Bioremediation

It means use of biological treatment system to destroy or reduce the concentration of hazardous wastes from contaminated site. E.g- use of microbes or higher plants, which are efficient in removing or destroying the hazardous wastes.

Biodegradation of Xenobiotic compound/ Synthetic organic compound.

Unnatural things, new to environment and microbes, e,g .Pesticides, Herbicides, Insecticides, Biocides, Preservatives, Polychlorobiphenyls(PCBs) , Chlorinated compounds such as trichloroethane, carbon tetrachloride , pentachlorophenol and Polyaromatic hydrocarbons(PAHs e.g- Phenonthrene,Anthracene, pyrene) These compounds are more complex than natural things, degraded slowly, compared to natural things, for e,g DDT requires 3-10 years for half of the compound to be broken down. The natural compound lignin is more complex and degrade slowly in the soil environment. The halogenated xenobiotic compound (e.g. DDT,dielderin,heptachlor.)are more resistant than non-halogenated compound. 2,4 – Dichlorophenoxyaceticacid is a biodegradable(2,4-D), addition of chlorine to 2,4-D it become recalcitrant.

Biochemical reactions – For biodegradation of xenobiotic compound require many number of enzymes and microorganisms. This degradradation occur either aerobically or anaerobically.The non –halogenated compound(mono, polycyclic aromatic hydrocarbon) degraded into catechol or protocatechuate.

Cleavage – Two cleavage occurs in catechol Ortho and Meta –cleavage. Both pathways lead to pyruvate,acetaldehyde,succinate and acetyl CoA, which can enter the Krebs Cycle. Both of these reactions are carried out by oxygenases and dioxygenases. The cleavage is due to incorporation of two hydroxyl groups by dioygenases. This process is found in bacteria, fungi and algae.

Halogenated xenobiotics are the main ingredients of herbicide,pesticides. Organisms capable of degrading this found in soil and sediment (bacteria,fungi and algae). The chlorinated compounds are degraded by monoxygenases and dioxygenases. Two steps involved – 1,Oxidative dehalogenation, here halogen is replaced by two hydroxyl ions. 2, Eliminative dehalogenation means removal of halogen and an adjacent ion.

Degradation of organochlorines e.g – Pentachlorophenol is a herbicide , fungicide and preservatives for preservation of wood. A number of microorganisms have been isolated which can degrade PCP under aerobic and anaerobic condition and include *Flavobacterium, Arthrobacter*, *Rhodococcus*, and the white rot fungus *Phanerochaete chrysosporium*. The breakdown of this compound consists of dechlorination and hydroxylation of the aromatic compound catalysed by oxygenase and deoxygenase.

Atrazine is a herbicide and is effective for broad leaf weeds. It had been employed for for some 40 years. Three steps are involved in the degradation of atrazine. The first two steps(Atrazine to cyanuric acid) are mediated(genes coded on a large plasmid) by *Clavibacter* and last step is(cyanuric acid to CO2 and NH3 mediated(plasmid) by *Pseudomonas sp.*

Bioremediation of hydrocarbons – mono, polycyclic hydrocarbons are degraded anaerobically, The reaction includes hydration,dehydration, reductivedehydroxylation, nitroreduction, and carboxylation , the final compound enter the Krebs cycle. Anaerobic degradation is slower than aerobic degradation.

Degradation of pentachlorophenol (PCP) is a highly chlorinated compound, under aerobic degradation produces CO_2 and H_20. Under anaerobic –CH_4 and CO_2.

Geobacter bacteria are tiny microbe that can play role in cleaning of uranium, this bacteria immobilize uranium , this bacteria have hair like nanowires appendages found on the outside of *Geobacter* responsible for cleaning up of uranium.

Biomagnification

An increase in the concentration of a chemical substance, such as pesticide, as the substance is passed to higher members of a food chain. E.g.- DDT dichlorodiphenyl trichloroethane conc is 0.3ppb in aquatic environment –plankton 30ppb –small fish 0.3ppm -large fish 3ppm – sea birds 30ppm. The conce-level is higher in birds compared to aquatic environment.

Natural attenuation

Different variety of physical, chemical or biological processes under favourable condition, act without human intervention to reduce the mass, toxicity, mobility, volume or concentration of contaminants in soil or ground water.

Polymerization

A chemical reaction in which one or two molecules combine to form large molecules. E.g – PHB formation in bacterial cell(*Rhizobium, Azotobacter, Azospirillum)* under excess concentration of carbon, low level of sulfur, phosphorous, nitrogen in the soil environment.

Phytoremediation

It refers to removal of contaminants and toxic metals from soil and aquatic environment by plants or the use of higher plants for bioremediation.

Naturally occurring plants for phytoremediation / hyperaccumulators.

Some plants have the ability to collect and accumulate heavy metals e.g- Fe,Mn,Zn,Cu,Mg,Mo,Ni, etc , which are essential for their growth and development, but also those which have no biological function e.g- Cd,Cr,Pb,Co,Ag,Se,Hg. Excessive level of these that occur in contaminated soils, that are toxic to higher plants, but some plants can tolerate these metals . These plants are called as hyperaccumulators.

