1.1 General

Both natural and anthropogenic activities, result in accumulation of wide ranges of toxic xenobiotic compounds in the environment when present in high concentration and thus cause a global concern [1]. Primarily, xenobiotics are those compound that are foreign to a living individual and have a tendency to accumulate in the environment. Solid as well liquid waste materials have developed over a years of human development. These materials were disposed off into rivers, lakes and seas or in burial sites, where they could be degraded naturally with times. It was easy and possible to manage this organic material by using physicochemical and environment dependent biodegradation. With the time the civilization increased and the population shifted from small localities to large communities where the management of the large quantity disposal became a genuine problem. Increased civilization initiated industrialization. This industrial revolution increased the demand of fuel and oils which resulted in increase in mining activities and generation of more industrial units. This industrial growth and their energy sources produced more contaminated atmosphere. Various textile industries have come up which resulted in generation of dye contaminated disposal that has polluted the natural water sources.

In the next step of development the conventional energy sources have been replaced by petroleum oil, which resulted in opening of the refineries. Subsequently, every industry was replacing their energy source from coal to oil. Most of these industries were dependent on petroleum based energy sources for some or all their activities. The lead containing gasoline in newly introduced motor vehicle produced extensive contamination of the environment. The unprecedented growth of population and industrial development that led to the formation of modern
civilization resulted in increased conventional waste products to a critical level as well as introduced a range of previously unknown synthetic chemicals for which community was unprepared.

The next development was in agricultural activities. The introduction of chemical fertilizers and insecticides has resulted in increased production of crops. However, such practices had an adverse effect on soil and ground water thereby disturbing the balance of ecosystem. In the 20\textsuperscript{th} century, raw material demand was increased for the newly immersing technologies, communication, and transport. Also, unknown strange synthetic production was also synthesized by incorporating into various metals, such as mercury and cadmium. Additionally, dielectric fluids like polychlorinated biphenyls, polychlorodioxins, were introduced principally in the electronic industry. Refrigeration and air-conditioning gas chlorofluorocarbons were newly introduced in market. Conclusively, the accumulation of recalcitrant xenobiotic compounds is due to continuous influx from population and industrial inputs that have created a serious impact on the pristine nature of our environment. Moreover, the pollutants in the environment are showing to various degradative forces. Most of the xenobiotics containing pesticides like DDT have long half-lives in the soil means their environmental persistence ranges over 3-10 years [2, 3]. The regulations are strictly enforced in developed countries like the United States and most of the European countries to meet the challenges of environmental contamination, these regulations often not in avoidable in most of the developing countries [2]. Moreover, in most of the developing countries unauthorized and unsafe dumpsites of harmful waste which are unlined, with no barrier between the waste and ground water containing solid or an unknown combination of unrelated harmful substances. The traditional means of disposing of solid and liquid wastes are
generating the secondary wastes. Hence cleaning up such sites is often not only technically challenging but also very expensive. Substantial anxiety encourages the implementation of waste management alternative with environmentally benign and economically feasible methodology. Inappropriate wastes management of industrial as well as household chemicals are increasing their dumping into water and hence accumulation in flora and fauna and contaminating aquatic environmental. These chemicals accumulate into the aquatic animals like fish. With direct ingestion by human it further accumulate into the tissues of human as well as other animals and creating health problems such as cancer, genetic mutations and teratogenic defects. They can also prevent photosynthetic by reducing the sunlight in the water and can interrupt water food webs.

Now, the fundamental questions are arising that how to dispose of the large extent of wastes that are repeatedly binge produced and how to remove the toxic compounds which are binge disposed off into the rivers site, hence creating the environmental threat to the soil and water systems [2]. It was many years after the introduction of many compounds that environmental problem caused by their use and careless disposal were first recognized.

Today mankind is struggling very hard to overcome this global problem [4]. Concern about the removal of the recalcitrant from the environment has led to extensive studies on their biodegradation. It is now known that microorganisms have the ability to reduce environmental contamination. Soil harbours a variety of microorganisms, which play a vital role in the clean up the environment. Using soil microorganisms as the machinery to clean up contaminant sites has given birth to new technology called, Bioremediation. Bacteria have the unique feature in rapidly adapting to limited nutrient supplies and occupying hostile environments. The
metabolic diversity and plasticity of bacteria in the face of environmental insult and limitations provide an immense reservoir of exploitable regulatory device and biochemical activities [5, 6]. Among these abilities, bacteria have the potential to biodegrade and hence to remove a wide variety of natural and manmade organic compounds discharged through geological cycles, urban and industrial activities.

A number of bioremediation approaches have been developed to tackle contaminating environment site. According to the basic principle to select the most appropriate strategy to treat specific site can be guided by considering the adaptability of the pollutant to biological transformation to less toxic products (biochemistry), the accessibility of the contaminant to microorganisms (bioavailability) and the opportunity for optimization of biological activity (bioactivity) [2].

Microbial strategies for the removal of environmental pollutants from waste streams can provide an attractive alternative to traditional methods. The word “xeno” means foreign and “biotic” related to living organism. A compound that is normal to one organism may be a xenobiotic to another. For example, antibiotics can be referred as xenobiotics in a human body because human body does not contain them or produce them naturally.

There are two types of xenobiotic compounds; they may be biodegradable or non degradable. Biodegradable xenobiotic compounds are those that get degraded by the action of microorganism or other reactions may be defined as weak xenobiotic, while non degradable xenobiotic compounds known as recalcitrant compounds are resistant to degradation by any reactions (Fig.1). The recalcitrant xenobiotic compounds can be grouped into various groups like halocarbons, polychlorinated biphenyls, oil mixtures, synthetic polymers, alkyl benzyl sulphonates, polycyclic
aromatic hydrocarbons (PAHs) etc. the recalcitrant xenobiotics are responsible to pollute the environment for eg. Plastics and nylon are synthetic polymers have higher resistant because it is insoluble in water, oil is also pollutant recalcitrant mixture complex mainly because of its insolubility in water and due to the toxicity of some of its compounds. Xenobiotic metabolism is the set of metabolic pathway that modify the chemical structure of xenobiotics; these pathways are a form of bioremediation present in all major groups of organisms. These reactions often act to detoxify poisonous compounds, however, in some cases; the intermediates in xenobiotic metabolism can themselves be the cause of toxic effects.

Xenobiotic metabolism is divided into three phases.

