CHAPTER 1

Introduction
1 Introduction

This chapter presents the introduction on farm chemicals (pesticides). The contamination of pesticides in environmental matrices, toxicity levels, their general degradation methodologies and the need for bioremediation were incorporated.

1.0 Environmental Pollution

Industrial growth in many developing countries is occurring under conditions of scarce resources and ineffective structures to address issues of health and safety at the workplace and outside. Poor countries are being squeezed between scarce financial resources, more competitive global markets, and an imperiled environment. In the developing world, it is not uncommon to find concentration of heavy industries in particular locations, designated by governments as "export-processing zones" or "development poles". But the rapid introduction of technologically complex and potentially hazardous processes into a social context which is not geared up to control the associated risks had been resulting in serious consequences to the environment and the health of people (Spiegel and Maystre, 2001).
Rapid industrialization had been leading to countless environmental hazards relating to land, air and water. The environment contaminated with toxic materials and other pollutants, had been resulting in serious health risks for humans and ecosystems. More extensive and intensive use of materials and energy created cumulative pressures on the quality of local, regional and global ecosystems (Spiegel and Maystre, 2006).

The problems of industrialization-induced environmental pollution are fundamentally life threatening and as such can and must be avoided. The severe problems brought in by environmental destruction can never be completely rectified because the culpable industrial organizations, the victims of environmental pollution, and the negatively affected society as a whole can never be fully freed from the deleterious results of such intrusions.

Pollution means any undesirable change occurring in the characteristics of the components of the environment (Diwaker Rao, 1986). These changes may be temporary or permanent depending on the extent to which pollution had taken place. Williamson gave a satisfactory definition of a “pollutant” and a “contaminant”. A contaminant is defined as anything added to the environment that causes a deviation from the geochemical mean composition. A pollutant is a contaminant responsible for causing some adverse affect on the environment (Harry, 1998).

Prior to 17th century there was pollution only due to garbage, human waste etc. But as the industrialization started in the later parts of the 18th century the situation took a different turn. All the countries in the world have been planning for the improvement of living standards of its citizens. This was brought about by striving for economic development in terms of massive infrastructure industrialization, extensive agriculture, development of service sector and so on (More, 1986). These measures, in turn, resulted in anthropogenic pressure on environment. In a developing country like India, population growth, increasing urbanization and industrialization are common phenomena and these
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had direct impact on the quality of air, water bodies, land, social and economic aspects of citizens and health of living beings (Rathi, 2001).

Rapid and indiscriminate industrialization became the order of the day. As a result pollution had taken a virulent form. Not only air, water and soil but also every component of the environment has been affected (Udeo Simonis, 1988). The levels of stack gases and particulate matter have been increasing in the atmosphere. The wastewaters emanating from the industries have been affecting the liner waters and also ground water aquifers. The solid garbage along with the municipal waste has been forming moulds of unwanted matter on the soil. In this connection, cases of environmental hazards which affected the human population and also animals to a greater extent could be quoted. The Minamata disease, which afflicted many Japanese in the Minamata Bay, was the result of indiscriminate discharge of organic mercury into the aquifers (Anil Kumar De, 1989). The Bhopal gas tragedy (Datta, 1991; Robert Desanto, 1986) was another testimony to the indifferent attitude of the industrial sector towards the environment. On that fateful day thousands of people in Bhopal became mute victims to the toxic gas emanating from the storage tank of one of the raw materials used in the factory. Hundreds of people died and many more were affected. The cost of inaction on conservation and environmental protection was enormous. The survey carried out by World Bank in October 1995 concluded that the average annual cost of environmental degradation in India is of the order of $ 9700 million. Surface water pollution (59%), soil degradation (20%) and urban air pollution (14%) are the major contributors to the environmental degradation (World Bank, 1995).

1.1 Classification of pollution

Historically and traditionally pollution sources were grouped according to the part of the environment most affected. Accordingly pollution might be classified into three types viz., water, air and land pollution (Mahagen, 1985).
1.1.1 Water pollution

The water bodies of the earth are being polluted in many ways. In general, the water bodies may be classified into the surface waters and ground waters. The surface water Viz., rivers, lakes, oceans were polluted due to irresponsible letting out of industrial and sewage discharges into them (Mai and Dale, 1977; Brown et al., 2002). In addition to this, pesticides used in the agriculture reach other locations through various physical transport processes and contaminate the environment especially the water bodies (Denner, 2000).

The ground water, contaminated by various pollutants, chemicals and toxic substances percolate deep into the soil and reach the water table. Ground water pollution had been resulting in the depletion of good potable water. Today, many people dump their garbage into streams, lakes, rivers, and seas, thus making water bodies the final resting place of cans, bottles, plastics, and other household products. The various substances that we use for keeping homes clean added to water pollution as they contain harmful chemicals (Wesley, 1999). Waste waters emanating from various industries and municipal waste waters were also contributing to water pollution to a large extent especially in a developing country like India. In many instances untreated or partially treated wastewaters are released into the environment.

Pollutants like pesticides, organic solvents like reaction intermediates, by-products, final products, floor washings and inorganic pollutants like heavy metals, anions, salts, from chlor-alkali industries were also major contributors to water pollution (Shiva, 2002).

1.1.2 Air pollution

The term air pollution may be broadly defined as the presence in the atmosphere of a substance or substances added directly or indirectly by an act of man in such amounts as
to affect humans, animals and vegetation. Pollution of air is occurring due to increased industrialization/urbanization apart from natural causes. The materials that occur in the troposphere in excess of normal (the harmless level) amounts might be considered as air pollutants (Walters, 2001). The sources for such materials may be natural or anthropogenic.

There are wide variations in the nature and extent of pollutants depending upon location and activities in and around it. Some of the pollutants are directly released into the troposphere and may be called as primary pollutants whereas there are other pollutants, which get synthesized in the atmosphere and may be called as secondary pollutants. Air pollution is the main problem of industrial regions because huge quantities of gaseous pollutants are released from factories manufacturing sulphuric acid, nitric acid, cement and foundries and power station plants. Vehicular pollution had also been contributing to a major extent to air pollution in the modern world. Air pollutants like oxides of sulphur and nitrogen, ozone and carbon monoxide cause health hazards (Gonzalez-Avalos and Ruiz-Uarez, 2001). It was reported that cattle manure released methane due to the dominant factors like temperature, moisture, the handling of manure, fermentation and moisture content of the excreta (Dubey, 2001). It was shown to have global warming potential of approximately 24.5% relative to carbon dioxide and is responsible for approximately 25% of the anticipated global warming (Caggiano et al., 2001).

