Bioremediation is a process used to treat contaminated media, including water, soil and subsurface material, by altering environmental conditions to stimulate growth of microorganisms and degrade the target pollutants. In many cases, bioremediation is less expensive and more sustainable than other remediation alternatives.Most bioremediation processes involve oxidation-reduction reactions where either an electron acceptor (commonly oxygen) is added to stimulate oxidation of a reduced pollutant (e.g. hydrocarbons) or an electron donor (commonly an organic substrate) is added to reduce oxidized pollutants (nitrate, perchlorate, oxidized metals, chlorinated solvents, explosives and propellants).In both these approaches, additional nutrients, vitamins, minerals, and pH buffers may be added to optimize conditions for the microorganisms. In some cases, specialized microbial cultures are added (bioaugmentation) to further enhance biodegradation. Some examples of bioremediation related technologies are phytoremediation, mycoremediation, bioventing, bioleaching, landfarming, bioreactor, composting, bioaugmentation, rhizofiltration, and biostimulation.

Need for bioremediation:

Synthetic pesticides contribute to the spectacular increase in the crop yield. Soil, groundwater and sediments are the ultimate sinks for these pollutants, where they are either broken down to simpler forms or remain persistent. Although they play a significant role in augmenting food production, their large scale and indiscriminate use has resulted in highly unstable ecosystem, development of resistance by insects, elimination of parasites, predators and pollinators, resurgence of minor pests and destruction of useful insects. Thus, looking towards the environmental concerns and health hazards resulting due to the continuous use of these noxious xenobiotic pesticides, it is highly desirable to detoxify them. Traditional methods of pesticide detoxification have relied on landfills and incineration, which generate secondary contamination problems due to leaching of pesticides into surrounding soil and groundwater supplies and production of potentially toxic by-product emissions. By contrast, bioremediation may be viewed as a more effective and environmentally benign clean-up technology since it results in partial or complete bioconversion of the organic pollutants to microbial biomass and stable non-toxic end-products. Rhizosphere harbours a variety of beneficial microorganisms having the potential for degradation of these pollutants. These indigenous rhizosphere competent microbial strains are being exploited these days for the degradation of organic pollutants through bioremedial processes. Microorganisms involve various biochemical mechanisms for degradation of pesticides such as oxidative transformations by synthesizing various enzymes, hydrolytic transformations, aromatic nitroreductive processes, carbon–phosphorus bond cleavage reactions, pesticide conjugation reactions and formation. Most of the pesticides are recalcitrant in nature and resist their biodegradation, while some are metabolized incompletely, and as a result, accumulate in the environment. Example -DDT,aldrin,chlordane,diazinone, malathion, parathion, 2,4-dicholophenoxyacetic acid(2,4-D),arazine,simazine,propazine etc.

MICROBIAL POTENTIAL FOR DEGRADATION OF PESTICIDES:

Microbial degradation of pesticides has been long recognized. Recent research has revealed a number of microbial systems capable of biodegradation of organic compounds. The natural capacity of microorganisms to degrade a large variety of synthetic herbicides and pesticides is the essence of microbial method for the degradation of soil contaminants (bioremediation). Another very interesting feature of microorganisms is that they can degrade some of the organic substances that are produced only synthetically. Although microbial degradation of pesticides does not always lead to detoxification, in many cases the products are much less hazardous and/or become susceptible to further degradation. The microbes having the potential for pesticide degradation are mainly bacteria, especially actinomycetes and cyanobacteria, algae and fungi. Twenty-eight of the bacterial genera that can utilize aliphatic hydrocarbons have been isolated and most common among them are the species of Pseudomonas, Alcaligenes, Bacillus, Arthrobacter, Brevibacterium, Flavobacterium, Klebsiella, Methylococcus, etc. Several fungi having pesticide degrading potential have also been identified, such as the species of Aspergillus, Candida, Fusarium, Penicillium, Trichoderma, Rhodotorula, Pleurotus, Phaenerochaete, etc. The biochemical processes induced by microorganisms under aerobic and anaerobic conditions are mineralization, detoxification, cometabolism and activation. Unlike organochlorine pesticides, organophosphate, carbamate and pyrethroid pesticides are biodegradable. The main detoxification processes are hydrolysis and oxidation, with hydrolysis being the most efficient route for all three types of pesticide. There are many advantages of bioremediation technology: (a) It harnesses natural biogeochemical processes.(b) It is a cost-effective alternative.(c) Toxic chemicals are destroyed or removed from the environment and not merely separated.(d) Low capital expenditure.(e) Less energy required when compared with other technologies.(f) Less manual supervision requirement.