In phase I, enzymes such as cytochrome P-450 oxidases introduce reactive or polar groups into xenobiotics. These modified compounds are then conjugated to polar compounds. In phase II reactions, these reactions are catalysed by transferases enzymes such as glutathione S-transferases. Finally, in phase III, the conjugated xenobiotics may be further processed, before being recognised by efflux transporters and pumped out of cell.

**Fig.1** Origin of different types of chemical compounds in the environment
1.2 Source of xenobiotics

Xenobiotics organic compounds (XOCs) originated from personal care products, pharmaceuticals, excreted hormones, household and industrial chemicals etc. the main source are households, institutions, agriculture and the industry [7, 8]. The total annual world production of synthetic organic chemicals is over 300 million tons [9] with more than 1000 new compounds every year, so that the actual diversity of chemicals reaches approximately 20 millions of substances. If not treated, all these compounds sooner or later appear in the environment. In fact, during the past three decades, research on the impact of chemical pollution has focused almost exclusively on the conventional ‘priority’ pollutants (i.e. persistent organic pollutants, POPs) and this has been extensively reviewed recently [10].

Today, these compounds are less relevant for many first world countries because emission have been substantially reduced through the adoption of appropriate legal measures and the elimination of many of the dominant pollution sources. The focus has consequently switched to compounds present in lower concentrations but which nevertheless might have the ability to cause harm [11]. One of the interesting characteristics of many of the chemicals that might cause this type of pollution is that they do not need to be persistent in the environment to cause negative effects [12]. This is because their high transformation and removal rates can be offset by their continuous introduction into the environment, often through sewage treatment works [13, 14]. In particular, volatile organic compounds (VOCs) of fuels and industrial solvents are commonly found contaminants in the subsurface, pesticides and phenols in groundwater. Persistent organic pollutants (POPs) are long lived organic compounds that become concentrated as they move through the food chain. They are also known as persistent toxins that bioaccumulate or as pseudo-
oestrogenic chemicals. They have toxic effects on animal reproduction, development, and immunological function.

In a recent study, a special attention has been focused on main concern organic environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs), eg anthracene, phenanthrene, fluoranthene and pyrene, and experimental approaches for degradation of these compounds by microorganisms.

In fact, although the risks associated with exposure to chemicals are probably most significant with regard to the natural environment, the public’s concern is understandably more focused on human exposure. However, the issue of xenobiotics (and their metabolites) in the environment, notably the aquatic compartment, has been a growth area in environmental chemistry for several years [15, 16].

To date, most of the published literature has addressed the occurrence of organic pollutants in sewage effluent and receiving waters. This is especially important for human health in areas that practice indirect water reuse, where sewage effluent is released to streams and rivers that are in turn used as a source of raw water for the production of notable supplies for communities living downstream [17]. From the recent environmental awareness and legislative restrictions on uncontrolled discharges of wastes, the best strategy for treatment of high toxic hazardous and xenobiotic wastes may be their treatment at source with a special stress put on the industry [18]. A large group of aromatic compounds generally not produced by natural processes has not to be completely degraded and mineralized because of their high persistence. This may be achieved through the development of particular solutions for each ecological problem.
1.3 Removal mechanisms of xenobiotics

The main removal mechanisms of xenobiotics from environmental west site are biological or chemical degradation, sorption, stripping and volatilization, a biotic hydrolysis and a biotic oxidation. The conventional treatment is not directly designed for removing xenobiotics organic compounds but to reduce concentration of several xenobiotics [19 - 22].

1.3.1 Biodegradation

Biodegradation is the process in which organic substances are broken down into smaller compounds by the microbial enzymes.

A biodegradation process in which sites contaminated with xenobiotics are cleaned up by means of bacterial bio-geochemical processes, preferably in situ, exploits the ability of microorganisms to reduce the concentration and/or toxicity of a large number of pollutants. It is an economical, versatile, environment-friendly and efficient treatment strategy, and a rapidly developing field of environmental restoration.

Biodegradation utilizes the microbial ability to degrade and/or detoxify chemical substances such as petroleum products, aliphatic and aromatic hydrocarbons (including polycyclic aromatic hydrocarbons and polychlorinated biphenyls), industrial solvents, pesticides and their metabolites, and metals. The presence of a large number of various bacterial species in nature can degraded variety of chemical pollutants. In natural waters and soils, the mineralization or complete biodegradation of an organic molecule is almost always a consequence of microbial activity [23].
Xenobiotic compounds can be degraded aerobically, with oxygen, or anaerobically, without oxygen the most rapid and complete degradation of the majority of pollutants is brought about under aerobic conditions [24].

1.3.1.1 Aerobic Biodegradation

Aerobic biodegradation is the breakdown of organic contaminates by microorganisms when oxygen is present, therefore the chemistry of the system, environment, or organism is characterized by oxidative conditions. The essential characteristics of aerobic microorganisms degrading organic pollutants are illustrated in (Fig.2 and Fig.3). Aerobic bacteria (aerobe) have an oxygen based metabolism. Aerobes, in a process known as cellular respiration, use oxygen to oxidize substrates in order to obtain energy. This process of funnelling compounds allows for common pathways in organisms that break down these substrates, for instance, many aromatic compounds are oxidized by the incorporation of oxygen into the ring cleavage. This can be accomplished via mono- or dioxygenases, which introduce one or two oxygen atoms, respectively, into the benzene ring. It is more common for organisms to employ dioxygenases to incorporate the entire oxygen molecule into benzene to form a cis-diol, which is then rapidly transformed [25]. Major breakdown intermediates of aromatic ring compounds are catechol or protocatechuate [26]. Once catechol is formed, the ring can be cleaved and broken into fragments that can be further degraded [27]. This oxidation can occur through ortho-fission (between the hydroxyl groups) or meta-fission (adjacent to one of the hydroxyl groups) [28]. The ortho-cleavage of catechol is catalyzed by catechol 1, 2-dioxygenase and generates cis, cis-muconic acid [29] which can enter β oxidation, with acetyl CoA and succinate CoA as products which can enter the citric acid cycle. Meta-cleavage of catechol is...
catalyzed by catechol 2, 3-dioxygenase and produces 2-hydroxymuconic semialdehyde [30] which is eventually broken into acetaldehyde and pyruvate.