1.1.3 Land Pollution

Pollution of land may occur due to urbanization, garbage, waste paper and packing materials, plastic waste, rejects from households and solid waste from industries and excessive use of chemicals like pesticides. Solid waste from industries if not treated suitably might pollute the water and air environment also (Bela Liptak, 1974; Jeevan Rao and Shanta Ram, 1993). Land pollution was increasing in alarming proportions with time. Both developed and developing countries were contributing significantly in this regard.
Domestic waste materials were dumped indiscriminately without appropriate treatment. The nature of landfills designed to reduce waste through generation of methane by anaerobic bacteria led to the production of methylated mercury compounds by the landfills of mercury-bearing materials like fluorescent lights, batteries, electric switches and thermometers (Lindberg et al., 2001). Indiscriminate use of pesticides resulted in the contamination of soils through the water from agricultural fields and rainwater (Walker and Hollis, 1994; Wikipedia, 2006).

1.2 Need for environmental pollution control

Pollution of environment was causing serious hazards to the living beings (Thrope, 1989; Sundesan and Raman, 1983). The twentieth century had been the proliferation of chemical products and wastes, many of which posed direct threat to the ecosystem and to the human health (Cralley, 1972). Governments, regulatory agencies, scientists and social organizations have been striving hard to keep the environmental pollution under control. Some of the important treatment methods, which are in practice for the degradation of pollutants, are discussed in the following pages.

1.3 Treatment methods

The treatment methods available for the degradation of the pollutants might be classified as physical, chemical and biological (Denis, 1974). Physical methods include adsorption, evaporation, incineration, landfill, composting and reverse osmosis. Chemical methods in vogue include solvent extraction, chemical oxidation (such as photo oxidation, hydrogen peroxide oxidation and ozonation), reduction, neutralization and complexation. Biological treatment methods include bio-sorption, biodegradation (with microorganisms like bacteria/fungi), activated sludge processes, aerated lagooning, bioremediation, aerobic and anaerobic digestion etc. Depending on the nature of the waste, a suitable treatment method has to be adapted.
1.4 Pesticides

Pesticides are used in many situations such as livestock farming, cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, roadsides, and recreational and industrial areas. They are a vital facet of farming in our daily life. Pesticides (or farm chemicals, agro chemicals or agvet chemicals) are those substances, which are used to control, destroy, repel or attract pests in order to minimize their detrimental effects (Brown et al., 2002). Pests are those organisms like weeds, insects, bacteria, fungi, viruses and animals, which adversely affect our way of life. They can reduce the quality and quantity of food produced by lowering production and destroying stored produce; they can harm our animals (like fleas, worms and diseases); they compete with humans for food and affect the health, welfare and way of life of people; they can destroy buildings (termites) and are a major cause of land degradation (noxious weeds, rabbits, feral pigs, etc). Pests were reported to be a major nuisance around our homes (prickles in the lawn, flies, etc). Activity of pests greatly increased the costs of farming (Brown et al., 2002)

Pesticides may be derived from inorganic sources (copper, sulphur), natural organic sources (plants) or synthesized in a laboratory. Many of these synthesized products mimic the activity of natural organic compounds

Without the use of farm chemicals, the production and quality of food would be severely jeopardized. It was estimated that food supplies would immediately fall to 30 to 40% due to the ravages of pests (Anon, 1990 and 1992). Only part of the problem is distribution and the ability to pay for purchases. Regardless of political ideology, environmental issues are becoming paramount in all economic systems from the very poor to the very rich. India, one of the world’s largest agricultural economies, is no exception. Demand for chemical pest control and resulting negative externalities expanded greatly during the last four decades (Chand et al., 1997).
Three decades ago, intensive and extensive cultivation of high yielding varieties of crops was introduced in India to increase food grain production. India has an agrarian economy, where the 1012.4 million population was dependent on agricultural commodities from 124.07 million hectares cropped area cultivated by 110.7 million producers (Prasad, 2001). The consumption of pesticides in India since 1955 is shown in Figure 1.1.

![Growth rate during 1955-56 to 1969-70: 18.42%](image)

**Figure 1.1: Consumption of pesticides in India**

The new crop varieties and cropping sequences, especially monocropping, along with injudicious and indiscriminate use of pesticides created many problems. Several hitherto unimportant pest species began to cause economic losses. Others, especially polyphagous insects, precipitated national problems (Srinivasa, 1993). One glaring example is the old world bollworm, *Helicoverpa armigera* (Noctuidae: Lepidoptera), which caused 66 per cent reduction in the yield of seed cotton in Andhra Pradesh State during the 1987-88-crop season. Previously, this pest seriously affected rabi crops such as chickpea and was commonly known as the gram pod borer. Similar outbreaks were reported for other
insects: Whitefly in 1984-85 and in 1985-86 caused damage to crops in South Indian states, and tobacco caterpillar in 1977-78 and in 1979-80 destroyed crops in the states of Tamil Nadu and Gujarat (Arunakumara, 1995). The agrochemical industry in India now produces 47,020 metric tons of pesticides (Directorate of Plant Protection and Quarantine (DPPQ), 2002). Although pesticide consumption in India was low (around 500 g per ha) compared to other countries like Japan (12 kg per ha) and Germany (3 kg per ha), problems in India resulting from unregulated use were quite alarming. The predominant classes of pesticides used in India (2001-02) are presented in Figure 1.2.

Figure 1.2: Pesticide consumption by different crops in India
1.4.1 Classification of pesticides

In agriculture the use of pesticides starts from the pre-sowing stage. Pesticides are broadly classified into five major groups (a) Insecticides (b) Fungicides (c) Rodenticides (d) Herbicides and (e) Fumigants. The predominant classes of pesticides used in India (during 2000-01) were insecticides, accounting for 61 per cent of total consumption, followed by fungicides (19 %) and herbicides (17 %). In India, most pesticide use is on cotton (45%), followed by rice (22%). The broad groups are further classified in terms of chemical identity and site of action (Bass, 1994). The classification of pesticides according to chemical identity is given in Figure 1.3. In terms of chemical identity all the five broad types of pesticides are classified into organic and inorganic pesticides. In terms of site of action the pesticides are classified as (a) Stomach poisons (b) contact poisons (c) Systamatic poisons (d) Fumigants (e) Repellents and (f) Attractants. This classification helps in relating various specialized products with the specific uses. (Figure 1.3).
Figure 1.3: Schematic representation on classification of pesticides according to chemical identity (Srivastava and Patel, 1990)
1.4.2 Advantages of using pesticides

Use of agro chemicals is an economical way of controlling pests. They require low labour input and allow large areas to be treated quickly and efficiently. It was estimated that for every dollar a farmer spends on farm chemicals he receives $4 return (Anon, 1990).