Degradation of hydrocarbon and oil spill:

Oil degrading organisms have evolved to use the hydrocarbons and organic compounds in petroleum as energy, and use molecular transfer mechanisms to denature these toxins. The aerobic and anaerobic properties of these microbes allow them to respire and ferment compounds as well, and this tends to result in the transformation of toxins into innocuous compounds. These compounds have more stable pH levels, increased solubility in water, and are less aggressive molecularly. It is known that the composition of oil-degrading microorganisms in marine ecosystems is originally less than 1%. When these organisms are given the necessary substrate, they tend to thrive and grow to almost 10% of the complete microbiome. Dependent on physical and chemical properties, petroleum-degenerative microorganisms take longer to degrade high-molecular weighted compounds, such as polycyclic aromatic hydrocarbons (PAH's). These microbes require a wide array of enzymes for the breakdown of petroleum, and require very specific nutrient composition to work at an efficient rate. Microbes work in a step-wise fashion to breakdown and metabolize the components of petroleum. Treatments that use breakdown processes most commonly use heat and chemicals to extend the efficacy. Microorganisms use many unique mechanisms to convert molecules and transfer electrons. Using aerobic respiration mechanism, petroleum substrate are oxidized by molecular oxygen to nitrogen Gas, hydrogen Sulfide,methane, metals, carbon dioxide and water. Through inorganic electron donation mechanism, ammonium, nitrite, iron, manganese are oxidize to form nitrate, nitrite, Iron, manganese, sulfate.By fermentation process,toxic petroleum compounds of organic nature are converted to harmless compounds, fermentation products.Microbes demobilize the iron, sulfate, mercury, chromium, uranium of oil spill to ferric hydroxide, sulfide, pyrite, reduced chromium,uraninite.By reductive dehalogenation,halogen compounds are converted to reduced contaminant.
Microorganisms play a vital role in bioremediation of heavy metal from the contaminated soil and waste water. Though when microorganisms especially bacteria are exposed to higher concentration of metal, it may have cidal effects on them. Microorganisms can interact with metals and radionuclides via many mechanisms, some of which may be used as the basis for potential bioremediation strategies. Mechanisms by which microorganisms act on heavy metals includes biosorption (metal sorption to cell surface by physico chemical mechanisms), bioleaching (heavy

metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes) intracellular accumulation and enzyme catalyzed transformation.

Biosurfactant:

Biosurfactants can be defined as the surface-active biomolecules produced by microorganisms with wide-range of applications. In recent years, due to their unique properties like specificity, low toxicity and relative ease of preparation, these surface-active biomolecules have attracted wide interest. Due to their unique functional properties, biosurfactants were used in several industries including organic chemicals, petroleum, petrochemicals, mining, metallurgy (mainly bioleaching), agrochemicals, fertilizers, foods, beverages, cosmetics, pharmaceuticals and many others. They can be used as emulsifiers as well as demulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients and detergents. The interfacial surface tension reducing ability of biosurfactants made them to play important role in oil recovery and bioremediation of heavy crude oil. The three major functions played by biosurfactants - 1.they were used to increase the surface area of hydrophobic substrates,2. biosurfactants also used to increase the bioavailability of hydrophobic substrates through solubilization/desorption,3.they also regulate the attachment and removal of microorganisms from the surfaces.Biosurfactants possess both hydrophilic and hydrophobic regions causing them to aggregate at interfaces between fluids with different polarities such as hydrocarbons and water,hence, decrease interfacial surface tension. They also found to be enhancing the nutrient transport across membranes and affect in various host-microbe interactions.When compared to chemical or synthetic surfactants, biosurfactants gained several advantages including their biodegradability, biocompatability and digestibility. The biosurfactants can be used in environmental cleanup by biodegradation and detoxification of industrial effluents and in bioremediation of contaminated soil. Their specificity and availability of raw materials also made them most preferred surfactants.

Properties: The unique and distinct properties of biosurfactants when compared to their chemically synthesized counterparts and broad substrate availability made them suitable for commercial applications. The distinctive features of microbial surfactants are related to their surface activity, tolerance to pH, temperature and ionic strength, biodegradability, low toxicity,emulsifying and demulsifying ability and antimicrobial activity.

Types of biosurfactants:

The chemically synthesized surfactants are usually classified according to their polarity, whereas, biosurfactants are generally categorized by their microbial origin and chemical composition as following.