**Fig.2** Main principle of aerobic biodegradation of hydrocarbons: growth associated processes.

**Fig.3** The *ortho* and *meta*-cleavage degradation pathway for PAHs
**1.3.1.2 Anaerobic Biodegradation**

Biodegradation of contaminated environmental by microbes in the absence of oxygen is known as anaerobic degradation. Anaerobic digestion is a series of processes in which microorganisms break down biodegradable material in the absence of oxygen. It is widely used to treat wastewater sludge and biodegradable waste because it provides volume and mass reduction of the input material. Theoretically, in subsurface sediments, anaerobic bacteria and anaerobic biodegradation of PAHs should play greater roles than the respective aerobic ones [31]. Some biologically related factors, such as bacterial population size, electron transport system (ETS) activities, Fe(III) utilization and anaerobic gas production, which are important in understanding the mechanisms involved in the biodegradation of PAHs under anaerobic condition.

The degradation of xenobiotic compounds by anaerobic microbes (e.g. *Clostridia*, *Desulfbacterium*, *Desulfovibrio*, *Methanococcus*, *Methanosarcina* and *Dehalogenating* bacteria) has been a subject of extensive research during the last two decades [32].

In the absence of molecular O$_2$, alternative used to oxidize aromatic compounds [33, 34] anaerobic consortia able to metabolize PAHs using nitrate or sulphate as an electron acceptor (Fig.4). Phthalate compound degradation is mainly carried out by anaerobic methanogens (*Methanospirillum hungatei*, *Methanoseta concilii*, *Syntrophobacter fumaroxidens*), producing acetate and methane as end products by decarboxylation initially, then reduction followed by ring cleave and ultimately pave to the b-oxidation pathway [35,33]. Benzene, toluene, ethylbenzene, and xylenes (BTEX) compounds act as carbon and energy sources for diverse anaerobic bacteria under nitrate-reducing, Fe (III)
reducing, sulphate-reducing and methanogenic conditions. Methyl tert-butyl ether (MTBE), a parallel contaminant with BTEX and PAHs in petroleum contaminated sites.

Fig.4 Anaerobic biodegradation of PAHs

1.3.2 Bioremediation

Waste products resulting from human activities have always been a serious problem. These waste products of recalcitrant chemicals have to removal from environment. There is a serial of studied to reduce the influence of these harmful chemicals. The main process is the biotic degradation or bioremediation. Bioremediation is the methodology or a biodegradation process of using microorganisms to degrade or remove hazardous chemicals or the industrial wastes to overcome the ecological hazards for clean environment [36]. Bioremediation is playing important role in deciding overall fates of organic pollutants. These degradation processes are following by chemical changes through changing their physiological properties or mineralization via TCA cycle by using these compounds as sole carbon and energy [3].
Microbial diversity and adaptability for adjustment to xenobiotics makes stronger among all living organisms to transfer xenobiotic compounds into natural eco-friendly metabolites [37, 38]. Due to the ability of metabolic diversity and plasticity, bacteria have the potential to degrade and hence to remove a variety of naturally occurring as well as synthetic aromatic compounds released through geochemical cycles, urban and industrial activities [2, 39]. Recent research has shown that the variety of soil microorganisms plays important role to clean up the environmental. It is now important to understand phenomenon of biodegradation/biotransformation of organic contaminants in the natural environment to understand microbial ecology, physiology and evolution for their potential in bioremediation [25, 40-43]. A process of biodegradation is nothing but bioremediation in which the sites contaminated with xenobiotics are cleaned up by means of bacteria biogeochemical metabolic processes. The technique of in situ bioremediation exploits the ability of microorganisms to reduce the concentration and/or toxicity of a large number of xenobiotic pollutants. It is an inexpensive, adaptable, environment-friendly and proficient treatment approach, and a rapidly developing field of environmental renovation. It also helpful in developing the microbial ability to degrade and/or detoxify chemical substances such as petroleum products, aliphatic and aromatic hydrocarbons (including polycyclic aromatic hydrocarbons and polychlorinated biphenyls), industrial solvents, pesticides and their metabolites, and metals [44]. Thus, the use of microorganisms for degradation of xenobiotic pollutants is now being increasingly useful technology of choice for clean up or restoration of contaminated/polluted sites as it can be self sustaining and economical [44]. Bacteria have the unique feature in rapidly adapting to toxic nutrients supplies and engaging antagonistic environments. The metabolic diversity and plasticity of bacteria in the
face of environmental disaster and limitations provide an immense reservoir of exploitable regulatory devices and biochemical activities [5, 6]. Using these abilities, range of naturally occurring as well as manmade aromatic compounds are discharged through geochemical cycles, urban and industrial activities are helping to develop the potential to degrade them [2, 39].

Biodegradation capacity of aromatic compounds processes was also performed by prokaryotes like archaea to eubacteria either as pure culture or in consortia. The universal bacteria genera in soil include *Acinatobacter, Agrobacterium, Alcaligenes, Arthrobacter, Bacillus, Brevibacterium, Cellulomonas, Clostridium, Corynebacterium, Flavobacterium, Micrococcus, Mycobacterium, Pseudomonas, Staphylococcus, Streptococcus, and Xanthomonas* are also useful to biodegradation of xenobiotics. These soil bacteria are very significant as they bring about the global cycling off carbon and other elements. The motivating features of these bacteria are that they have the capacity to take advantage of this compound as sources of energy and carbon. Most the time microbial consortia were applied for clean up to contaminated site to enhance biodegradation. Where single bacterium is does not possess the enzymatic ability to degrade most of the organic compounds in a contaminated site.

1.3.2.1 Classifications of bioremediation:

Biotransformation is the alteration of contaminant molecules into less or non-hazardous molecules [45].

Biodegradation is the breakdown of organic substances in smaller organic or inorganic molecules. [45].

Mineralization is the complete biodegradation of organic materials into inorganic constituents such as CO$_2$ or H$_2$O [45].
1.4 Factors influencing microbial degradation of xenobiotics

Various factors can affect the biodegradation processes, they depend on the nature of chemical molecules to be degraded (e.g. molecule size, charge, number and position of functional groups, solubility and toxicity) as well as the environmental conditions. For instance, some molecular features can increase recalcitrance [9] and environmental factors influence the growth of organisms, the availability of xenobiotics and more subtly, they can affect the gene expression [46, 47].

Therefore, basic information is required to achieve a successful biotreatment:

- Nature of the xenobiotic chemicals,
- Concentration of the xenobiotic chemicals,
- Presence and activity of the xenobiotic degrading microorganisms,
Appropriate conditions of cultivation which are required for the growth of the target microorganisms. Whenever they are identified and controllable, chemical and environmental factors should be taken into account during the engineering development process and all along with the xenobiotic degradation [48].