Heavy metals	**Hyperaccumulator vascular plants**
Cadmium	*Brassica juncea*
Copper	*Aeollanthus spp*
Cobalt	*Cyanotis longifolia*
Lead	*Armeria maritima*
Manganese	*Alyxia rubicaulis*
Nickel	*Alyssum spp*
Zinc	*Viola calaminaria*

Types.

1, Phytoextraction – phytoaccumulation, removal of contaminants and its storage in plants.

2,Phytodegradation - uptake and degradation of organic compounds.

3,Phytovolatilization – volatilization of pollutants into the atmosphere.

4, Phytostabilization - Transformation of toxic chemicals to non toxic chemicals. E.g $–Cr^{4+}$ to Cr^{3+}.

Phytoextraction

e.g – pollutants like TNT, TCF and BTEX are removed by this method. Sun flower roots can concentrate uranium 30,ooo fold from contaminated water. A fern *Dicropteris dichotoma* can accumulate Lanthanum, Cerium, Praseodynium and Neodynium.

Four grasses – Vetiver grass, Bahia grass(*Paspalum notatum*) , St Augustine grass and bana grass were used to removal of cadmium, lead from mineand TNT from soil. The reduction conc from gm level to mg.

Aquatic plants Parrot feather, Creeping primrose and water mint are used to romove Fe, Zn, Cu and Hg from polluted water.

Water hyacinth (*Eichhornia crassipes*) is used to treat tannery and diary wastes. The aquatic plant Myriophyllum spicatum can remove TNT. The Poplar trees trichloroethane from contaminated water.

These are metal (Cadmium)resistance plants *Salix, Betula, Alnus* and *Populas.*

Metals extracted plants – *Alyssum spp,* is a phytomining plants. It is used for extaction high grade metal from low grade ore.

Phytodegradation

Organic pollutants and toxic metals are degraded by plants. The plant produces various kinds of enzymes for degradations purposes. The enzymes includes nitroreductases, dehalogenases, laccase, peroxidase and nitrilase are involved for degradation of TCE,TNT,PAHs, and PCBs into CO2, ammonium, and nitrate. Oxygenation is a mechanism involved in herbicide and pesticide degradation and makes the molecules more soluble in water and therefore more suitable for attack.

Phytovolatiliztion

Some plants convert the harmful metal ions into volatile compound, which can reduce toxicity and evaporated through the stomato. E.g –TCE can be volatilized into methyl t-butyl ether by eucalyptus. Selenium converted to dimetylselenide by mustard , methylmercury converted to mercury vapour by tobacco.

Phytostabilization

Green plants can stabilize the soil, prevent the mobilization of soil and metals . The rhizosphere bacteria can convert the toxic Cr^{6+} into Cr^{3+}. *Lolium perenne* is an excellent soil stabilizer.

Rhizofilteration

It means removal of contaminants and toxic metals from flowing water by plant roots. It is also meant for destruction of pathogens, nutrient removal , metal uptake and stabilization.

e.g –construction of wetland system. It consists of common reed *Phragmites* sp, which can grow in fresh or brackish water. The reed bed consists of clay,soil lined with polypropylene in order to stop leakage into subsoil . The reed grow upto 1.5 meter in length. The BOD of the waste is reduced from 1006 mg/l down to 56mg/l. Other e.g includes *Typha latifolia, Salix atrocinerea* are used as rhizofilteration that can remove 60% of organics, 30% of nutrients, and 90% fecal contamination.

Rhizostimulation – The high level of microbes associated with plant roots is due to high levels of nutrients that are exuded or released from the roots. Some bacteria contain the enzyme ACC-

1, aminocyclopropane-1- carboxylic acid synthase , which convert ACC to ethylene, CO_2 and cyanide.

Phycoremediation

Macro and micro algae are used for nutrient removal, *Chlorella vulgaris. C.minuta, C.sorokiniana, Scenedesmus platydiscus, S.dimorphus* are used for removal of ammonium,nitrite and orthophosphate from waste water.

Bioremediation of soil

Any undesirable changes in environment is called as pollution. Substances which causes pollution is pollutant. Pollution is always decreases the life span of humans.

Three methods are used

1, The placement of contaminated soil (6 to 9 inches) over /on the top of gravel/soil bed . The nutrients, microbes, water are sprayed over the bed, which is periodically tilled for the microbes to get oxygen.

2, Soil-slurry bio-treatment – use of vessel containing contaminated soil+ water , nutrients and aeration are periodically supplied.

3, Bioreactor technology.

In Situ bioremediation of contaminated soil – 1, Inoculation with non-indigenous bacteria.

2, Introduction of H_2O_2 – It will limit the growth of indigenous bacteria that involved bioremediation of contaminated soil

3,Treatment of contaminants in bioreactors.

Contaminants soil is treated in a bioreactor supplied with nutrients, water, oxygen and microbes.