1. Glycolipid: They are carbohydrates linked to long-chain aliphatic acids or hydroxyaliphatic acids by an ester group. Biosurfactants are majorly glycolipids. Among the glycolipids, the best known are rhamnolipids, trehalolipids and sophorolipids.
2. Lipopeptides and lipoproteins.
3. Surfactin.
4. Lichenysin.
5. Fatty acids, phospholipids and neutral lipid.

Polymeric biosurfactants: These are the best-studied polymeric biosurfactants including emulsan, liposan, alasan, lipomanan and other polysaccharides protein complex.

Particulate biosurfactants: These form the extracellular membrane vesicles partition to form a microemulsion which plays an important role in alkane uptake by microbial cells.

Source of biosurfactants:

Bacterial biosurfactants: Microorganisms make use of a wide range of organic compounds as a source of carbon and energy for their growth. When the carbon source is in an insoluble form like a hydrocarbon, microorganisms make possible their diffusion into the cell by producing a variety of substances, the biosurfactants. Some of the bacteria and yeasts excrete ionic surfactants which emulsify the CxHy substance in the growth medium. A few examples of this group of biosurfactant are rhamnolipids that are produced by different Pseudomonas spp. or sophorolipids that are produced by several Torulopsis spp. Some other microorganisms are able to change the structure of their cell wall which are achieved by them by producing nonionic or lipopolysaccharides surfactants in their cell wall. Some examples of this group are: Rhodococcuserythropolis and various Mycobacterium spp. and Arthrobacter spp. which produce nonionictrehalosecorynomycolates. There are lipopolysaccharides, such as emulsan, produced by Acinetobacter spp. and lipoproteins such as surfactin and subtilisin that are produced by Bacillus subtilis. Fungal biosurfactants: Where the field of production of biosurfactants by bacterial species is well explored, relatively fewer fungi are known to produce biosurfactants. Among fungi, Candida bombicola , Candida lipolytica,Candidaishiwadae , Candida batistae,Aspergillusustus and Trichosporonashii are the explored ones. Many of these are known to produce biosurfactant on low cost raw materials. The major type of biosurfactants produced by these strains is sophorolipids (glycolipids).Candida lipolytica produces cell wall-bound lipopolysaccharides when it is growing on n-alkanes.

Applications of biosurfactant: Food industries: The surfactants can have various other functions in food industries, apart from their obvious role as agents that decrease surface and interfacial tension, thus facilitating the formation and stabilization of emulsions. For example, to control the aggregation of fat globules,stabilization of aerated systems, improvement of texture and shelf-life of products containing starch, modification of rheological properties of wheat dough and improvement of constancy and texture of fat-based products . In bakery and ice-cream formulations, biosurfactants act by controlling the consistency, slowing staling and solubilizing the flavour oils; they are agents during cooking of fats and oil. Improvement in the stability of dough,volume, texture and conservation of bakery products is obtained by the addition of rhamnolipid surfactants. The study also suggested the use of rhamnolipids to improve the properties of butter cream and frozen confectionery products. L-Rhamnose has substantial potential as a forerunner for flavouring.

Removal of oil and petroleum contamination: Recent research findings confirmed the effects of biosurfactant on hydrocarbon biodegradation by increasing microbial accessibility to insoluble substrates and thus enhance their biodegradation. Biosurfactants increase the apparent solubility of these organic compounds at concentrations above the Critical Micelle Concentration (CMC) which enhance their availability for microbial uptake.For these reasons, inclusion of biosurfactants in a bioremediation treatment of a hydrocarbon polluted environment could be really promising, facilitating their assimilation by microorganisms.

Bioremediation of toxic pollutants: Bioremediation involves the acceleration of natural biodegradative processes in contaminated environments by improving the availability of materials (e.g. nutrients and oxygen), conditions (e.g., pH and moisture content) and prevailing microorganisms. Thus, bioremediation usually consists of the application of nitrogenous and phosphorous fertilizers, adjusting the pH and water content, if necessary, supplying air and often adding bacteria. The addition of emulsifiers is advantageous when bacterial growth is slow (e.g. at cold temperatures or in the presence of high concentrations of pollutants) or when the pollutants consist of compounds that are difficult to degrade, such as PAHs. Bioemulsifiers can be applied as an additive to stimulate the bioremediation process, however with advanced genetic technologies it is expected that the increase in bioemulsifier concentration during bioremediation would be achieved by the addition of bacteria that overproduce bioemulsifiers. This approach has been recently used successfully in the cleaning of oil pipes.