Production per labour unit has increased while production costs and energy inputs were lower. A suitable farm chemical is available for most pest problems with variations in activity, selectivity and persistence. The best product can be chosen for the situation. This allowed more flexibility in management options and better timeliness of pest control. Farm chemicals ensure a plentiful supply and a variety of high quality, wholesome food at a reasonable price. Modern society demands nutritious food free from harmful organisms and blemishes. Ornamental horticulture also requires unblemished and pest-free plants and flowers. This would be very difficult without farm chemicals. Farm chemicals are frequently used to prevent pest problems from occurring, e.g. preventing weeds in gardens and lawns; treatment of export and import produce to prevent the spread of pests; treatment of stored products to prevent pest attack and destruction during storage (Subra and Waters, 1993). Without farm chemicals the treatment of spiders, cockroaches, fleas on pets, etc, would be most difficult. If no farm chemicals were available to control environmental pests like noxious weeds, feral animals, etc, our environment would have suffered very badly and the food grain production would have been much lower. Use of herbicides to control crop weeds reduced the need for cultivation, thus reducing land degradation (Colston, 1990).

1.4.3 Adverse effects of pesticides

Despite their many advantages, there are some potential hazards and risks when using farm chemicals. These risks may be associated with all chemicals whether they be industrial chemicals, pesticides, household products or even natural chemicals found in the environment. Undesirable side effects of farm chemical use usually stems from a lack of understanding of the impact of the chemical on the environment, compounded by
indiscriminate and overuse of the product. Non-target organisms, including predators and parasites of pests, could also be affected by chemical application (Spencer and Cliath, 1975). The reduction of these beneficial organisms could result in changes in the natural biological balances. Losses of honeybees and other pollinating insects could also be a problem. During application the pesticides caused severe damage and residue problems in crops, livestock, waterways and the general environment. Care in the methods of application and the weather conditions under which it was carried out can reduce drift. Environmental pollution from careless application and runoff resulted in wildlife and fish losses. Residues in food for humans and feed for livestock were the consequence of direct application of a chemical to the food source, and biomagnification of the chemical along the food chain (USEPA, 1998).

1.4.4 Fate of pesticides in the Environment

Main contribution of pesticides for the pollution of environment was from their use in the agricultural fields and from wastewaters released out of pesticide manufacturing industries without appropriate treatment. Pesticides transportation after they were applied to agricultural land involved several simultaneous processes, including emission, wash off, degradation, sorption/desorption, volatilization, leaching, runoff, and plant uptake (Tinsley, 1979). It is important to examine those components to gain insights into the pesticide movement in the environment. Pesticide emission is the releasing process of pesticides into the air from agricultural fields. Following pesticide application to agricultural lands as spray or soil incorporation, the pesticide are emitted from plant canopy or soil surface to the atmosphere. The amount of pesticide emissions usually depended on the vapor pressure and heat of vaporization of the chemicals, the partition coefficient between the atmosphere and any other phase and the air flow mass which transported the airborne chemicals (Haque and Freed, 1974). In addition to these factors, pesticide emission was also affected by the method of pesticide application (Figure 1.4).
Considerable research work was carried out to estimate the emission factors of different pesticides (Berdowski et al., 1997; Li et al., 2000; Scholtz et al., 1997 and 1998; Bass and Huygen, 1992; Baas, 1994; Baart et al., 1995). Pesticides applied on leaves or crop residues were subject to interception by the plant material. These pesticides remain on the plant surface until they were removed by rainfall or volatilization. The wash off of these pesticides amended the pesticide load in the soil. Thus the amount of pesticide available for movement by runoff and sediment was altered. Several studies were conducted on the movement of pesticides from plant material (Martin et al., 1978; Baker and Sheirs, 1985; Willis et al., 1986).

Pesticides and other organic molecules in soils can be adsorbed/desorbed by soil particles. Adsorption refers to the adhesion or attraction of the ions or molecules to the surface of the particles. The degree of adsorption and desorption between the soil and the pesticide influenced the bioactivity, leachability, and degradability of these chemicals in a given environment, and affected their distribution through the soil profile. Adsorption was affected by soil characteristics such as content of organic matter, pH, particle size distribution of the soil, temperature, and moisture content (Dao and Lavy, 1978; Wauchope and Myers, 1985). Pesticide characteristics such as molecular structure,
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electrical charge, and solubility might also affect pesticide adsorption (Sonon and Schwab, 1995; Helling, 1971; Moreale and Van Bladel, 1980). Volatilization is the diffusion of pesticides through the soil to the soil surface and the movement of a pesticide into and through the atmosphere. Volatilization of pesticides was observed to depend on the vapor pressure and heat of vaporization of the chemical, the partition coefficient between the atmosphere and any other phase, and the air flow mass which, transported the airborne chemical (Haque and Freed, 1974; Azevedo, 1998). In addition to these factors, pesticide volatilization was affected by the modes of pesticide application. The spray was more susceptible to volatilization than soil incorporation (Spencer and Cliath, 1975; Hamaker, 1972; Spencer et al., 1973). Volatilization of a pesticide from soil was controlled by many variables, including temperature, soil properties, water content of the soil and nature of the pesticide – particularly its solubility and its degree of adsorption (Hamaker, 1972; Spencer et al., 1973).

Whether a pesticide reached the ground water depended not only upon its movement through the soil, but also upon its disappearance from the soil. If the rate of degradation was sufficiently rapid compared to the rate of leaching, the chemical disappeared before it could reach the ground water and, therefore, would not pose that environmental problem (Hamaker, 1975).

Pesticides carried by surface runoff from agricultural areas constituted a significant portion of the pesticide pollutant loading of surface water bodies. The transport of pesticides in surface runoff depended on the form in which the compounds existed in the water and the hydrodynamics of the system (Larson et al., 1997; Huber and Heaney, 1982; Reckhow et al., 1985).

For most pesticides, the potential for surface loss or leaching to groundwater depended mainly on the half-life, solubility, and adsorption coefficient. In general, pesticides with a
long half-life had a higher potential of reaching surface or groundwater because they were exposed to the hydrologic forces for a longer period of time (Thomas et al., 1992). Pesticides, which were insoluble or have high adsorption coefficients tend to remain near the soil surface and are more susceptible to surface loss. Soluble pesticides with low adsorption coefficients had high leaching potentials (Alexander, 1999).

Soil type is one of the major factors determining how much water percolates through the soil profile and how much runs off the surface (Figure 1.5). The permeability of the soil determined the potential for water transport through the soil profile. This could be inferred from soil texture (USEPA, 1989; Williams et al., 1988; Wagenet et al., 1989; Walker and Porter, 1989). The USDA Soil Conservation Service had developed a data base which rates soils for their potential for surface loss and pesticide leaching capacity (Harold, 1990).

1.5 Treatment methods of pesticides

A suitable treatment method was developed depending on the nature of the pesticide and its persistence in the environment (Frere, 1975). Pesticide degradation refers to the
breakdown of pesticides within the environment. Pesticides were susceptible to photochemical, chemical, and microbial decomposition (Figure 1.6).

