: Intrinsic bioremediation is satisfactory at some places but it is a slow process due to poorly adapted microorganisms, limited ability of electron acceptor and nutrient, cold temperature and high concentration of contaminants. When site conditions are not suitable, bioremediation requires construction of engineered systems to supply materials

that stimulate microorganisms. Engineered in situ bioremediation accelerates the desired biodegradation reactions by encouraging growth of more microorganisms via optimizing physic-chemical conditions. Oxygen and electron acceptors and nutrients promote microbial growth in surface.

(2) Ex situ Bioremediation

Ex situ bioremediation involves the removal of waste materials and their collection at a place to facilitate microbial degradation. Ex situ bioremediation technology includes most of disadvantages and limitations. It also suffers from costs associated with solid handling process e.g. excavation, screening and fractionation, mixing, homogenizing and final disposal. On the basis of phases of contaminated materials under treatment ex situ bioremediation is classified into two: (i) solid- phase system (including land and soil piles) i.e. composting, and (ii) slurry-phase systems (involving treatment of solid-liquid suspensions in bioreactors).

(a)Solid-phase treatment: It includes organic wastes (e.g. leaves, animal manures and agricultural wastes), and problematic wastes(e.g. domestic and industrial wastes, sewage sludge and municipal solid wastes). The traditional clean-up practice involves the informal processing of the organic materials and production of composts which may be used as soil amendment.

(i) Composting: It is a self-heating, substrate-dense, managed microbial system, and one solid-phase biological treatment technology which is suitable to the treatment of large amount of contaminated solid materials. However, many hazardous compounds are resistant to microbial degradation due to complex chemical structure, toxicity and compound concentration that hardly support growth. Microbial growth is also affected by moisture, pH, inorganic nutrients and particle size.

Composting can be done in open system i.e. land treatment, and in closed system. The open land system can be inexpensive treatment method, but the temperature fluctuates from summer to winter. Therefore, rate of biodegradation of waste materials declines. Secondly, land treatment system may become oxygen limited, depending on amount of substrate, depth of waste, application, e.t.c. However, efficiency of open treatment system can be increased by passing air. This approach is referred to as engineered soil piles and forced aeration treatment. The closed treatment is preferred over the open land system because controlled air is supplied to maintain the microbial activity. As a result microbial growth and volatilization of hazardous compounds, internal temperature gradually rises. Therefore, use of blowers for air circulation and exhaust for removal of toxic wastes are set up in closed system. Ventilators supply oxygen and remove heat through evaporation of water.

(b)Slurry-phase Treatment: The contaminated solid materials (soil, degraded sediments etc.), microorganisms and water formulated into slurry are brought within bioreactor i.e. fermenter. This slurry-phase treatment is a triphasic system involving three major components: water, suspended particulate matter and air. Here water serves as suspending medium where nutrients , trace elements, pH adjustment chemicals and desorbed contaminants are dissolved. Suspended particulate matter includes biologically inert substratum consisting of contaminants (soil particles) and biomass attached to matrix or free in suspending medium. Air provides oxygen for microbial growth. The objectives of bioreactor designing are to (i) alleviate microbial growth limiting factors in soil environment such as substrate, nutrients and oxygen availability. (II) promote suitable environmental conditions for microbial growth such as moisture, pH and temperature, and (iii) minimize mass transfer limitations and facilitates desorption of organic materials from the soil matrix. Biologically, there are three types of slurry-phase bioreactors: aerated lagoons, low-shear airlift reactor, and fluidized-bed soil reactor. The first two types are in use of full scale bioremediation, while the third one is a developmetal stage.

(I) Aerated Lagoons: Nutrients and aeration are supplied to the reactor. Mixers are fitted to mix different components and from slurry, whereas surface aerators provide air required for microbial growth. The process may be single-phase or multistage operation. If the wastes contains volatiles, this reactor is not appropriate.

(II) Low-shear Airlift Reactors: They are useful when waste contains volatile components; tight process control and increased efficiency of bioreactors are required. In this bioreactor, pH, temperature, nutrient addition, mixing and oxygen can controlled as desired.

(c) Factors Affecting Slurry-phase biodegradation are (i) pH (optimum 5.5-8.5), (ii) moisture content, (iii) temperature (20-30^{0}C), oxygen (aerobic metabolism preferred), (iv) ageing, (v) mixing (naturally and air mixing), (vi) nutrients (N, P and micronutrients), (vii) microbial population (naturally occurring microorganisms are satisfactory, genetically engineered microorganisms for layer compounds may be added)., and (viii) reactor operation (batch and continuous cultures).