Usual factors which play a significant role during biological treatments are:

- Compound bioavailability
- pH and temperature of the medium
- Nutrients
- Oxygen availability
- Residence time (dilution rate)
- Temperature
- Salinity
- Chemical and physical properties

Laboratory experiments, which aim to study the xenobiotic biodegradation, are preferably carried out at pH and temperature which are optimal for the cultivated microorganisms in order to maximize their degradation activity. Many bacteria have their optimum pH near neutrality and are mesophilic, whereas, fungi prefer more acidic environments [49]. However, under suboptimal environmental conditions, an increased resistance of microorganisms can be achieved by control of the bacterial cells [50]. This is interesting for wastewater treatment. Improving the screening of these parameters will turn out as an improved ability to perform environmental risk estimations, to design environmental strategies and to optimize wastewater treatment.
1.5 Enzymes involved in biodegradation of xenobiotics

1.5.1 Azoreductase

Azoreductase is a biotransformation enzyme plays an important role in degradation of nitro compounds as well as azo compounds. Azoreductase are involved in the bioremediation by bacteria of azo dyes and azo compounds found in waste water [51]. Bacterial degradation of azo dyes is often initiated by cleavage of azo (N=N) bonds by azoreductase which are followed by the aerobic degradation of resulting amines [52]. Decolorization of azo dyes requires initial cleavage of azo group (N=N) bond breakage which generates the toxic aromatic amine, which are subsequently degraded by aerobic biodegradation [3]. Electron donation effect of the functional group on the substrate assist to the azoreductase enzymes for cleaving the N=N bond from the parent azo dyes compounds by ping-pong mechanism [53]. Moreover, it is an oxidoreductase protein responsible for the degradation of azo compounds. Azoreductase catalyzes the reductive cleavage of azo groups (-N=N-) utilizing NADH or NADPH as an electron donor by means of the flavin but that final step in transfer of electrons occurs non-enzymatically so referred to as non enzymatic azo reduction and it is mediated by flavoprotiens in microbial electron transport chain. This flavoprotiens catalyses the generation of reduced flavin (FAD or FMN) by re-oxidation of reduced NADH or NADPH. The reduced flavin transfer electrons to azo compound which is the terminal electron acceptors, thereby reducing azo bonds and being concurrently deoxidised [52]. In vitro azoreductase has functions as nitroreductase and flavin mononucleotide (FMN) reductase. Recently it is shown that the nitroreductase have degraded various nitro compounds like 2, 4, 6-trinitrotoluene [54, 55].
1.5.2 Cytochrome P-450

Cytochrome P-450 (CYP-450) (Fig.6) is used to refer to a large and diverse group of enzymes that it is requires a sulphur atom legated to the iron and forms carbon monoxide complex in reduced state. The function of most CYP enzymes metabolizes a wide variety of xenobiotic compounds such as drugs, wide variety of organic compounds and carcinogens, as well as endobiotics such as prostaglandins and steroids. Cytochrome P-450 enzymes were first discovered by Garfinkel and Klingenberg in 1955 in rat liver microsomal and they are characterized by an intense absorption band at 450 nm in the presence of carbon monoxide. Ryan and Engel [56] further provided the evidence for its possible relation with enzymatic hydroxylation. Omura and Sato [57] later named the substance cytochrome P-450 from the position of absorption of CO-complex. Involvement of cytochrome P-450 in the reaction of mixed function oxidase system and activation of oxygen was established by Estabrook et al [58]. Solubilisation of the hemoprotein resulted into the loss of the typical absorption of CO-complex at 450 nm. With concomitant shift to 420 nm, this was referred as inactive or cytochrome P-420. Presence of cytochrome P-450 is not confined to mammalian species. It is also found in birds [59, 60], fishes [61] plants and bacteria [62]. Cytochrome P-450 monooxygenase system is highly nonspecific. Though the reason for this is unknown, the enzymes in the system with endogenous substrate and important functions in the synthesis of steroids or hormones are more specific. Well-defined products are produced for the functioning of the organisms. However, in the biotransformation of the xenobiotics, introduction of the oxygen atom at any position in the molecule is usually sufficient to increase the hydrophilic properties of these compounds. This reaction facilitates its excretion from the body.
Several investigators have attempted to find out the possible explanation for the substrate non-specificity of this enzyme. It was found that several forms of cytochrome P-450 (isozymes), with slightly different but overlapping substrate specificities exist [63, 64]. The relative distribution of different forms of cytochrome P-450 is attributed to genetic and environmental influence [65]. Sex, species, age, strains, nutritional status and exposure of organism to various chemicals explained the multiplicity of the cytochrome P-450 [62, 66]. It is well known that various cytochrome P-450 so far characterized are different from each other by molecular weight, amino acids composition and terminal amino acids [62, 67, 68]. Even antibodies prepared against electrophoretically homogenous form of particular cytochrome P-450 do not cross react with other forms of cytochrome P-450. However, the spectral properties are almost identical due to small but significant differences between the various forms [67, 66]. The heme is bound to cytochrome P-450 to form a single polypeptide chain with molecular weight varying from 40,000 to 60,000 Da. The similarity in the optical spectra could be explained on the basis of essentially identical coordination sphere of heme [69].

**Fig.6** Cytochrome P-450 oxidases the important enzyme in xenobiotics metabolism
1.5.2.1 Classification of cytochrome P-450

Cytochrome P-450 can be divided into four classes depending on how electrons from NAD(P)H are delivered to the catalytic site [70].

**Class-I.** These types of proteins require both a FAD-containing reductase and an iron sulfur redoxin (ferredoxin).

**Fig.7** Class I Cytochrome P-450

**Class-II.** These types of proteins require only an FAD/FMN-containing P-450 reductase for transfer of electrons.

**Fig.8** Class II Cytochrome P-450

Flow of electrons normally takes place from FAD to FMN and then to the P-450 component in two sequential one-electron-step. Eukaryotic cytochrome P-450 (microsomal) is mostly membrane bound [71, 72], the richest source being the endoplasmic reticulum and mitochondria [73]. Bacterial cytochrome P-450 is not membrane bound. It is actually solubilized in the cytosol [74, 75]. The molecular weight of cytochrome P-450 falls in the range between 50000 D to 60000 D. The
prokaryotic P-450 is monomeric [76], as compared to eukaryotic cytochrome P-450, which is highly associated under normal condition [77].