Figure 1.6: Pesticide movements in the environmental matrices (Frere, 1975)

1.5.1 Chemical degradation

Chemical degradation, including hydrolysis, oxidation, reduction and ionization, occurred through the presence of excess acidity or alkalinity, and was therefore related to pH. As the soil pH became extremely acidic or alkaline, microbial activity decreased, but such conditions might result in increased chemical degradation (Soulas, 1982).

1.5.1.1 Hydrolysis

Hydrolysis is the breaking of bonds in a molecule due to reaction with water. Typically a compound was altered in a hydrolytic reaction by the replacement of some chemical group of the compound with a hydroxyl group. The hydrolysis reactions were catalyzed by the presence of hydrogen or hydroxide ions, and hence the reaction rate was strongly dependent on the pH of the system. Neely, (1985) listed several functional groups that
were susceptible to hydrolytic reactions, including amides, carbamates, carboxylic acid esters, epoxides, lactones, phosphoric acid esters, and sulfonic acid esters.

1.5.2 Photochemical degradation

Photochemical degradation (photolysis) is the degradation process whereby radiant energy in the form of photons breaks the chemical bonds of a molecule (USEPA, 1987). It involves direct absorption of photons by the molecule. Indirect photolysis involves the absorption of energy by a molecule from another molecule that absorbed the photons. Mill and Mabey (1985) described the types of photolysis reactions affecting a variety of compounds including chloroaromatics, ketones, and aldehydes. Generally, the factors affecting photochemical degradation of pesticides include the intensity of the sunlight, length of exposure, the application site and/or method, and the chemical properties of the pesticide.

1.5.3 Biological degradation

Biological degradation was the result of microbial metabolism of pesticides, and was often the main method of pesticide degradation in soils (Haque and Freed, 1974). It occurred when fungi, bacteria, and other microorganisms in the soil use pesticides as food or other energy source and consume the pesticides along with other sources of food or energy. Experimental parameters viz., soil content of organic matter, moisture, temperature, extent of aeration, and pH affect microbial degradation (Sanseverino et al., 1994). Microbial activity was high in warm, moist soils with neutral pH. Depending on the specific chemical, the biological degradation might be very fast due to the presence of enzymes; for other compounds the process might be very slow. Various kinetic formulations were proposed, including first- and second-order forms. The rate coefficients were known to be a function of temperature, pH, and available nutrients. The second-order kinetic formulations described the degradation rate as a function of the
concentration of the compound and the size of the bacterial population, which was changing as the compound degraded. A variety of organic compounds could be subjected to biodegradation and a discussion was provided by Klecka, (1985). Pesticide degradation, as affected by various soil parameters, was studied by several researchers. Kells and co-authors, (1980) and Moreale, (1980) studied the influence of soil pH and soil temperature, which affected the rates of both microbial growth and death, influenced the rate of pesticide degradation (Figure 1.7). This relationship was expressed in terms of the Arrhenius equation, which held that the degradation rate always increased with increasing temperature. However, the pesticide-degradation decreased as temperature increased, if the temperature was optimum (Li, 2001).

The effects of soil moisture and temperature on the degradation of carbofuran and 2,4-D was reported by Ou and co-authors, (1982) and Parker and Doxtader, (1983) respectively. Zhang and co-authors, (2002) and Parker and Doxtader, (1983) also examined the kinetics of pesticide degradation at constant temperature and varied temperatures.

Figure 1.7: Breakdown of Pesticides in soil matrix (Klecka, 1985)
Although many consumers were concerned about ingestion of pesticides with food, only a few of the synthetic organic compounds manufactured were studied in plant uptake investigations (Kaufman, 1983). The most important factors were the plant species, growth stage, and intended use (Finlayson and MacCarthy, 1973). Soil characteristics such as pH, temperature, clay fraction, moisture content, and particularly content of organic matter also influenced the uptake of pesticides by plants (Finlayson and MacCarthy, 1973). In addition, the type of pesticide, the pesticide formulation, the method of application, and the mode of action affected plant uptake (Kaufman, 1983; Kirkwood, 1983). Biological treatment was used primarily to remove the biodegradable organic substances (colloidal or dissolved) in wastes. Basically, these organic substances were converted into gases that might escape to the atmosphere, and into biological cell tissue that could be removed by settling (Najeeb, 2003).

The biological treatment methods are broadly classified into aerobic and anaerobic methods.

1.5.3.1 Aerobic Biological Treatment

Organic waste was introduced into a reactor where an aerobic bacterial culture carries out the conversion by oxidation. The aerobic environment in the reactor was achieved by the use of diffused or mechanical aeration. The final end products were oxidized to very low energy levels that result in more stable end products without any damage to the environment and without creating a nuisance conditions. Composting was considered as one of the aerobic biological treatment methods of solid waste (Tchobanglous and Burton, 1991). The major parameters influencing aerobic (composting) process are strength of organic wastes, polymeric structure of waste, dissolved oxygen control, moisture content of the waste, pH and the degree of mixing efficiency in the system.
1.5.3.2 Anaerobic Biological Treatment

Anaerobic decomposition of organic wastes involves metabolic processes that are less efficient than aerobic metabolism. Molecular oxygen and nitrate must not be present as electron acceptor. Sulfate (SO$_4^{2-}$), carbon dioxide and reduced organic compounds serve as electron acceptors in the process. The reduction of sulfate results in production of hydrogen sulfide (H$_2$S). Anaerobic treatment releases methane at the end of the reaction (Davis and Cornwell, 1991; Venkata Mohan et al., 2006a). The growth rate and metabolic rates of anaerobic bacteria are slow compared to aerobes. (Venkata Mohan and Sarma, 2003).

The degradation of organic compounds (can be expressed as COD or BOD) involves hydrolysis, acidogenesis, and methanogenesis phases, by combined activities of four kinds of anaerobic bacteria. The polymeric substrate like cellulose, protein and fat are firstly hydrolyzed by extra cellular enzymes of the cells of the microorganisms because of large size of the substrate (Najeeb, 2003).

1.5.3.3 Importance of biological treatment methods

Advantages of the biological treatment include reduced volume in the waste material, stabilization of the waste, destruction of pathogens in the waste material, and biogas for energy use. The end products of the biological treatment can, depending on its quality, be recycled as fertilizer and soil amendment. Efficient treatment method for waste requires substantial reduction of the content of organic materials, as well as other pollutants (Nitrogen, Phosphorus etc.). Biological treatment, in which bacteria degrade the organic compounds, was the most efficient and cost-effective wastewater treatment method. Important advantages of biological treatment are (Pittier and Chudoba, 1990).
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• Low capital and operating costs compared to those of chemical-oxidation processes

• Degradation of organics, to relatively safe compounds.

• Removal of reduced inorganic compounds, such as sulfides and ammonia, and total nitrogen removal possible through denitrification

• Operational flexibility to handle a wide range of flows and wastewater characteristics

Biological treatment technologies rely on the biodegradative activities of microorganisms and focus on enhancing existent but slow biodegradation processes in nature or technologies that bring chemicals into contact with microorganisms in some type of reactor that allows rapid transformation. In many instances the focus of attention is on existing sites of pollution and such technologies are encompassed by the term "bioremediation". The term is an apt one because a remedy is being applied to a problem.