BIOREMEDIATION OF HYDROCARBONS

Petroleum and its products are hydrocarbons. These two have much economic importance. Oils constitute a variety of hydrocarbons viz., xylanes, napthalenes, octanes, camphor, etc. If present in the environment these cause pollution. For example, during cold war between Iraq and America, millions of gallons of petroleum was leaked into sea which resulted in fish mortality. In toxic environment, microorganisms act only if the conditions e.g. temperature, pH and inorganic nutrients are adequate. Oil is insoluble in water and less dense. It floats on water surface and forms slicks. The microorganisms which are capable of degrading petroleum include pseudomonas, various corynebacteria, mycobacteria and some yeasts. However, there are two methods for bioremediation of hydrocarbons/oil spills, by using mixture of bacteria, and using genetically engineered microbial stains.

(a) Use of Mixture of Bacteria: Mixture of bacteria have successfully been used to control oil pollution in water supply or oil spills from ships. Artificially well characterized mixture of bacterial strains along with inorganic nutrients such as phosphorus and nitrogen are pumped into the ground or applied to oil spill areas as required.

(b) Use of Genetically Engineered Bacterial Strains: In 1990, the USA Government use superbug for cleaning up of an oil spill in water of state of Texas. Superbug was produced on a large scale in the laboratory, mixed with straw and dried. The bacteria laden straw can be stored until required. When the straw was spread on the over oil slicks, the straw soaked up the oil into non-polluting and harmless products.

BIOREMEDIATION OF INDUSTRIAL WASTES

A variety of pollutants are discharged in the environment from a large number of industries. For example, textiles industry alone contributes a significant amount of pollutants to water bodies such as enzymes, acids, alkali, alcohols, phenols, dyes, heavy metals, radionucliods, etc. Trades of Zinc, cadmium, mercury, copper, chromium, lead are found in dyes. It has been reported that actinomycetes show a higher capacity to bind metal ions as compared to fungi and bacteria. In addition, uptake mechanism of living and dead cells differ. Due to these differences they have potential application in industries. The living microbial cells accumulate metals intracellularly at a higher concentration, whereas dead cells precipitate metals in and around cell walls by several metabolic processes. Dead biomass immobilized on polymeric membrane absorbs uranium well, and immobilized Aspergillus oryzae cells on reticulated foam particles have been used for Cd removal. Aspergillus niger biomass contains up to 30% of chitin and glucan. Chitin phosphate and chitosan phosphate of fungi absorb greater amount of U than Cu, Cd, Mn, Co, Mg and Ca.

Bioremediation of Heavy metals: Various approaches have been made to detoxify and clean up these metals such as: use of certain chemical which in turn cause secondary

pollution, and physical methods that requires large inputs of energy/expansion materials. Another option is the use of different type of microorganisms such as algae, fungi and bacteria that removes metals from solution.

Metal-microbe Interactions and Mechanism of Metal Removal: On the basis of localization site of the metal, microbial interactions with heavy metal can classified into different categories such as extracellular, exocellular, and intracellular where organisms mobilize, immobilize, transform, precipitate, accumulate, coordinate, exchange, adsorb the metals and can form complexes. Microorganisms remove metals by the following mechanisms, adsorption (negatively charged cell surfaces of microorganisms bind to the metal ions), complexation (microorganisms produce organic acids e.g. citric acid, oxalic acid, lactic acid, malic acid and gluconic acid. Biosorption of metals also takes place due to carboxylic groups found in microbial polysaccharides and other polymers, precipitation (some bacteria produce ammonia, organic bases or H_2S which precipitates metals as hydroxides or sulfates. For example, Desulfovibro and Desulfotomaculum transform SO_4 to H_2S which promotes extracellular precipitation of insoluble metal. Volatilization (some bacteria causes methylation of Hg^{2+} and converts to dimethyl mercury which is a volatile compound).

Fungi are also capable of accumulating heavy metals in their cells.

Fungi involved in metal removal from industrial wastewater.

Microorganisms	Metals removed
Aspergillus niger	Cu, Cd, Zn
A. Oryzae	Cd
Penicillium spinulosum	Cu, Cd, Zn
Rhizopus arhizus	U
Saccharomyces cerevisiae	U

BIOAUGMENTATION (USE OF BLENDS OF MICROOGANISM)

Acceleration of biodegradation of specific compounds by inoculating bacterial cells is called bioaugmentation. Bacterial cells contain specific plasmid which encodes enzymes for degradation of those compounds. A variety of plasmid have been reported from Alcaligens, Acinetobacter, Kiebsiella, Arthrobacter and Pseudomonas. It is applicable in: (a) the increased BOD removal in waste water plants, (b) reduction of sludge volume by about 30% after addition of selected microorganisms, (c) use of mixed cultures in sludge digestion, (d) biotreatment of hydrocarbon wastes and (e) biotreatment of hazardous wastes.

Some demerits of bioaugmentation are: (a) need of an acclimation period prior to onset of biodegradation, (b) a short survival or lack of growth of microbial inocula in the seeded bioreactors, and (c) sometimes negative or non-conclusion of some commercial products.