Bioremediation of water

Two methods- 1, Providing more oxygen to reduce the BOD, 2, Use of bioreactors

GEM and its role in bioremediation.

Recombinant bacteria can be obtained by genetic engineering techniques or by natural genetic exchange between bacteria. The genetic engineered microorganisms have higher degradative capacity. GEM have the capacity to clean-up process at lower cost. GEM have degradative (catabolic genes).

Examples : 1, *Pseudomonas sp*. B13 degrade mono/ dichlorobenzoates.

2, *P.putida* degrade 4- ethylbenzoate.

3, *P.putida* KT2442 degrade toluene/ benzoate.

4. *P.sp* FRI degrade chloro, methylbenzoates

5, *P. sp* LB400 degrade polychlorinated biphenyls

6, *E.coli* JM106 degrade PCB, benzene, toluene.

7, *P. pseudoalkaligenes* KF707-d2 degrade TCE, toluene, benzene.

8, *E, coli* FM5/Pky287 degrade TCE, toluene.

9, *P.putida* TVA8 degrade TCE,BTEX. benzene, toluene, ethylbenzene and xylene

10, *P. fluorescens* HK44 degrade naphthalene, anthracene and phenanthrene.

11, *B.cepacia* BRI600IL degrade 2,4- dichlorophenoxyacetic acid.

11, *P. fluorescens* 10586/pUCD607 degrade BTEX

12, *P.strain Shk1* degrade Cd, dinitrophenol, hydroquinone.

The gene code for catabolic process is isolated and inserted into broad host range plasmid and transfer to specific host. The transformed host is GEM.

Role of mobile genetic elements for degradation of xenobiotics

Plasmids : 1, *Achromobacter sp* LBSICI contain $_{P}$ss60 degrade 4. Chlorobenzoate.

2, *Alcaligenes sp* BR60 contain $_{P}$BRC60 degrade 3. Chlorobenzene.

3, *Burkholderia sp* PS12 contain $_{P}$ps12-1 degrade 1,2,4,5- Tetrachlorobenzene.

Transposns : 1, *Pseudomonas sp* p51 contain Tn5280 degrade chlorobenzene.

2, *Alcaligenes* sp BR60 contain Tn5271 degrade chlorobenzene.

3, *Pseudomonas putida* PP3 contain DEH degrade chlorinated aliphatic acids

4, *P.putida* mt-2 contain Tn 4651 degrade toluene, xylene.

5, *P.putida* G7 contain Tn4655 degrade naphthalene.

Construction of hydrocarbon degrading bacterium. Camphor, octanol,xylene and naphthalene.

GEM for PAH in soil

Pseudomonas fluorescens contain r DNA plasmid carries *lux* gene isolated from *Vibrio fischeri* can degrade naphthalene , anthracene and phenanthrene.

GEM for treating oil-spills

The strain was developed by Dr. Ananda Chackrabarty in USA . Pseudomonas which was capable of degrading 2,4,5- trichlorophenoxyacetic acid. The strain contained two plasmids each providing separate hydrogen degradative pathway.

GEM for sequestering heavy metals.

The gene code for metallothionein from a mouse to *Ralstonia eutropha* a natural inhabitant of soil become sequestering cadmium.

Biostimulation

It refers to modification of environment to stimulate existing bacteria to degrade the pollutants. This can be done by addition of rate of limiting nutrients and electron acceptors such as phosphorous, nitrogen, oxygen,or carbon. Biostimulation can be enhanced by bioaugmentation.

Recently a number of products have been introduced which allow popular use of bioremediation using biostimulation.

Bioaccumulation

It means an increase in the concen.of a chemicals in a biological organisms over time, compared to chemical concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down or excreted.

Uptake - it means entrance of a chemical into an organism- such as by breahing,swallowing or absorbing it through the skin.

Storage – temporary deposition of a chemical in body tissue or in an organ

Bioconcentration – is the specific bioaccumulation process by which concentration of a chemical in an organisms becomes higher than is conc. In the air or water around the organisms.

Biomagnification –

Bioaccumulation process – it is a normal and essential process for the growth of organisms. All animals, including humans, daily bioaccumulate many vital nutrients, such as vitamins, A,D, and K, and trace minerals and essential fats and aminoacids.

Uptake – it means passing of chemicals fromenvironment into an organisms cells, uptake is a complex process, which is still not fully understood. Scientist have learned that chemicals tend to move or diffuse passively from high con to low concen.

Storage – Some chemicals are attracted to certain sites and by binding to proteins or dissolving in fats they are temporarily stored.

One factor important in uptake and storage in water solubility, usually compounds that are highly water soluble have low potential to storage. Cobalt which binds very tightly and specific to sites in the liver. Similar process for mercury, copper, cadmium and lead.

The fat loving chemicals pass into organisms s cells through the fatty layer of cell membrane until they are stored in fatty tissues.

Elimination - chemical that loving fat are slowly eliminated from the body compared to water soluble chemicals.

Immobilized microbes in bioremediation.