1.6 Bioremediation

"Remediate" means to solve a problem, and "bio-remediate" means to use biological organisms to solve an environmental problem such as contaminated soil or groundwater (Alexander, 1996). In a non-polluted environment, bacteria, fungi and other microorganisms are constantly at work breaking down the organic matter. When waste water or soil containing an organic pollutant like oil is subjected to biological treatment, some of the microorganisms would die, while others capable of eating the organic pollution would survive. Bioremediation works by providing these pollution-eating organisms with fertilizer, oxygen, and other conditions that encourage their rapid growth (Potter et al., 1997; Sanseverino et al., 1994). These organisms would then be able to break down the organic pollutant at relatively faster rate.
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Bioremediation of a contaminated site typically works in one of the two ways. In the case described above, ways were found to enhance the growth of whatever pollution-eating microbes might already be living at the contaminated site (Strzempka et al., 1997; Wang and Bartha, 1994). In the second and less common case, specialized microbes were added to degrade the contaminants. Bioremediation provides a good cleanup strategy for some types of pollution, but as one might expect, it may not work for all (Venkata Mohan et al., 2006b). For example, bioremediation may not provide a feasible strategy at sites with high concentrations of chemicals that are toxic to most microorganisms. These chemicals include metals such as cadmium or lead, and salts such as sodium chloride.

Nevertheless, bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. Depending on the site and its contaminants, bioremediation might be safer and less expensive than alternative solutions such as incineration or landfilling of the contaminated materials (Lessard et al., 1995). It also had the advantage of treating the contamination in place so that large quantities of soil, sediment or water do not have to be dug up or pumped out of the ground for treatment. Bioremediation of contaminated sites is a new field of endeavor and many new developments in the field took place (Hyzy and Schepart, 1995).

The goal of bioremediation is to degrade the organic pollutants to concentration levels that are either undetectable or, if detectable, to concentrations below the limits established as safe or acceptable by regulatory agencies. Bioremediation was used for the destruction of chemicals in soils, ground water, wastewater, sludges, industrial waste systems and gases. Certain criteria must be met for bioremediation to be seriously considered as a practical means for treatment (Chang and Davis, 1976).
• Microorganisms must exist that have the needed catabolic activity.
• Those organisms must have the capacity to transform the compound at reasonable rates and bring the concentration to levels that meet regulatory standards.
• They must not generate products that are toxic at concentrations likely to be achieved during the remediation.
• The site must not contain concentrations or combinations of chemicals that are markedly inhibitory to the biodegrading species.
• The target compound(s) must be available to the organisms.
• Conditions at the site or in a bioreactor must be made conductive to microbial growth or activity.
• The cost of technology must be less expensive than other available technologies that can also destroy the chemical.

1.6.1 Principles of bioremediation

Microorganisms must be active and healthy in order for bioremediation to take place. Bioremediation technologies assist growth of microorganisms and increase microbial populations by creating optimum environmental conditions for them to detoxify the maximum amount of contaminants. The specific bioremediation technology used is determined by several factors, for instance, the type of microorganisms present, the site conditions, and the quantity and toxicity of contaminant chemicals. Different microorganisms degraded different types of compounds and survived under different conditions (Compeau et al., 1991). Indigenous microorganisms are those microorganisms that were found already living at a given site. To stimulate the growth of these indigenous microorganisms, the proper soil temperature, oxygen, and nutrient content might have to be provided (Barnes et al., 1994; DeFilippi, 1994).

If the biological activity needed to degrade a particular contaminant was not observed in the soil at the site, microorganisms from other locations, whose effectiveness was tested,
can be added to the contaminated soil. These are called *exogenous* microorganisms. The soil conditions at the new site should be adjusted to ensure that the exogenous microorganisms thrived (Gokcay and Onerci, 1994). Bioremediation can take place under aerobic and anaerobic conditions. In aerobic conditions, microorganisms use available atmospheric oxygen in order to function. With sufficient oxygen, microorganisms converted many organic contaminants to carbon dioxide and water (Tyagi and Couillard, 1991). Anaerobic conditions support biological activity in which no oxygen was present. The microorganisms break down the chemical compounds present in the soil to release the energy they need. Sometimes, during aerobic and anaerobic processes of breaking down the original contaminants, intermediate products that are less or more toxic than the original contaminants were formed. Literature survey on implementation of bioremediation techniques for treating different environmental matrices from toxic materials was consolidated and presented in Table 1.1. Bioremediation applications fall into two broad categories: *in situ* or *ex situ*. *In situ* bioremediation treated the contaminated soil or groundwater at the same place where contamination was detected. *Ex situ* bioremediation processes required excavation of contaminated soil or pumping of groundwater before they could be treated (Dablow et al., 1997).
Table 1.1: Treatment of some toxic chemicals using bioremediation techniques

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<th>Pollutant</th>
<th>Organisms or culture</th>
<th>Overall efficiency of degradation</th>
<th>Reference</th>
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<tbody>
<tr>
<td>2-Chlorophenol</td>
<td>Anaerobic sludge from a swine</td>
<td>&gt;90%</td>
<td>Chang et al., 2004; Chang et al., 2003</td>
</tr>
<tr>
<td>4-Chlorophenol</td>
<td>Bacterial consortium from rhizosphere of <em>Phragmites australis</em></td>
<td>70-100%</td>
<td>Caldeira et al., 1999; Carvalho et al., 2001</td>
</tr>
<tr>
<td>2,4-Dichlorophenol</td>
<td><em>Pseudomonas putids</em></td>
<td>~100%</td>
<td>Kargi and Eker, 2005</td>
</tr>
<tr>
<td>2,4,6-Trichlorophenol</td>
<td><em>Pseudomonas sp.</em>, <em>Rhodococcus sp.</em></td>
<td>~100%</td>
<td>Puhakka et al., 1995</td>
</tr>
<tr>
<td>2,3,4,6-Tetrachlorophenol</td>
<td><em>Pseudomonas sp.</em>, <em>Rhodococcus sp</em></td>
<td>~100%</td>
<td>Puhakka et al., 1995</td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td><em>Pseudomonas sp.</em>, <em>Rhodococcus sp</em></td>
<td>~100%</td>
<td>Puhakka et al., 1995</td>
</tr>
<tr>
<td>Pyrene, phenanthrene</td>
<td><em>Polaromonas sp.</em>, <em>Sphingomonas sp.</em>, <em>Alcaligenes sp.</em>, <em>Caulobacter</em> and <em>Variovorax sp.</em></td>
<td>~50% (pyrene) ~98% phenanthrene</td>
<td>Eriksson et al., 2002</td>
</tr>
<tr>
<td>O-Cresol, naphthalene, phenol, 1,2,3-trimethylbenzene</td>
<td><em>Pseudomonas fluorescens</em></td>
<td>Not determined</td>
<td>Vayenas et al., 2002; Yamaguchi et al., 1999</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>Providnecia stuartii, <em>Pseudomonas cepacia</em></td>
<td>~100%</td>
<td>Jin et al., 1998</td>
</tr>
<tr>
<td>Toulene</td>
<td>Secondary sludge from wastewater treatment plant; various aerobic and anaerobic bacteria</td>
<td>~84%; 65%</td>
<td>Parvatiyar et al., 1996; Arcangeli and Arvin, 1995</td>
</tr>
<tr>
<td>Acid Orange 10,14</td>
<td><em>Methylosinus trichosporium</em></td>
<td>~60%</td>
<td>Zhang et al., 2002</td>
</tr>
<tr>
<td>Everzol Turquoise Blue G</td>
<td><em>Coriolus versicolor</em></td>
<td>~82%</td>
<td>Kapdan and Kargi, 2002</td>
</tr>
<tr>
<td>MCPP; 2,4-D</td>
<td>Mixed culture of herbicide-degrading bacteria</td>
<td>MCPP (partial); 2,4-D (complete)</td>
<td>Oh et al., 1994</td>
</tr>
</tbody>
</table>
1.6.2 In Situ Bioremediation of Soil