Use of Enzymes

Several enzymes have been detected in wastewaters such as catalase, phosphate esterases and amino-peptidases. These enzymes can be added to fresh waters to improve biodegradation of xenobiotic compounds. For example, parathion hydrolases (isolated from Pseudomonas and Flavobacterium) have been used to cleanup the containers of parathion and detoxification of waste containing high concentration of organophates. Some specific enzymes, the extracellular fungal laccases can be used for the treatment effluents generated by the pulp and paper industry because this enzyme can be useful for

dechlorination of chlorinated phenolic compounds or oxidation or aromatic compounds even at adverse environmental conditions such as low pH, high temperature, presence of organic solvents, etc.

BIOFILTERATION

It is a new technology used to purify contaminanted air evolved from volatile organic compounds by involving microorganisms. It is the oldest biotechnological method for removal of undesired foul gas components from air. Since 1920. Biofilters were used to remove compounds that have odour from wastewater treatment plants or animal farming. It could be achieved by digging trenches, laying an air distribution system and refilling the trenches with permeable soil, wood chips and compost. However biofilteration is not suitable for highly halogenated compounds e.g. trichloroethylene, trichloroethane and carbon tetrachloride; due to its low aerobic degradation. Also the size of a biofilteration in inversely proportional to the degradation rate. Biofilteration is done by using biofilters

BIOLOGICAL CONTROL OF PLANT PATHOGENS, PESTS AND WEEDS

Biological control method is such a technique which involves disease control by some biological agent(s) (living micro- or macroorganisms, other than disease causing organisms (the pests) and damanged plants (the hosts). Biological control is defined as any condition under which or practice whereby , survival or activity of a pathogen is reduced through the agency of any other living organisms except man himself with the result that there is reduction in incidence of disease caused by pathogen. Pathogens is an organism or a virus, capable of causing disease in a particular host or range of hosts. A unified concept of biological control is given by Baker and Cook (1974) as the reduction of inoculum density or disease-producing activity of a pathogen or parasite in its active or dormant state, by one or more organ isms, accomplished naturally or through manipulation of environment, host or antagonist, or by mass introduction of one or more antagonists.

Aims and Objectives of Biological Control

(i) reduction of inoculum of the pathogen through disease survival between crops, decreased production or release of viable propagules or decreased spread by mycelial growth

(ii) reduction of infection of the host by the pathogen and

(iii) reduction of severity of attack by the pathogen.

A variety of soil microorganism are present in the rhizosphere and rhizoplane regions of plant. The increase in microbial number and their activity have been referred to as rhizosphere effect. It is caused by secretion of growth promoting (root exudates) and casting of sloughed off root tissues by plants in soil during their growth phases. Nowadays manipulation of soil environment has become a tool for biological control of soil borne plant pathogens. Several methods have been developed which bring about artificial manipulation of rhizosphere, phyllosphere and soil environment. Consequently number of antagonistic microorganism is increased. This can be done by (i) artificial introduction of antagonists in soil or spraying these antagonists on the aeriel parts of plants, (ii) modification of soil environment by organic amendments, (iii) green manuring, changing soil pH, C: N ratios, temperature, and (iv) adding the selective chemicals or heat treatment of plant tissues.

Antagonism- The Mechanism of Biocontrol

Biological control is principally achieved through antagonism (the inhibitory relationships between microorganisms including plants) which involves: amensalism i.e. antibiosis and lysis, (ii) competition, and (iii) parasitism and predation.

AMENSALISM: This is a phenomenon where one population adversely affects the growth of another population whilst itself being unaffected by the other population. Antibiosis is a situation where the matabolites secreted by organism A inhibit organism B, but organism A is not affected.

Applications of Biological Control in Field

Practical application is the translation of in vivo conditions. Owing to variations in environmental factors, sometimes , antagonistic potential and production of mutants adjustable to stress conditions are being carried out in most countries. Some of the methods available now which (i) can decrease virulence of the pathogens, (ii) increase hosts resistance, and (iii) stimulate the antagonistic potentialities and intensify their activity. These can be achieved by one or more methods which are:

Examples of biological control of soil-borne pathogens

Plant pathogens	Diseases	Methods applied for control
Cephalosporium gregantum	Brown stem of soybean	Five years cropping of maize before soybean
Fusarium oxysporum f. pisi	Fusarium wilt of pea	Crop rotation with cereals or rape
Meloidogyne incognita	Root knot of tomato	Incorporation of chopped alfalfa hay
Pythium ultinum	Seedling blight of lettuce	Incorporation of cereals or cruciferous cover crop into soil 2 weeks prior to planting

CROP ROTATION

Crop rotation is the method of rotating crop in a field, followed by other crop, alteration in gross microbial community in soil is done. By doing so, inoculum density of a pathogen is lowered. Microbial shift results in increased no of beneficial microbes, and decreased no of pathogens e.g. rotation of wheat with leguminous crops affects take-all of wheat. Leaf spot of pea nut caused by Cercospora sp. Is reduced by 88% on rotation with maize or soybean.