Immobilized microbes can break down harmful chemicals such as phthalates in China. The microbes might be used to treat industrial waste water and so prevent these materials from entering the environment. Phthalates are widely used as additive in polymer prepararation.

University in Beijing - microbes that can digest phthalate, found in groundwater, river water, drinking water, open ocean water, soilhumates, lake sediments and marine sediments. This microbial strain can quickly metabolize the phthalate and convert it into the raw materials for microbial growth and reproduction.The phthalate digesting microbe can be immobilized on a ceramic honeycomb support. The immobilized cells can degrade 100 mg/ liter phthalate to less than 1.o milligrams/ liter within two days of treatment .The degradation rate was two and half times faster with immobilized microbes.

Immobilized materials includes – nanoporous, polymer beads is Bio-Beds, microbes with bio beds can break down perchlorate, methyl-terbutyl ether, chlorinated solvents and other biodegradable contaminants in treating contaminated ground water.

Mucor sp. immobilized on maize cob, filamentous fungi can degrade benzol(a) pyrene, degradation rate is compared with free fungus. The degradation rate was higher in immobilized fungus 68% within 42 days than free mobile fungus 52%.The SEM analysis showed that immobilized microstructure was suitable for Mucor sp., immobilized on MC.

Gaseous bioremediation

Air contains various contaminants that include volatile organic compound, sulphur dioxide, nitrous oxide, CFC, greenhouse gases such as CO_2 and methane.These contaminants originate from different source, v.o.comp, derive from industries, S.dio. from the combu.of sulphur containing oils and coals. CO2 fr. comb.of fossil fuels.

There are number of bioreactor designs available for the removal of gaseous pollutants, The basic principles are to pass the polluted gas through a vessel in which the pollutants can be transferred to a water medium where they can be degraded by microorganisms.

These are as follows: biofilters, trickling biofilters, bioscrubbers, membrane bioreactor and activated sludge.

In bioreactor the gas is passed through a bed of porous materials, which contains microbes, immobilized in the bed. The bed needs to be humidified in order to keep the microorganisms viable, large volume of gas can be treated, good removal up to 99% and low cost operation. The disadvantage are the designs are still developing.

Biofilters – is used for removal of v.o.c.. , here bed bioreactor or open soil/ gravel bed imm.with micro, where the conta. gas is passed into the base of the bed , as they pass through the soil it will degrade the v.o.c by micro.

1, Polluted air + water---------- soil/gravel immo .with.micro---------- air free from v.o.c.

2, Pollu air + water ----------packing/support materials e.g.peat, wood bark, compost, leaves,soil, high porosity plastic materials, humidity 10-50% , coated with micro. ------- clean air.

Trickling biofilter.

Poll.air ----------packing materials + micro poured with nutria.med and recycled ----- clean air.

Burkholderia cepacia G4 -used to treat trichloroethylene.

Bioscrubbers

Pollu/cont. air----------medium contain.nutr+micro------trickling down the column cont.packing materials-------clean air.

Membrane bioreactor –

Poll/con.air ------- Semi-permeable membrane have specified pore/selective (silicon rubber or polysulphone) ----- biofilm in suspension/immobilized in another side with nutrients ----clean air.

Activated sludge – a.s. tank is used to treat pollu/cont.air contains micro.nutri,supplied with oxygen to mic for speed up degrada.of poll.air.It will remove H_2S, 99% removal.

Thiobac- rem-ammonia,H2S.

Thio,Xanthomo,Hyphomicrobium, Arthrobacter oxydans for rem of BTEX, dichloroethane,styrene, *Alcaligenes xylosoxidans* can rem.toluene,

Bioremediation of metals

Both chemical and biological processes can not degrade metals, metals which causes pollution in aquatic environments or effuluents from industries or mining, that includes zinc, copper, lead, cadmium and mercury. The following methods have been used to treat metal containing waste water from industries.

Biosorption – means biological materials can adsorp a variety of metals. The uptake of metals from wastewater by living materials can be two types – Active and passive or both,

P.uptake of metal ions is independent of cellular metabolisms and involves binding metals ions to the poly anionic cell wall or by ions exchange mediated by extracellular polysaccharides. The p.uptake occur within 5-10 minutes. But this is very much affected by physical condition like pH and ionic strength. P.uptake is reversible occur in both living and dead materials.

A.uptake of metals is slower than p.utake. It is dependent on cellular metabolisms and is affected by metabolic inhibitors and temperature, it is more complex processes, mediated by specific proteins such as metallothioneins or contained in the vacuole, both p.up and a.up can occur in same time, A.uptake of metals ions are non –specific,

Refer diagram,

Macroalgae – both living and non-living materials to adsorp metals, it has used two types of cycles for adsorption and deadsorption. The non –living microalgae has been used to remove cadmium, The alga ***Ecklonia radiate*** is used to remove copper. It contain metal binding protein metallotheioneins. Some bacteria possesses peptides on their cell walls that bind the metal ion. ***E.coli*** has two hexahistidine clusters on the surface.

Three methods contain immobilized microbes used to remove metals,

1, fixed-bed

2,fluidized- bed

3, Rotating disc.