In situ techniques do not require excavation of the contaminated soils so may be less expensive, create less dust, and result in release of smaller quantities of contaminants than ex situ techniques. Also, it is possible to treat a large volume of soil at once. In situ techniques, however, were found to be slower than ex situ techniques. They were most effective at sites with permeable (sandy or uncompacted) soil (Baker et al., 1994).

The goal of aerobic in situ bioremediation was to supply oxygen and nutrients to the microorganisms in the soil. Aerobic in situ techniques vary in the way they supply oxygen to the organisms that degrade the contaminants (Baldwin et al., 1997; Litherland and Anderson, 1997). Two such methods were bioventing and injection of hydrogen peroxide. Oxygen could be provided by pumping air into the soil above the water table (bioventing) or by delivering oxygen in the liquid form as hydrogen peroxide. In situ bioremediation did not work well in clays or in highly layered subsurface environments because oxygen cannot be evenly distributed throughout the treatment area (Buchanan et al., 1997). In situ remediation often required years to reach cleanup goals, and depended mainly on the biodegradability of the contaminants (Dybas et al., 1997; Loehr and Webster, 1997).

1.6.3 Ex Situ Bioremediation of Soil

Ex situ techniques were faster, easier to control, and used to treat a wider range of contaminants and soil types than in situ techniques (Anderson, 1995). However, they required excavation and treatment of the contaminated soil before and, sometimes, after the actual bioremediation step (Figure 1.8).
Introduction

The general principles of ex situ bioremediation are as described above. However, there are a number of specific techniques (Lynch and Genes, 1989).

- The construction of bio-piles involving the excavation of soils and the introduction of nutrients, moisture and aeration via pipework to promote biodegradation.

- Land farming comprises the excavation and spreading of the contaminated soils in a thin layer, which is then ploughed and tilled to improve aeration.

- Windrow turning involves the excavation of the contaminated soils, which are then formed into windrows of between 1m to 2 m in height. The windrows are turned over using agricultural machinery.

- Bio remediation in the slurry phase involves mixing of the contaminated soils with water, to which nutrients and/or oxygen is added (with pH control) followed by dewatering.

Generally these techniques have a beneficial effect on the soil structure (not slurry treatment) and are suitable for organic and cyanide contamination in granular soils.

The technology may be cost effective but it may not be applicable for some contaminants (eg metals) or with clay/silt soils where there is a high groundwater table. Some of the techniques require large process areas and long time periods to achieve acceptable concentrations. Ex situ techniques include solid-phase bioremediation and slurry-phase bioremediation.
1.6.3.1 Solid phase bioremediation

Solid-phase bioremediation is equipped with collection systems to prevent any contaminant from escaping the treatment. In this method, moisture, heat, nutrients and oxygen are controlled to enhance biodegradation of the substrate. The remediation may take longer times if the above parameters are not controlled properly. Solid phase systems are relatively simple to operate and maintain, require a large amount of space, and cleanups require more time to complete than with slurry-phase processes. Solid phase soil treatment processes include landfarming, soil biopiles, and composting (Gibson, 1984; Azad, 1976).
The soils are excavated and spread on a pad with a built-in system to collect any "leachate" or contaminated liquids that seep out of the contaminant soaked soil. The soils are periodically turned over to mix air into the waste. Aeration is provided by pulling air through the heap with a vacuum pump. Moisture and nutrient levels are maintained at levels that maximize bioremediation. The soil heaps can be placed in enclosures. Volatile contaminants were easily controlled since they were usually part of the air stream being pulled through the pile (Sayler, 1991; Walker and Keith, 1992). In some cases, reduction of contaminant concentrations were attributed more to volatilization than biodegradation (Venkata Mohan et al., 2004 and 2006b). When the process was conducted in enclosures to control escape of volatile contaminants, volatilization losses were minimized (Paul Cheremisinoff, 1990).

Biodegradable waste was mixed with a bulking agent such as straw, hay, or corncobs to make it easier to deliver the optimum levels of air and water to the microorganisms. Three common designs were static pile composting (compost was formed into piles and aerated with blowers or vacuum pumps), mechanically agitated in-vessel composting (compost was placed in a treatment vessel where it was mixed and aerated), and windrow composting (compost was placed in long piles known as windrows and periodically mixed by tractors or similar equipment) (Sayler, 1991; Walker and Keith, 1992).

1.6.3.2 Slurry phase bioremediation

The bioavailability of polluted soil compounds is strongly affected by a number of phenomena. Sorption of organic compounds onto mineral and organic matter surfaces, and micropores is an important one among them. When hydrocarbons remain in soil for some time, there is a time-dependent decline in bioavailability. This slow process, called aging, decreases the biodegradation rate or a complete resistance of the molecules to microbial degradation. Explanation of the desorption-biodegradation process by means of spiked compounds mislead the analysis, since those compounds did not behave similar
to aging compounds (Carmichael et al., 1997). Since desorption was the limiting step in the hydrocarbon biodegradation (Carmichael et al., 1997), it is necessary to improve mass transfer. This could be done by means of slurry bioreactors.