**Class-III.** These types of cytochrome P-450 do not require ancillary electron transport proteins. It is self-sufficient to carry out the oxidative reactions in the presence of reduced pyridine nucleotide [78, 79]. This cytochrome P-450 does not require molecular oxygen nor electron donor for catalysis. It catalyzes the rearrangement or dehydration of alkylhydroperoxides or alkylperoxides initially generated by dioxygenases [80]. These enzymes are involved in the synthesis of signaling molecules, such as prostaglandins in mammals and jasmonate in plants.

**Class IV.** This class of enzyme receives electrons directly from NAD(P)H. This unique fungal cytochrome P-450 is soluble and reduces NO generated by denitrification to N$_2$O [80].

Class III and IV cytochrome P-450 might be considered as the remains of the most ancestral forms of cytochrome P-450 in detoxification of harmful activated oxygen species.

### 1.5.2.2 Bacterial cytochrome P-450

Bacteria possess the first type of cytochrome P-450. Most of the pioneer work on bacterial cytochrome P-450 studies is being done in *Pseudomonas putida*, which is used as a reference in the study of other species. Bacterial cytochrome P-450 systems consist of a NAD(P)H or NADH ferredoxin reductase, containing an FAD prosthetic group, a [2Fe-2S] ferredoxin-type iron sulphur protein and the P-450 component [81, 82]. The cytochrome P-450 heme proteins are of molecular weight around 50 kDa and house the catalytic site that binds substrate and atmospheric oxygen. Ferredoxin is a small hydrophilic protein. It contains one [2Fe-2S] cluster per molecule and serves as one-electron carriers. The two atoms of iron are bridged...
by two labile sulphide atoms and coordinated to the protein by four sulphide ligands, contributed by four-cysteine residue. They are reddish in color [83, 84].

Cytochrome P-450\textsubscript{cam} from \textit{Pseudomonas putida} was the first bacterial P-450 discovered and has been thoroughly characterized [85, 86]. It is a 45 kDa protein which catalyzes the stereo and regio-specific hydroxylation of camphor at its 5-exo position. To bring about the hydroxylation reactions, it requires the participation of two other proteins, putidaredoxin (11726 Da ferredoxin) and putidaredoxin reductase (48000-Da FAD-containing NADH-specific). They act as redox partners in the monooxygenation system. Cytochrome P-450\textsubscript{cam} is a 414-amino acid enzyme [87, 88]. The major difference between eukaryotic cytochrome P-450 and P-450\textsubscript{cam} is that the amino-terminal portion which attaches the former to the membrane (microsomal or mitochondrial) is absent in the latter [89]. Electron flow in cytochrome P-450\textsubscript{cam} system occurs as follows. NADH reduces FAD component of NADH-putidaredoxin reductase to FADH\textsubscript{2}, which reduces the iron cluster of putidaredoxin and electrons shuttle from NADH-putidaredoxin reductase to the P-450 component. The electron transfer proteins are all soluble and do not appear to be membrane associated [90, 91]. Cytochrome P-450 from \textit{Bacillus megaterium} catalyzes the hydroxylation of long chain fatty acids having a mass of 118 kDa. It exhibits the activities of both P-450 hydroxylase and NADPH-cytochrome P-450 reductase [92, 93]. Such composite P-450 enzymes are not found in eukaryotes [94]. This system is composed of large soluble proteins with a cytochrome P-450 reductase domain and a cytochrome P-450 domain, each of which are homologous to the microsomal P-450 system components [95, 96, 97]. The \textit{Bacillus megaterium} P-450 CYP102 has the NADPH cytochrome P-450 reductase fused to the cytochrome P-450 in a single gene. Cytochrome P-450BM-3 is one of the three cytochrome P-450 isolated from \textit{Bacillus megaterium}. 

23
Cytochrome P-450BM-3 is a class II P-450, since it obtains reducing equivalents from a FAD/FMN diflavin reductase, similar to a microsomal P-450 system. However, cytochrome P-450 BM-3 is unique in that the diflavin reductase domain is fused into a single polypeptide, giving a self-sufficient enzyme requiring only O2 and NADPH to carry out ω-1, ω-2 and ω-3, hydroxylation or epoxidation of long chain fatty acids. The electron transport component of the P-450sca system from *Streptomyces carbophilus*, NADH-P-450sca reductase is a single polypeptide containing FMN and FAD (Fig.9). This reductase resembles the eukaryotic reductases but its size, 51 KD, is smaller than the sizes of its eukaryotic counterparts [98, 99]. Cytochrome P-450nor is a nitric oxide reductase from a denitrifying fungus *Fusarium oxysporium* [100]. This enzyme carries out reaction without the mediation of accessory proteins and do not possess any monooxygenase activity but is able to reduce nitric oxide (NO) form to dinitric oxide (N2O) directly using NADH as electron donor. Oxygen is not required as NO binds to the ferric heme Fe3+ directly unlike in a normal P-450. No heme reduction occurs.

\[
2\text{NO} + \text{NADH} + \text{H}^+ \rightarrow \text{N}_2\text{O} + \text{H}_2\text{O} + \text{NAD}^+ 
\]

![Fig.9 Bacterial and microsomal cytochrome P-450.](image)
1.5.2.3 Reactions catalyzed by cytochrome P-450.

Bacterial cytochrome P-450 generally catalyzes the hydroxylation of various substrates such as camphor, phenolics, and herbicides. Microsomal cytochrome P-450 plays key roles in the synthesis and interconversion of steroids and detoxification of many drugs. The hydroxylation reactions are also known as monooxygenation reactions, as the enzyme catalyzes the incorporation of only one atom of molecular O\(_2\) into substrate, while reducing the second into H\(_2\)O with the following stoichiometry:

\[
RH + O_2 + NAD(P)H + H^+ \xrightarrow{\text{RH + O}_2 + \text{NAD(P)}H + \text{H}^+} \text{ROH} + \text{H}_2\text{O} + \text{NAD(P}^+)\]

Each cycle of monooxygenation requires two electrons from pyridine nucleotides, NADH or NADPH. The function of the electron transport proteins of cytochrome P-450 system is to accept the two electrons from NAD(P)H and to transfer them one at a time to the P-450 component during the monooxygenase reaction [101-106]. The biotransformation reactions carried out by cytochrome P-450 are epoxidation, peroxycyclation, N-, S-, and O-dealkylation, N- and S-oxidation, desulfuration, reduction of nitro and azo groups, as well as, N-oxides, peroxides, and epoxides, deamination, dehalogenation, isomerisation and nonhydrolytic carbon-carbon bond cleavage [103,106-108]. Bacterial cytochrome P-450 is generally more substrate specific, typically acting on only a few substrates and exhibiting high specificities for the position attacked and the stereochemistry of the reaction [109, 110].