Extracellular precipitation

Desulfotomaculam is a bacterial strain used to remove metals from industries and mining industries. This bacteria generate hydrogen sulphide using sulphate in the presence of simple organic carbon lactic acid. This hydrogen sulphide react with any metals forming insoluble metal sulphide.

$H_2S + Cu^{2+}$ -------- $CuS + 2H^+$

Hydrogen sulphide is corrosive, poisounous, Green, purple sulphur bacteria can oxidize H_2S into elemental sulphur. Another method is sand filter incubated with bacteria can adsorb and precipitate m

Immobilized microbes in Bioremediation

Petroleum hydrocarbons are the most common environmental pollutants in the world and oil spills pose a great hazard to terrestrial and marine ecosystems. Oil pollution may arise either accidentally or operationally whenever oil is produced, transported, stored and processed or used at sea or on land. Oil spills are a major menace to the environment as they severely damage the surrounding ecosystems. To improve the survival and retention of the bioremediation agents in the contaminated sites, bacterial cells must be immobilized. Immobilized cells are widely tested for a variety of applications. There are many types of support and immobilization techniques that can be selected based on the sort of application. In this review article, we have discussed the potential of immobilized microbial cells to degrade petroleum hydrocarbons. In some studies, enhanced degradation with immobilized cells as compared to free living bacterial cells for the treatment of oil contaminated areas have been shown. It was demonstrated that immobilized cell to be effective and is better, faster, and can be occurred for a longer period

Keywords: Application, bacteria, biodegradation, crude oil, soil.

INTRODUCTION

There is a growing public concern as a wide variety of toxic organic chemicals are being introduced inadvertently or deliberately into the environment. Petroleum hydrocarbons are one of the common examples of these chemicals, which enter the environment frequently and in large volumes through numerous pathways [1].

Oil contamination has become a global problem in industrialized and developing countries. It is one of the most dangerous pollution factors known today. It can cause a threat to the environment. It is very feared by environmen-talists and it's very hard to control if it spills out [2, 3].

There are a lot of methods for treating petroleum conta-minated sites such as mechanical and chemical methods, but these methods generally are expensive and have limited effectiveness. On the other hand, bioremedia-tion is the promising technology for the reduction of these petroleum

pollutant areas since it is cost-efficient and will result in to complete mineralization. Bioremediation is a process that degrades environmental pollution by microorga-nisms [4, 5].

In the last few years, the application of biotechnological processes that involvesing microorganisms with the objective of solving environmental pollution problems, is rapidly gro-wing. The researchers have proved that biological methodology is versatile, high stability, broad applications in various areas, economical and efficient for the remediation ofpetroleum [6]. One of the key points'spoints for bioremediation is maintaining high biomass of bacterial populations. To improve the survival and retention of the bioremediation agents in the contaminated sites, bacterial cells must be immobilized. Immobilized cells have been extensively used in the production of useful chemicals, treatment of wastewaters and bioremediation of pollution cause of its longer operating lifetime and enhanced stability and survival of the cells [7, 8].

The use of immobilized cells has been investigated as an alternative technology for environmental applications. These biocatalysts can offer the possibility of a wider and more economical exploitation in industry, waste treatment, medicine, and development of bioprocess and monitoring devices like the biosensor [9].

The many advantages of immobilized cell systems have been reported and a few of the major reasons are listed below [10]:

Providing high biomass.

Providing cell reuse and reducing the costly processes of cell recovery and cell recycle.

Elimination of cell washout problems at high dilution rates.

High flow rates allow high volumetric productivities.

Providing suitable micro environmental conditions.

Improving genetic stability.

Protection against shear damage.

High resistance to toxic chemicals, pH, temperature, solvents and heavy metals.

Decline of maturation time for some products.

Go to:

Definition of immobilization

An immobilized molecule is one whose movement in space has been restricted either completely or to a small limited region by attachment to a solid structure. In general the term immobilization

refers to the act of the limiting movement or making incapable of movement [11]. Immobilization can be occurred for enzymes, cellular organelles, animal and plant cells.

Recently the immobilization of whole cells have been developed as biocatalysts in environmental pollutions when are used for multi enzyme systems. They can be classified to three physiological states consist of dead, living and growing states. So we must choose the state more suitable for the application purpose [12]. There are many different the immobilization of whole cell with other biocatalysts such as enzymes. The heat stability and functional stability of immobilized microbial cells have more than enzyme systems. They are not needful tThe processes for the extraction, separation and purification of the enzymes from the cells are not necessary. Not only enzymes due to their protein structure have less stability in extreme conditions, but also in enzymatic systems unwanted reactions can occurbe occurred [13].

The field for immobilization of whole cell, field of their usageis varied from food industry to biomedical sciences. Microorganisms survived on a carrier can be used in continuous and semi-continuous production processes (biosynthesis of vitamins, amino acids, organic acids, production of monoclonal anti-bodies, recovery of heavy metals, whole cell enzymatic reaction and ethanol fermentation), allowing for significant cost decrease due to refillable biocatalyst [14, 15].