The concept of bioslurry phase reactor is a significantly recent area of interest to environmental scientists especially in bioremediation of soil. It utilizes naturally occurring bacteria (native microflora) or a specific strain which is inoculated having particular metabolic property to convert hazardous organic compounds to carbon dioxide and water present in the solid, liquid or solid-liquid interface under predefined optimized controlled conditions (Venkata Mohan et al., 2004 & 2006c). The objectives of bioreactor designing are (a) to alleviate microbial growth limiting factors in soil environment such as substrate, nutrients and oxygen availability, (b) to promote suitable environmental conditions for bacterial growth such as moisture, pH, temperature, and (c) minimize mass transfer limitations and facilitate desorption of organic material from the soil matrix. Slurry-phase treatment systems therefore offer special advantages for the treatment of contaminated soil, especially in areas where, for example, low environmental temperatures adversely affect biodegradation rates. Bioslurry phase reactors allow optimization of conditions conductive to biodegradation, viz., mixing facilities aeration and enhance the contact between the substrate, soil particles and the solution. Conditions can be further optimized for biodegradation by controlling pH and temperature and by providing nutrients and other specialized amendments, such as addition of surfactants (Troy, 1994). Bioslurry phase bioremediation involves the treatment of contaminated soil materials (soil, sediment, sludge) in a closed system (bio reactor in triphasic system involving three major components: water, suspended particulate matter and air. It is considered to be high rate biological system economically used for highly concentrated compounds in less contaminated area. Water serves as a suspending medium where nutrients, trace elements, chemicals used for pH adjustment and desorbed contaminants are dissolved. Suspended particulate matter includes a biologically inert substratum consisting of contaminants (soil particles) and biomass attached to soil matrix, which is
free in suspending medium and air, provides oxygen for bacterial growth. Biocatalysts employed for decontamination include indigenous microorganisms, specially selected microorganisms, which are capable of rapidly and efficiently degrading targeted pollutants. In general the rate and extent of biodegradation is more manageable and hence more predictable with bioreactors than with other bio treatment processes such as in situ and soil phase bioremediation. Use of slurry phase reactors is an innovation in soil bioremediation.

Slurry phase biological treatment could be a rapid process compared to other biological treatment processes, particularly for contaminated clays. The success of the process is highly dependent on the specific soil and chemical properties of the contaminated material. This technology is particularly useful where rapid remediation is a high priority.

A good design would mean that solids are kept in suspension and not allowed to settle. However high solid concentration reduces oxygen transfer rate. The configuration of bioslurry phase reactor was having significant influence on the slurry phase homogeneity and decontamination and detoxification process (Fava et al., 2000; Shailaja et al., 2006). Different modes of bioslurry operations were tested in the laboratory and pilot scale, which include batch systems (Jerger et al., 1994; Glaser et al., 1995; Zhang et al., 2000), cyclic batch mode/sequence batch reactor (Irvine et al., 1993; Chunlong et al., 2001; Cassidy and Irvine, 1997), continuous flow reactor (Geerdink, 1995), and tanks-in-series (Kleijntjens, 1991). Different modes of bioslurry operations were tested in the laboratory and pilot-scale, which include (Jerger et al., 1994; Glaser et al., 1995), soil slurry-sequencing batch reactor (SS-SBR) (Irvine et al., 1993), continuous stirred tank reactor (CSTR) (Geerdink, 1995), and tanks- in-series (Kleijntjens, 1991). SS-SBR and CSTR operations were also used (Eweis et al., 1998).

Oxygen requirement, oxygen transfer rate, particle size, solid liquid ratio, reactor design, operation conditions, type of inoculum, nature of substrate etc., have significant influence
Introduction

on the process of the bioslurry reactor performance. Batch reactors are more appropriate when the volume of the soil to be treated is relatively small and the contaminant present is relatively toxic and inhibitory to biodegradation. Reported data on slurry reactors used for the degradation of some of the toxic compounds are presented in the Table 1.2.

Table 1.2: Reported slurry reactors used for the degradation of toxic compounds

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pollutant</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,4,6-Trinitro Toluene</td>
<td>Arienzo et al., 2000</td>
</tr>
<tr>
<td>2</td>
<td>Tetra chloroethylene</td>
<td>Legg, 1983</td>
</tr>
<tr>
<td>3</td>
<td>Pyrene</td>
<td>Ramirez et al., 2001</td>
</tr>
<tr>
<td>4</td>
<td>Ozone decomposition</td>
<td>Lim et al., 2002</td>
</tr>
<tr>
<td>5</td>
<td>Naphthalene</td>
<td>Park et al., 2002</td>
</tr>
<tr>
<td>6</td>
<td>PAH</td>
<td>Kim et al., 2001;</td>
</tr>
<tr>
<td>7</td>
<td>Poly chlorobenzene</td>
<td>Baha, 2002</td>
</tr>
<tr>
<td>8</td>
<td>Chlopyriphos</td>
<td>Venkata Mohan et al., 2004</td>
</tr>
<tr>
<td>9</td>
<td>Flourene</td>
<td>Garibsu and Alkorta, 1999</td>
</tr>
<tr>
<td>10</td>
<td>Phenantherene</td>
<td>Woo et al., 2001</td>
</tr>
</tbody>
</table>

The first field-scale bioslurry process described in the literature that was used to remediate nitro aromatic soils was the sequencing anaerobic bioremediation process (SABRE). It was initially used to treat the nitroaromatic herbicide dinoseb (2,4- dinitro-6- sec butylphenol, (Crawford et al., 1995; Roberts et al., 1993; Stevens, 1989; USEPA, 1990 and 1995). Water was added to the excavated soil in bioslurry treatment and the reactor was stirred for proper mixing. Oxygen was provided with compressed air diffusers or surface agitators. Slurry reactor designs included mixing-tank, airlift, fluidized bed, rotating drum, and lagoon (Cookson, 1995). Petroleum hydrocarbons, solvents, polycyclic aromatic hydrocarbons (PAH), pesticides, and pentachlorophenol and associated chlorinated aromatics used in wood preservation were successfully treated by bioslurry technology (USEPA, 1990; Cookson, 1995; Eweis et al., 1998).
Bioslurry systems were maintained under aerobic or anaerobic conditions and inoculum was added if the soil being treated had low populations of organisms capable of degrading the chemicals interest. (Black et al., 1994). This technique is known as bioaugmentation. In this technique microorganisms were added to enhance a specific biological activity. It had been in practice for years in a number of areas, including agriculture and forestry (Jasper, 1994) and wastewater treatment (Rittmann and Whiteman, 1994; Portier et al., 1988; Pritchard, 1992).