Treatment of soil with Selected Chemicals: The idea of recolonization and survival of treated soil with some chemicals by microorganism was given by Bliss in 1951. He found that in soil treated with carbon disulpide for the control of Armillaria mellea from infected citrus root, population of T. virde rapidly increased. Consequently, A. mellea was killed directly by fungicidal action of carbon disulphide and necrotrophic activity of T. viride.

Use of Biofertilizer as Biocontrol of Plant Diseases: Diatotrophic bacteria have been reported to control many plant disease. Treatment with Azotobacter of rice, soyabean and cotton seeds resulted in reduced seedling mortality by inhibiting the pathogen. The biocontrol mechanisms have been explained by two facts: (i) antagonism, and (ii) development of host resistance against pathogens. Antagonisms takes place by secretion of antibiotic substances and toxic metabolites, release of acids that make unfavourable pH for growth, competition for nutrients or stimulation of host defence mechanism or direct parasitism. Moreover, activity of antagonists can be stimulated by adopting both seed dressing and chemical treatment methods where individually seed treatment (seed dressing with microorganism) or chemical treatment fails to control the disease.

Soil inoculum: Soil is a unique habitat which harbours a vast majority of microorganisms in a continuous dynamic state by actions and interactions. Now-a-days researchers on introduction of antagonists in soil for disease control are in progress. Damping off of seedlings caused by Pythium ultimum and R. solani was reduced by introduction of Bacillus or Streptomyces species into the steamed soil

Genetic Engineering of Biocontrol Agents

Occurrence of a no of interactions among the microorganisms provides a broad opportunity for genetic engineering of microbial biocontrol agents directed at plant pathogens or other microorganisms against pests and weeds. Several biocontrol agents have been successfully employed for such purposes in experimental or commercial agriculture. The effectiveness of biocontrol agents can be intensified by gene splicing, gene cloning and transformations. It is however, obvious that bacteria pathogenenicity on plant is determined by several genes. Certain genes encodes enzymes involved in biosynthesis of phytotoxins, growth hormones or enzymes capable of degrading plant cell wall or other constituents.

Since these genes are positively needed for pathogensis, their activation would destroy the organisms, their pathogenic potential or reduce virulence.

BIOLOGICAL CONTROL OF WEEDS

Weeds are unwanted plants which grow in agricultural fields, ponds, lacks etc. and have bad effects on flora and fauna present/growing in their vicinity. However, in agricultural fields, nutrients supplied to specific crop are absorbed by weeds and results in poor supply of nutrients to crop plants. Similarly, in paddy fields, they do so. Larvae of the harmful insects e.g. malaria mosquito survives around the aquatic weeds growing in ponds or lacks. Although synthetic herbicides have been formulated which have phytotoxic and mutagenic effects on many agricultural plants and in turn enter in animal or human system. Therefore use of synthetic herbicides is being discouraged in many countries. An alternative method of control of weeds have been developed which is the use of microbial herbicides or bioherbicides.

Pathogens of aquatic and terrestrial weeds

Plant species	Pathogens
A. Aquatic weeds	Cyanophages (virues) and bacteria
Algae (Cyanobacteria)	
Alligator weeds (Atternathea philoxeroides)	Stunt virus
Hydrilla (Hydrilla verticillata)	Aspergillus sp., Penicillin sp., Phytophthora parasitica.
Salvinia (Salvina sp.)	
Water hyacinth (Eichhornia crassipes)	Acremonium natum, Alternaria eichhorniae
Water lettuce (Pisina stratiotes)	Virus
B. Terrestrial Weeds	
Sida spinosa	Colletotrichum malvarum
Sorghum halepense	C. graminicola
Crotalaria spctabilis	C. dematium
Diospyros virigiana	Acremonium diospyri
Pathenium hysterophorus	Selerotium rolfsii, Fusarium oxysporum

BIOFERTILIZER

In recent years, use of microbial inoculants as a source of biofertilizers has become a hope for most of countries, as far as economical and environmental viewpoints are concerned. Biologically fixed nitrogen is such a source which can supply an adequate amount of nitrogen to plants and other nutrients to some extent. It is a non-hazardous way of fertilization of field. Moreover, biologically fixed nitrogen consumes about 25-30% less energy than normaly done by chemical process. The term biofertilizer denotes all the

nutrients inputs of biological origin for plant growth. Here biological origin should be referred to as microbiological process synthesizing complex compounds and they are further release into outer medium, to close vicinity of plant roots which are again taken up by plants. Therefore the appropriate term for biofertilizer should be microbial inoculants. As bacteria and cyanobacteria are known to fix atmospheric nitrogen, both bacteria and cyanobacteria are widely used as biofertilizer. Biofertilizer-wise crop response showed that the contribution of nitrogeneous biofertilizers was in theorder of Azolla> Rhizobium> cyanobacteria> Azospirillum> Azotobacter. Use of phosphatic biofertilizers (PSM) also showed increased crop response. But nitrogenous biofertilzer +PSM resulted in higher cop response. Beside benefiting N and P nutrition, the microbes benefited plant growth and increased crop yield due to secretion of phytohormones like auxins, Giberellins, cytokinins. Some microbes also acts as plant growth-promoting rhiozobacteria which control plant pathogens also.