History of immobilization

Immobilization is a natural phenomenon existing in the globe. Radwan et al. [16] have provided evidence that the immobilization principle is already found in nature, as microalgal samples collected along the Gulf coast were covered by biofilms of oil utilizing bacteria that help degrade hydrocarbons found in seawater.

Biofilms are surface-attached microbial communities consisting of multiple layers of cells embedded in hydrated matrices [17]. Biofilms spread on surfaces or within natural structuresincluding such as body, a tooth, grains, glass, a water pipe or conduit, etc. [18].This natural phenomenon encouraged humans to utilize it for his their services. In the 1969 for the first time, enzyme immobilization was applied for continuous production of L-amino acids from acyl DL-amino acids. by By immobilizinged aminoacylase enzyme and since the late 1970s, immobilization techniques have been used extensively in many laboratories [19].

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Carrier selection

The selection of carrier is very important for use in immobilization. Carriers must be have the following criteria [20]:

non toxic, non polluting, non biodegradable.

High cell mass loading capacity.

High mechanical, biological and chemical stability.

Long shelf life.

Adequate function groups.

Low cost price.

Optimum diffusion distance from flowing media to center of carrier.

Easy separation of cells and carrier from media.

Easy to handle and regenerate.

The type of support media used for anoxic biomass immobilization can affect the efficiency of a bioreactor. The number of cells adhering to the support depend on the kind of support.

Immobilization supports are commonly divided into two main groups: organic and inorganic. Organic carriers are such as modified celluloses, dextran, chitosan and agarose, and inorganic carriers are such as zeolite, clay, anthracite, porous glass, activated char-coal, and ceramics [21].

Organic materials are more abundant than inorganic carriers and can be obtained with strictly controlled porosity, but they are usually very sensitive to pressure or pH, and in many cases to both of them. On the other hand, Inorganic supports generally have one major advantage over other materials, namely, their toughness and etc. Most inorganic supports are totally inert, resistance to temperature, pH, chemicals, microbial degradation, and also crushing or abrasion. Given that they do not normally have to be pro-duced by the end-user and may well be naturally occuring occurring (e.g. sand used in methanogenic fluidized beds), the inorganic supports also lendthemselves more conveniently to scale-up [22, 23].

Organic carrier can be divided into natural and synthetic polymers. Some examples of natural carriers that can be used as support include alginate, carrageenan, agar, agarose, chitosan and chitin. A variety of synthetic polymers such as acrylamide, polyurethane, polyvinyl and resins are also used for immobilization [24].

Alginates (polymers made of different proportions and sequences of mannuronic and guluronic acids extracted from brown algae) are the polymers of choice in most systems of immobilization because they are easy to handle, nontoxic to humans, the environment, and the entrapped microorga-nisms, legally safe for human use, available in large quantities and inexpensive. From

a physiological perspec-tive, a major advantage of alginate is that immobilized cells do not suffer extreme changes in physicochemical condition during the procedure of immobilization and the gelis trans-parent and permeable [25].

Types of Immobilization

Many different forms of cell immobilization have been used including Adsorption, Covalent Binding, Entrapment and Encapsulation (Fig. 11). Among these methods the Entrapping has been widely investigated [26].

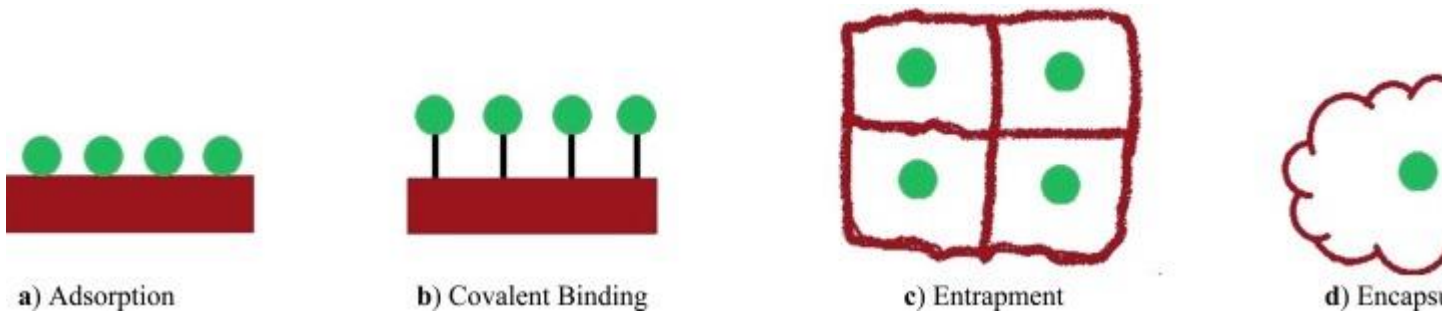

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Fig. (1)

Types of Immobilization.