1. Aromatic hydroxylation

\[
\text{C}_6\text{H}_5\text{X} \xrightarrow{(O)} \text{HOC}_6\text{H}_4\text{X}
\]

2. Aliphatic hydroxylation or acyclic oxidation

\[
\text{R-CH}_3 \xrightarrow{(O)} \text{R-CH}_2\text{OH}
\]
3. N-Dealkylation

\[ R-\text{NH-CH}_3 \xrightarrow{(O)} (R-\text{NH-CH}_2\text{-OH}) \rightarrow R-\text{NH}_2 + \text{HCHO} \]

4. O-Dealkylation

\[ R-\text{OCH}_3 \xrightarrow{} (R-\text{OH-CH}_2\text{-OH}) \rightarrow R-\text{OH} + \text{HCHO} \]

5. Deamination

\[ R_2\text{CH-NH}_2 \rightarrow R_2\text{C-(OH)NH}_2 \rightarrow R_2\text{C=O} + \text{NH}_3 \]

6. Sulfoxidation

\[ R-S-R' \rightarrow (R-\text{SOH-R'}) + R-\text{SO-R'} + \text{H}^+ \]

7. N-Oxidation

\[ (\text{CH}_3)_3\text{N} \rightarrow (\text{CH}_3)_3\text{N-OH}^+ \rightarrow (\text{CH}_3)_3\text{NO} + \text{H}^+ \]

1.5.2.4 Cytochrome P-450 dependent monooxygenation

Generally the steps involved in the hydroxylation reactions are showing in (Fig.10):

1. Binding of substrate to the oxidized cytochrome P-450.
2. Reduction of NAD (P) H or NADH cytochrome P-450 ferredoxin reductase by NAD (P) H or NADH.
3. Reduction of cytochrome P-450 substrate complex by ferredoxin.
4. Addition of oxygen molecule to reduced cytochrome P-450 substrate complex.
5. Reduction of oxygenated reduced cytochrome P-450 substrate complex by another electron, probably from NAD(P)H or NADH cytochrome P-450 reductase.
6. Decomposition of oxygenated reduced cytochrome P-450 substrate complex to hydroxylated substrate oxidized cytochrome P-450 and water.
1.5.3 Modulation of mixed function oxidase system

Miller et al. [110] and Brown et al. [111] reported the stimulatory effects of foreign compounds on liver microsomal enzyme and were further examined in more detail by Remmer et al. [112-113]. Many other investigators then explored this phenomenon thoroughly [114-118] and found its relation with increased biosynthesis of microsomal drug metabolizing enzymes, which was termed as induction. Cytochrome P-450 consists of several forms, which possesses different specificities for a given substrate [119,120]. The different forms of cytochrome P-450 are induced by different inducers [121-123]. The alteration of any specific forms of cytochrome P-450 may thus be responsible for the metabolism of certain substrate. The mechanism underlying the inductive effect of different xenobiotics may vary from each other and this formed the basis of classification of enzyme inducers. The differences in the type of inducers are with respect to their carbon monoxide different spectrum, ethyl isocyanide differences spectrum, substrate specificity and genetic control of cytochrome P-450 induction. Phenobarbital, a broad spectrum inducer, causes increase in electron transport components like cytochrome P-450, NADH cytochrome-c-reductase, increase in cytochrome P-450 substrate complex, its rate of reduction and other enzyme involved in drug metabolism [124 - 126]. Polycyclic hydrocarbons, such as 3-methyl cholanthrene, a narrow spectrum inducer, induce the formation of another variety of cytochrome P-450 called cytochrome P-448. It has different affinities for various substrates than the usual form [127- 130]. DDT, polychlorinated biphenyls, halogenated hydrocarbons and organophosphates are potent stimulators of microsomal enzymes [131- 133]. Various environmental factors alter the activities of the cytochrome P-450 [134]. Some substances inhibit the metabolism of the chemical by combining reversibly with cytochrome P-450 [135].
Some inhibitors, such as piperonylbutoxide are converted to metabolites that apparently have a higher affinity for the cytochrome P-450 than does the parent compound. [136]. Some chemicals cause the destruction of cytochrome P-450. For example, carbon tetrachloride causes the destruction of cytochrome P-450 [137,138], presumably by causing the peroxidation of microsomal lipids. Some salts, like nickel chloride and cobalt chloride inhibit ALA-synthetase [139, 140], which is the rate limiting enzyme in the heme biosynthesis. They induce the heme oxygenase, which is the heme-degrading enzyme. As a result, the rate of enzymatic degradation of cytochrome P-450 is decreased [141], whereas, allylic compounds such as allylisopropylacetamide cause the destruction of cytochrome P-450 by an unknown mechanism.

Fig.10 Mechanism of cytochrome P-450 dependent monooxygenation.
1.6 Alkaliphilic bacteria

There are no precise definitions of what characterizes an alkaliphilic or alkalitolerant organism, several microorganisms exhibit more than one pH optimum for growth depending on the growth conditions, particularly nutrients, metal ions, and temperature. In this review, therefore, the term “alkaliphilic” is used for microorganisms that grow optimally or very well at pH values above 9, often between 10 and 12, but cannot grow or grow only slowly at the near neutral pH value of 6.5 (Fig.11). The first paper concerning an alkaline enzyme of alkaliphilic microorganisms was published in 1971 [142].

![Fig.11 The typical pH dependency of the growth of alkaliphilic and neutrophilic bacteria (By KOKI HORIKOSHI 1999).](image)

The aspects that have received the most attention in recent years include

(i) Extra cellular enzymes and their genetic analysis.


(iii) The taxonomy of alkaliphilic microorganisms.

1.6.1 Distribution of Alkaliphilic

Alkaliphiles consist of two main physiological groups of microorganisms; alkaliphiles and Haloalkaliphiles. Alkaliphiles require an alkaline pH of 9 or more
for their growth and have an optimal growth pH of around 10, whereas Haloalkaliphiles require both an alkaline pH (pH 9) and high salinity (up to 33% NaCl) wt/vol. Alkaliphiles have been isolated mainly from neutral environments, sometimes even from acidic soil samples and feces. Haloalkaliphiles have been mainly found in extremely alkaline saline environments, such as the Rift Valley lakes of East Africa and the western soda lakes of the United States.