BACTERIA INOCULANTS

Bacterization is a technique of seed-dressing (as water suspension) e.g. Azobacter, Bacillus, Rhizobium etc. It has been proved that bacteria can successfully be established in root region of plants which in turn improve the growth of hosts. Moreover, information gathered on associate symbiosis (i.e. symbiosis between roots of grasses and Azospirillum) has increased the interest on this bacterium to be used as seed inoculant for cereals.

Azospirillum Inoculants

Soil pH governs Azospirillum distribution.Soil pH between 5.6 and 7.2 promotes nitrogenase activity, whereas pH below 5-6 does not encourage nitrogenase activity in soil. By using semi-solid sodium malate enrichment medium Azospirillum could be isolated from Digitaria decumbens. The essential requirements for its isolation are the surface sterilization of roots by 70% ethanol and creation of micro-aerophilic conditions in the medium.

Isolation of Azospirillum: it can be isolated from plant roots as well as soil samples. For isolation from plant roots, a suitable host plant is selected and taken out of soil. Its root system is washed with running tap water and roots are cut into 0.5cm long pieces. Roots are sterilized with 0.1%$Hgcl_2$ solution for one minute. Therefore, these are serially washed with sterile distilled water. One or two pieces of roots are placed on sterile and cool down semi-solid agar medium containing sodium malate in screw-capped tubes. Inoculated tubes are incubated at 28-30^{0}C for 2days. Just 1-2cm below from the surface medium, white pellicles of Azospirillum can be observed. Thereafter, the nitrogen-free malate medium becomes blue in colour which represents the presence of Azospirillum.

Characteristics of Azospirillum Strains:

Azospirillum is a Gram-negative, motile, vibroid bacterium. It consists of poly-β-hydroxybutyrate (PBH) granules. It behaves as highly aerobic when grown in ammonium-containing medium, and found as micro-aerophilic when grown in nitrogen-free medium. The carbon sources that provide energy are malate, succinate, lactate and pyruvate. Hence, it grows well on media containing the carbon sources. It grows moderately on galactose or acetate-containing medium and poorly on glucose or citrate-containing medium.

Preparation of Carrier-based Inoculants

Several low cost and locally available carriers have been evaluated for Azospirillum: for example farmyard manure (FYM)+ soil, FYM-charcoal, peat, etc. Properly sterilized soil and FYM in the ratio 1:3 proved as the best carrier for Azospirillum. The harvested broth inoculum is mixed with FYM+ soil carrier till 40% moisture is obtained. The carrier-based inoculant is packed in polythene bags. The bags are stored at 4^{0}C for about days. Method of carrier-based inoculant production is the same as described for Rhizobium and Azobacter. It is applied in field for various crops.

Seedling Treatment

Slurry of Azospirillus inoculant is prepared by mixing 1kg of inoculant with 40litres of water. The roots of transplanted seedlings are dipped in slurry for 15-20minutes. Then seedlings are transplanted in field.The remaining slurry is spread in field.

Seed Treatment

Slurry of Azospirillum inoculant is prepared by mixing with water in a container. Seeds to be sown in a field are soaked in slurry at 2kg inoculant per hectare overnight. Then the seeds are sown.

Crop Response

It benefits the plant growth and increases the crop yield by improving root development, mineral uptake and plant-water relationship. Beside nitrogen fixation, it also produces the growth-promoting substances like IAA and giberelins that enhance the plant growth.

PHOSPHATE SOLUBILISING MICROORGANISMS (PHOSPHATE BIOFERTILIZER)

Phosphorus is the second vital nutrient next to nitrogen required for growth of microorganisms and plants. But most P is not available to paints. Only 1-2% P is supplied aboveground parts of the plants. Therefore to meet out the P demand of plant, exogenous source of P is applied to plant as chemical fertilizers. One of the most common forms of phosphate fertilizer is the superphosphate (single or triple). The basic raw material for phosphate fertilizer is the rock phosphate. But rock phosphate is not recommended to apply directly due to agronomic problems being as a raw material. There are several phosphate solubilizing microorganisms present soil e.g. the species of Bacillus, Pseudomonas, Penicillin, Aspergillus, etc.

Isolation of PSM

Pikovskaya medium is prepared, mixed with 5% gum Arabic, autoclaved and dispensed in petri plates. A small amount of soil is collected from field and serially diluted in known volume of water. Each plate is inoculated with 1ml of soil-water suspension. Plates are incubated at 28^0C for about 4-5 days. Only PSM grow and from colony which can be identified due to formation of clear zone around each colony. Because PSM utilize $Ca_3(PO_4)_2$ and form clear zone. Such colonies are picked up, purified and preserved for further use. Starter culture is prepared by inoculating the fresh Pikovskaya broth and incubated on a rotatory shaker at 28^0C.