Adsorption

This reversible method for the immobilization of cells is based on the physical interaction between the microorganism and surface of water-insoluble carriers. It is most commonly used for adherence of cell. Immobilization by adsorption is mild, quick, simple, economically advantageous, no need for chemical additives. Moreover it is, easy to perform the process and with thepossibility of reloading of the support. In the interaction between microorganism and the surface of the matrix, weak forces are involved include hydrogen bonds, ionic bonds, hydrophobicbonds and van der Waals forces. It has the disadvantage that the adsorbed enzyme may leak from the carrier during use due to a weak binding force between the enzyme and the carrier. Disadvantages of cells immobilized using the adsorption technique is a the very high rate of leakage from matrix due to weak interactions, unstable interactions, no possibility to control the loading, so the reproducibility is also low [27, 28].

Covalent Binding

The covalent Binding is areversibleeversible immobilization that based on covalent bond formation between activated inorganic carrier and cell in the presence of a binding (cross linking) agent. Covalent method of immobilization is mainly used for enzyme immobilization but it is rarely applied in whole cell immobilization because the toxicity of the coupling agents often RESULTS in loss of cell viability or enzyme activity [29].

Go to:

Entrapment

Entrapment method is an irreversible immobilization that is based on capturing of particles or cells within a support matrix or inside a hollow fiber. In this type of technique creates a protective barrier is created around the immobilized microbes and prevents the cells leakage from the polymers into surrounding medium while allowing mass transfer of nutrients and metabolites. Entrapment is the mostly applied in cell immobilization. The advantage of entrapment of cell immobilization method is that it is fast, cheap and mild conditions are required for the reaction process. The main disadvantages of this technique are costs of immobilization, injury of support material during usage diffusion limitations, deactivation during immobilization and low loading capacity as biocatalysts [30]. Various types of supports have been used such as agar, chitosan, alginate, celite, carrageenan, cellulose and its derivatives, collagen, gelatin, epoxy resin, photo cross-linkable resins, polyester, polystyrene, polyurethane and acrylic polymers [31]. These matrixes have porous structure, and thus the pollutant and various metabolic products could easily diffuse through into the matrix. Immobilized particle-size to support material pore-size ratio probably is the most important parameter. When the pores are too big the material is leaksing, what which also decreases the loading [32].

Go to:

Encapsulation

Encapsulation is another irreversible technique similar to entrapment. This method can be achieved by enveloping the biological components within various forms of spherical semi permeable membranes with a selective controlled permeability [33].

The ratio of size of pore of membrane to size of core material is a significant factor in this phenomenon. This limited availability to the microcapsule inside is one of the main advantages of microencapsulation, due to protection of the biocatalyst from the extreme conditions. As most immobilization methods, it prevents biocatalyst leakage, increasing the process efficiency of the processas a result [34].

Go to:

Application of immobilization techno-logy for use in bioremediation of pollu-tants

The increasing of environmental pollution and the treating contaminated sites are necessary atat present [35]. This review focuses more on the remediation of oil contaminated areas. Bioremediation of crude oil using immobilized cells is rarely studied. All of the methods of immobilization such as Adsorption, Covalent Binding, Entrapment and Encapsulation were tried for bioremediation of crude oil. The high immobilization efficiency of the cells onto the immobilization material and the high affinity between the hydrophobic immobilization material and the substrates caused excellent degradation. Increasing availability of the substrates for the cells and a better interaction between the substrates and the immobilized cells synergistically resulted in developing the degradation rate [36].

Omar and Rehm [37] demonstrated that Candida parapsilosis and Penicillium frequetans when immobilized on granular clay in columns, effectively degraded n-alkanes. They observed that residuals of C12 to C18 alkanes in immobilized bacterial cells system are 13.4 to 32.3% whereas in free bacterial cells system is are 85.9 and 98.9%. Davis and Westlake [38] reported that immobilization of cells onto inert surfaces increased available surface area to facilitate growth of biomass and also enhance degradation rate.

Obuekwe and M. Al-Muttawa [39], an Arthrobacter sp. and a Gram-negative bacillus isolated from Kuwait oil lakes,and then these bacteria incubated with sawdust, Styrofoam or wheat bran, as carriers, under low nutrient conditions, stable exopolysaccharide mediated immobilized cultures were formed. The authors tested the ability to survive and degrade hydrocarbons for 6 weeks at 45 ◦C. Suspensions of free cells degraded less crude oil than freshly immobilized cells.

In other study Quek et al. [40] have reported the immobilization and performance of Rhodococcussp. F92 on polyurethane foam (PUF) in the bioremediation of petroleum hydrocarbons. The immobilized wcells could toere able to degrade a variety of petroleum products such as Arabian light crude, Al-Shaheen crude, diesel and oil slops.

Radwan et al. immobilized oil-utilizing bacteriain biofilms coating macroalgae. This natural immobilization can be protected the bacteria from being washed out and diluted also provided with oxygen, and probably nitrogenous and phosphorus compounds and vitamins for oil-utilizing bacteria [16].