1.6.2 Aerobic alkaliphiles

Alkaliphilic microorganisms coexist with neutrophilic microorganisms, as well as occupying specific extreme environments in nature (Fig.11) illustrate the relationship between the occurrence of alkaliphilic microorganisms and the pH of the sample origin. To isolate alkaliphiles, alkaline media must be used. Sodium carbonate is generally used to adjust the pH to around 10, because alkaliphiles usually require at least some sodium ions. Table the makeup of an alkaline medium suitable for their isolation. The frequency of alkaliphilic microorganisms in neutral “ordinary “organic substrates such as Casamino Acids, yeast extract, peptone, meat extract, tryptone, and casein. DNA-DNA hybridization and 16S rRNA partial sequences indicated that the new isolate belongs to the genus *Thermococcus* and represents a new species, *Thermococcus acidaminovorans*. An outstanding review on anaerobic alkalithermophiles has recently been published by Wiegel. [143].

Alkaliphilic microorganisms offer a multitude of actual or potential applications in various fields of biotechnology, not only do many of them produce compounds of industrial interest, but they also possess useful physiological properties, which can facilitate their exploitation for commercial purposes. [144].
1.6.3 The alkaline lake of Lonar

The alkaline lake of Lonar is situated in the Maharashtra State of India (19°58', long. 76°34'). It is a unique inland saline lake in Asia and only the third in the world. The lake is 400 km away from Pune and 553 km away from Mumbai. Based on geological studies [145] it is postulated that the lake originated as a meteorite impact crater ~50-60 thousand years ago. The Lonar crater is the only crater in basaltic rock on earth. The lake has a circular periphery and is situated in a hollow, 0.14 km below the ground level with an amphitheatre of vertical cliffs (Fig.12). The diameter of the lake around the top of the banks is about 2 km, while at the bottom it is 1.2 km. Alkalinity of the lake is attributed to the high content of sodium carbonate [145] and it was used previously as a source of washing soda. Water enters the lake via rain, ground water seepages, and the springs sited in the cliffs at the edge of the lake. It does not receive any industrial discharges. The lake water is alkaline, having an average pH of 10.5 which is the highest known in the world. It was therefore assumed that the micro flora present in the lake might be alkaliphilic, growing at a pH of 9.0-10.0.

Fig.12 Alkaline Crater Lake of Lonar, M.S India.
1.7 Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are the most commonly found environmental pollutants that belong to hydrophobic organic compounds (HOCs) based on their properties. The fate of PAHs in nature is of great environmental and human health concern due to their carcinogenic, mutagenic and teratogenic properties [146-148], as well as their high concentration and frequency found in the environmental [149, 150]. Some PAHs metabolites bind to protein, DNA, and RNA and adducted compounds may cause damage to cell and cause carcinogenic effects [151]. These facts make PAHs priority pollutants needed to be controlled [152,153].

Sixteen PAHs are listed and regulated by Environment Protection Agency (EPA) as priority pollutants due to their toxicity and mutagenicity as shown in (Fig.13). During the last three centuries a relationship between higher incidence of cancer in urban and industrial areas than rural areas, and the exposure of humans to PAHs have prompted considerable research on the sources, occurrence, bioaccumulation, metabolism, and disposition of these pollutants in aquatic and terrestrial ecosystems [154-156].
Fig. 13 Chemical structure of some PAHs.

1.7.1 Toxicity of PAHs

It has long been known that PAHs can have serious deleterious effects to human health [157], with the physician John Hill first recognising the link between the use of snuff (a product made from ground or pulverised tobacco leaves) and nasal cancer in 1761 [157]. Following this discovery, research into the toxic effects that PAHs have upon mammalian health has continued, with many PAHs displaying acute carcinogenic, mutagenic and teratogenic properties. Benzo [a] pyrene compound is known to be one of the most potently carcinogenic of all known PAHs [158]. When ingested, PAHs are rapidly absorbed into the gastrointestinal tract due to their high lipid solubility [157]. A major route of PAH uptake is via dermal absorption as highlighted by a study of 12 coke-oven workers [159]. An estimated 75% of the total absorbed amount of PAHs (specifically pyrene) entered the body through the skin, highlighting this as a major exposure route of PAHs. The rapid
absorption of PAHs by humans results in a high potential for biomagnification in the food chain. In general the greater the number of benzene rings, the greater the toxicity of the PAH [155]. PAHs are also suspected carcinogens but are not thought to be genotoxic unless they are ‘activated’ by mammalian enzymes to reactive epoxides and quinones. This occurs via a cytochrome P450 monooxygenase enzyme-mediated reaction that oxidises the aromatic ring to form epoxide and diol–epoxide reactive intermediates. It is reported that these intermediates may undergo one of at least four different mechanisms of oxidation and/or hydrolysis before the intermediates combine with and/or attack DNA to form covalent adducts with DNA. DNA adducts can lead to mutations of the DNA, resulting in tumours [160].

1.7.2 Chemical and physical properties of PAHs

Polycyclic aromatic hydrocarbons (PAHs) contain carbon and hydrogen atoms with fused benzene rings in linear, angular or cluster arrangements. PAHs are nonpolar hydrophobic organic compounds and rapidly become associated with soil particles or sediments, where they may become buried and persist for long periods. PAHs are low molecular weight (LMW) containing 2 or 3 benzene rings and higher molecular weight (HMW), containing four or more fused benzene rings, pose the greatest hazard to both environmental and human health [161] this is a significance of the resistance of PAHs to degradation processes [162], their high affinity for organic matter [163] and their low water solubility [164]. Properties of some PAHs as shown in (Table.1) the solubility of PAHs decrease as the number of benzene ring increase. Even though PAHs have low solubility in water, their dissolution can contaminate large amount of ground water for long periods [165]. Their lipophilicity, environmental persistence, and genotoxicity increase as the molecular size of PAHs
increase up to four or five fused benzene rings, and their toxicological concern shifts towards chronic toxicity, primarily carcinogenesis [166,147].