Production of Carrier-based Inoculants

Different carriers (e.g. wood charcoal, peat mixture or mixture of wood charcoal and soil) are used for inoculum production. The carrier is powdered, neutralized, sterilized and mixed with broth inoculant. Carrier and inoculant are properly mixed till 40% moisture is attained. This mixture is left for curing by leaving it in a sterile chamber. Then it is filled in polythene bags at 200g/packet) and stored at 15-20^0C

Crop Response

The PSM solubilize 20-30% phosphate which is then absorbed by plant. Consequently, plant growth is increased. The PSM can be used for all types of plants because they are heterotrophs.

GREEN MANURING

Green manure is a farming practice where leguminous plant which has derived enough benefits from its association with appropriate species of Rhizobium is ploughed into the soil and then a non legume is grown and allowed to take the benefits of already fixed nitrogen. E.g. of some leguminous plants are: Cyamopsis pamas, Glycine wightii etc.

Contribution of Nitrogen by some of the nodulated legumes when used as green manure/cover crop.

CYANOBACTERIAL INOCULANTS

In water-logging condition, the cyanobacteria multiply, fix atmospheric nitrogen and release it into the surroundings in the form of amino acids, proteins and other growth promoting substances.

Algalization: It denotes the process of application of blue-green algal culture in field as biofertilizer. The main objectives are: To develop low cost indigenous technology for mass production of cyanobacteria, to isolate regional specific fast growing and better nitrogen fixing strains, to study the benefits on both economy and ecology. As a result of these studies cyanobacteria biofertilizer was found very useful, especially for small and marginal farmers of the country with the view point of both economy and ecology.

MYCORRHIZAL FUNGI AS BIOFETERLIZER

Mycorrhiza (fungus roots) is a distinct morphological structure which develops as a result of mutualistic symbiosis between some specific root-inhabiting fungi and plant roots. Plants which suffer from nutrient

scarcity, especially P and N, develop mycorrhiza i.e. plants belong to all groups e.g. herbs, shrubs, trees, aquatic, xerophytes, hydrophytes or terrestrial ones. In most of the cases plant seedling fails to grow if the soil does not contain inoculum of mycorrhizal fungi. In recent years, use of artificially produced inoculum of mycorrhizal fungi has increased its significance due to its multifarous role in plant growth and yield, and resistance against climatic and edaphic stresses, pathogens and pests.

BENEFITS OF BIOFERTILIZERS

(i)It is a low cost and easy technique, and can be used by small and marginal farmers

(ii)It is free from pollution hazards and increase soil fertility.

(iii)Cyanobacteria secrete growth promoting substances like IAA, IBA, NAA, amino acids, proteins, vitamins, etc.

(iv)Canobacteria can grow and multiply under wide pH range of 6.5-8.5. Therefore, they can be used as the possible tool to reclaim saline or alkaline soil because of their ameliorating effect on the physic-chemical properties of the soil.

(v)Rhizobial biofertilizer can fix 50-150kg N/ha/annum.

(vi) Azobacter and Azospirillum, besides supplying N to soil, secrete antibiotics which act as pesticides

(vii)Azolla supplies N, increases organic matter and fertility in soil and shows tolerance against heavy metals.

(viii)The biofertilizer increase physic-chemical properties of soil such as soil structure, texture, water holding capacity, cation exchange capacity and pH by providing several nutrients and sufficient organic matter.

(ix)The mycorrhizal biofertilizers make the host plants available with certain elements, increase longevity and surface area of roots, reduce plants response to soil stresses and increase resistance in plants. In general, plant growth, survival and yield are increased.

BIO GAS

In 1776, for the first time, the Italian physicist, Volta, demonstrated methane in the marsh gas, generated from organic matter in bottom sediments of ponds and streams. Under anaerobic conditions, the organic materials are converted through microbiological reactions into gases (fuel) and organic fertilizer (sludge). The mixture is composed of 63% methane, 30% CO_2, 4%N and 1% hydrogen sulphide and traces of hydrogen, oxygen and carbon monoxide. Methane is the main constituent of biogas. It is also referred to as biofuel, sewerage gas, bioenergy and fuel of the future. About 90% of energy of substrate is retained in methane. Biogas is used for cooking and lighting purposes in rural sector. It is devoid of smell and burns with a blue flame without smoke.

Benefits from Biogas Plants

In Asia, biogas is used mainly for cooking and lighting purposes. In addition, there are many other advantages in installing the biogas plants. It is used in internal combustion engines to power water pumps and electric generators. It is also used as fuel in fuel type refrigerators. Sludge is used as fertilizer. The most economical benefits are minimizing environmental pollution and meeting the demand of energy for various purposes.