Gentili et al. [41] used chitin and chitosan flakes for immobilization of Rhodococcus corynebacterioides QBTo. This supports are natural, nontoxic, nonpolluting and biodegradable that are obtained from shrimps and crabs. The R. corynebacterioides QBT oimmobilized on chitin and chitosan flakes increased significantly the crude oil biodegradation.

Wiesel et al. [42] observed that a mixed bacterial culture immobilized on granular clay exhibited good growth, and demonstrated equivalent degradation potential of polyaromatic hydrocarbons (PAHs) compared to freely suspended cells in their model soil system.

Diaz et al. [43] used immobilized the bacterial consortium MPD-M on polypropylene fibers for biodegradation of crude oil in water with salinities varying from 0 to 180 g L-1. They observed the immobilized cells significantly increased the biodegradation rate of crude oil compared with free-living cells, the bacterial consortium MPD-M was highly stable in immobilized systems and it was not greatly affected by addition in salinity, also the biodegradation of pristanepristine (PR) and phytane (PH) and of the aromatic fraction was also increased using cells immobilized on polypropylene fibers.

Xu and Lu [44] demonstrated that oil removal in a crude oil-contaminated soil was increased by application of hydrocarbon-degrading bacteria immobilized on peanut hull powder as biocarrier. This biocarrier provides large surface area and strong adsorption capability, in addition improves oxygen diffusion and enhancesed dehydrogenase activity in soil.

Oil-degrading ability of the immobilized bacterial consortium in cocopeat, rice hull powder and sodium alginate capsules was compared by Nunal et al. [45]. They reported that immobilization of the oil-degraders on the surface of cocopeat higher oil reduction, compared to encapsulation in sodium alginate gel. higher oil reduction by the cocopeat-immobilized cells is presumably due to highly sustained microbial population attached to surface of the biocarrier, providing protective niche, the porous nature of cocopeat might allow efficient substrate diffusion, slow release of nutrients, and acceleration of oxygen transfer, thus providing a favorable niche for hydrocarbon utilization. In addition to the encapsulated bacterial cells might not be allowed to replicate inside the alginate matrix and subsequent release into the medium [45].

Cocquempot et al. [46] examined that immobilized cells in PUF better than of immobilized in alginate because of storage stability and microbial activity. The use of polyurethane foams developed due to wide range of porosity, mechanical properties, hydrophobicity and hidrophilicity of polyurethane foams. For example Oh et al. [47] immobilized Yarrowia lipolytica in polyurethane foams for degradation of crude oil.

Liang et al. [48] compared amount of degradation of crude oil in contaminated soil with free-living bacterial cultures and activated carbon biocarrier. RESULTS revealed that immobilization in activated carbon biocarrier increased the biodegradation of crude oil, bacterial population and total microbial activity due to improvement the oxygen, nutrient mass transfer and water holding capacity of the soil.

Immobilized cells are being used in biodegradation of another compounds. Some immobilized cells for use in biodegradation compounds are given in Table 112. Recently, Maliji et al. [49] used natural support such as luffa and sponge for immobilization of ***Bacillus cereus*** in diesel Oil degradation. This adsorption system could control pollution and also be easilyy used and with low costs.

Table 1

Some immobilized cells for use in biodegradation compounds.

Compounds Degraded	Carriers	Microorganisms	REFERENCES
acrylamide	alginate	Pseudomonas sp. and Xanthomonas maltophilia	[54]
Cadmium and Zinc	alginate	Pseudomonas fluorescens G7	[55]
2-chloroethanol	sand	Pseudomonas putida US2	[56]
cyanuric acid	Granular clay	Pseudomonas sp. NRRL B-12228	[57]
Diesel oil	Polyvinyl alcohol	Hydrocarbon-degrading bacteria	[58]
Ethylbenzene	Alginate, agar, polyacrylamide	Pseudomonas fluorescens-CS2	[59]
Mercury	alginate	nitrogen-fixing bacteria (NFB)	[60]
naphthalene	alginate, agar and polyacrylamide	Pseudomonas sp. strain NGK 1	[61]
p-Nitrophenol	diatomaceous earth	Pseudomonas sp.	[62]
pentachlorophenol	polyurethane	Flavobacterium sp.	[63]
Pentachlorophenol	alginate	Phanerochaete chrysosporium	[64]
Pentachlorophenol	k-Carrageenan	Pseudomonas sp. UG30	[65]
phenol	Polyvinyl alcohol (PVA)	Acinetobacter sp.strain PD12	[66]
phenol	agar	methanogenic consortium	[67]
Phenol,trichloroethane	Chitosan	Pseudomonas putida BCRc14349	[68]

Compounds Degraded	Carriers	Microorganisms	REFERENCES
sodium cyanide and acetonitrile	alginate	Pseudomonas putida	[69]
Sodium dodecyl sulfare (SDS)	polaycrylamide	Pseudomonas C12B	[70]
2,4,6-trinitrotoluene (TNT)	alginate	Arthrobacter sp.	[71]
2,4,6-Trichlorophenol	k-Carrageenan/gelatin gel	Microbial consortium	[72]

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In some study it wasdemonstrated that the tolerance ability into difficult conditions of immobilized ce