1.7 Bioaugmentation

Bioaugmentation may be defined as the addition of pregrown microbial cultures to enhance microbial populations at a site to improve contaminant clean up and reduce clean up time and cost. Biodegradation was the major process affecting natural attenuation of contaminants (Alan and Taloat, 2003; Venkata Mohan et al., 2005. During the process contaminants were metabolized into less toxic or non-toxic compounds by naturally occurring organisms (Maxwell and Baqai, 1995; Neralla et al., 1995). Often these natural processes were slow and there was a requirement to increase the rate of biodegradation. Bioaugmentation is the addition of native or non-native microbial cultures or “inocula” to the matrix to enhance or replace the native microbial population. Indigenous or native microbes were those that occur naturally at a site (Shailaja et al., 2006). They are usually present in very small quantities and may not be able to prevent the spread of the contaminant. In some cases, native microbes do not have the ability to degrade a particular contaminant. Bioaugmentation offered a way to provide specific microbes in sufficient numbers to complete the biodegradation (Shin and Crawford, 1995; Briglia, 1994; Riggle, 1995; Dave et al., 1995; Lamar et al., 1994). The re inoculation of soil with indigenous microorganisms directly isolated from the same soil was also carried out and was included in the term bioaugmentation (Phelps et al., 1994; Otte et al., 1994; Venkata Mohan et al., 2006c).
For compounds that were considered relatively recalcitrant but generally 'available', bioaugmentation was demonstrated to be beneficial (e.g. 2,4,6-trinitrotoluene (Shin and Crawford, 1995), carbon tetrachloride (Dybas et al., 1995; Witt et al., 1995). Bioaugmentation provides certain advantages over biostimulation in cases where pollutant toxicity or lack of appropriate microorganisms (both quantity and quality) are important. Success of bioaugmentation depended on the understanding of the bioavailability of the pollutant, the survival and activity of the added microorganism(s) or its genetic material, and the general environmental conditions that control soil bioremediation rates (Vogel, 1996).

Bioaugmentation was a subject of several reviews in the literature. The recent review by Gentry and co-workers, 2004 was probably the most up to date; this provided an extensive survey of new bioaugmentation strategies. The authors emphasized the methodology and the tools developed recently to increase the persistence and the activity of exogenous microorganisms and/or genes after their introduction into the environment (Lendvay et al., 2003; Silva et al., 2001; Bouchez et al., 2000).

1.7.1 Bioaugmentation options

The concept of introduction of non-indigenous microorganisms into different natural or engineered environments is not entirely new. It is being practiced in agriculture and in some wastewater treatment sequences. However, bioaugmentation remains, to a large extent, an experimental methodology for in situ bioremediation of contaminated sites. There were only a few well documented field applications cited in the literature (Dybas et al., 2002; Major et al., 2002), as the vast majority of reports in the literature dealt with laboratory-scale (microcosm) or demonstration-scale (mesocosm) studies. Several possibilities exist for the introduction of exogenously grown strains, either singly or in the form of consortia, into a polluted environment slated for bioremediation. One of the most common options, tested primarily in the laboratory, was the addition of a pre-
adapted pure bacterial strain as, for example, in the case of inoculation of wastewater treatment plants or soils with chloroaromatic degraders (Dybas et al., 2002; Fantroussi et al., 1999; Lalthier et al., 2005). Second option was the addition of pre-adapted consortia, as in the case of addition of degrading enrichment cultures to contaminated soils (Lendvay et al., 2003; Da Silva and Alvarez, 2004; Goux et al, 2003); Third one was the introduction of genetically engineered bacteria to avoid the accumulation of potentially toxic or dead-end intermediates of target pollutants, as in the case of hybrid strains that were constructed to ensure simultaneous mineralization of both chloro- and methyl-aromatics (Moslemy et al., 2002; Erb et al., 1997). Yet another one was the addition of biodegradation-relevant genes that were packaged in a vector in order to transfer by conjugation into microorganisms already present in the biotope under remediation (Dejonghe et al., 2001). Using as criteria the type of biotope targeted (e.g. soil, activated sludge or groundwater/aquifer), the pollutant(s) slated for treatment, and the form of the biotic vehicle used (e.g. pure strain, consortium or gene vector), a tabulation is provided (Table 1.3) that contains the most characteristic studies that have appeared in the past few years.

A recent report (Da Silva and Alvarez, 2004) showed successful bioaugmentation using anaerobic consortia to enhance the degradation of benzene–toluene–ethylbenzene–xylene (BTEX)–ethanol mixtures under methanogenic conditions that were characteristic of sites contaminated by ethanol-blended gasoline. Significant benzene biodegradation (up to 88%) was observed only in the column bioaugmented with the benzene-enriched methanogenic consortium, and this removal efficiency was sustained for one year (Da Silva and Alvarez, 2004).
### Table: 1.3: Different bacteria used for bioaugmentation technique in remediating toxic substances

<table>
<thead>
<tr>
<th>Pollutant</th>
<th>Genera</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenanthrene</td>
<td>Acromonas</td>
<td>Ding et al., 2002</td>
</tr>
<tr>
<td>Flourene, phenanthrene</td>
<td>Arthrobacter</td>
<td>Cadellas et al., 1997</td>
</tr>
<tr>
<td>Naphthalene, phenanthrene, pyrene</td>
<td>Bacillus</td>
<td>Watanabe, 2001</td>
</tr>
<tr>
<td>Fluoranthene, pyrene, chrysene, coronene, benz[a]anthracene</td>
<td>Burkholderia</td>
<td>Juhasz et al., 1997; Watanabe, 2001</td>
</tr>
<tr>
<td>Pyrene, chrysene</td>
<td>Cycloclasticus</td>
<td>Watanabe, 2001</td>
</tr>
<tr>
<td>Pyrene, chrysene</td>
<td>Flavobacterium</td>
<td>Watanabe, 2001</td>
</tr>
<tr>
<td>Naphthalene, phenanthrene, fluoranthene, pyrene, chrysene, benz[a]anthracene</td>
<td>Mycobacterium</td>
<td>Kelley and Cerniglia, 1995; Schneider et al., 1996</td>
</tr>
<tr>
<td>Chrysene</td>
<td>Rhodococcus</td>
<td>Watanabe, 2001</td>
</tr>
<tr>
<td>2,3-dimethlnaphthalene, fluorene</td>
<td>Sphingomonas</td>
<td>Siddiqi et al., 2002</td>
</tr>
<tr>
<td>Fluoranthene, chrysene, benz[a]anthracene, pyrene</td>
<td>Stenotrophomonas</td>
<td>Juhasz et al., 2000; Watanabe, 2001</td>
</tr>
<tr>
<td>Naphthalene, phenanthrene</td>
<td>Vibrio</td>
<td>Hedlund and James, 2001</td>
</tr>
<tr>
<td>Naphthalene, phenanthrene</td>
<td>Streptomyces</td>
<td>Chun et al., 2001</td>
</tr>
</tbody>
</table>

However, recent studies on bioaugmentation were encouraging and thus bioaugmentation might well be used as a rational methodology for site remediation, subject to a thorough understanding of the site's ecology and of the local physicochemical constraints. Under such circumstances, bioaugmentation could emerge as one of only a few environmentally friendly techniques available for pollution abatement. (Fantroussi et al., 2005). Keeping in view the importance of degradation of toxic compounds in the environment, the present studies have been undertaken. The following chapter gives a bird's eye view of the literature survey on pendimethalin and also presents the objectives of the research work.