Biogas Production: Anaerobic Digestion

Anaerobic digestion is carried out in air tight cylindrical tank which is known as digester. A digester is made up of concrete bricks and cement or steel. It has a side opening (charge pit) into which organic materials for digestion are incorporated. There lies a cylindrical container above the digester to collect the gas. In biogas plant, a concrete tank is built up which has the concrete inlet and outlet basins. Fresh cattle dung is deposited into a charge pit, which leads into the digestion tank. Dung remains in tank. After 50, sufficient amount of gas is accumulated in gas tank, which is used for house-hold purposes. Digested sludge is removed from the basin and is used as fertilizer. Usually digesters are buried in soil in order to benefit from insulation provided by soil. In cold climate, digesters can be heated by the installation provided from composting for the agricultural wastes.

Anaerobic digestion is accomplished in 3 stages, solubilization, acidogenesis and methanogenesis. These stages are characterised by 3 groups of bacteria.

a) Solubilization: It is the initial stage, when feedstock is solubilized by water and enzymes. The feedstock (cattle dung and other organic polymers) is dissolved in water to make slurry. The

complex polymers are hydrolysed into organic acids and alcohols by hydrolytic fermentative bacteria which are mostly anaerobes.

b) Acidogenesis: During this stage, the second group of bacteria i.e. facultative anaerobic and H_2 producing acidogenic bacteria convert the simple organic material via oxidation/reduction reactions into acetate, H_2 and CO_2. These substances serve as food for the final stage. Fatty acid other acids from H_2 and CO_2 via acetogenic hydrogenation.

c) Methanogenesis: This is the final stage of anaerobic digestion where acetate and H_2 plus CO_2 are converted by methane producing bacteria (methanogens) into methane, carbon dioxide, water and other products.

Different species of methanogens are involved in breakdown of complex organic matter into acetate or other organic acids. Acetate is one of the substrates of methanogenic bacteria. Hydrogen with CO_2 is a general substrate for methanogenesis. Numbers of these bacteria differ with types of substrates. For example, counts of 10-10^6 per ml and 10^5-10^8 per ml of hydrogen utilizing bacteria were determined from the pig waste and sewage sludge digesters, respectively.

i. Methanogens: Methanogens are a unique group of bacteria. They are obligate anaerobes and have slow growth rate. They play a major role in breakdown of substrate into gas form. They are the only organisms which can anaerobically catabolize acetate and H_2 to gaseous products in the absence of exogenous electron acceptors other than CO_2 or light energy. In their absence, effective degradation would cease because of accumulation of non-gaseous, reduced fatty acid and alcohol products of the fermentative and other H_2 using bacteria that have almost the same energy content as the original organic matter.

ii. Mechanism of methane formation. Metabolically the methanogens are very peculiar. Carbon dioxide fixation, Calvin cycle, serine or hexulose pathway are absent in them. The mechanism of methane formation is not well understood. Ralph wolfe (1979) found that several new coenzymes are involved which are not present in any other group of bacteria. These coenzymes are methyl coenzymes M, hydroxyl methyl coenzyme M, coenzyme F420, coenzyme F430, component B, corrinoids, methanofuran or carbon dioxide reducing factor and methanopterin and formaldehyde activating factor.

Biogas Production from Different Feedstocks

Although there are various types of biomaterials different in chemical composition, yet gobar (cow dung) is most popular in India. Besides gobar, other materials which would be successful in the coming years are agricultural wastes, municipal wastes, industrial wastes and some of the aquatic biomass.

a) Salvinia: This fern can be a feedstock material for biogas production. Fermentation of Salvinia starts within 7-9 days on putting under water in suitable container. Biogas yield is about 0.1 litres/kg fresh weight for 4 weeks. Air dried weed produces about 1 litre/kg for 90days and fermentation is continued for 3 months. Thereafter gas yield gradually declines.

Daily requirement of gas is 0.4 litre per capita. Two tonnes of air dried Salvinia, therefore, can meet the fuel requirement of a small family for 3-5months. Special advantage of using Salvinia is that unpleasant odours do not come out. This will certainly provide cheap and clean burning fuel and reduce the increasing pressure on the forest.

b) Water Hyacinth: This weed can be used for the production of laboratory and domestic fuel. Its decomposition rate is higher than that of cow dung for gas production. Water Hyacinth is totally decomposed within 3 days in summer while cow dung takes 8 days. The ratios between total gas

evolved by water hyacinth and cow dung under the identical conditions in summer and winter are 5:3 and 5:1, respectively.

Factors Affecting Methane Formation

(i)Slurry: proper solubilization of organic materials (the ratio between solid and water) should be 1:1 when it is house hold things.
(ii) Seeding: In the beginning, seeding of slurry with small amount of sludge of another digester activates methane evolution. Sludge contains acetogenic and methanogenic bacteria.
(iii)pH: For the production of sufficient amount of methane, optimum pH of digester should be maintained between 6-8 as the acidic medium lowers methane formation.
(iv)Temperature: Flunctuation in temperature reduces methane formation, because of inhibition in growth of methanogens. In case of mesophilic digestion, temperature should be between 30^0C and 40^0C but in case of thermophilic ones, it should be between 50^0C and 60^0C.