**Table.1** Physicochemical properties of some PAHs

<table>
<thead>
<tr>
<th>PAH</th>
<th>Chemical formula</th>
<th>M.W  (g/mol⁻¹)</th>
<th>Solubility In water (mg/l)</th>
<th>M.P °C</th>
<th>B.P °C</th>
<th>Log (Kₗₗ)</th>
<th>Vapour pressure</th>
<th>Ionization potential (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>C₁₀H₈</td>
<td>128.18</td>
<td>3.0</td>
<td>80</td>
<td>218</td>
<td>3.58</td>
<td>12.0</td>
<td>8.13</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>C₁₂H₈</td>
<td>154.20</td>
<td>3.6</td>
<td>95</td>
<td>279</td>
<td>3.92</td>
<td>4.02</td>
<td>7.86</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>C₁₄H₁₀</td>
<td>178.24</td>
<td>1.10</td>
<td>101</td>
<td>340</td>
<td>4.46-4.63</td>
<td>0.0161</td>
<td>7.91</td>
</tr>
<tr>
<td>Anthracene</td>
<td>C₁₄H₁₀</td>
<td>178.24</td>
<td>0.045</td>
<td>216</td>
<td>340</td>
<td>4.45</td>
<td>0.001</td>
<td>7.43</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>C₁₆H₁₀</td>
<td>202.26</td>
<td>0.25</td>
<td>109</td>
<td>384</td>
<td>5.22</td>
<td>0.001</td>
<td>7.95</td>
</tr>
<tr>
<td>Pyrene</td>
<td>C₁₆H₁₀</td>
<td>202.26</td>
<td>0.1-0.12</td>
<td>145</td>
<td>404</td>
<td>5.88-6.7</td>
<td>0.0006</td>
<td>7.53</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>C₁₈H₁₂</td>
<td>228.30</td>
<td>0.01</td>
<td>158</td>
<td>400</td>
<td>5.9</td>
<td>20.0×10⁻⁷</td>
<td>7.7</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>C₂₀H₁₂</td>
<td>252.32</td>
<td>0.001-0.006</td>
<td>179</td>
<td>495</td>
<td>5.79-6.4</td>
<td>7.0×10⁻⁷</td>
<td>7.6</td>
</tr>
</tbody>
</table>

**1.7.3 Metabolism of PAHs**

There are two essentially different mechanisms in the aerobic metabolisms of PAHs by microorganisms (Fig.14), specific details of bacterial and fungal. The basis of these mechanisms is the oxidation of aromatic ring, followed by the systematic breakdown of the compound to PAHs metabolites and carbon dioxide (CO₂) and H₂O. But in an aerobic metabolism of PAHs is occur via the hydrogenation of the aromatic ring.

PAHs- degrading microorganisms are distributed in the natural environment such as in soil, sediment, wood materials. Many PAH-contaminated sites host active populations of PAH-degrading bacteria and fungus.
1.7.3.1 Bacterial Metabolism of PAHs

The principal mechanism for the aerobic bacterial metabolism of PAHs is the initial oxidation of the benzene ring by the action of dioxygenase enzymes to form cis-dihydrodiols. These dihydrodiols are dehydrogenated to form dihydroxylated intermediates, which can then be further metabolised via catechols to carbon dioxide and water. The metabolic pathways and enzymatic reactions involved in the microbial degradation of anthracene have been studied in detail with an example pathway of anthracene degradation given in (Fig.14). There is a large diversity of bacteria that are able to oxidise anthracene and other PAHs using dioxygenase enzymes, including organisms from the genus *Pseudomonas* and *Rhodococcus*. A few bacteria are also capable of oxidising PAHs by the action of the cytochrome P-450 monooxgenase enzyme to form trans-dihydrodiols such as *Mycobacterium sp* [167] these are minor mechanisms compared with the activity of the dioxygenase enzymes [168]. The toxicity of anthracene and other PAHs metabolites generated during bacterial degradation have been little studied. The metabolites of anthracene, such as anthracene dihydrodiols, have higher water solubility than anthracene.

1.7.3.2 Fungal Metabolism of PAHs

Fungi are also known to degrade polycyclic aromatic hydrocarbons (PAHs) (Fig.14). They use another mechanism for degradation of hydrocarbons differs of bacterial mechanism, and they are therefore maybe able to degrade the hydrocarbon compound left by the normally faster degrading bacteria. This can be useful for the five-ring PAHs, which are only poorly degraded by bacteria. Fungi secrete extracellular oxidizing enzymes for degradation of lignin [169]. These enzymes are able to make reactive peroxide from oxygen [170]. Especially white rot fungi are able to degrade lignin. Lignin is a complex random molecule containing a lot of
aromatic groups. That fungi are able to degrade lignin makes them also possible candidates for PAH degradation. The degrading enzymes lignin peroxidase by manganese peroxidase has shown to be able to degrade some model lignin compounds. Peroxidase has showed to be involved in degradation of PAH to quinones [171]. Fungi do not degrade the hydrocarbons completely to CO\(_2\) as bacteria mechanism. In the highest conversion of hydrocarbons shown only 19% was converted [170]. Instead they form a range of degradation products which are solved in the aqueous phase or become bound to organic fraction in the soil. For benzo (a) pyrene it was found that nearly all degradation product was bound to the compost fraction used in that experiment. The degradation rate of benzo (a) pyrene was found to be double of the degradation rate in the culture without the fungi. After a month the degradation stopped, which is suggested to be due to nutrient limitation [172]. The presence of fungi and bacteria in one culture leads to an increase in degradation. The culture showed a degradation rate which was higher than the total amount of degradation in a bacterial and a fungi culture separately. Bacteria which have shown no growth on benzo (a) pyrene seems in presence of fungi able to grow. A degradation rate of 27% in 56 days was found for benzo[a]pyrene and 19% for dibenz [a, h] anthracene in the combined culture. The influence of the lignolitic enzymes on the degradation product can be seen in (Fig.14) [173]. Degradation mechanisms of bacteria and fungi show a remarkable difference with cis-trans hydroxylation for fungi and cis-cis hydroxylation for bacteria [174].
1.8 Aims and objectives

Based on an extensive literature review on biodegradation of xenobiotics contaminated environmental, the present study focused on biodegradation of polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene, anthracene, fluoranthene and pyrene by alkaliphilic and neutrophilic microorganisms.

Therefore the present studies are planned:

1. To isolate and identify the alkaliphilic bacterial strain from alkaline pristine lake of Lonar, (MS) Buldana, India.

2. To isolate and identify the neutrophilic bacterial strain from textile finishing industrial waste disposal site, Ichalkaranji, India.
3. To study the degradation potential of three benzene rings polycyclic aromatic hydrocarbons (phenanthrene, anthracene) by alkaliphilic and neutrophilic bacteria.

4. To study the degradation potential of four benzene rings polycyclic aromatic hydrocarbons (fluoranthene, pyrene) by alkaliphilic and neutrophilic bacteria.

5. Identification of intermediate metabolites by UV visible spectrophotometer, FTIR, GC-MS chromatography and $^1$HNMR.

6. To study the effect of different experimental parameters and supplemented of carbon and nitrogen sources on biodegradation of PAHs.