Nitrogen concentration: Excess amount of nitrogen inhibits growth of bacteria, and thereby lowers methane production. Therefore, use of such materials should be discouraged.
(vi)Carbon-nitrogen (C:N) ratio: Improper C:N ratio lowers methane production. Maximum digestion occurs when C:N ratio is 30:1. Amendment of nitrogen or carbon substrates should be done exogenously according to chemical nature of substrates used in fermentation.
(vii)Creation of anaerobic conditions: It is obvious that methane production takes place in strictly anaerobic condition, therefore, the digesters should be totally airtight. In Indian conditions, digesters are buried in soil.
(viii)Addition of algae: Ramamoorthy and Sulochana (1989) have an enhancement in biogas production from cow dung on addition of green alga, Zygogonium sp. The amount of biogas produced from alga was twice (344m/g dry algae) of that obtained from cow dung (179/g dry cow dung) alone.

BIOFORMATICS

Bioformatics is the application of information sciences (mathematics, statistics and computer sciences) to increase our understanding of biology. It is also the management and analysis of biological information stored in databases.
A database is a repository of sequences (DNA or amino acids) which provide a centralized and homogeneous view of its contents. The repository is created and modified through a database management system (DBMS). Every data item in the database is structured according to a scheme. The contents of database can be accessed through a graphical user interface (GUI) that allows browsing through the contents of the repository very much similar as one may browse through the books in the library.

Classification of Databases

The databases are broadly classified into two categories: sequence databases (that involves the sequences of both proteins and nucleic acids), and structural databases (that involves only protein databases). In addition, It is also classified into 3 categories (a)primary database, (b)secondary database, and (c)composite database
Primary databases contain information of the sequence or structure alone of either protein or nucleic acid e.g. protein sequences. Primary database tools are effective for identifying the sequence similarities, but analysis of output sometimes difficult and cannot always answer some of the more sophisticated questions of sequence analysis.
Secondary databases contain derived-information from the primary databases. For example, information on conserved sequence, signature sequence and active sites residues of protein families by using SCOP, eMOTIF etc. It more useful than the primary databases.

A moderate database pertaining to protein sequence and structural correlations on the Net was established by Bairoch (1991). This database was called PROSIT which later on was strengthened with a database on sequence analysis and comparison of protein sequences known as SEQUANALREE.

SEQUENCES AND NOMENCLATURE

Indirectly the sequence data means the structure of biopolymer, and structure expresses the function. It shows a reductionist approach. Therefore, the sequence data can be used as context free.

The IUPAC Symbols

The International Union of Pure and Applied Chemistry (IUPAC) have made certain recommendations. The nomenclature system in bioformatics is based on these recommendations. Language used in bioformatics is given below:

Alphabet	Nucleotides
Words	Gene (prokaryotes)
	Introns (eukaryotes)
Sentence	Operon (prokaryotes)
	Gene (eukaryotes)
Punctuation	Regulatory gene
Chapter	Chromosome

Types of Sequences used in Bioformatics

There are different types of sequences which are known to have genetic information. Therefore, such sequences are used in bioformatics. Such sequences are below:

(i)Genomic DNA: The genomic DNA acts as the reservoir of genetic information of all organisms

(ii)cDNA: The double stranded molecules prepared by using mRNA as template and reverse transcriptase are called cDNA. These are expressed genes of genomic DNA. By using cDNA molecules, substantial number of sequences have been determined and deposited in database.

(iii)Organellar DNA: Eukaryotic cells consist of different types of organelles e.g. chloroplast, mitochondria, Golgi complex, nucleus, etc. In eukaryotes genomic DNA is found in nucleus and organellar DNA molecules are located in mitochondria and chloroplasts.

The DNA molecules of these organelles are usually circular and double stranded of varying size besides a few exceptions.

Use of Bioinformatics Tools in Analysis

By using bioinformatics tools different types of analysis of biological data can be done.

i. Processing Raw Information: The biological information hidden in DNA/RNA and protein sequences are generated experimentally. These are called raw information. Using bioinformatics tools these informations are processed into genes and proteins and a relationship is established between a gene and a protein. Phylogenetic relationships can also be among the species of organisms.
ii. Genes: Using bioinformatics tools such as GenMark (for bacteria), and GenScan (eukaryotes) gene prediction is carried out in organisms. GenScan can identify introns, exons, promotor sites and poly A signals and other gene identification algorithms.
iii. Proteins: By using computer programmes, protein sequence can be deduced from the predicted genes.
iv. Regulatory Sequences: Using computer programmes, the regulatory sequences can also be identified and analysed.
v. Phylogenetic Relationships: There are a few web-based applications that will allow you to carry out phylogenetic analysis over the web.